อนุกรมวิธานและซิสเทแมติกส์ของไส้เดือนดิน
สกุล *Amynthas* Kinberg, 1867 และ *Metaphire* Sims and Easton, 1972 ในประเทศไทย

นางสาวเอื้องฟ้า บรรเทาวงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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TAXONOMY AND SYSTEMATICS OF TERRESTRIAL EARTHWORM GENERA *Amynthas* Kinberg, 1867 AND *Metaphire* Sims and Easton, 1972 IN THAILAND

Miss Ueangfa Bantaowong

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	TAXONOMY AND SYSTEMATICS OF TERRESTRIAL EARTHWORM GENERA *Amynthas* Kinberg, 1867 AND *Metaphire* Sims and Easton, 1972 IN THAILAND
By	Miss Ueangfa Bantaowong
Field of Study	Biological Sciences
Thesis Advisor	Professor Somsak Panha, Ph.D.
Thesis Co-Advisor	Professor Samuel James, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science
(Associate Professor Polkit Sangvanich, Ph.D.)

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.....Chairman
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.....Thesis Co-Advisor
(Professor Samuel James, Ph.D.)

.....Examiner
(Assistant Professor Tosak Seelanan, Ph.D.)

.....Examiner
(Assistant Professor Chirasak Sutcharit, Ph.D.)

.....Examiner
(Piyoros Tongkerd, Ph.D.)

.....External Examiner
(Professor Visut Baimai, Ph.D.)

เอื้องฟ้า บรรเทาวงษ์ : อนุกรมวิธานและซิสเทแมติกส์ของไส้เดือนดินสกุล *Amynthas* Kinberg, 1867 และ *Metaphire* Sims and Easton, 1972 ในประเทศไทย (TAXONOMY AND SYSTEMATICS OF TERRESTRIAL EARTHWORM GENERA *Amynthas* Kinberg, 1867 AND *Metaphire* Sims and Easton, 1972 IN THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร.สมศักดิ์ ปัญหา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.แซมูเอล เจมส์, 147 หน้า.

ได้ทำการศึกษาและวิเคราะห์อนุกรมวิธานและซิสเทแมติกส์ของไส้เดือนดินสองสกุลหลักในวงศ์ Megascolecidae ในประเทศไทย จากรายงานก่อนหน้านี้ยืนยันจำนวนทั้งสิ้น 29 ชนิด โดยพบ 18 ชนิดในสกุล *Amynthas* และพบ 11 ชนิดในสกุล *Metaphire* จากการเก็บตัวอย่างเพิ่มเติมอีก 123 พื้นที่ และตรวจสอบลักษณะสัณฐานสำคัญได้แก่ ลักษณะของช่องเปิดเพศผู้ ปุ่มยึดขณะผสมพันธุ์ ถุงเก็บสเปิร์ม อวัยวะสืบพันธุ์เพศผู้และต่อมสร้างน้ำเลี้ยงเซลล์สืบพันธุ์เพศผู้ ต่อมลูกหมาก และไส้ติ่ง ทำให้สามารถจำแนกได้เพิ่มจำนวนขึ้นอีกรวมเป็น 44 ชนิด และยังเป็นชนิดที่ยังไม่บรรยายลักษณะ 3 ชนิด ในจำนวนนี้มีที่รายงานครั้งแรกในประเทศไทย 2 ชนิด คือ *Amynthas andersoni* (Michaelsen, 1907) และ *A. corticis* (Kinberg, 1867) และพบไส้เดือนที่มีการแพร่กระจายกว้างอยู่ 3 ชนิด คือ *A. corticis* (Kinberg, 1867), *A. morrisi* (Beddard, 1892) และ *A. gracilis* (Kinberg, 1867) นอกจากนี้ยังได้ทำการบรรยายลักษณะของไส้เดือนดินเพิ่มเติมอีก 14 ชนิด ประกอบด้วยสกุล *Amynthas* 7 ชนิด และสกุล *Metaphire* 7 ชนิด อีกทั้งยังได้ทบทวนการบรรยายลักษณะของชนิดต้นแบบของสกุล *Amynthas* คือ *Amynthas aeruginosus* Kinberg, 1867 ปัจจุบันไส้เดือนดินทั้งสองสกุล *Amynthas* และ *Metaphire* มีสมาชิกโดยรวมเป็น 26 และ 18 ชนิด ตามลำดับ

ผลการวิเคราะห์โดยใช้ลำดับเบสของยีน COI และยีน ITS2 โดยใช้ไส้เดือนดินที่ยืนยันได้ 27 ชนิด ประกอบด้วย ไส้เดือนในสกุล *Amynthas* 15 ชนิด และสกุล *Metaphire* 12 ชนิด ซึ่งเกือบจะสอดคล้องกับการจัดจำแนกโดยใช้สัณฐานวิทยา นอกจากนี้ยังสังเกตเห็นความเป็นไปได้ของการมีสปีชีส์ซ่อนเร้นในไส้เดือนชนิด *Metaphire houlleti* ซึ่งจำแนกได้สามกลุ่มสปีชีส์ ความสัมพันธ์ทางวิวัฒนาการในกลุ่มไส้เดือนสกุล *Amynthas* และ *Metaphire* นั้น ยังไม่สามารถอธิบายได้ชัดเจน ทั้งสองสกุลไม่แสดงผลแบบชาติพันธุ์เดียวแต่เป็นแบบหลายชาติพันธุ์ หรือแบบคู่ขนาน อย่างไรก็ตามความสัมพันธ์ที่ใกล้ชิดสามารถพบเห็นได้ในบางชนิด เช่น ชนิด *Amynthas thakhantho* มีความสัมพันธ์ใกล้ชิดกับ *A. turris* เช่นเดียวกับ *Metaphire peguana* และ *M. bahli* ที่มีความสัมพันธ์ทางวิวัฒนาการเป็นแบบชาติพันธุ์เดียว ดังนั้นการเพิ่มจำนวนตัวอย่าง พื้นที่ศึกษา และยีนอาจช่วยให้การวิเคราะห์ความสัมพันธ์ทางวิวัฒนาการมีความชัดเจนมากยิ่งขึ้น

สาขาวิชา วิทยาศาสตร์ชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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UEANGFA BANTAOWONG: TAXONOMY AND SYSTEMATICS OF TERRESTRIAL EARTHWORM GENERA *Amynthas* Kinberg, 1867 AND *Metaphire* Sims and Easton, 1972 IN THAILAND. ADVISOR: PROF. SOMSAK PANHA, Ph.D., CO-ADVISOR: PROF. SAMUEL JAMES, Ph.D., 147 pp.

Taxonomy and systematics of two main earthworm genera of the family Megascolecidae has been carried out and analyzed in Thailand. The previous reports confirmed the total number of 29 nominal species which 18 belonging to the genus *Amynthas* and 11 to the genus *Metaphire*. After making additional 123 collecting sites and carefully examining principal morphological characters such as the male pore area, spermathecae, genital markings, testes, seminal vesicles, prostate gland and intestinal caeca. The total number of 44 nominal species and three undescribed have been confirmed and reported. Of which, two are newly record to Thailand: *Amynthas andersoni* (Michaelsen, 1907) and *A. corticis* (Kinberg, 1867), while three are considered to be peregrine species. There are *A. corticis* (Kinberg, 1867), *A. morrisi* (Beddard, 1892) and *A. gracilis* (Kinberg, 1867). In additional, fourteen species have been described containing seven from each genus. The re-descriptions of the type species of the genus *Amynthas*, *A. aeruginosus* Kinberg, 1867 has also been written. The total number has finally been clarified as 26 and 18 species of the genera *Amynthas* and *Metaphire* respectively.

The phylogenetic analysis using COI and ITS2 genes has been analyzed and using 27 operational taxonomic units (OTUs), consisting of 15 species of *Amynthas* and 12 species of *Metaphire*. The results were largely congruent with morphological identified species. However, some possible cryptic speciation has been noticed in *Metaphire houlleti* species complex, which probably contains at least three distinct species. Phylogenetic relationships between the two genera still have not been resolved clearly. The relations are not monophyly, but interpreted as polyphyly and/or paraphyly groups. However the closed relationships have been found in many cases such as *Amynthas thakhantho* and *A. turris*; *Metaphire peguana* and *M. bahli* which showing monophyletic relationships. The adding taxa and sampling localities and also genes may be interpreted the better distinct phylogenetic analysis.

Field of Study: Biological Sciences
Academic Year: 2015

Student's Signature .....
Advisor's Signature .....
Co-Advisor's Signature .....

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CHAPTER 1
Introduction

Terrestrial earthworms are belonged to a major class Oligochaeta, containing members that occupy broad ranges of habitat, and are especially abundant in terrestrial environments. This group show a high diversity, which comprises of more than one third of annelid members at approximately 15,000 species. The principal characteristics are cylindrical body with bilateral symmetrical, all external segments bearing with setae except the first two segments. Hermaphrodite with cross fertilization through reciprocal copulation is the well-known behavior in normal earthworms. A remarkable part of the body calls “clitellum” is one of the important portion. It is a thickened, swollen epidermis, and develops in the shape of a ring or saddle around a specific part of the anterior part of the body when the worm becoming maturity, and cocoons production for covering fertilized eggs before egg laying is the main function of clitellum. (Edwards, 2004).

In terrestrial ecosystems, earthworms play very important roles in the soil quality improvement such as increasing pores for aerating, enhancing microbial activities in increasing organic matters. The main activities of earthworms, e.g. feeding and casting affect the soil involving ingestion of soil and mixing of the main soil ingredients of mineral particles and humus; the production of castings of a fine crumb structure which are ejected on the soil surface by some species; the construction of burrows that enhance aeration, drainage and plant root penetration; and the creation of suitable habitats for the smaller scale soil fauna and micro-organisms (Edwards and Bohlen, 1996; Lavelle et al., 2004; Lee, 1985).

The location of Thailand is in the heart of a biodiversity hotspot, however the terrestrial earthworms in Thailand have been poorly surveyed. The classical research by Gates in 1972 reported more than 170 species in Burma, of which the genera *Amynthas* and *Metaphire* are dominant containing almost half of the existing species.

James (2004) estimated the occurrences of 250 species in the Philippines and also estimated over 200 Thai indigenous species compared with 55 recognized species to date (Bantaowong et al, 2014, 2015a, 2015b). The genera *Amynthas* Kinberg, 1867 and *Metaphire* Sims and Easton, 1972 are the most diverse genera which belong to the family Megascolecidae, and are the most dominant genera in Thailand. The current records confirmed 26 and 18 species respectively occurring in many areas. However, the classification of both genera is still problematic because most of the distinguished characters of the two genera are still overlapping. The classical diagnostic character to distinguish the two genera is the occurrence of copulatory pouches of the male pores, present only in *Metaphire* but absent in *Amynthas*. Various species show wide variation in reproductive organ structures, which are the key characteristics for taxonomic analysis, for examples the location and number of genital markings, the male pores region, form of spermathecae, prostate glands. Somatic characters such as hearts and intestinal caeca are also used. Recently molecular analysis has been widely used for taxonomy and phylogenetic studies in many animals, including earthworms. Some species of *Amynthas* have been re-assigned into *Metaphire* by using of morphology-based taxonomy together with molecular data.

In this study, traditional morphology-based taxonomy and systematics of the genera *Amynthas* and *Metaphire* have been compared with DNA sequence data from mitochondrial and nuclear genes.

Literature Review

Over the centuries approximately 5,000 species of earthworms have been described, and the estimated total global species diversity exceeds 7,000 species (Fragoso et al., 1999; Lavelle and Lapied, 2003; Reynolds, 1994). The genera *Amynthas* and *Metaphire* are the most diverse groups belonging to the family Megascolecidae. They are dominant in Asia and Southeast Asia, consisting more than 400 and 200 species respectively (Sims and Easton, 1972). The two genera are known to be occurred sympatrically in a wide range of habitats and soils and often making systematic

controversy (Bantaowong et al., 2011a; Bantaowong et al., 2011b; Prasankok et al., 2013).

Comprehensive taxonomic surveys of Asian oligochaetes, particularly terrestrial earthworms, had been initially conducted by Stephenson (1923) with a publication under the monograph series 'Fauna of British India'. Later, Gates (1972) made some revision and reported more than 170 species of earthworms from Burma and adjacent areas. The first study of Thai earthworms was recorded by Stephenson (1931) Stephenson in 1931. *Pheretima hupbonensis* (=*Amynthas hupbonensis*) from Chonburi province was described. Later, Gates (1939) listed 24 nominal species from various parts of Thailand i.e. Chiang Rai, Chiang Mai, Phrae, Lumphun, Yala and Bangkok. The publication of Gates in 1972 also included 27 species from Thailand. Moreover Blakemore (2006) updated a checklist of Thai earthworms and confirmed of 28 recognized species.

Some Thai scientists have conducted earthworm taxonomic studies and gradually published some papers and reports as follows: Kosavititkul (2005) reported 13 species from Khao Yai National Park, of which six species are from the genus *Amynthas* and three species are from the genus *Metaphire* with three unidentified species and the exotic *Pontoscolex corethrurus*; Chantaravisoot (2007) reported 40 terrestrial earthworm species collected from various parts of Thailand and investigated some available museum specimens. Ten species are from the genus *Amynthas*, 28 species are from the genus *Metaphire* and two are from the genus *Pithemera*. However, there were many specimens still unidentified. Somniyam and Suwanwaree (2009) recorded 21 species from Nakhon Ratchasima province including seven *Amynthas*, five *Metaphire*, three species of *Drawida* (Moniligastridae), three exotic species of genus *Dichogaster* (Benhamiidae) and many still unidentified. The very recent records are from Blakemore (2011) who described *Amynthas siam* from Sakon Nakhon province; Bantaowong et al. (2011a, 2011b, 2014) described a species of *Metaphire* and eight species of the genus *Amynthas* from some areas of north and northeast Thailand. Some *Amynthas* species were incorporated as the first record of Thai earthworms in

the International Barcode of Life (IBOL). The current record of Thai terrestrial earthworms species is 45 nominal species, of which *Amynthas* contains 23 species and 11 species belonging to the genus *Metaphire*.

The classification of the two genera is still problematic due to morphological similarity. Sims and Easton (1972) noticed that the preservation may cause evagination of copulatory pouches and, therefore, a false resemblance to the superficial male pores of *Amynthas*. These authors assumed to be a taxon belong to *Amynthas* unless copulatory pouches are proven; several members of the *Metaphire hilgendorfi* species-complex are provisionally removed from *Amynthas* based on these characters (Blakemore, 2003). Moreover, some earthworms are also characterized as parthenogenetic based on the absent of male organs which is a particularly big problem in complexes of very similar species containing both amphimictic and parthenogenetic species (Blakemore, 2003; Blakemore et al., 2006; Cosin et al., 2011). stated that several synonyms added up the possibility of the occurrence of species-complexes which may be involved with parthenogenetic effects. Even though the fully developed reproductive organs showed no sign of male sterility, many authors are still not confident for the separation of taxa because they tend to vary intraspecifically (Blakemore, 2002; Chang et al., 2007; Gates, 1972). The more collecting events in several different localities and many more specimens with various study dimensions are needed to be carried out in order to solve these questions.

Molecular techniques have been widely used for phylogenetic analysis in all organisms including the Oligochaeta. Some molecular markers such as COI and 16S rRNA are appropriate genes that most experts have used for phylogenetic analysis and interpretation of earthworm evolutionary relationships. There are some recent studies that proved this to be an effective technique for systematic clarification for examples: Chang and Chen (2005) compared morphological differences between *Amynthas yuhsii* and *A. formosae*, and also used cytochrome c oxidase subunit I (COI) to analyse the status of the two species. They suggested the distinct reproductive isolation of the two species and also suggested to move them to the genus *Metaphire*. Huang et al. (2007)

attempted to analyse the partial COI sequences from 86 specimens of 28 Chinese earthworm species using 566 bps portion of the mitochondrial genome to serve as DNA barcode which supposed to be reliably discriminate species of earthworms. They concluded that the DNA barcode is a powerful tool for identifying earthworm species and also be a great assistant for traditional morphological taxonomy. Chang et al. (2008) investigated *Metaphire formosae* species group by using COI, 16S rRNA and ND1 sequencings. The results indicated that the 13 morphotypes of the *Metaphire formosae* species group formed a clade, including a cryptic species.

Furthermore, James et al. (2010) used the DNA barcoding for evaluation of cryptic diversity in *Lumbricus terrestris* which later designated a neotype for *Lumbrucus terrestris* and a lectotype of *L. herculeus*. Minamiya et al. (2011) clarified the phylogenetic relationships of *Amynthas vittatus* with different degree of degraded reproductive organs by using mitochondrial DNA sequencing.

Rationale

The terrestrial earthworm genera *Amynthas* Kinberg, 1867 and *Metaphire* Sims and Easton, 1972 are the most diverse groups in Southeast Asia belonging to the family Megascolecidae which reported occurring in almost the same localities and habitats. They are quite well-known for local people in utilizing both worm genera in various ways such as improvement of soil quality, decomposing, organic fertilizers production. The diagnostic taxonomic characters are copulatory pouches locating near the male pores in *Metaphire*, but these characters are superficially occurred in *Amynthas*.

Since Thai earthworms have been initially studied and reported by Gates in 1939. The members in genera *Amynthas* and *Metaphire* are majority which sometimes making classification problematic with the congeneric from other nearby regions as published in such Malaysia and Laos (Hong, 2010; Hong et al., 2014). The two genera are important for ecosystem analysis as well as zoogeographic analysis, and many species have been suggested as Southeast Asian origination or even Thai born species.

Morphological taxonomy is becoming complicated after making large scale surveys in various geographic areas. Species complex is showing evolutionary in progress of both common genera. To solve those mentioned problems, molecular phylogeny using DNA sequencing has been designed after morphological identification, and after molecular phylogeny results, the morphological revisit are expected to critically observe. The new multidisciplinary results will improve the systematic interpretation of the two common earthworm genera.

Objectives

1. To revise the taxonomy of the terrestrial earthworm of the genera *Amynthas* and *Metaphire* in Thailand using morphological characteristics.

2. To reconstruct the molecular phylogeny of the earthworm genera *Amynthas* and *Metaphire*.

Anticipated benefit

A reliable taxonomy and systematics is crucial to ensuring sustainable harvest of exploited earthworm species and to distinguish alien species. These will be of great benefit to agro-ecosystem in Thailand.

CHAPTER 2

Four new species of the earthworm genus *Amynthas* Kinberg, 1867 with redescription of the type species (Clitellata: Megascolecidae)

Ueangfa Bantaowong[1,2], Pattana Somniyam[3], Chirasak Sutcharit[2], Samuel W James[4] & Somsak Panha[2*]

[1] Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

*[2] Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; Email: somsak.pan@chula.ac.th (*corresponding author)*

[3] Faculty of Agriculture, Uttaradit Rajabhat University, Uttaradit 53000, Thailand

[4] Department of Biology, University of Iowa, Iowa City, Iowa, USA 52242

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Abstract

The type species of the genus *Amynthas* Kinberg, 1867 was re–investigated using type specimens from Biozentrum Grindel und Zoologisches Museum, University of Hamburg and Swedish Museum of Natural History. Also we described four new species of the genus *Amynthas* from Thailand, two species of the *aelianus* species group, namely *A. arenulus* Bantaowong & Panha, new species and *A. longicaeca* Bantaowong & Panha, new species, and two species of the *corticis* species group, *A. thakhantho* Bantaowong & Panha, new species and *A. phucheefah* Bantaowong & Panha, new species, *Amynthas arenulus* and *A. thakhantho* occur in sandy habitats of dry dipterocarp forest and deciduous respectively, and especially in the modified highland paddy fields, while the following two species, *A. longicaeca* and *A. phucheefah* occur in deciduous forest reserve areas in clay–rich topsoil.

Key words. *Amynthas*, Clitellata, Systematics, Biodiversity, Thailand

Introduction

The terrestrial earthworm genus *Amynthas* Kinberg, 1867 is the largest genus of the family Megascolecidae, containing 22 species groups with over 400 recognized species but also large numbers of synonyms (Blakemore, 2004, 2007; Sims and Easton, 1972). After visiting Biozentrum Grindel und Zoologisches Musuem, University of Hamburg, Germany in September 2010 and September 2011, we had the chance to study most of the type specimens including the type species *Amynthas aeruginosus* Kinberg, 1867, and we also studied a type specimen of *A. aeruginosus* sent from Swedish Museum of Natural History on loan.

In Thailand, the current records of the genus *Amynthas* confirmed 19 described species occurring in many areas, whereas a total of 41 recognized species of terrestrial earthworms have been reported (Bantaowong et al., 2011a; Blakemore, 2006, 2011; Gates, 1939, 1972; Kosavititkul, 2005; Somniyam and Suwanwaree, 2009). In this paper we describe four new *Amynthas* species collected recently from various parts of

Thailand (Fig. 2.1), which were firstly classified in *aelianus* and *corticis* species groups, characterized by spermathecal pores in 6/7–8/9 and 5/6–8/9, respectively. The addition of these new species brings to 45 the total number of terrestrial earthworm species known so far from Thailand. The four new species are described by U. Bantaowong and S. Panha, who are the taxonomic authorities for *A. arenulus* Bantaowong & Panha, new species, *A. longicaeca* Bantaowong & Panha, new species, *A. thakhantho* Bantaowong & Panha, new species, and *A. phucheefah* Bantaowong & Panha, new species.

Materials and Methods

The earthworms collected were anesthetized in 30% (v/v) ethanol, fixed in 10% (v/v) formalin, and preserved in 75% (v/v) ethanol. The descriptions are based on observations of dorsal dissections. Dimensions and segment counts are from clitellate adult specimens under the Olympus SZX7 stereoscopic light microscope. Illustrations were made of the body segments and the distinct external characters and internal organs.

The type specimens of the type species of *Amynthas* from Swedish Museum of Natural History (SMNH), Stockholm, Sweden, were critically examined. The specimens and type specimens of the *corticis* and *aelianus* species groups housed at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH) have been critically studied and also used to compare with the new species in this report. We have carefully studied in detail all morphological characters, made new illustrations and redescribed the type species of the genus *Amynthas* in this paper.

Holotype and paratype specimens of the new described species were deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ). Additional paratypes will be deposited in the Natural History Museum (NHMUK), London, at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH), and the Raffles Museum of Biodiversity Research Collection, Singapore (ZRC).

Anatomical abbreviations: fp, female pore; gm, genital marking; gmg, genital marking gland; ic, intestinal caeca; mp, male pores; pg, prostate gland; sc, spermathecae; sp, spermathecal pores; sv, seminal vesicles.

Systematics

Family Megascolecidae Rosa, 1891

Genus *Amynthas* Kinberg, 1867

Type species. *Amynthas aeruginosus* Kinberg, 1867, by monotypy.

Diagnosis. Perichaetine megascolecids with large number of setae distributed around segmental equators. Clitellum annular in XIV–XVI. Female pore single, medio–ventral in segment XIV. Spermathecal pores small or large, usually paired, intra– or intersegmental within the range 4/5–8/9. Male pores paired in segment XVIII, superficial on porophore, copulatory pouches absent. Genital markings present or absent. Dorsal pores present. Ovaries paired in XIII. Spermathecae usually paired. Nephridia usually absent from the spermathecal ducts. One gizzard, behind septum 7/8. Intestine begins in or behind XV. Intestinal caeca present usually originating in XXVII. Single pair of racemose prostate glands with duct joining vas deferens within body cavity. Mostly holandric, rarely proandric or metandric. Seminal vesicles small to large in one or two of segments XI–XII, and sometimes XIII. Last pseudohearts in XIII. Genital marking glands stalked or sessile, present or absent, usually associated with genital markings.

Amynthas aeruginosus Kinberg, 1867

Fig. 2.2

Amyntas aeruginosus Kinberg, 1867: 101. Type locality: Guam, sub lapidibus prope rivulum. Michaelsen, 1899a: 434–437, fig. 2. Michaelsen, 1899b: 4.

Perichaeta aeriginosus: Beddard, 1891: 278.

Pheretima aeruginosa: Michaelsen, 1900: 253.

Amynthas aeruginosus: Sims and Easton, 1972: 211.

Material examined. A holotype was not designated, and the original description was based on more than one specimen (see Kinberg, 1867: 101; Michaelsen, 1899). The syntype lot from the Swedish Museum of Natural History consists of 4 specimens in ethanol. The complete and sexually mature specimen, which is similar to the original description and a later description in Michaelsen (1899a: 434, fig. 2.2) is designated here as the lectotype SMNH 154.1 (Figs. 2.2A–C). The other three specimens, two complete and immature, and one fragmented and mature specimen from the same lot are recognized as the paralectotypes SMNH 154.2. In addition, we examined one specimen originally from the Kinberg type series, which was examined by Michaelsen (1899a: 434) and housed in Biozentrum Grindel und Zoologisches Museum, University of Hamburg. It is now recognized as paralectotype ZMH V5221 (Figs. 2.2D–F). This designation is to preserve the stability of the name and verify the unique characters of the type species, which were not mentioned in the original description.

Diagnosis. Quadrithecal with spermathecal pores in 7/8/9. Male pores paired, superficial in segment XVIII, each pore situated in the center of small round flat disc, genital markings lacking. Each spermatheca a large transversely oval sac and tubular diverticulum, its proximal end with a neck–like constriction. Intestinal caeca simple. Multilobed prostate gland with stout duct.

Description of lectotype. Dimension; 95 mm by 4.5 mm at segment X; body cylindrical with 99 segments. Setae regularly distributed around segment equators, numbering 49 at VIII, 57 at XX, 4 between male pores, setae formula AA:AB:ZZ:ZY=1.3:1:1:1 at XIII. Clitellum annular XIV–XVI, setae absent. Single female pore at XIV. Prostomium epilobic. Male pores are superficial in segment XVIII, 0.17

circumference apart ventrally; distance between male pores 2.5 mm, each pore situated in the center of small round flat disc. Genital markings absent. Spermathecal pores two pairs in 7/8–8/9, 0.16 circumference apart ventrally, distance between spermathecal pores 2.3 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, simple and extend anteriorly to XXV. Typhlosole rudimentary. Lymph glands not observed. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles paired in XI–XII. Prostate glands paired in XVIII, multi–lobed radiating fan–like from ental end of prostatic duct; ducts stout, thick muscular. Ovaries in XIII. Spermathecae two pairs in VIII and IX, ampulla large sac–shaped, diameter slightly greater than ampulla axis length, with a stout stalk, as long as ampulla. Diverticulum tubular, its proximal end with a neck–like constriction and iridescent.

Variation. The lectotype measures 95 mm body length, with 99 segments; the body lengths of four paralectotypes are 84, 85, 92, 100 mm, with 92, 94, 95, 96 segments.

Remarks. Michaelsen (1899) records the history of *A. aeruginosus* type series, which composed of 5 spirit-preserved specimens. The adult and complete syntype specimen was examined and was used for the redescription of internal characters, but it was not designated as a lectotype (ICZN, 1999; Michaelsen, 1899). We have examined all the type specimens and found the pseudo hearts and gizzard of Michaelsen's dissected specimen were missing. Therefore another complete adult specimen of Kinberg's collection is here designated as the lectotype.

The description and measurements given herein are in agreement with those of Kinberg (1867) and Michaelsen (1899). However, the species description published by Sims and Easton (1972) are slightly incongruence by having genital markings and 0.22 circumference apart ventrally of male pores. This different is clearly due to an expansion of the description to include another two nominal species, *A. upoluensis*

(Beddard, 1887) and *A. rennellanus* (Gates, 1959), which were recognized as junior synonyms (see Lee, 1981; Sims and Easton, 1972). Currently, these two nominal species are widely accepted as separate species (Blakemore, 1997; Gates, 1937; Gates, 1959; Michaelsen, 1913). *Amynthas rennellanus* differs from *A. aeruginosus* by having single, presetal genital markings in some of IX–XI and XVII–XXI while, *A. upoluensis* from Western Samoa is distinct from *A. aeruginosus* in the presence of genital markings on VII–VIII and XVII–XIX.

Amynthas aeruginosus is still known only from the type specimens. Michaelsen (1899) provided a brief illustration only of a spermatheca, but not other important features such as the male genital field, prostate glands and intestinal caeca. In this study, the completed description with schematic figures of the external ventral view and internal dorsal view are provided.

***Amynthas arenulus* Bantaowong & Panha, new species**

Figs. 2.3, 2.11A, B, 2.12; Table 2.1

Material examined. Holotype: One semi-clitellate (CUMZ 3299) Ban Khok Pho, Prasat, Surin, Thailand, (14° 33’ 5.33”N, 103° 22’ 21.79”E), 172 m in elevation, coll. S. Panha, U. Bantaowong, C. Sutcharit, R. Chanabun & W. Siriwut, 15 October 2012. 16 paratypes: 10 adults (CUMZ 3300), 2 adults (ZMH), 2 adults (NHMUK), and 2 adults (ZRC), same collection data as for holotype.

Other material examined. 2 adults (CUMZ 3301), Khao Sala Temple, Buachet, Surin, Thailand, (14° 25’ 9.7”N, 103° 56’ 0.7”E), 340 m in elevation, 16 October 2012. 3 adults and 1 juvenile (CUMZ 3302), paddy field in Num Yuen, Ubon Ratchathani (road no. 2248, about 18 km from Kantharalak, Sisaket), Thailand, (14° 28’ 10.1”N, 104° 52’ 33.2”E), 191 m in elevation, 17 October 2012. 5 adults (CUMZ 3303), Kaeng Lam Duan Waterfall, Nam Yuen, Ubon Ratchathani, Thailand, (14° 26’ 6.2”N, 105° 06’ 17.0”E), 164 m in elevation, 17 October 2012.

Diagnosis. Large; length 267–465 mm. Male pores paired, superficial in segment XVIII, each on large transversely elliptical disc, male aperture inconspicuous, genital markings absent. Spermathecal pores paired in segments 6/7–8/9, Spermathecae large bulb–shaped ampulla, small tube–like diverticulum adherent to ampulla on its entire length. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, its duct flanked by large sessile glandular masses on body wall.

Etymology. The specific epithet is from the Latin for a sandy place. This referred to the find–grained sandy area modified as a highland rice paddy system, which is the habitat of new species.

Description of holotype. Dimensions; 465 mm by 13.3 mm at segment VII, 15.7 mm at segment XX, 14.5 mm at clitellum; body cylindrical with 176 segments. Setae regularly distributed around segmental equators, numbering 68 at VII, 101 at XX, 26 between male pores, setae formula AA:AB:ZZ:ZY=1:1:2:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with setae.

A pair of male pores located ventro-laterally in XVIII, 0.41 circumference apart ventrally, distance between male pores 18 mm, porophores large transversely elliptical discs, surrounded by elevated rim. The indistinct male apertures located at the outer edges of each porophore. No genital markings are observed. Three pairs spermathecal pores, transverse slits, in furrows 6/7–8/9, ventral, distance between each pair about 0.38 body circumference ventrally apart, distance between spermathecal pores 15 mm. No genital markings in the spermathecal pore area.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and bend to XXIII. Typhlosole simple fold one–fourth lumen diameter, begins in XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles

paired in XI–XII, are large. Prostate glands well developed, large, extending anteriorly to segment XVII, posteriorly to XXII. Prostate duct flanked by large sessile glandular masses on body wall.

Ovaries in XIII. Three pairs spermathecae in VII–IX. Ampulla large sac–shape, duct relatively stout, diverticulum adherent to duct and ampulla on its entire length, slender with a thin stalk, chamber a dilated, elongate bulb.

Variation. The holotype measures 465 mm body length with 176 segments; the sixteen paratypes range in size from 267–340 mm (±26.62) body length with 133–169 segments.

Distribution. Surin, Sisaket and Ubon Ratchathani.

Habitat. They live in the sandy top soil at about 20–30 cm depth, in a highland paddy system modified from dipterocarp forest. Some forest patches are still present near the paddy fields.

Remarks. *Amynthas arenulus* new species is sexthecal with spermathecal pores in 6/7–8/9. The *Amynthas* species with these characters were formerly classified in the *sieboldi* species group (Sims and Easton, 1972), however Easton (1981) transferred *A. sieboldi* (Horst, 1892) to the genus *Metaphire*, so the species group name is no longer appropriate. Thus James et al. (2005) have critically investigated and proposed the *aelianus* species group after *A. aelianus* (Rosa, 1892), to replace the *sieboldi* species group name. This is one of many modifications of the group names of Sims and Easton (1972); among those are the use of confirmed senior synonyms *corticis* species group (replacing *diffringens*) and *gracilis* species group (replacing *hawayanus*).

The *aelianus* species group consists of more than 50 species and also included nine recently described species from Taiwan and one species from Thailand (Blakemore, 2011; James et al., 2005; Shen et al., 2003; Sims and Easton, 1972; Tsai et

al., 2010; 1999). In Thailand, only 2 species within this species group were reported from northeastern Thailand, *A. fucosus* (Gates, 1933) and *A. siam* Blakemore, 2011. However, *A. arenulus*, new species is distinguished from the above related species in Thailand by the larger body with no genital markings while *A. fucosus* has two pairs genital markings at 17/18, 18/19 and *A. siam* has single pair between the male pores.

Amynthas arenulus is also fairly similar to *A. osmastoni* (Michaelsen, 1907) from Burma, and *A. burchardi* Michaelsen, 1899 from Sumatra, in body size, but it is easily distinguished by having no genital markings, whereas the latter two species have genital markings. Moreover, the distance between the male pores as a fraction of the estimated circumference of segment XVIII is 0.25 and 0.28 in *A. osmastoni* and *A. burchardi*, respectively while *A. arenulus* new species this distance is 0.41 body circumference for the holotype. In addition, *A. arenulus*, new species has unique spermathecae, the diverticulum being adherent to the duct–ampulla axis along its whole length, while in other *Amyntha*s species, the diverticulum is usually free of the duct and ampulla except at a single point of origin (Table 2.1).

***Amynthas longicaeca* Bantaowong & Panha, new species**

Figs. 2.4, 2.11C, D; Table 2.1

Material examined. Holotype: One adult (CUMZ 3304) Phu Lan Kha National Park, Nong Bua Daeng, Chaiyaphum, Thailand, (16° 00’ 01.70”N, 101° 52’ 35.40”E), 660 m in elevation, coll. S. Panha, R. Chanabun, P. Tongkerd and W. Siriwut, 17 September 2011. One adult paratype (CUMZ 3305), same collection data same as for holotype.

Diagnosis. Large; length 230–278 mm. Male pores paired, superficial in segment XVIII, somewhat convex, with a large crescent shape genital marking. Spermathecae three pairs, in segments VII–IX, ampulla oval, with duct shorter than ampulla. Diverticulum very small with ovate knob, length less than half of ampulla. Intestinal caeca long,

simple. First dorsal pore in 12/13. Prostate glands large, prostatic duct flanked by large sessile glandular masses on body wall.

Etymology. This species was named after the characteristic long intestinal caeca.

Description of holotype. Dimensions; 278 mm by 10.1 mm at segment VII, 9.2 mm at segment XX, 8.2 mm at clitellum; body cylindrical with 160 segments. Setae regularly distributed around segmental equators, numbering 53 at VII, 81 at XX, 25 between male pores, setal formula AA:AB:ZZ:ZY=1:1:2:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

A pair of male pores located ventro-lateral in XVIII, 0.40 circumference apart ventrally, distance between male pores 9 mm. Male pores superficial, somewhat convex, located between small crescent genital markings. The paired median markings large crescent shape, 3–4 setal intervals distant from male aperture and separated from each other midventrally by a distance about equal to 8 intersetal intervals. Spermathecal pores three pairs, transverse slits, in furrows 6/7–8/9, ventral, paired pores about 0.45 body circumference ventrally apart, distance between spermathecal pores 10 mm. No genital markings in the spermathecal pores region.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–12/13 thin. Gizzard large behind 7/8, intestinal origin in XV; the intestinal caeca simple and long located in XXVII to XXIV, when fully extended they reach as far forward as the prostate duct at XVIII. Typhlosole simple, one third intestinal diameter beginning at XXVII. No lymph glands observed. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles paired in XI–XII, large. Prostate glands well developed, separated into two major lobes, extending XVI–XXI. Prostate duct long, slender and hairpin shape, flanked by large sessile glandular masses on body wall.

Ovaries in XIII. Spermathecae three pairs in VII–IX. The ampulla large oval, with duct shorter than ampulla, diverticulum small ovate knob, stalk very short, nephridia present on diverticulum and on segmental chambers in VII and VIII.

Variation. The holotype measures 278 mm body length with 160 segments; the paratype is 230 mm long with 115 segments.

Distribution. The new species is known only from the type locality.

Habitat. Top soil at about 20 cm depth in the dipterocarp forest at elevation 660 meters of Phu Lan Kha National Park, Chaiyaphum, at pH 7, silt loam soil.

Remarks. *Amynthas longicaeca* new species is easily distinguished from the other two earthworms of the *aelianus* species group reported from Thailand, namely *A. fucosus* (Gates, 1933) and *A. siam* Blakemore, 2011 of 3–6 mm body width, while the current new species is about 10 mm in diameter. This new species is quite similar to *A. burchardi* from Sumatra and *A. osmastoni* from Burma in body dimensions but differs in having crescent shaped genital markings in the male pore region while *A. burchardi* has a group of small circular papillae in mid-ventral XVIII and *A. osmastoni* has genital markings in the spermathecal pore region (Table 2.1).

***Amynthas burchardi* Michaelsen, 1899**

Fig. 2.5; Table 2.1

Amyntas burchardi Michaelsen, 1899b: 88, fig. 14. Type locality: Bindjey Estate, Sumatra.

Amynthas burchardi: Sims and Easton, 1972: 237.

Material examined. Syntype from ZMH (V 3864. Fig. 2.5): One adult in ethanol which was dissected by Michaelsen.

Remarks. *Amynthas burchardi* differs from *A. arenulus* new species and *A. longicaeca*, new species by the having a mid-ventral group of 40 small circular papillae present in XVIII, which are related to the stalked genital marking glands. The spermathecal diverticulum is long and slender with an ovate sperm chamber (Table 2.1).

***Amynthas osmastoni* (Michaelsen, 1907)**

Fig. 2.6; Table 2.1

Pheretima osmastoni Michaelsen, 1907: 163, fig. 11. Type locality: Port Blair, South Andaman. Gates, 1972: 204.

Amynthas osmastoni: Sims and Easton, 1972: 237.

Material examined. Syntype from ZMH (V 7171. Fig. 2.6): Four adults in ethanol (two specimens dissected by Michaelsen).

Remarks. *Amynthas osmastoni* differs from *A. arenulus* new species and *A. longicaeca* new species by the presence of a group of small round genital papillae in transverse rows, postsetal, median in VIII or XII or XIII. It also differs by having nephridia on spermathecae and on the segmental chambers in VI–XIV. The sessile genital marking glands are another difference from the new species mentioned above (Table 2.1).

***Amynthas phucheefah* Bantaowong & Panha, new species**

Fig. 2.7; Table 2.2

Material examined. Holotype: One adult (CUMZ 3306), Phu Chee Fah, Thoeng, Chiang Rai, Thailand, (19°48’ 47.0”N, 100° 26’ 20.4”E), 1205 m elevation, coll. S. Panha, U.

Bantaowong, C. Sutcharit, P. Tongkerd, R. Chanabun, P. Pimvichai and P. Prasankok, 24 October 2008. 2 paratypes (CUMZ 3307) same collection data as for holotype.

Diagnosis. Large; length 215–314 mm. Male pores paired, superficial in segment XVIII. Small circular genital markings widely paired on 19/20–24/25. Spermathecal pores paired in segments 5/6–8/9, spermathecae large sac–shaped ampulla with nephridia on the duct, diverticulum long, loosely coiled, intestinal origin at XV, intestinal caeca simple, first dorsal pore in 12/13. Holandric. Prostate glands large, small sessile genital marking glands corresponding to external genital markings.

Etymology. This species was named after the type locality, Mt. Phu Chee Fah.

Description of holotype. Dimensions; 314 mm by 8.7 mm at segment VII, 8.2 mm at segment XX, 8.0 mm at clitellum; body cylindrical with 132 segments. Setae regularly distributed around segmental equators, numbering 71 at VII, 68 at XX, 19 between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

Male pores paired, located ventro-laterally in XVIII, 0.31 circumference apart ventrally, distance between male pores 6.5 mm. Male pores superficial on XVIII, each pore situated on a top of small and elongated oval papilla surrounded by 3–4 circular ridges. Genital markings widely paired on 19/20–24/25, in line with male pores, each one slightly elevated with a thick circular margin, and depressed in center. Single genital marking present on the left side of 25/26. Spermathecal pores four pairs, transverse slits, in furrows 5/6–8/9, ventral, distance between pairs about 0.3 body circumference ventrally apart, distance between spermathecal pores 10 mm. No genital markings on the spermathecal pores region.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestine begins in XV. Typhlosole simple fold one–quarter lumen diameter from

XXVII, the intestinal caeca originate in XXVII, simple and extend to XXIII. Oesophageal hearts four pairs in X–XIII. Holandric, testes in X and XI. Seminal vesicles small in XI and large asymmetrical in XII–XIV. Prostate glands extend from XVII to XXI. Prostate duct U shape. Genital marking glands small sessile, corresponding to each external genital marking.

Ovaries paired in XIII. Spermathecae four pairs in VI–IX. Ampulla large sac. Diverticulum long loosely looped, and tufted nephridia present on ducts of the spermathecae in VIII–IX.

Variation. The holotype is 314 mm long with 160 segments; the two paratypes are 215 and 227 mm long with 130 and135 segments, respectively.

Distribution. The new species is known only from the type locality. Our collections in nearby areas have found some earthworm species of the genus *Metaphire*.

Habitat. The new species exhibited swarming in October 2008 at 1,205 m asl, and most of them were juveniles. The reason is still unknown. We collected some specimens by chance. The worms emerged from the top soil of deciduous forest and tried to migrate across a road to another side of the mountain.

Remarks. *Amynthas phucheefah*, new species would have been classified in the *diffringens* species group in Sims and Easton (1972), but the group has been renamed as the *corticis* species group, which is the most diverse group of the genus *Amynthas* with more than 90 species names (Sims and Easton, 1972). Some previously recorded species from Thailand exhibit 4 pairs of spermathecal pores in 5/6–8/9. These are *A. alexandri* Beddard, 1900, *A. mekongianus* (Cognetti, 1922), *A. exiguus* (Gates, 1930), *A. manicatus* (Gates, 1931), *A. corticis* (Kinberg, 1867), *A. comptus* (Gates, 1932), and *A. longicauliculatus* (Gates, 1931). Within the *corticis* species group in Thailand, the first two species lack genital markings, while the current newly described species shows six

pairs of genital markings at the male pores lines. The three latter nominal species are smaller in body width (2.5–3 mm) compared with 8.7 mm of the new species and the last two species have genital markings in 18/19–20/21 and prostate glands small and confined to XVIII, and no nephridia on the spermathecal ducts while the new species has six pairs of genital markings in 19/20–24/25, prostate glands in XVII–XXI, and nephridia present on spermathecal ducts (Table 2.2).

***Amynthas thakhantho* Bantaowong & Panha, new species**

Figs. 2.8, 2.11E, F; Table 2.2

Material examined. Holotype: One adult (CUMZ 3308), Wat Tham Phra, Tha Khantho, Kalasin, Thailand, (16°51’ 57.10”N, 103° 15’17.60”E) 220 m in elevation, coll. P. Tongkerd, P. Pimvichai, B. Kongim & N. Nantarat, 15 October. 2010. 10 paratypes: 7 adults (CUMZ 3309), 1 adult (ZMH), 1 adult (NHMUK), and 1 adult (ZRC), same collection data as for holoype.

Diagnosis. Large; length 320–402 mm. Male pores paired, superficial in segment XVIII, each on a transversely oval, slightly raised areas, four pairs of postsetal genital markings on segments XVII, XIX, XX and XXI. Spermathecal pores paired in segments 5/6–8/9. Spermathecae large sac–shaped ampulla, diverticulum long, coil or zigzag. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, its duct long slender, paired sessile genital marking glands on XVII, XIX, XX and XXI.

Etymology. This species was named after Tha Khantho, Kalasin, the type locality of the new species.

Description of holotype. Dimensions; 332 mm by 11.1 mm at segment VII, 10.7 mm at segment XX, 10.5 mm at clitellum; body cylindrical with 198 segments. Setae regularly distributed around segmental equators, numbering 108 at VII, 105 at XX, 23

between male pores, setal formula AA:AB:ZZ:ZY=1:1:2:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

Male pores paired located ventro-laterally in XVIII, 0.27 circumference apart ventrally, distance between male pores 8.5 mm. Male pores, superficial, small transversely oval, slightly raised. Four pairs of genital markings, postsetal on segments XVII, XIX, XX and XXI, in line with male pores. Four pairs spermathecal pores in 5/6–8/9, ventro-lateral, depressed in furrows, almost invisible, distance between the pores about 0.27 body circumference ventrally apart, distance between spermathecal pores 8 mm. No genital markings in or near spermathecal segments.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestine begins at XV; the intestinal caeca originate at XXVII, simple and extend to XXIII. Typhlosole rudimentary. Lymph glands begin on dorsal intestinal wall of XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels in X and XI. Large seminal vesicles paired in XI–XII. Prostate glands divided into numerous main lobes, extending from the segment XVI to segment XIX. Prostate duct long, slender with hairpin bend. Sessile genital marking glands present corresponding to each external genital marking in XVII, XIX, XX and XXI.

Ovaries in XIII. Spermathecae four pairs in VI–IX. Large sac ampulla with a long slender duct, clearly marked off from ampulla. The diverticulum slender and long with elongated oval seminal chamber, the distal half of its stalk zigzag or coiled.

Variation. The holotype measures 332 mm body length with 198 segments; the nine paratypes range in size from 320–402 mm (±31.86) body length with segments varied from 196–207. The genital markings on segments XVII, XIX, XX are present in all individuals, with an additional pair in XXI (5), or unpaired in XVI (3), XXI (2), and XXII (1).

Distribution. The new species is known only from the type locality.

Habitat. Top soil at about 15 cm depth, at pH 7, loamy soil. Worms produce columnar or tower-like castings about 20 cm high and 4 cm diameter.

Remarks. *Amynthas thakhantho* new species is similar to *A. phucheefah* new species with regard to holandry, spermathecal pores in 5/6–8/9, and the body size, but differs in male pore structure, and number and arrangement of genital markings in the male field. *Amynthas thakhantho* has four pairs of postsetal genital markings on segments XVII, XIX, XX, XXI, whereas *A. phucheefah* new species has six intersegmental pairs on 19/20–24/25 (Table 2.2). Otherwise it also differs from other regional members of the *corticis* species group by a combination of body size and male field characters, as discussed for *A. phucheefah.*

***Amynthas jacobsoni* (Michaelsen, 1922)**

Fig. 2.9; Table 2.2

Pheretima jacobsoni Michaelsen, 1922: 34, Type locality: Indonesia.
Amynthas jacobsoni: Sims and Easton, 1972: 235.

Material examined. Syntype from ZMH (V 9294. Fig. 2.9): One adult in 70% ethanol deposited at Biozentrum Grindel Zoologisches Museum, University of Hamburg, Germany.

Remarks. *Amynthas jacobsoni* differs from *A. phucheefah* new species and *A. thakhantho*, new species by having a group of 6 small circular papillae present on male pores area, which correspond to the sessile genital marking glands (Table 2.2).

***Amynthas suctoria* (Michaelsen, 1907)**

Fig. 2.10; Table 2.2

Pheretima suctoria Michaelsen, 1907: 165, fig. 12. Type locality: Andaman Islands.
Stephenson, 1922: 434, fig. 1; 1923: 311, fig. 123.
Amynthas suctoria: Sims and Easton, 1972: 235.

Material examined. Syntype from ZMH (V 7168. Fig. 2.10): Three adults and one sub-adult in 70% ethanol deposited at Biozentrum Grindel Zoologisches Museum, University of Hamburg, Germany.

Remarks. *Amynthas suctoria* differs from *A. phucheefah* new species and *A. thakhantho*, new species by lacking of genital markings at male pores area, but having a large pair of sessile genital marking glands (Table 2.2).

Discussion

The descriptions of the new species of *Amynthas* are based on specimens collected on various occasions in various habitats. The four new species belong to two species groups and are relatively large compared to most Thai *Amynthas* species. Two species, *Amynthas longicaeca* new species and *A. phucheefah* new species were found in protected areas in which soils are still fertile containing clay–rich topsoil, but the other two species, *A. arenulus* new species and *A. thakhantho* new species were found in modified dipterocarp and deciduous forest areas used for rice and other economic plant cultivation. Soil textures are sandy in the cultivated areas with lower silt content, and farmers typically improve them by adding both organic and chemical fertilizers in each cropping season. Soil pH is close to 7 in both natural and modified areas. *Amynthas arenulus* has the largest body among the four new species, and as shown in Figure 2.11, it lives on the earthen barriers adjacent to paddy fields during cropping but moves to the paddy areas after harvesting. The area was formerly dipterocarp forest on sandy soil, but after modification the soil fertility is low. Individual casts deposits are usually separated by about 30–40 cm. The low mound-like casts exhibit the sandy soil texture of the sites and are the characteristic cast shape of this species

(Fig. 2.11B). The worms live in shallow topsoil at about 20–30 cm where mostly juveniles were collected during cultivating in rainy season (June to October). Adults appear during the dry season before harvesting (end of October to January). Although juveniles are quite tolerant of chemical agriculture, the trend of organic farming probably poses fewer challenges to earthworm populations. Worms can consume organic matter such as crop residues or materials added by the farmers, enhancing its decomposition rate, and the transport and dispersion of the organic matter, and enrichment of the soil microorganisms through casting. In addition, while the worms move throughout the soil they mechanically improve the soil aeration property, and so improve soil fertility for the next crop cycle.

Amynthas thakhantho also occurs in agricultural areas, formerly deciduous forest which produces a different leaf litter quality from dipterocarp forest. However after conversion to agriculture the soil lost clay particles and became more sandy. This does not seem to deter the earthworms of these areas. The photos in Figure 2.11E and F show *A. thakhantho* is numerous in the farm lands cultivated according to organic farming protocols. The unique tower casts are spaced about 30 to 50 cm apart. The other two new species were found in nature reserve areas in which soil conditions are still unmodified from original, pH close to 7 and high in clay. *Amynthas longicaeca* lives in dry dipterocarp forest with abundant leaf litter. It also produces tower-like cast mounds and the casts are separated from each other by 30 to 70 cm (Figs. 2.11C, D) within a grass community. We have no precise habitat information of *A. phucheefah*, because this species was collected during its annual emergence in October, 2008. Most emerged worms were juveniles. The location is in a nature reserve area under deciduous forest with fertile clay-rich soil. October is just the end of the rainy season and becoming dry. We suggest that worms move to the more humid areas in order to better survive the dry 5–6 months before the next rainy season.

Acknowledgments

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Table 2.1. The comparison of characters among *A. arenulus* new species, *A. longicaeca* new species and others *aelianus* species group in Thailand, Burma and Sumatra *A. burchadi* Michaelsen, 1899, *A. osmastoni* (Michaelsen, 1907), *A. fucosus* (Gates, 1933) and *A. siam* Blakemore, 2011

Characters	*A. arenulus*	*A. longicaeca*	*A. burchadi*	*A. osmastoni*	*A. fucosus*	*A. siam*
Body length (mm)	465	278	270	250–320	120	73
Body width (mm)	13.3	10.1	9	10–11	6	3
Segment number	176	160	126	126–148	114	nd
Spermathecal pores	6/7/8/9	6/7/8/9	6/7/8/9	6/7/8/9	6/7/8/9	6/7/8/9
First dorsal pore	12/13	12/13	13/14	12/13	12/13	12/13
Setal number						
vii	68	53	40	nd	nd	nd
xx	101	81	49	65–84	nd	nd
Between male pores	26	25	13	10–20	29	nd
Genital markings						
Preclitellum	absent	absent	absent	median in VIII	absent	absent
Postclitellum	absent	crescent shape	group of small circular	absent	paired at 17/18,	paired at XVIII
Spermathecae	large sac	oval	oval	spherical	flatten	spherical
Diverticulum	slender	small ovate	long slender	tubular	tubular and coiled	convoluted
Prostate gland	XVII–XXII	XVI–XXI	XV–XX	XV–XXIV	XVII–XX	nd
Intestinal caeca	simple	simple	simple	simple	simple	simple
Type locality	Thailand	Thailand	Sumatra, Indonesia	South Andaman Islands	Burma	Thailand

nd=no data

Table 2.2 The comparison of characters among *A. phucheefah* new species, *A. thakhantho* new species and others *corticis* species group in Thailand and nearby countries.

Characters	*A. phucheefah*	*A. thakhantho*	*A. alexandri*	*A. mekongianus*	*A. longicauliculatus*	*A. comptus*	*A. jacobsoni*	*A. suctoria*
Body length (mm)	314	332	145	1 meter	170	>86	137	75–135
Body width (mm)	8.7	11.1	4–9	8	7	6	7	4.5–7
Segment number	132	198	133	370	138	>120	90	103–123
Spermathecal pores	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9
First dorsal pore	12/13	12/13	12/13	10/11	12/13	12/13	nd	12/13
Setal number								
vii	71	108	33–43	96–114	63–71	41	nd	60-70
xx	68	105	58–72	96–106	96	89–102	nd	75
Between male	19	23	13	10	29	31	3	2
Genital markings								
Preclitellum	absent	absent	absent	absent	absent	absent	absent	absent
Postclitellum	paired on	paired on XVII,	absent	absent	paired on 18/19–	three trios on	cluster on XVIII	absent
Spermathecae	large sac	large sac	oval	sacciform	spherical	nd	oval	pear-shaped
Diverticulum	loosely looped	zigzaged	moniliform	clavate	saccular	slender	intimately	bent
Prostate gland	XVII–XXI	XVI–XIX	XVII–XX	XVIII	XVIII	XVIII	XVII–XX	XVII–XIX
Intestinal caeca	simple	simple	simple	simple	simple	simple	simple	simple
Type locality	Thailand	Thailand	India	Mekong river,	Burma	Burma	Indonesia	Andaman

nd=no data

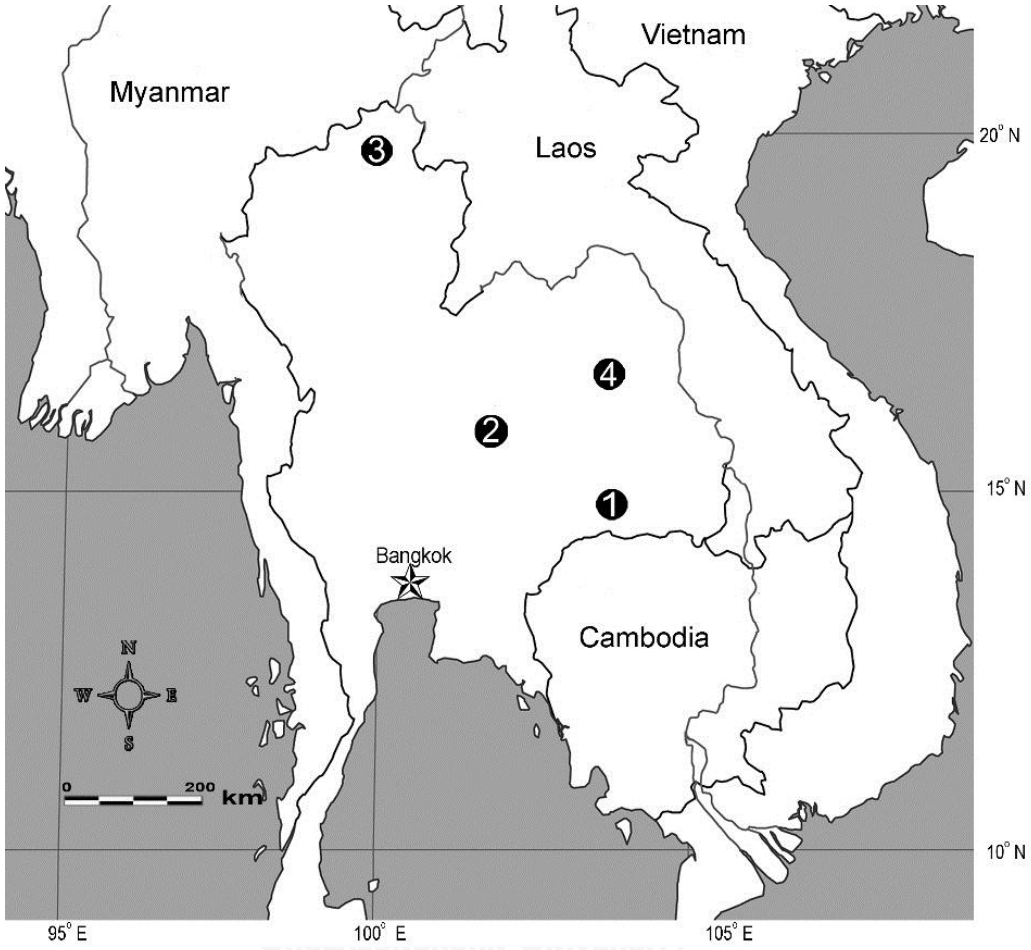

Figure 2.1 Map of type localities of *Amynthas arenulus* new species from Ban Khok Pho, Surin (1), *Amynthas longicaeca* new species from Phu Lan Kha National Park, Chaiyaphum (2), *Amynthas phucheefah* new species. from Phu Chee Fah, Chiang Rai (3), and *Amynthas thakhantho* new species from Tha Khantho, Kalasin (4).

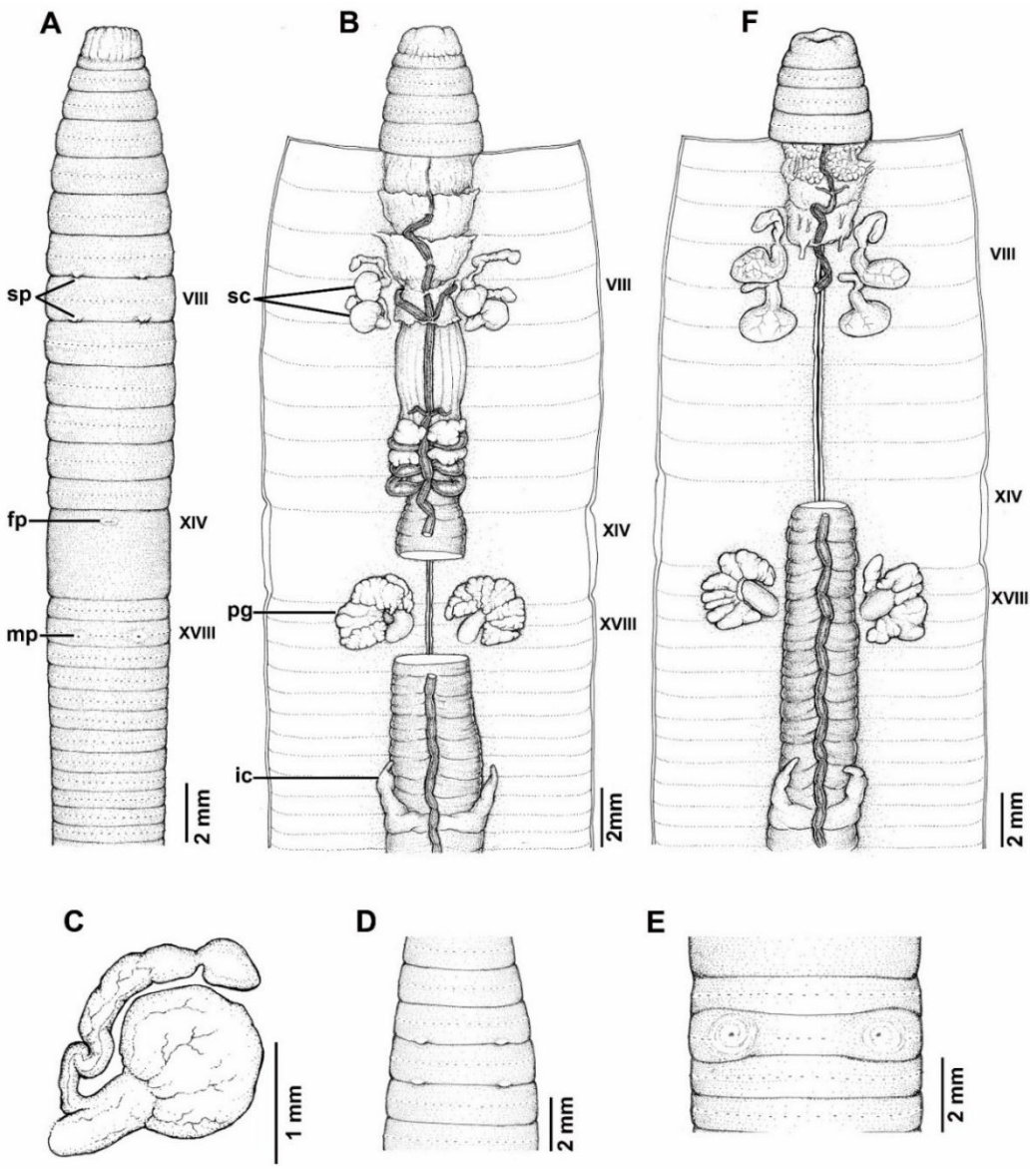

Figure 2.2 External and internal morphology of *Amynthas aeruginosus*, the type species, A–C, lectotype (SMNH 154), A, external ventral view; B, internal dorsal view; C, spermathecae, D–F, paralectotype (ZMH V5221), D, spermathecal pores; E, male pores and F, incomplete internal dorsal view.

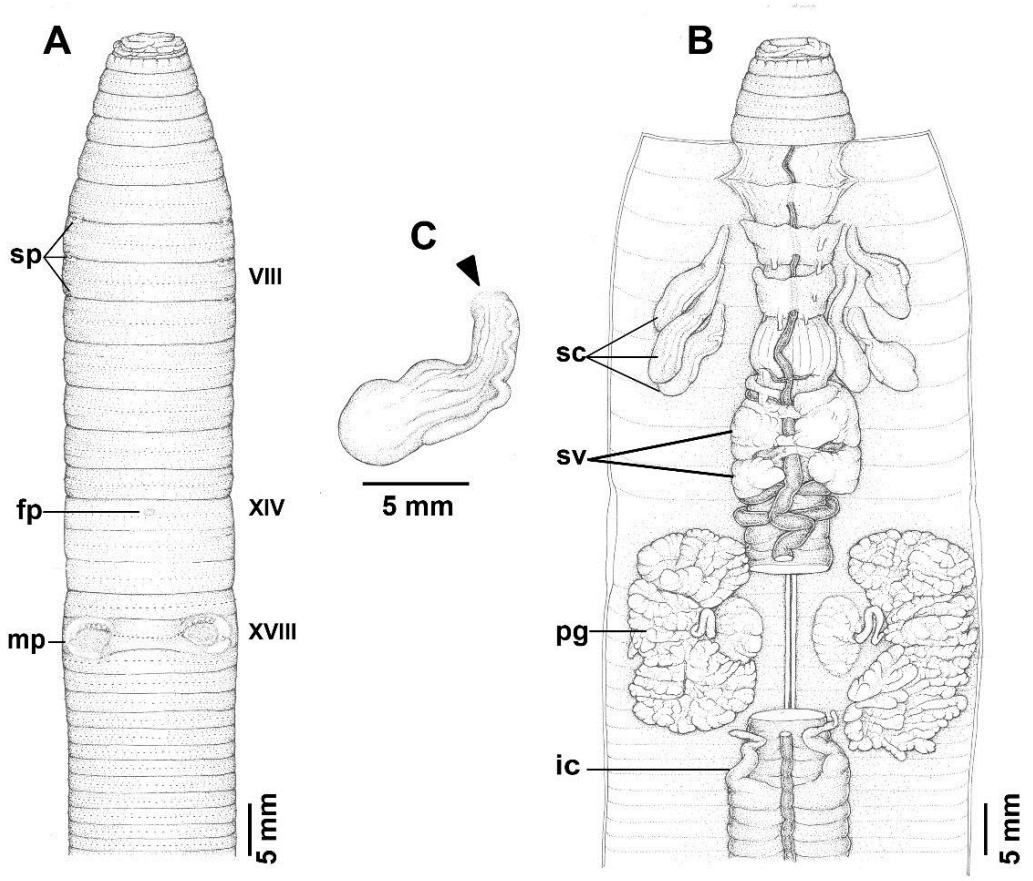

Figure 2.3 External and internal morphology of holotype (CUMZ 3299) of *Amynthas arenulus* new species: A, external ventral view, B, internal dorsal view and C, spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

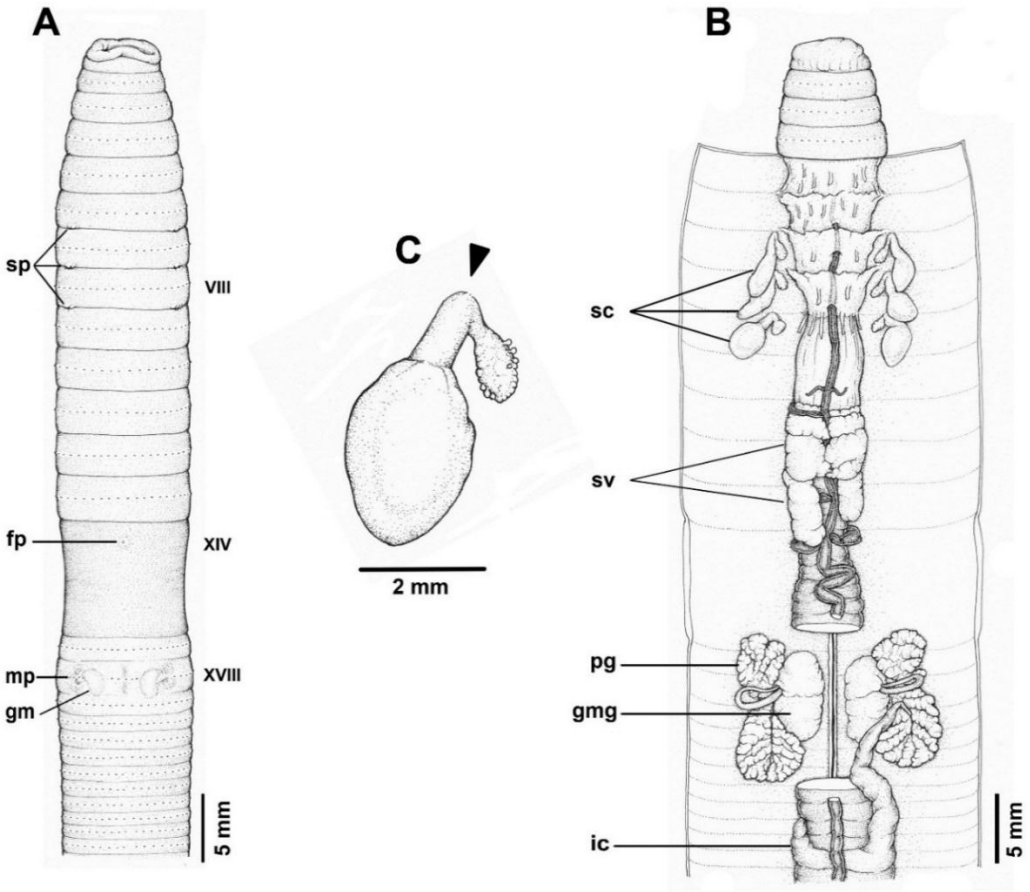

Figure 2.4 External and internal morphology of holotype (CUMZ 3304) of *Amynthas longicaeca* new species: A, external ventral view, B, internal dorsal view and C, spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

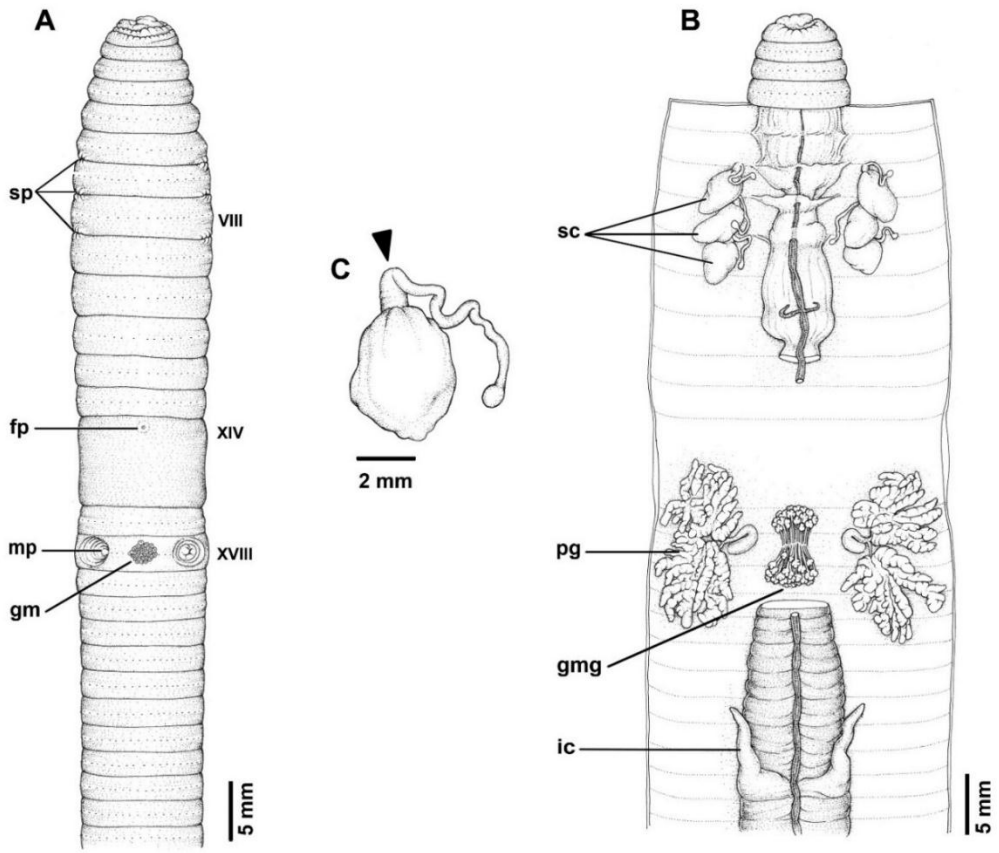

Figure 2.5 External and internal morphology of syntype (ZMH V3864) of *Amynthas burchardi*: A, external ventral view, B, internal dorsal view and C, spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

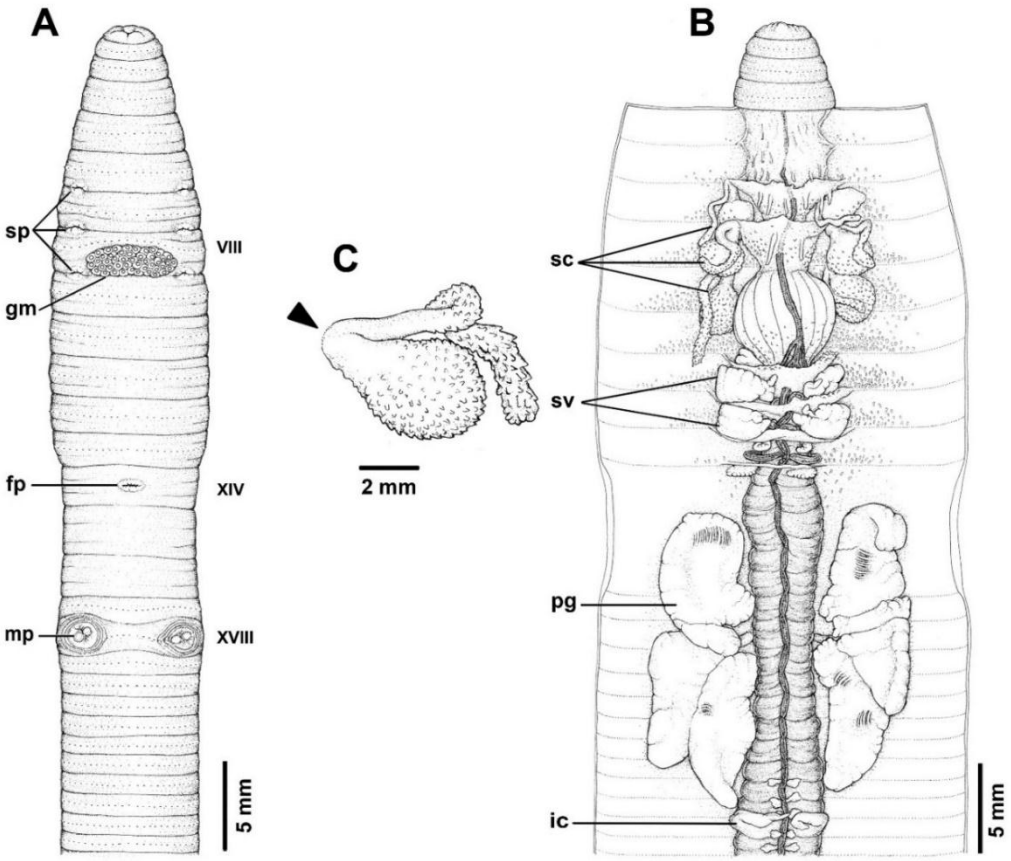

Figure 2.6 External and internal morphology of syntype (ZMH V7171) of *Amynthas osmastoni*: A, external ventral view, B, internal dorsal view and C spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

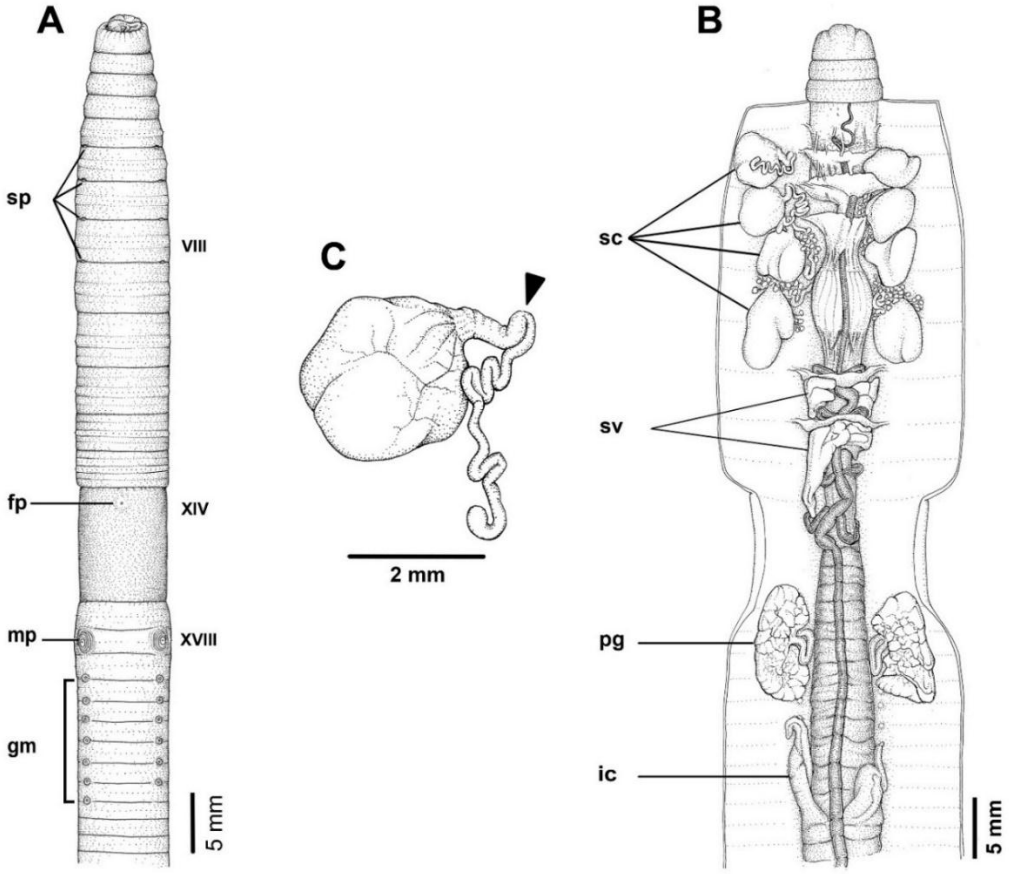

Figure 2.7 External and internal morphology of holotype (CUMZ 3306) of *Amynthas phucheefah* new species: A, external ventral view, B, internal dorsal view and C, spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

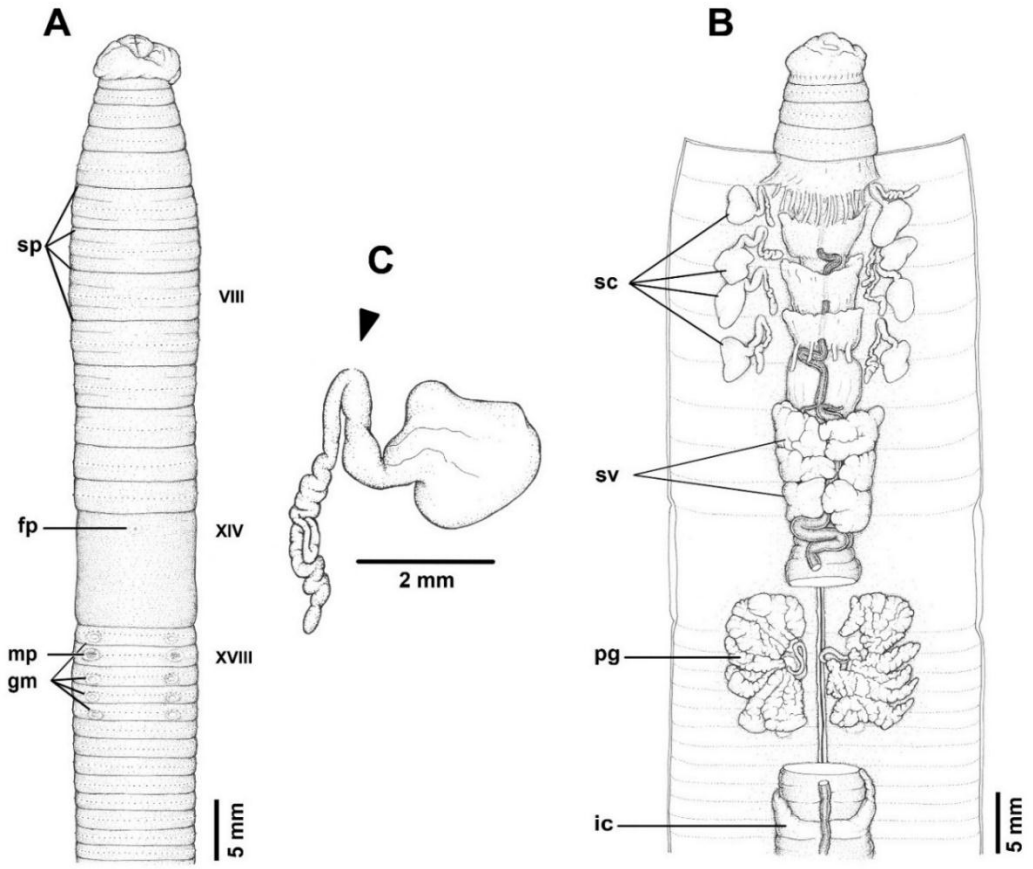

Figure 2.8 External and internal morphology of holotype (CUMZ 3308) of *Amynthas thakhantho* new species: A, external ventral view, B, internal dorsal view and C, spermathecae, and dark arrow indicates the connection of the spermathecae and spermathecal pore.

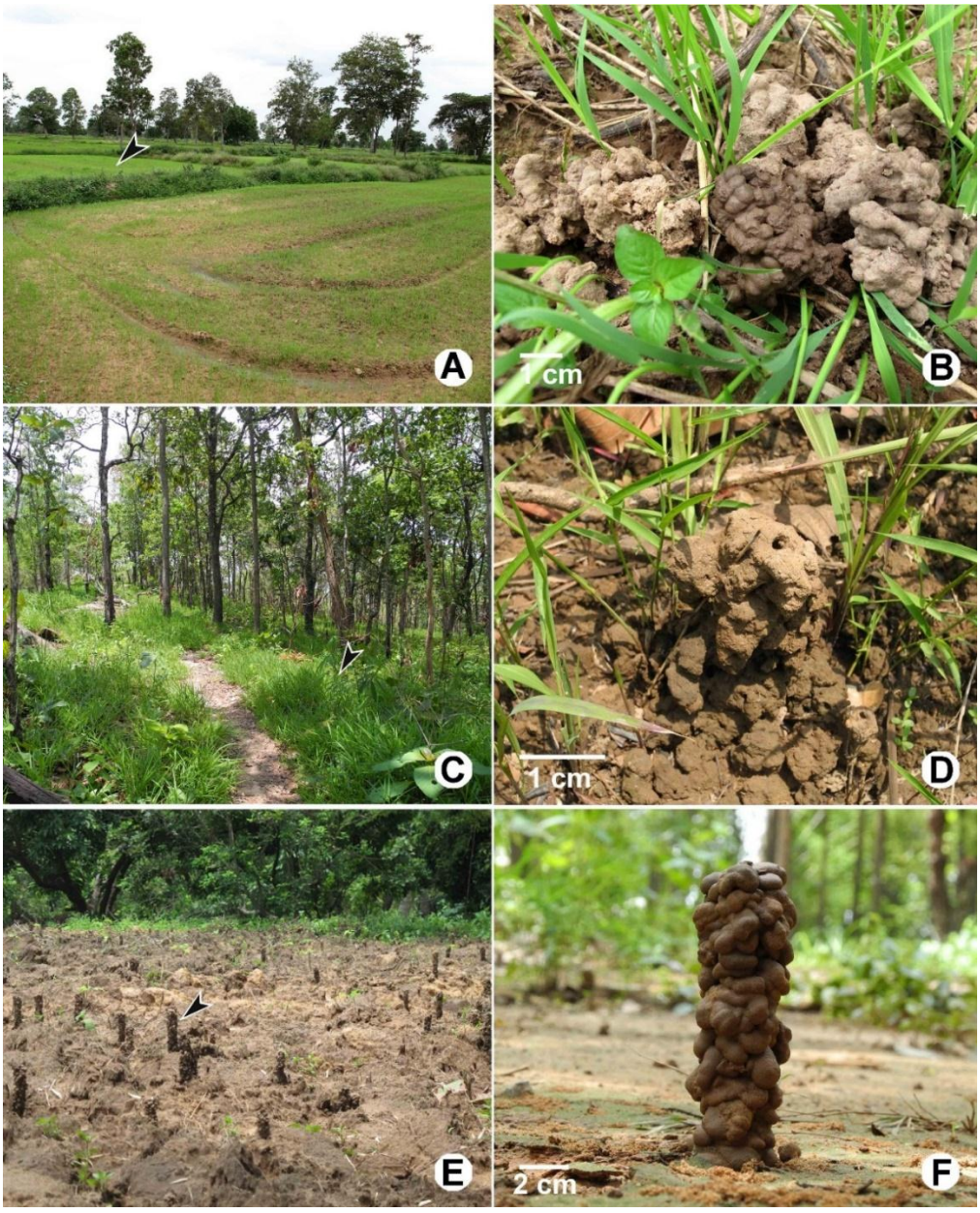

Figure 2.9 Photographs showing typical habitat types of the new species: A and B, *Amynthas arenulus* new species, (A) earthen dyke around paddy field and (B), casting. C and D, *Amynthas longicaeca* new species, (C) dipterocarp forest and (D), casting. E and F *Amynthas thakhantho* new species, (E) organic gardening and (F) tower–like casting.

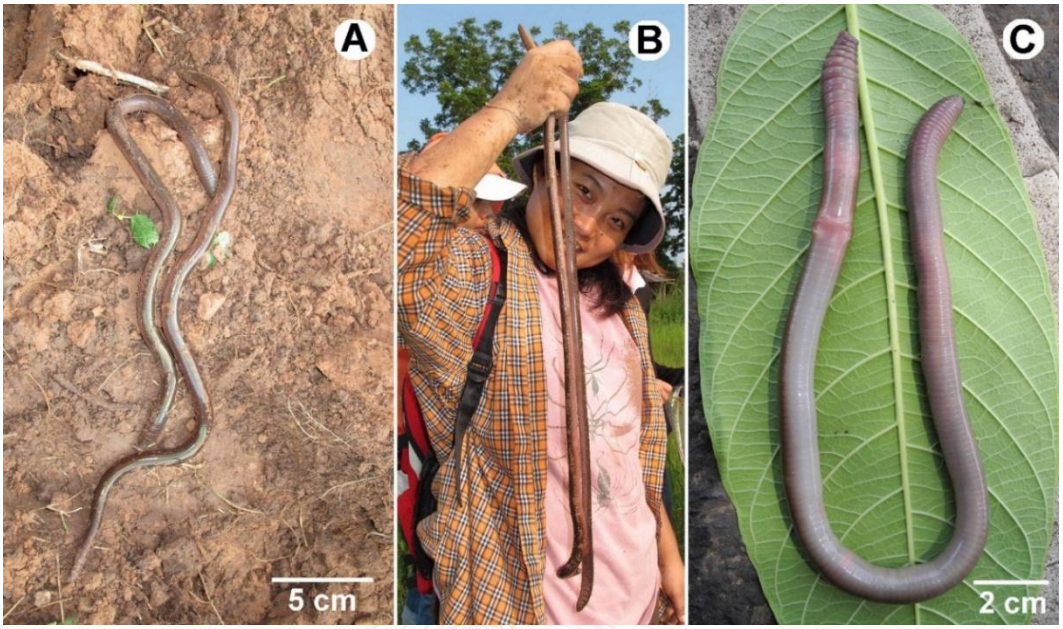

Figure 2.10 Photographs of *Amynthas arenulus* new species. A and B, living specimen from the type locality showing the coloration, C, specimen from Sisaket, just after anesthesis step in 30% (v/v) ethanol showing the coloration of the ventral side.

CHAPTER 3

Three new earthworm species of the genus *Amynthas* Kinberg, 1867 from Thailand (Clitellata: Megascolecidae)

Ueangfa Bantaowong[1,2], Samuel W. James[3] & Somsak Panha[2,*]

[1]Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok, 10330 Thailand

[2]Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand.

[3]Department of Biology, University of Iowa, Iowa City, Iowa, USA 52242

[*]Corresponding author: somsak.pan@chula.ac.th

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Abstract

This paper describes three new species of earthworms belonging to the genus *Amynthas* (Megascolecidae: Oligochaeta) from Thailand. They are *A. turris* n. sp., *A. nangrongensis* n. sp., and *A. khaohayod* n. sp. which are octothecate, quadrithecate and bithecate, respectively. They were determined to be new after comparing the three new species with the other members of their respective species groups based on their original descriptions and some reference materials from Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany and the Natural History Museum, London. The new species differ from other *Amynthas* in their pattern of genital markings and the appearance of the male field and shape of spermathecae as well. The description with illustrations of three new species are provided.

Key words: *Amynthas*, Earthworm, Taxonomy, New species, Thailand

Introduction

Thailand is located in the tropical area between 5° 37′ and 20° 27′ north latitudes, and between 97° 22′ and 105° 37′ east longitudes. It includes an area of approximately 500,000 square kilometers, and lies at the zoogeographical crossroads of South East Asia. Continental Thailand comprises two major biogeographical regions. They are the Indochinese region in the North and the Sundaic region in the South. Apart from the effect of these two regions within the Indomalayan Realm, some elements of Thailand's flora and fauna are also influenced by biogeographical characteristics of the Indian and Palearctic region (MacKinnon and MacKinnon, 1986). Consequently, its supports a variety of tropical ecosystems, forming highly diverse habitats for terrestrial animals including earthworms.

Up to date, forty five species of terrestrial earthworm are already known from Thailand, and mostly they belong to the genera *Amynthas* and *Metaphire* of the family Megascolecidae. Of these 23 species or 50% are *Amynthas*. These species occur in

virtually all habitats including natural habitats and agricultural areas, even in mud or sand of banks of the Mekong River, like *A. mekongianus*, and are also found from sea level to high mountains (Bantaowong et al., 2011a; Bantaowong et al., 2011b; Bantaowong et al., 2014; Blakemore, 2011; Gates, 1939, 1972). Here we report one new species from each of the *Amynthas corticis*, *morrisi* and *minimus* species groups, from localities at Phitsanulok, Nakhon Nayok and Chonburi provinces, (Fig. 3.1).

Material and Methods

Earthworms collected were killed in 40% (v/v) ethanol, photographed, transferred to 10% (v/v) formalin for fixation, and then transferred to 70% (v/v) ethanol for longer term preservation and subsequent morphological studies. The preserved specimens were examined by external observation of characters and by dorsal dissection.

The descriptions of each species were made during observation under the Olympus SZX7 stereoscopic light microscope. Drawings were made of the external characters and internal organs. In addition, type and other reference material from the following collections was reinvestigated: the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (ZMH), and the Natural History Museum, London (NHMUK). We also compared these specimens to published descriptions of relevant *Amynthas*.

Specimens are deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ), the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (UHH), and the Natural History Museum, London (NHMUK).

Anatomical abbreviations: fp, female pore; ic, intestinal caeca; mp, male pores; pg, prostate gland; sc, spermathecae; sp, spermathecal pores; sv, seminal vesicles.

Systematics

Genus ***Amynthas*** Kinberg, 1867

Type species *Amynthas aeruginosus* Kinberg, 1867, by monotypy.

***Amynthas turris* Bantaowong & Panha, new species**

Figs. 3.1 and 3.2

Description of Holotype: Dimensions; 190 mm by 7.6 mm at segment X, 7.7 mm at segment XX, 7.6 mm at clitellum; body cylindrical with 156 segments. Setae regularly distributed around segmental equators, numbering 48 at VII, 60 at XX, 14 setae between male pores, setae formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

Male pores are in XVIII situated close to the lateral border on slightly elevated horizontal triangular area on the setal line. Each male aperture is in a small demarcated tubercle within a distinctly delimited, longitudinally elliptical set of skin folds, whose margin is prominently elevated from the body surface, and separated by 14 setae. Distance between male pores 7.5 mm, 0.28 circumference apart ventrally. Two pairs of oblong- shaped genital markings in XVII and XIX in line with the male pores. Spermathecal pores paired in 5/67/8/9, 0.30 circumference apart ventral; distance between spermathecal pores 7.5 mm.

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent, 10/11–13/14 thin. Gizzard large behind 7/8, intestinal origin in XV, no lymph glands observed. Typhlosole small from XXVII. Intestinal caecum originated from XXVII extending forward to XXI, simple. Hearts esophageal in X–XIII. Holandric; testes and funnels in ventrally joined sacs in X–XI. Seminal vesicles paired in XI–XII. Prostates in XVI–XX; prostatic ducts long slender, bent into hairpin shape. Sessile genital marking glands present and corresponding to each external genital marking in XVII and XIX.

Ovaries in XIII. Spermathecae four pairs in VI–IX; ampulla an elongate sac, distended, with relatively slender duct as long as ampulla. Diverticulum is twisted into a compact mass of coils, stalk attached to duct near body wall.

Variation: The holotype measures 190 mm body length with 156 segments; the eleven paratypes range in size from 160–190 mm (177±10.43 mm) body length with 143–169 segments.

Type locality: Tham Pha Tha Phon non-hunting area, Noen Maprang district, Phitsanulok province, Thailand, 16° 30’ 19.4” N, 100° 39’ 39.0” E, 110 meters elevation (18 November 2012).

Etymology: The specific epithet “*turris*” in Latin word mean “tower”. This name refers to the ecological characteristics of this species as making tower-like castings.

Type material: The holotype (CUMZ 3397) and seven paratypes (CUMZ 3398) are deposited in Chulalongkorn University, Museum of Zoology. Another two paratypes will be deposited in the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (ZMH), and another two paratypes in the Natural History Museum, London (NHMUK).

Habitat: Found in the top soil at about 15 cm depth, the soil surface covered with the leaf litter of a deciduous limestone forest, mostly disturbed. The soil was carefully dug close to the tower-like casts, which are about 10 cm in height and 3 cm in diameter

Diagnosis: Length 160–190 mm. Male pores, paired, superficial in segment XVIII, each on a horizontal triangular area, slightly raised areas, two pairs of genital markings on segment XVII and XIX. Spermathecal pores paired in segments 5/6–8/9. Spermathecae elongate sac ampulla, diverticulum twisted into a compact mass of coiled. Holandric,

intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, ducts long and hairpin shape. Paired sessile genital marking glands on XVII and XIX.

Remarks: *Amynthas turris* n. sp. is one of the medium-sized *Amynthas* and belongs in the *corticis* species group (Sims and Easton, 1972). The characteristic male field with two pairs of genital markings and sessile genital marking glands and simple intestinal caeca are fairly similar to some *corticis* species group members in Indo-Burma. There are three species or species groups which have genital markings at male pore area and has sessile genital marking glands with simple intestinal caeca: *Amynthas rodericensis* (Grube, 1879), *A. suctoria* (Michaelsen, 1907) and *A. andersoni* group (Gates, 1972). The present species can easily be separated from *A. rodericensis* by the position of spermathecal pores in the ventral side while *A. rodericensis* has spermathecal pores in the dorsum. The difference between this species and remaining groups are the locations of genital markings. The present species has two pairs of genital markings in XVII and XIX while *A. suctoria* has only one pair in segment XVIII and the genital markings of *A. andersoni* group are unpaired, or if paired, it will be present in XVIII or behind XVIII.

Comparing with others *corticis* group members in Thailand, mostly they show more than two pairs of genital markings at the male pore lines in *A. longicauliculatus* (Gates, 1931), *A. comptus* (Gates, 1932), *A. phucheefah* Bantaowong & Panha, 2014 and *A. thakhantho* Bantaowong & Panha, 2014, whereas some species; *A. alexandri* Beddard, 1900 and *A. mekongianus* (Cognetti, 1922) lack genital markings. The remaining members; *A. corticis* (Kinberg, 1867), *A. exiguus* (Gates, 1930) and *A. manicatus* (Gates, 1931) have the genital markings at the male pore area on segment XVIII while the present species has two pairs of genital marking in XVII and XIX.

Although some morphological characters are apparently plastic such as spermathecal pores position, the genital markings at the male pore area, and similar tower-liked cast. These characters are similar to those of *A. thakhantho*. However, the shape of spermathecae in *A. turris* is clearly differ from *A. thakhantho* by the elongated

sac ampulla, diverticulum twisted into a compact mass of coils, and spermathecae of *A. thakhantho* is a large sac ampulla with slender, long coiled diverticulum. The male field of *A. turris* is still more convex than in *A. thakhantho* and presenting only two pairs of genital markings in segment XVII and XIX while *A. thakhantho* has four pairs of genital markings in XVII, XIX, XX and XXI. The additional habitat records showed that *A. turris* found in limestone forest at Phitsanulok while *A. thakhantho* found in non-limestone agricultural field which 400 kilometer distance from *A. turris* separated by Pethchaboon Mountain range.

***Amynthas nangrongensis* Bantaowong & Panha, new species**

Figs. 3.1 and 3.3

Description of Holotype: Dimensions; 173 mm by 7.7 mm at segment X, 8.0 mm at segment XX, 7.5 mm at clitellum; body cylindrical with 127 segments. Setae regularly distributed around segmental equators, numbering 44 at VII, 76 at XX, 27 setae between male pores, setae formula AA:AB:ZZ:ZY= 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

A pair of indistinct rounded male porophores in XVIII, 0.29 circumference apart ventrally; distance between male pores 7.5 mm. Genital markings consist of two pairs of elongated oval, diagonally placed markings one pair anterior and one posterior to setae of XVIII, just medial to male pores. Entire male field slightly elevated as a rounded rectangular area. Spermathecal pores paired in 5/6–6/7, each small, with wrinkled margins, about 0.15 circumference apart ventrally; distance between spermathecal pores 3.5 mm.

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent 10/11–13/14 thin. Gizzard large behind 7/8, intestinal origin in XV, no lymph glands observed. Typhlosole small from XXVII. Intestinal caecum originating in XXVII extending forward to XXI, simple finger-shape. Hearts esophageal in X–XIII. Holandric; testes and funnels in ventrally

joined sacs in X–XI. Seminal vesicles paired in XI–XII. Prostates in XVI–XXII; prostatic ducts long slender with hairpin bend. Genital marking glands are sessile and corresponding to each external genital marking in XVIII.

Ovaries in XIII. Spermathecae two pairs in VI–VII; ampulla an elongate sac, twisted, with relatively slender duct as long as ampulla. Diverticulum compact zigzag bound together as a tube shape.

Variation: The holotype measures 173 mm body length with 127 segments; the four paratypes range in size from 170–248 mm (204.3±39.8 mm) body length with 125–138 segments.

Type locality: Nangrong Waterfall, Nakhon Nayok province, Thailand, 14° 19’ 45.3” N, 101° 19’ 6.8” E, 54 meters elevation (5 October 2013).

Etymology: This species was named after the type locality, Nangrong Waterfall.

Type material: The holotype (CUMZ 3399) and two paratypes (CUMZ 3400) are deposited in Chulalongkorn University, Museum of Zoology. Another paratype will be deposited in the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (ZMH).

Habitat: Found in the top soil at about 10-15 cm depth, the soil surface covered with leaf litter of deciduous forest which originated at the Nangrong Waterfall area.

Diagnosis: Medium size, length 170–248 mm. Male pores paired, superficial in segment XVII, each on a rounded porophore, two pairs of genital markings pre- and post-setal in XVIII. Spermathecal pores paired in 5/6–6/7. Spermathecae elongated sac ampulla, diverticulum tubular. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, ducts long, two pairs of sessile genital marking glands in XVIII.

Remarks: *Amynthas nangrongensis* is a quadrithecate earthworm belonging to the *morrisi* species group of the genus *Amynthas* (Sims and Easton 1972). This group is from the Oriental region; those having genital markings near the male pore areas include *A. morrisi* (Beddard, 1892), *A. pallidus* (Michaelsen, 1892), *A. insulae* (Beddard, 1896), *A. incongruus* (Chen, 1936), *A. monoserialis* (Chen, 1938) and *A. tripunctus* (Chen, 1946). However, the present species can be easily distinguished from all above related species by having two pairs of genital markings pre- and post-setal in XVIII and without genital markings near the spermathecal pores. All the others mentioned above have both pre-and post-clitellar genital markings, and none has the pattern of *A. nangrongensis*.

The genital markings and their arrangement in this species are somewhat similar to those of *A. fucosus* (Gates, 1933) which has been reported from Nakhonratchasima province, in north-eastern Thailand but the latter has three pairs of spermathecal pores in 6/7–8/9 (Gates, 1933) and so belongs to the *aelianus* species-group (Sims and Easton, 1972).

***Amynthas khaohayod* Bantaowong & Panha, new species**

Figs. 3.1 and 3.4

Description of Holotype: Dimensions; 230 mm by 7.5 mm at segment X, 6.8 mm at segment XX, 7.1 mm at clitellum; body cylindrical with 137 segments. Setae regularly distributed around segmental equators, numbering 113 at VII, 105 at XX, no setae between male pores, setae formula AA:AB:ZZ:ZY= 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

Male pores on the ventro-lateral sides of XVIII, about 0.18 circumference ventrally apart; distance between male pores 4 mm. Each pore on inner wall of a large

granular which protrude outside the body wall. Spermathecal pores paired in 5/6, 0.40 circumference apart ventrally; distance between spermathecal pores 8 mm.

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent, 10/11–13/14 thin. Gizzard globular behind 7/8, intestinal origin in XV, no lymph glands observed. Typhlosole small from xxvii. Intestinal caecum originates in xxvi extending forward to XXII, long finger-shape. Hearts esophageal in X–XIII. Holandric; testes and funnels in ventrally joined sacs in X–XI. Seminal vesicles paired in XI–XII. Prostates glands and ducts in XVIII, extending between XVII–XXI; prostatic ducts tightly folded twice, ducts are surrounded by large sessile genital marking glands.

Ovaries in XIII. Spermathecae one pair in VI; ampulla is large and spatulate, with stout duct shorter than ampulla. The diverticulum as long as ampulla, with roughly elliptical terminal chamber.

Variation: The holotype measures 230 mm body length with 137 segments; the length of nine paratypes varies from 140-190 mm, the number of segments from 100-140. The first dorsal pore is in 12/13 in all of specimens.

Type locality: Khao Hayod temple, Chonburi province, Thailand, 18° 22’ 11.1” N, 100° 50’ 23.2” E, 607 meters elevation (30th September 2010).

Etymology: This species was named after the type locality Khao Hayod.

Type material: The holotype (CUMZ 3401) and five paratypes (CUMZ 3402) are deposited in Chulalongkorn University, Museum of Zoology. Another two paratypes will be deposited in the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (ZMH), and two paratypes in the Natural History Museum, London (NHMUK).

Habitat: Found in the top soil at about 10-15 cm depth in the limestone forest at Khao Hayod temple.

Diagnosis: Length 140-230 mm. Male pores paired in XVIII, each pore on inner wall of the large granular and protruding porophore. Spermathecal pores paired in 5/6, spermathecae spatulate shaped ampulla**,** diverticulum long and roughly elliptic terminal chamber, intestinal caeca simple, first dorsal pore in 12/13. Holandric. Prostate glands as large as genital marking glands, its duct flanked by large sessile glandular masses on body wall.

Remarks: *Amynthas khaohayod* n. sp. is one of 14 bithecal members of the *minimus* species group (Sims and Easton, 1972). This group has never been recorded in Thailand before, but three species occur in Burma; *A. minimus* (Horst, 1892), *A. nugalis* (Gates, 1931) and *A. papilio* (Gates, 1930). The present species can be separated from first two species by the larger body in 140–230 by 5–7.5 mm while *A. minimus* and *A. nugalis* range in size from 20–56 by 1.5–2.5 mm. The present species appears to be closely related to *A. papilio* (Gates, 1930), but it is separated easily by the aspects of male pore region because male pores of *A. papilio* are on large flat genital markings, but in this species the male pores and genital markings protrude. Moreover, *A. papilio* has slender, small genital marking glands while the present species has large sessile glandular masses.

Another related species of the *minimus* species group is the Indonesian *Amynthas lompobatangensis* (Michaelsen, 1899). The present species can be easily distinguished by having no paired genital papillae at the male pore area and by having the sessile genital marking glands. In contrast *A. lompobatangensis* has two pairs of genital markings on segments XVII and XIX but lacks genital marking glands.

***Amynthas lompobatangensis* (Michaelsen, 1899)**

Fig. 3.5

Pheretima lompobatangensis Michaelsen, 1899: 33, fig. 5. Type locality: Celepes, Indonesia

Amynthas lompobatangensis: Sims and Easton, 1972: 236

Material examined. Syntype from ZMH (V 5184, fig. 3.5): One adult and one sub-adult in 70% ethanol deposited at Biozentrum Grindel Zoologisches Museum, University of Hamburg, Germany.

Remarks. *Amynthas lompobatangensis* differs from *A. khaohayod* n. sp. by having two pairs of genital papillae present in XVII and XIX, but lacking genital marking glands. The spermathecae have a transversely oval ampulla with a small, short diverticulum.

Discussion

From the current study, there are a total of approximately 55 species described from Thailand (Bantaowong et al., 2015a; Bantaowong et al., 2014). The total land area of Thailand is approximately 500,000 sq. km compare to Burma that is about 657,740 sq. km but has over double the number of described species (129, Gates, 1972; Reynolds, 2009). The terrestrial earthworms of Thailand are still poorly known, with only a small fraction of the species in this country having been described properly. Many of these species are known only from a single locality or very few localities, so range boundaries can be tentatively outlined in very few cases. These huge gaps in knowledge, prevent a proper zoogeographic analysis. Further extensive and detailed taxonomic studies are especially needed to improve the situation.

Acknowledgements

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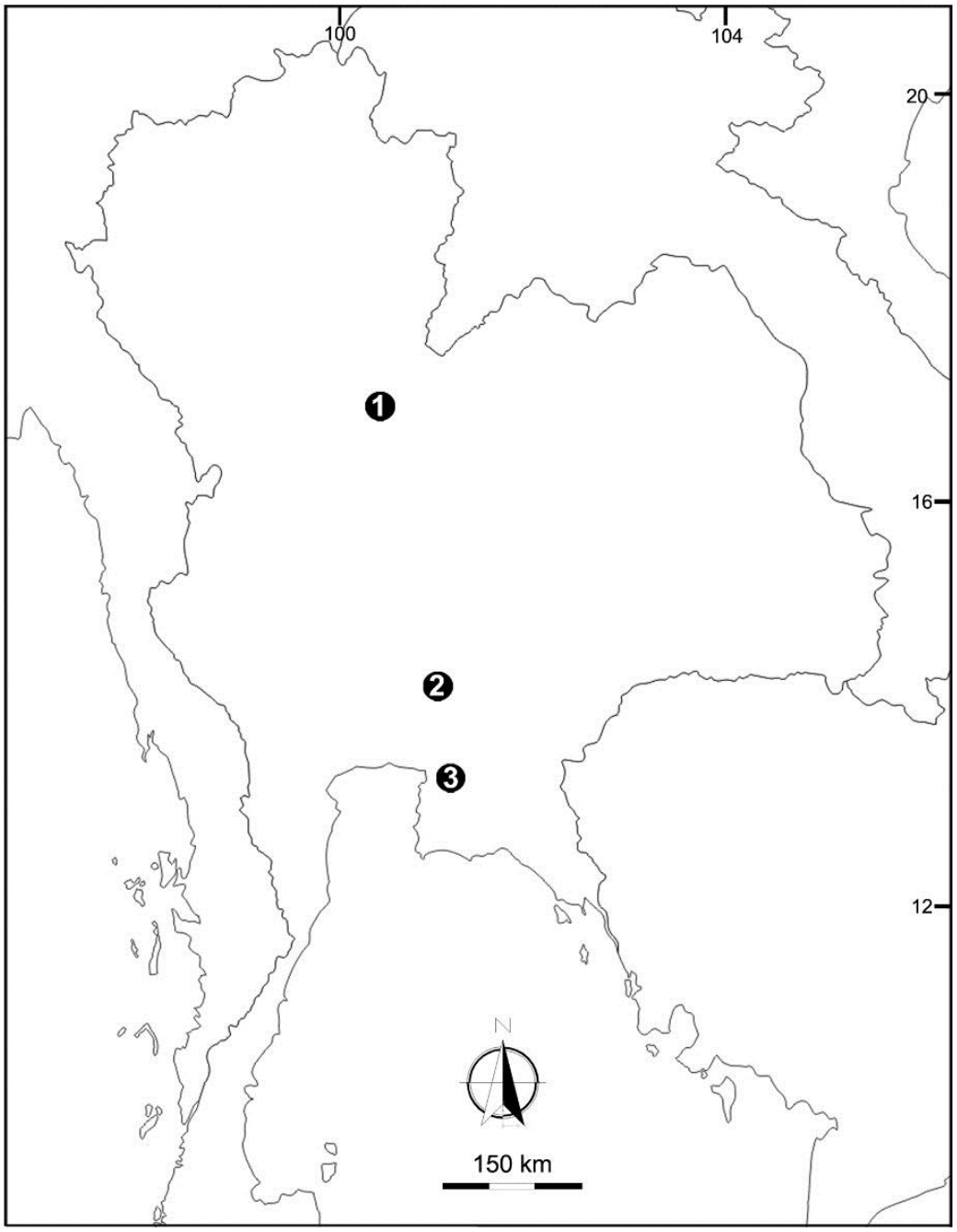

Figure 3.1 Map of type locality of (1) *Amynthas turris* n. sp. from Phitsanulok province, (2) *Amynthas nangrongensis* n. sp. from Nakhon Nayok province, (3) *Amynthas khaohayod* n. sp. from Chonburi province.

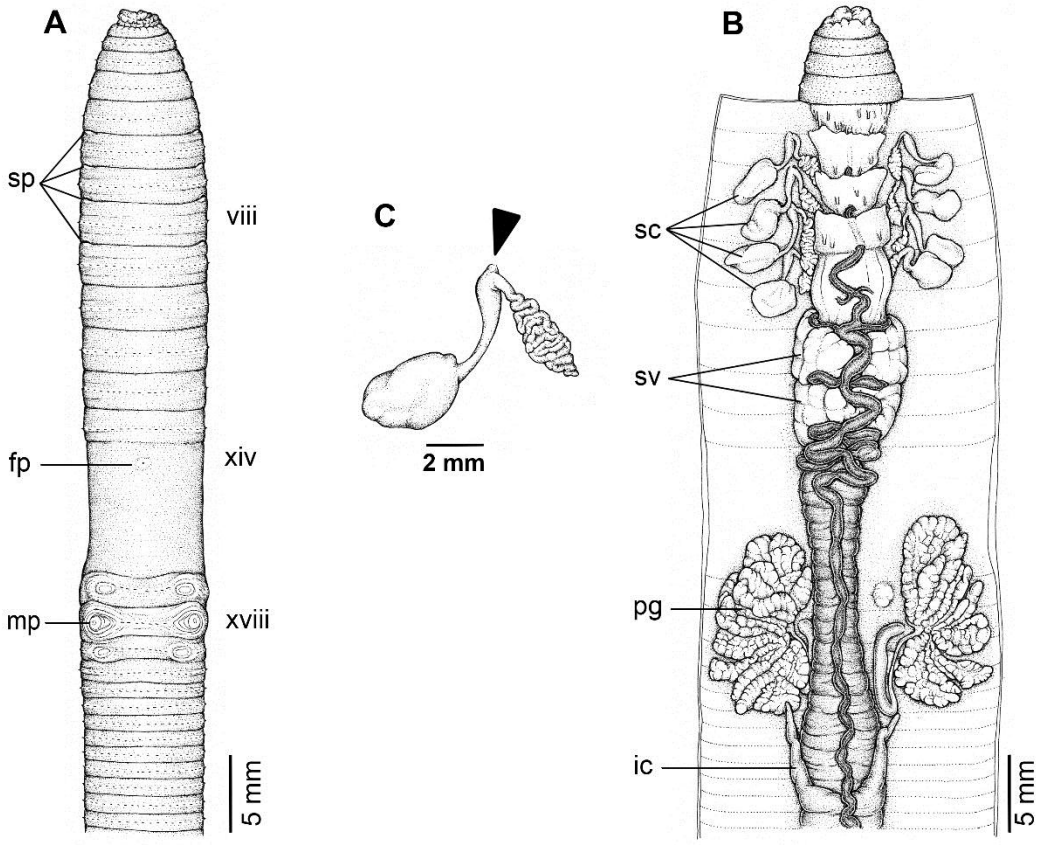

Figure 3.2 External and internal morphology of holotype (CUMZ 3397) of *Amynthas turris* n. sp. (A) External ventral view, (B) internal dorsal view and (C) spermatheca, and black arrow indicates the connection of the spermatheca and spermathecal pore.

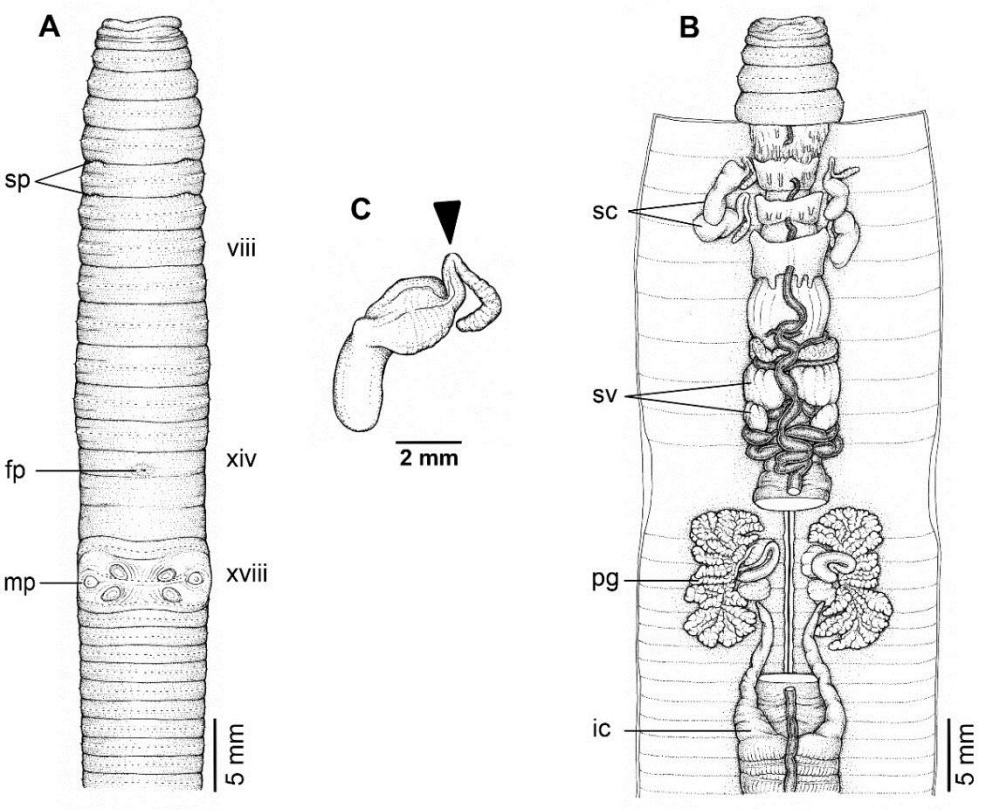

Figure 3.3 External and internal morphology of holotype (CUMZ 3399) of *Amynthas nangrongensis* n. sp. (A) External ventral view, (B) internal dorsal view and (C) spermatheca, and black arrow indicates the connection of the spermatheca and spermathecal pore.

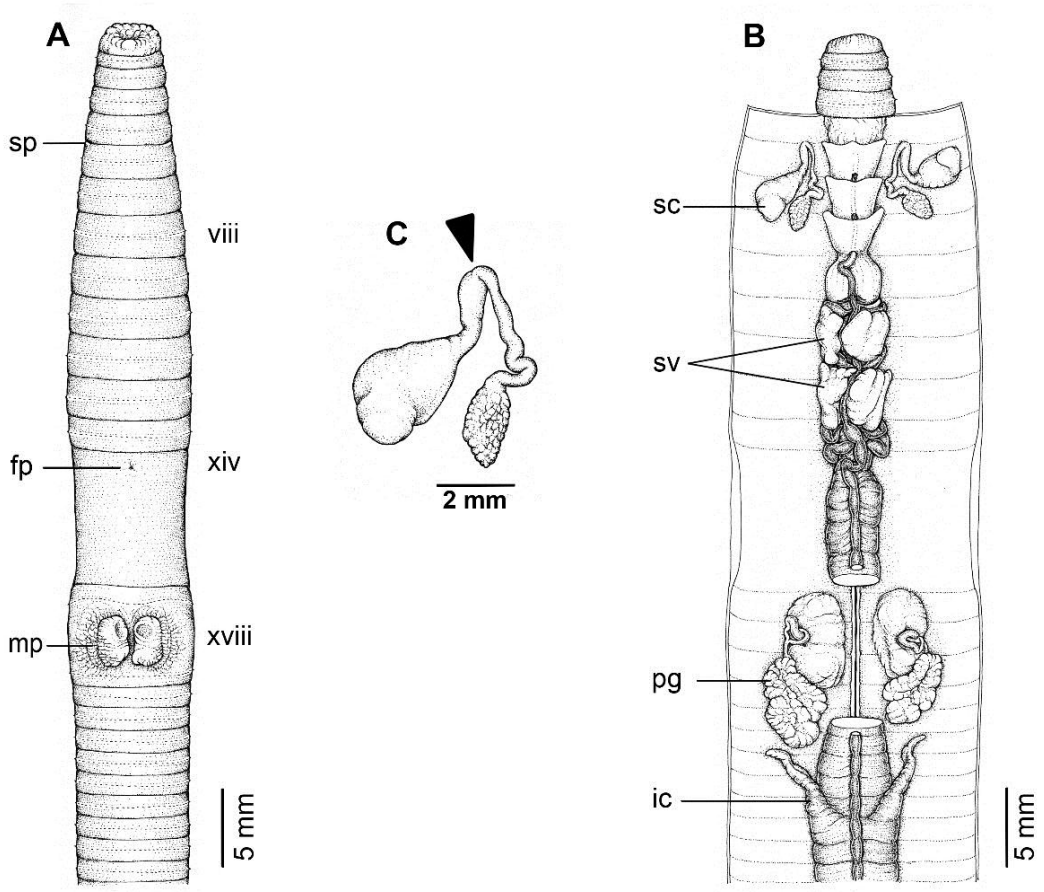

Figure 3.4 External and internal morphology of holotype (CUMZ 3401) of *Amynthas khaohayod* n. sp. (A) External ventral view, (B) internal dorsal view and (C) spermatheca, and black arrow indicates the connection of the spermatheca and spermathecal pore.

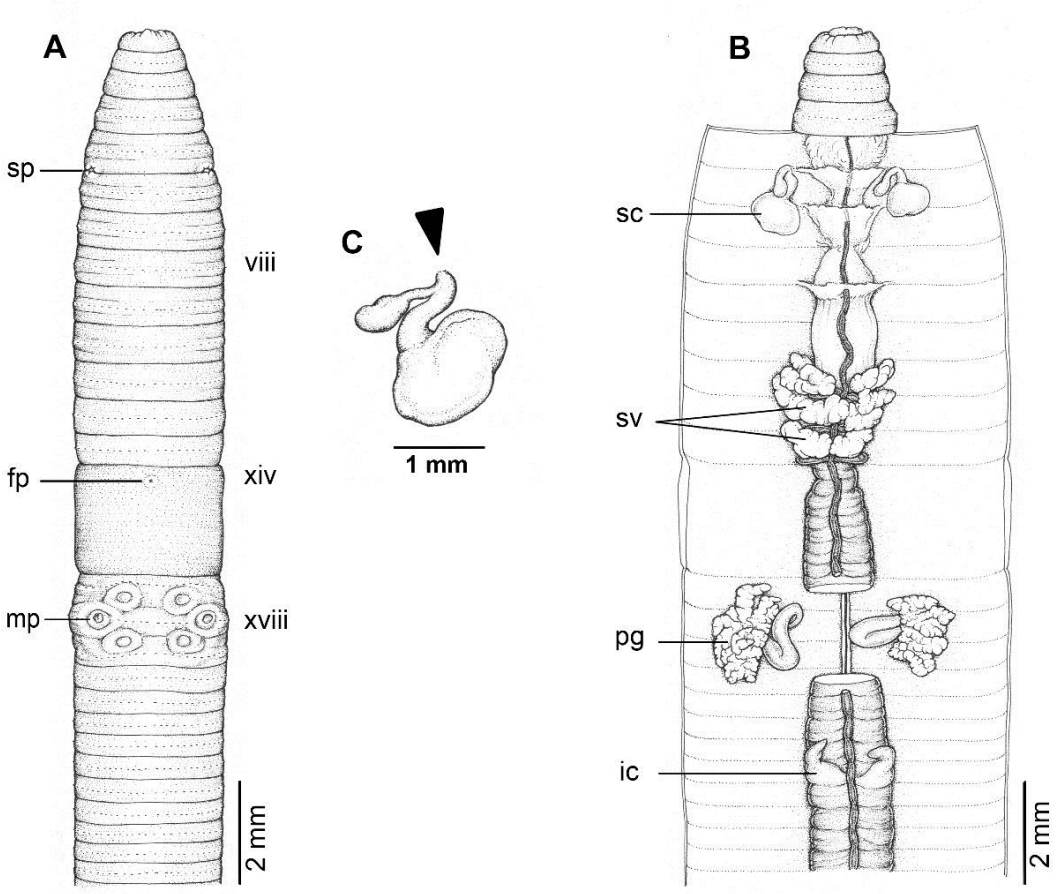

Figure 3.5 External and internal morphology of type (ZMH V 5184) of *Amynthas lompobatangensis*. (A) External ventral view, (B) internal dorsal view and (C) spermatheca, and black arrow indicates the connection of the spermatheca and spermathecal pore.

CHAPTER 4

Seven new species of the earthworm genus *Metaphire* Sims & Easton, 1972 from Thailand, with redescriptions of some species (Clitellata: Megascolecidae)

Ueangfa Bantaowong[1,2], Ratmanee Chanabun[3], Samuel W James[4] & Somsak Panha[2,5]

[1] Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[2]Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; e-mail: somsak.pan@chula.ac.th and uengfa@yahoo.com

[3] Program in Animal Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand, e-mail: cratmanee@yahoo.com

[4]Department of Biology, University of Iowa, Iowa City, Iowa, USA 52242; e-mail: samuel-james@uiowa.edu

[5]Corresponding author

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Abstract

Earthworm specimens collected from various parts of Thailand were found to contain seven new species of the genus *Metaphire* Sims & Easton, 1972. These were *M. songkhlaensis* sp. n. and *M. trangensis* sp. n. in the octothecal *pulauensis* species group, *M. khaoluangensis* sp. n. and *M. orientalis* sp. n. in the sexthecal *houlleti* species group, *M. doiphamon* sp. n. in the sexthecal *peguana* species group, *M. saxicalcis* sp. n. in the quadrithecal *planata* species group and *M. surinensis* sp. n. in the bithecal species group. Lectotypes were designated for *M. pulauensis* (Beddard, 1900) and *M. dunckeri* (Michaelsen, 1902), while *M. baruana* (Stephenson, 1932), *M. perichaeta* (Beddard, 1900) and *M. planata* (Gates, 1936) are redescribed in this study in order to cover all the significant morphological features with additional illustrations presented, and also to clarify the superficial resemblance to the current proposed new species. The new descriptions and comparisons are presented and discussed.

Key words: *Metaphire*, Clitellata, Taxonomy, Thailand

Introduction

The genus *Metaphire* Sims and Easton, 1972 is one of the largest amongst the earthworm family Megascolecidae that dominates East and Southeast Asia. This genus currently contains 170 nominal species within 21 species groups and is characterized by the presence of copulatory pouches containing the male pores, intestinal caeca originating in the 27th segment, and a lack of nephridia on the spermathecal ducts (Sims and Easton, 1972).

Several surveys of terrestrial earthworms in Thailand have been published (Bantaowong et al., 2011a; Bantaowong et al., 2011b; Bantaowong et al., 2014; Blakemore, 2011; Gates, 1939; 1972; Somniyam and Suwanwaree, 2009), but many localities in Thailand and nearby areas and various types of microhabitats are still unexplored. Recently, Bantaowong et al. (2014) summarized the knowledge about the Thailand earthworm fauna and reported 45 species, of which the genus *Metaphire* is

one of the most abundant, diverse and widespread throughout Thailand. *Metaphire* is represented by the 10 nominal species of *M. anomala* (Michaelsen, 1907), *M. bahli* (Gates, 1945), *M. bipora* (Beddard, 1900), *M. grandipenes* Bantaowong & Panha, 2011, *M. houlleti* (Perrier, 1872), *M. peguana* (Rosa, 1890), *M. perichaeta* (Beddard, 1900), *M. planata* (Gates, 1926), *M. posthuma* (Vaillant, 1868) and *M. virgo* (Beddard, 1900). Among these species, *M. peguana* is one of the most common indigenous species in Thailand (Bantaowong et al., 2011b; Prasankok et al., 2013).

The recently collected specimens of this study were found to be very similar to species described previously from nearby localities in North Malaysia, South Thailand and some parts of Myanmar (Burma), but their identifications remained tentative due to a lack of detail in the original descriptions. Thus, the type and other reference material of the species in question were reinvestigated during visits to the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH) and the Natural History Museum (NHMUK), London, in 2012 and 2014, so that they could be redescribed to the same level of detail as in our material. As a result, our specimens were determined to belong to seven new species, morphologically similar but significantly different from those species described previously from the same or nearby areas.

The present study contains the descriptions of these seven new species of *Metaphire* from various parts of Thailand (Figure 4.1) and the redescriptions of the following species: *Metaphire pulauensis* (Beddard, 1900), *M. dunckeri* (Michaelsen, 1902), *M. baruana* (Stephenson, 1932), *M. perichaeta* (Beddard, 1900) and *M. planata* (Gates, 1936). We further designate lectotypes of *M. dunckeri* and *M. baruana*.

Materials and methods

The earthworms were collected by digging and hand-sorting from several localities in Thailand in 2012–2014 (Figure 4.1). They were anesthetized in 30% (v/v) ethanol, fixed in 10% (v/v) formalin and preserved in 75% (v/v) ethanol. The specimens were examined under an Olympus SZX7 stereoscopic light microscope. The

descriptions are based on observations of dorsal dissections of the specimens. In the illustrations, we have included both dorsal and ventral views containing important features. Some of the specimens in the same lot were preserved in 95% (v/v) ethanol for further molecular phylogenetic studies.

Some specimens and type specimens housed at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH) and the Natural History Museum (NHMUK), London were critically studied for comparison with the new species in this report.

The type series are deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ). Additional paratypes will be deposited in the Natural History Museum (NHMUK), London, and at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH).

Anatomical abbreviations: fp, female pore; gm, genital marking; gmg, genital marking gland; ic, intestinal caeca; mp, male pores; pg, prostate gland; sc, spermathecae; sp, spermathecal pores; sv, seminal vesicles.

Taxonomic part

***Metaphire songkhlaensis* Bantaowong & Panha sp. n.**

Fig. 4.2; Table 4.1

Material examined. Holotype: clitellate (CUMZ 3373), Thailand, Songkhla, Hat Yai, Literary Botanical Garden, 07° 01’ 11.1” N, 100° 17’ 34.1” E, 89 m above mean sea level (amsl), 17 January 2014, leg. U. Bantaowong, C. Sutcharit, J. Tubtimon & W. Siriwut. Paratypes: 2 adults (CUMZ 3374), 1 adult (NHMUK), same data as holotype.

Etymology. The species is named after the province where it was collected.

Diagnosis. Large; length >165 mm. Male pores paired, prominent in XVIII, genital markings 5–7 papillae in transverse rows on VIII–IX, XIX–XXII. Spermathecal pores paired at 5/6–8/9, spermathecal ampulla elongated, sac-shaped, diverticulum rod shaped. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, genital marking glands sessile.

Description of holotype. Dimensions: 300 mm by 9.0 mm at VII, 9.0 mm at XX, 10.0 mm at clitellum; body cylindrical with 116 segments. Setae regularly distributed around segmental equators, numbering 71 at VII, 74 at XX, 20 between male pores, setae formula AA:AB:ZZ:ZY = 2:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI.

Male apertures prominent on ventro-lateral sides in XVIII, 0.22 circumference apart ventrally, distance between male pores 6 mm, the small penes within small copulatory pouches are everted and erupted entirely through a male pore. Genital markings arranged in transverse rows, pre-setal in XIX–XXII, post setal in XXI, XXII, each row consisting of 5–7 papillae. Four pairs spermathecal pores, each marked by a whitish spot in furrows 5/6–8/9, ventral, distance between each pair about 0.37 circumference apart ventrally, distance between spermathecal pores 11 mm. Genital papillae arranged in transverse rows centered on mid-ventral line, pre-setal in VIII, IX, post setal in VIII, IX; four per row in VIII, 5 per row in IX.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXIV. Typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sac in X and XI, sacs not connected. Seminal vesicles paired in XI–XII, are large, well developed. Prostate glands well developed, large, occupying segments XVII to XIX. Prostatic duct coiled in the middle portion, copulatory pouch locates within the body wall and well developed conical penis present, and extends outwards and passes through the sleeve to opening. Genital marking glands sessile, corresponds with external genital papillae.

Ovaries and funnels in XIII. Four pairs spermathecae in VI–IX. Ampulla elongated saccular-shape, with relatively short duct, and terminal rod shaped diverticulum, with irregular stalk.

Variation. The holotype is the only complete specimen, with 300 mm body length and 116 segments; all three paratypes are incomplete specimens, longer than 165 mm and with more than 89 segments. The most significant variation is the number of genital papillae on each segment: 3–9 papillae on pre-clitellum and 6–8 papillae on post-clitellum. One paratype specimen has additionally one papilla in VII and three in XXIII.

Distribution. This species lives in the top soil at about 10–15 cm depth, in the watercourse where it contributed to sedimentary organic matter accumulation, in the literary botanical garden.

Remarks. *Metaphire songkhlaensis* sp. n. is octothecal with spermathecal pores in 5/6–8/9, keying out with the *pulauensis* species group in Sims and Easton (1972) that consists of two species members, *M. pulauensis* (Beddard, 1900) and *M. baruana* (Stephenson, 1932) from Malaysia, and with body lengths of 120–165 mm.

The main difference between the present species and these two known species is the position of the genital markings. *Metaphire songkhlaensis* sp. n. has genital markings situated in segments VIII, IX, XIX–XXII, while *M. baruana* has genital markings only in the male pore area, on segment XVIII, and *M. pulauensis* presents genital markings in VII, VIII, IX, XVII and XVIII. Another difference is the distance between the male pores as a fraction of the estimated circumference of segment XVIII: 0.25 in *M. baruana* and *M. pulauensis*, while in *M. songkhlaensis* this distance is 0.22 of the body circumference for the holotype. Table 4.1 contains information on the characters used to separate the members of the *pulauensis* species group.

Metaphire trangensis **Bantaowong & Panha sp. n.**

Fig. 4.3; Table 4.1

Material examined. Holotype: clitellate (CUMZ 3375), Thailand, Trang, Na Yong, Peninsular Botanical Garden, 7° 32’ 57.1” N, 99° 46’ 58.3” E, 9 m amsl, 16 January 2014, leg. U. Bantaowong, C. Sutcharit, P. Pimvichai & W. Siriwut. Paratypes: 21 adults (CUMZ 3376), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Etymology. This species is named after the province where it was collected.

Diagnosis. Medium size; length 120–169 mm. Male pores paired, round opening wrinkled radially around its margin in segment XVIII, genital markings absent. Spermathecal pores paired in 5/6–8/9. Spermathecae oval ampulla, and an ellipsoidal terminal knob shaped diverticulum. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 11/12. Prostate glands large, its duct C-shape, with round copulatory sac.

Description of holotype. Dimensions; 155 mm by 7.3 mm at segment VII, 7.3 mm at segment XX, 7.6 mm at clitellum; body cylindrical with 100 segments. Setae regularly distributed around segmental equators, numbering 70 at VII, 88 at XX, 20 between male pores, setae formula AA:AB:ZZ:ZY = 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI.

Male pores, with transversely oval opening wrinkled radially around its margin, on XVIII, 0.27 circumference apart ventrally, distance between male pores 6 mm. Spermathecal pores four pairs, epidermis around each pore slightly wrinkled, in furrows 5/6–8/9, ventral, pores about 0.40 body circumference ventrally apart, distance between spermathecal pores 10 mm. No genital markings in the spermathecal pores region.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXI. Typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands large, extending anteriorly to segment XVII, posteriorly to XXI. Prostate duct C-shape, coiled around round copulatory sac in segment XVIII; coiled clockwise left side, anti-clockwise right side.

Ovaries in XIII. Spermathecae four pairs in VI–IX. The ampulla large oval with duct shorter than and sharply marked off from the ampulla, diverticulum ellipsoidal with tubercle of circular outline and slightly dilated to form a small ovate knob.

Variation. The holotype measures 155 mm body length with 100 segments; the twenty five paratypes range in size from 120–169 mm (142.53 ± 15.24) body length with 80–152 segments. The prostate glands and intestinal caecum position varied from segments XVI–XXII and XXVII–XXIV, respectively.

Distribution. Known only from the type locality, and they live in the top soil at about 10–15 cm depth.

Remarks. This species also keys out with the *pulauensis* species group, but it is easily distinguished from the two former species by the absence of genital markings in the area of the male pores and the space between the male pores measuring 0.27 body circumference, with 20 setae between male pores. In contrast, *M. pulauensis* has genital markings in the area of spermathecal pores and male pores, male pores are 0.25 body circumference apart ventrally with 13 setae between male pores. *M. baruana* has genital markings in area of the male pores, which are 0.25 body circumference apart ventrally, and there are 17 setae between male pores. Furthermore, *M. trangensis* sp. n. is distinguished from all other member of the

pulauensis group by elongate caeca, prostatic ducts coiled around the copulatory bursae, and relatively large copulatory bursae.

***Metaphire pulauensis* (Beddard, 1900)**

Fig. 4.4; Table 4.1

Amyntas pulauensis Beddard, 1900: 904, fig. 5.

Pheretima pulauensis Stephenson, 1932: 233, fig. 10.

Metaphire pulauensis Sims and Easton, 1972: 239.

Material examined. The specimen that contained all the important morphological features and matched the original description is designated herein as the lectotype NHMUK 1924.3.1.214-215.1. The type locality is Pulau, Bideng, Kedah, Malay Peninsular. Paralectotypes: NHMUK 1924.3.1.214-215.2, 1 adult. NHMUK 1904.10.5.1002, 1 adult; same locality as lectotype.

Description of lectotype. Dimensions; 165 mm by 6.0 mm at segment VII, body cylindrical with 110 segments. Setae regularly distributed around segmental equators, setae formula AA:AB:ZZ:ZY = 1.5:1:1.5:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI.

Male pores on XVIII, puckered radially around the margins, 0.25 body circumference ventrally apart, distance between male pores 3 mm, with 10 setae intervening. Spermathecal pores inconspicuous, four pairs in 5/6–8/9, about 0.33 body circumference ventrally apart, distance between spermathecal pores 4 mm. Genital papillae aggregated together to form slightly transversely oval patches, arranged mid ventral, pre setal of VII, VIII, IX, XVII and XVIII.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXIV. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed

in paired sacs in X and XI. Seminal vesicles paired, present only in segment XI and another pair in segment XII had been removed. Prostate glands large, occupying XVII–XX. Prostate duct short, slender, slightly curved and embed on a small round copulatory sac in segment XVIII. Sessile genital marking glands present corresponding to external genital markings in VII, VIII, IX, XVII and XVIII.

Ovaries in XIII. Spermathecae four pairs in VI–IX. The ampulla elongated, with duct shorter than and sharply marked off the ampulla, diverticulum has one-third length of ampulla, as lender stalk, and twisted in to short loops.

Variation. Body length of paralectotypes range from 143–160 mm with 104–110 segments.

Remarks. The original description (Beddard, 1900) was said to be based on three specimens and included details of the reproductive organ such as male field area, genital papillae and shape of the spermathecae, together with an illustration of the external ventral view. However, other significant characters were not described, such as the testes and distance of the male pores and spermathecal pores relative to the body diameter. Later, Stephenson (1932) gave an emended description that included an illustration of the spermathecae, but this description was based on only two specimens. In this paper, the specimen that contained the important morphological features that matched the original description is here designated as the lectotype, and the schematic drawings of both the external ventral and internal dorsal views are given.

The type locality of *M. pulauensis* in northern Malaysia is quite close to the type localities of *M. songkhlaensis* sp. n. and *M. trangensis* sp. n. in southern Thailand, and superficial morphological investigation may identify all of them as *M. pulauensis.* However, *M. pulauensis* can be easily distinguished from *M. songkhlaensis* and *M. trangensis* by the genital markings on segments VII, VIII, IX, XVII and XVIII, while *M. songkhlaensis* has no genital markings on segments XVII and XVIII, and *M. trangensis* lacks genital markings altogether.

This species is known only from its type locality in Pulau Bidan, Kedah, Malaysia, as in the original description.

***Metaphire baruana* (Stephenson, 1932)**

Fig. 4.5; Table 4.1

Amyntas bosschae Horst, 1892 sensu Beddard 1900, nec Horst (Stephenson, 1932)
Pheretima baruana Stephenson, 1932: 209. Fig. 3.
Metaphire baruana Sims and Easton, 1972: 239.

Material examined. Holotype NHMUK 1924.10.5.14.1 (designated by Stephenson 1932). The type locality is Khota Baru, Malay Peninsula. Non-type specimen: one semi-clitellate (NHMUK 1924.10.5.14.2) same locality as lectotype.

Description of holotype. Dimensions: 132 mm by 6.0 mm at segment VII, body cylindrical with 127 segments. Setae regularly distributed around segmental equators. There are no setae gaps. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 10/11. Clitellum annular XIV–XVI with no setae.

Male pores on small protuberances on XVIII, 0.25 body circumference apart ventrally, distance between male pores 4 mm, 17 setae between them. One circular genital marking on the inner slope of the left male pore. Spermathecal pores inconspicuous, four pairs in 5/6–8/9, about 0.30 body circumference apart ventrally, distance between spermathecal pores 4 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originate in XXVII, simple, extends to XXIV. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles had been removed. Prostate glands large, occupying XVI–XX. Prostate duct short, C-shaped, glandular mass on and between basal duct and the nerve cord.

Ovaries in XIII. Four pairs spermathecae in VI–IX. The ampulla pear-shaped, gradually merging into duct, diverticulum slender, slightly dilated to form a small ovoid knob.

Variation. Body length of paralectotype is 120 mm with 125 segments.

Remarks. Stephenson's description is based on one of the two specimens from Khota Baru, Malaysia, that Beddard (1900) had identified as *Amyntas bosschae* Horst, 1892; this specimen, as he wrote, "... may be considered as the type, ..." (Stephenson, 1932: 209). Since no further mention is made of the second specimen, it should be considered as not belonging to the type series (see above: "non-type reference material"). The original description is detailed but only the spermathecae were illustrated. In this study, the description with schematic drawings of both the external ventral and the internal dorsal views are given.

Khota Baru, north Malaysia, the type locality of *M. baruana*, is quite close to Songkhla and Trang, south Thailand, the type localities of the two new species *M. songklaensis* sp. n. and *M. trangensis* sp. n., and the newly collected specimens of the two new species could superficially be identified as *M. baruana.* However, the distinct genital marking in the male pores area of *M. baruana* is absent in the two new species *M. songkhlaensis* and *M. trangensis*, this being the most significant difference of the new species to *M. baruana*.

This species is known only from its type locality in Kota Bharu, Malaysia, as in the original description.

***Metaphire khaoluangensis* Bantaowong & Panha sp. n.**

Fig. 4.6; Table 4.2

Material examined. Holotype: clitellate (CUMZ 3377), Thailand, Nakhon Si Thammarat, Nopphitam, Ban Khlong Phod, 8° 48’ 8.4” N, 99° 35’ 18.6” E, 140 m amsl, 17 January

2013, leg. U. Bantaowong, C. Sutcharit & W. Siriwut. Paratypes: 22 adults (CUMZ 3378), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Other material examined. 2 adults (CUMZ 3379), Thailand, Nakhon Si Thammarat, Nopphitam, Krungching Waterfall, 8° 43’ 34.8” N, 99 39’ 58.4” E, 208 m amsl, 17 January 2013. 9 adults (CUMZ 3380), Thailand, Nakhon Si Thammarat, Nopphitam, Tham Lod, 8° 47’ 17.2” N, 99° 38’ 40.1” E, 97 m amsl, 17 January 2013. 4 adults (CUMZ 3381), Thailand, Phatthalung, Srinagarindra, Tham Phutthakodom, 7° 33’ 38.5” N, 99° 53’ 5.6” E, 54 m amsl, 16 January 2013.

Diagnosis. Medium size; length 130–265 mm. Male pores paired, with puckered margin on segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–8/9. Spermathecae elongate sac-like ampulla, with short duct, diverticulum slender, constricted with round seminal chamber. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands well developed, its duct embed in the copulatory sac.

Etymology. This new species was named after Mt. Khaoluang where it was collected.

Description of holotype. Dimensions; 220 mm by 10.0 mm at segment VII, 9.0 mm at segment XX, 9.0 mm at clitellum; body cylindrical with 119 segments. Setae regularly distributed around segmental equators, numbering 36 at VII, 51 at XX, 14 setae between male pores, setae formula AA:AB:ZZ:ZY = 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XV.

Male pores conspicuous, with puckered margin, separated from each other by 0.28 circumference ventrally, distance between male pores 7 mm. Spermathecal pores three pairs in 6/7–8/9, epidermis around each pore slightly wrinkled, about 0.44 circumference apart ventrally, distance between spermathecal pores 11 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originate in XXVII, simple, extend to XXI. Typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate gland occupying segments XVI to XIX. Prostatic duct slightly muscular, closely attached to lateral side of copulatory sac.

Ovaries in XIII. Three pairs spermathecae in VII–IX. Ampulla elongate, sac-like, with short duct; diverticulum slender, its proximal end with a neck-like constriction; total length slightly less than ampulla.

Variation. The holotype measures 220 mm body length with 119 segments; body length of paratypes 130–265 mm (186.4 ± 34.7), segments 70–131. Some prostate glands in XVI–XX, intestinal caeca often shorter than in holotype, ending in XXIII.

Distribution. Known from the type locality and one additional locality in Phatthalung province, the two sites being separated by approximately 240 km.

Remarks. This species is sexthecal, with spermathecal pores on 6/7–8/9, and no genital marking, so it belongs to the *houlleti* species group that consists of more than 30 species. In Thailand, this group has only three reported species of *M. houlleti* (Perrier, 1872), *M. virgo* (Beddard, 1900) and *M. perichaeta* (Beddard, 1900). *Metaphire khaoluangensis* can be distinguished from the first two species in Thailand by the spermathecal shape and the distance between male pores as a fraction of the estimated circumference. This measure is 0.28 in *M. khaoluangensis*, but 0.30 and 0.33 in *M. houlleti* and *M. virgo*, respectively. Other differences from *M. houlleti* and *M. virgo* are: first dorsal pore in 12/13 (11/12 in *M. houlleti* and *M. virgo*) and spermathecal ampulla large and elongate (spherical and small sac in *M. houlleti* and *M. virgo*, respectively). The new species does not have the contorted diverticulum stalk enveloped in connective tissue as found in *M. houlleti*, and also lacks the typhlosole

present in *M. houlleti*. *Metaphire virgo* is further distinguished from the new species by wider male pore spacings and a spermathecal diverticulum stalk with multiple folds. Both *M. houlleti* and *M. virgo* tend to have genital markings bearing stalked glands in association with spermathecae and with the copulatory sacs, whereas the new species lacks these characters. This new species is fairly similar to *M. perichaeta* in male pore spacing (0.28 body circumference), but it is distinguished by elongated spermathecae, with slender diverticulum and last hearts in XIII compared to reverse pear-shape spermathecae, with coiled diverticulum and last hearts in XII in *M. perichaeta*. A comparison of characters between *M. khaoluangensis* and other related species is presented in Table 4.2.

***Metaphire orientalis* Bantaowong & Panha sp. n.**

Fig. 4.7; Table 4.2

Material examined. Holotype: clitellate (CUMZ 3382), Thailand, Rayong, Khao Cha Mou, Wat Ma Dua, 13° 03’ 14.1” N, 101° 36’ 46.6” E, 343 m amsl, 10 November 2013, leg. S. Panha, U. Bantaowong, P. Tongkerd & W. Siriwut. Paratypes: 5 adults (CUMZ 3383), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Other material examined. 2 adults (CUMZ 3384), Thailand, Rayong, Klaeng, Wat Tham Naramitr, 12° 58’ 20.2” N, 101° 39’ 54.3” E, 34 m amsl, 10 November 2013. 3 adults (CUMZ 3385), Thailand, Rayong, Mueang, Wat Khao Sab, 12° 36’ 48.1” N, 101° 23’ 23.0” E, 9 m amsl, 10 November 2013. 77 adults (CUMZ 3386), Thailand, Rayong, Mueang, Khao Laem Ya-Mu Koh Samet National Park, 12° 35’ 16.7” N, 101° 25’ 10.9” E, 15 m amsl, 10 November 2013. 57 adults (CUMZ 3387), Thailand, Chonburi, Bo Thong, Khao Cha Ang On, 13° 12’ 0.7” N, 101° 34’ 43.6” E, 82 m amsl, 24 September 2012. 2 adults (CUMZ 3388) Thailand, Chonburi, Bo Thong, Khao Ha Yod, 13° 09’ 46.4” N, 101° 35’ 52.6” E, 79 m amsl, 9 November 2013. 4 adults (CUMZ 3389), Thailand, Chonburi, Si

Racha, Chan Ta Then Waterfall, 13° 14’ 12.7” N, 101° 2.0’ 28.2” E, 168 m amsl, 13 August 2010.

Etymology. This new species was named for its broad distribution in the eastern part of Thailand.

Diagnosis. Medium sized; length 100–148 mm. Male pores paired, slight ridge on ventro-lateral side of segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–8/9. Spermathecae with elliptic ampulla, diverticulum slender, with small round knob. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands several lobules, ducts not very long.

Description of holotype. Dimensions: 166 mm by 6.0 mm at segment VII, 6.1 mm at segment XX, 5.8 mm at clitellum; body cylindrical with 118 segments. Setae regularly distributed around segmental equators, numbering 34 at VII, 60 at XX, no setae between male pores, setae formula AA:AB:ZZ:ZY = 1.5:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XV.

Each male pore in a groove or furrow across the porophores, from lateral edge towards median edge, represented by a slight ridge on ventro-lateral side of XVIII, about 0.30 circumference apart ventrally, distance between male pores 5 mm, the median region between male pores depressed. Three pairs spermathecal pores, each marked by a whitish spot in furrows 6/7–8/9, ventral, distance between each pair 5 mm, about 0.30 of the body circumference apart ventrally.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXII. Typhlosole one-fourth lumen diameter, begins in XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in ventral paired sac in X and XI, sacs not connected. Seminal vesicles paired in XI–XII. Prostate gland well developed, located in XVII to XXIII, divided into several lobules. Prostatic duct not very long, greater

diameter towards body wall, usually with a deep loop in the middle, copulatory pouch invisible or other projections, well developed penis absent.

Ovaries in XIII. Three pairs spermathecae in VII–IX. Ampulla elliptic with a short duct. The diverticulum stalk slender, as long as amuplla, with a small rounded seminal chamber.

Variation. The holotype measures 166 mm body length with 118 segments; all nine paratypes ranged in size from 100–148 mm (122.6 ± 12.8) body length with 110–120 segments. The prostate glands and intestinal caecum locations varied from segments XVI–XXII and XXVII–XXIII, respectively.

Distribution. Known only from Rayong and Chonburi provinces, located in eastern Thailand.

Remarks. This species also keys to the *houlleti* species group in Sims and Easton (1972). Within the *houlleti* species group in Thailand, which consists of *M. houlleti*, *M. virgo, M. khaoluangensis* and *M. perichaeta*, the new species can be distinguished by the much more ventral placement of the spermathecal pores, the features of the male field including that the lateral slits associated with the male pores, absence of setae between male pores, and absence of an internally visible copulatory sac (Table 4.2).

***Metaphire perichaeta* (Beddard, 1900)**

Fig. 4.8; Table 4.2

Amyntas perichaeta Beddard, 1900: 896.

Pheretima perichaeta Stephenson, 1932: 227, fig. 8; Gates, 1939: 103.

Metaphire perichaeta Sims and Easton, 1972: 238.

Material examined. Holotype: one clitellate (NHMUK 1924.3.1.231; Fig. 4.8). The type locality is State of Patthalung, Malay Peninsula.

Description. Dimensions; 160 mm by 5.0 mm at segment VII, body cylindrical with 118 segments. Setae regularly distributed around segmental equators AA:AB:ZZ:ZY = 1:1:2:1. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with no setae.

Male pores conspicuous with transversely slit-like aperture and discharge into the copulatory pouches on XVIII, 0.28 body circumference ventrally apart, distance between male pores 3.5 mm, 12 setae intervening between them. No genital markings. Spermathecal pores inconspicuous, three pairs in 6/7–8/9, about 0.30 body circumference apart ventrally, distance between spermathecal pores 3.7 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXIV. Oesophageal hearts three pairs in X–XII. Holandric; testes and funnels in X and XI. Seminal vesicles in XI and XII. Prostate glands occupying XVII–XIX. Prostate duct curved, embed on round copulatory sac that is confined to XVIII.

Ovaries in XIII. Four pairs spermathecae in VI–IX. Ampulla reversely pear-shaped, with curl at the margin. Diverticulum slender and greatly coiled towards distal end.

Remarks. The description and measurements given herein are in agreement with those of Beddard (1900) and Stephenson (1932). However, Stephenson (1932) provided a brief illustration only of a spermathecae, and soother important features, such as the male genital characters of the prostate glands and the intestinal caecum, were not provided. In this study, the description with schematic illustrations of both the external ventral and internal dorsal views are given.

Metaphire perichaeta was first recorded from the State of Phatthalung (previously spelled Patalung), Malay Peninsula, but the locality is actually in Thailand, and *M. khaoluangensis* sp. n. is also found in Phatthalung. The types of *M. perichaeta* were

reinvestigated because of the close similarity of this species to *M. orientalis* sp. n. and *M. khaoluangensis* sp. n. Differences of *M. perichaeta* are as follows: last oesophageal hearts in XII; spermathecae reversely pear-shaped; prostate glands and intestinal caeca smaller than in the two new species; prostate glands situated on three segments in XVII–XIX (more than three in the new species) and intestinal caeca in XXVII–XXIV (XXVII–XXII in the new species). Moreover, *M. perichaeta* has a narrow break in the ring of setae on the dorsal surface, while *M. orientalis* has a break on the ventral surface, and *M. khaoluangensis* has no break in the ring of setae.

This species is known only from its type locality in Phatthalung province, Thailand, as in the original description, but without any coordination details.

***Metaphire doiphamon* Bantaowong & Panha sp. n.**

Fig. 4.9

Material examined. Holotype: clitellate (CUMZ 3390), Thailand, Chiang Rai, Thoeng, Phu Chee Fah, 19° 48’ 47.0” N, 100° 26’ 20.4” E, 1205 m amsl, leg. U. Bantaowong, C. Sutcharit, & P. Tongkerd, 19 November 2012. Paratype: 1 adult (CUMZ 3391), same data as holotype.

Etymology. This species is named after the Doi Phamon mountain range, where it was collected. Noun in apposition.

Diagnosis. Large; length 255–269 mm. Male pores paired, transverse openings in XVIII, genital markings paired on 17/18–18/19. Spermathecal pores paired in 6/7–8/9. Spermathecae with ovoid ampulla, diverticulum thread-like, slightly curved at tip. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands and ducts two branches, with white glandular mass on body wall where duct ends.

Description of holotype. Dimensions: 255 mm by 9.9 mm at segment VII, 11.0 mm at segment XX, 10.0 mm at clitellum; body cylindrical with 142 segments. Setae regularly distributed around segmental equators, numbering 49 at VII, 84 at XX, 21 between male pores, setae formula AA:AB:ZZ:ZY = 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with the row of setae in the middle line.

Male apertures as two transverse openings situated on the ventro-lateral sides of segment XVIII, about 0.26 circumference apart ventrally, distance between male pores 8 mm, each male pore in a very shallow rudimentary copulatory chamber that is like a skin fold, surrounded by 3–4 closely adjacent concentric circular ridges. Genital markings paired, transversely elliptical, 4–5 intersetal intervals wide, located in intersegments of 17/18 and 18/19, in line with male pores. Three pairs spermathecal pores, transverse slits in furrows 6/7–8/9, ventral, distance between each pair 9 mm, about 0.30 body circumference apart ventrally.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXII. Typhlosole simple rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands are usually confined to XVII–XIX, separated into two racemose lobes. Prostate duct divided into two small branches, one to each lobe. No copulatory sac visible, but there is a white glandular mass on the inner surface of the body wall, where prostatic ducts enter the parietes, and in the area of the genital markings.

Ovaries in XIII. Spermathecae three pairs in VII–IX. Ampulla ovoid, duct stout, one-third as long as ampulla and sharply marked off from ampulla. Diverticulum long and slender, thread-like, without a terminal enlarged seminal chamber, slightly curved in tip.

Variation. Holotype measures 255 mm body length with 142 segments; paratype is 269 mm body length with 142 segments.

Distribution. This species is known only from the type locality, where it coexists with *Amynthas phucheefah* Bantaowong & Panha, 2014.

Remarks. *Metaphire doiphamon* is sexthecal and presents genital markings at 17/18 and 18/19, which makes it key to the *peguana* species group that consists of three other species of *M. bahli* (Gates, 1945), *M. peguana* (Rosa, 1890) and *M. saigonensis* (Omodeo, 1957). Of these, *M. peguana* and *M. bahli* have been recorded in Thailand, whereas *M. saigonensis* is currently placed in synonymy with *M. peguana* (Bantaowong et al., 2011b; Blakemore, 2002; Gates, 1939; Prasankok et al., 2013; Somniyam and Suwanwaree, 2009).

This new species is distinguished from the other members of the species group by its body length of 255 mm, the form of the genital markings and the bifurcate prostatic ducts. The genital markings are transversely oval in *M. doiphamon*, they lack pores or other openings and are associated with sessile glandular masses, while the genital markings of *M. peguana* and *M. bahli* are circular to oval, invaginated or pouch-like, and with stalked glands. Both of them have a smaller body size (range of 110–240 mm) and fewer segments. Furthermore, *M. doiphamon* also has unique prostate glands and ducts, being divided into two main lobes each with a large and long duct, while the two former species have regular prostate glands, each consisting of a single racemose mass with many small ductlets.

***Metaphire saxicalcis* Bantaowong & Panha, sp. n.**

Fig. 4.10

Material examined. Holotype: clitellate (CUMZ 3392), Thailand, Chonburi, Bo Thong, Khao Cha Ang On, 13° 12’ 0.7” N, 101° 34’ 43.6” E, 82 m amsl, 24 September 2012, leg. S. Panha, U. Bantaowong, R. Chanabun & W. Siriwut. Paratypes: 6 adults (CUMZ 3393), 1 adult (ZMH), 1 adult (NHMUK), same data as holotype.

Other material examined. 4 adults (CUMZ 3394), Thailand, Chonburi, Bo Thong, Khao Ha Yod, 13° 9’ 46.5” N, 101° 35’ 52.3” E, 93 m amsl, 24 September 2012.

Diagnosis. Large; length 254–377 mm. Male pores paired, with transversely slit-like aperture and puckered-margin on segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–7/8. Spermathecae with large paddle-shaped ampulla, duct thin, with large saccular body at its base. Diverticulum slender, very long, its length as long as main part of spermatheca, enlarged spindle-like seminal chamber. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, its elongate spindle-shaped duct connected to center of the large circular copulatory sac.

Etymology. The specific epithet is from the Latin for a limestone forest, which is the habitat of the new species.

Description of holotype. Dimensions: 377 mm by 9.6 mm at segment VII, 9.5 mm at segment XX, 9.6 mm at clitellum; body cylindrical with 151 segments. Setae regularly distributed around segmental equators, numbering 89 at VII, 96 at XX, 20 setae between male pores, setae formula AA:AB:ZZ:ZY = 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XV.

Male pores very conspicuous, transversely slit-like apertures with puckered margins on ventro-lateral sides of segment XVIII, about 0.28 circumference apart ventrally, distance between male pores 8 mm. Spermathecal pores two pairs ventral lateral, each pore with transverse slits on somewhat raised glandular areas in furrows 6/7–7/8, about 0.34 body circumference apart ventrally, distance between spermathecal pores 10 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to

XXII. Typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes small, two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands in XVI–XXI, expanded into a fan-shaped outline wrapped around copulatory sacs. Duct large and long, looped in a hairpin-shape with enlarged spindle-shaped ectal half, enters into the large round cushion-like copulatory sac. Ovaries in XIII. Spermathecae large, in VII and VIII; ampulla large paddle-shaped, with saccular body at the base of spermathecal ducts, duct thin and not sharply marked off from the ampulla. Diverticulum slender, very long, length as long as main part of spermathecae, slightly enlarged and spindle-like seminal chamber has pebbly outer texture.

Variation. Holotype measures 377 mm body length with 151 segments; all eight paratypes range in size from 254–285 mm (271.6 mm ± 21.9 mm) body length with segments varying from 147–157. Most characters of the paratypes are quite similar to the holotype, but setae between male pores vary from 11 to 18, and prostate glands and intestinal caeca situate on segments XVI–XXII and XXVII–XXIII.

Distribution. Known only from the type locality and one further site from Khao Ha Yod, Chonburi, which is located approximately 15 km south-west of the type locality.

Remarks. *Metaphire saxicalcis* sp. n. keys to the *planata* species group in Sims and Easton (1972). This group consists of six species; *M. decipiens* (Beddard, 1900), *M. dunckeri* (Michaelsen, 1902), *M. parvula* (Ohfuchi, 1956), *M. sintangi* (Michaelsen, 1922), *M. ferdinandi* (Michaelsen, 1891) and *M. planata* (Gates, 1926). *M. saxicalcis* is easily distinguished from the above related species by the larger body length of 254–377 mm, while the first four species have body lengths of 50–70 mm and the two remaining species range from 70 to 190 mm. None of these six species have the saccular body at the spermathecal ducts that we observed in *M. saxicalcis*. Only one species within

this group has been reported from Thailand, *M. planata*, a species with stalked genital marking glands at the spermathecae.

Metaphire saxicalcis is also fairly similar to *M. dunckeri* (Michaelsen, 1902) from Malaysia, in the location of the spermathecal pores in 6/7–7/8 and the absence of genital markings, but it is easily distinguished by the size difference, 377 mm body length versus 58 mm in *M. dunckeri*. Moreover, in *M. saxicalcis* the copulatory sacs are surrounded by the prostate gland, whereas in *M. dunckeri* the prostate glands are situated posterior to the copulatory sacs. A type-based redescription of *M. dunckeri* is given below.

***Metaphire dunckeri* (Michaelsen, 1902)**

Fig. 4.11

Pheretima dunckeri Michaelsen, 1902: 9–11.

Metaphire dunckeri Sims and Easton, 1972: 239.

Material examined. The specimen that contained the important morphological features and matched the original description is designated herein as the lectotype ZMH (V5876.1; Fig. 4.11). Paralectotype: 3 adults (ZMH V5876.2), same locality with lectotype.

Description of lectotype. Dimensions; 58 mm by 2.5 mm at segment VII, body cylindrical with 87 segments. Setae regularly distributed around segmental equators, no setal gaps. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI with no setae.

Male pores puckered radially around its margin, on XVIII, 0.20 body circumference apart ventrally, distance between male pores 0.9 mm. Spermathecal pores are wrinkled, conspicuous, two pairs in 6/7–7/8, about 0.25 of the body circumference ventrally apart. Distance between spermathecal pores 1 mm. No genital markings.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXV. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands large, occupying XVIII–XXIII. Prostate duct is short, embed on a large round copulatory sac in segment XVIII.

Ovaries in XIII. Two pairs spermathecae in VII–VIII. Ampulla spherical with stout duct, same size as ampulla. Diverticulum convoluted stalk bound into a solid mass, with small oval knob.

Remarks. This species was originally described based on five specimens; four dissected specimens are extant at the ZMH. Some important organs were noticed as missing, such as the gizzard, seminal vesicle and spermathecae, in two specimens. So, the specimen that contained the important morphological features and matched the original description is here designated as the lectotype. The description and schematic drawings of both the external ventral and internal dorsal views are given.

The main different characters between *M. dunckeri* and *M. saxicalcis* sp. n. are the absence of an associated saccular body at the spermathecae, a stout, large spermathecal duct, and the prostate glands are posterior to the copulatory sac.

This species is known only from its type locality in Lubuk Paku, Pahang, Malaysia, as in the original description.

***Metaphire planata* (Gates, 1926)**

Fig. 4.12

Pheretima planata Gates, 1926: 212; 1930: 320; 1931: 405; 1932: 411; 1939: 103; 1972: 211.

Metaphire planata Sims and Easton 1972: 239.

Material examined. Type from NHMUK (1928.4.2.1; Fig. 4.12). One semi-clitellate in ethanol, which was dissected.

Description. Dimensions; 125 mm by 4.8 mm at segment VII, body cylindrical with 138 segments. Setae regularly distributed around segmental equators AA:AB:ZZ:ZY = 1:1:1:1. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI with visible setae.

Male pores conspicuous on XVIII, transversely slit-like apertures puckered radially around its margin, 0.27 body circumference apart ventrally, distance between male pores 4 mm, and 11 setae intervene between them. No genital markings. Two pairs spermathecal pores, transverse slits, in furrows 6/7–7/8, ventral, distance between each pair about 0.25 body circumference ventrally apart, distance between spermathecal pores 4 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXII. Oesophageal hearts four pairs in X–XII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles in XI and XII small. Prostate glands small, occupying XVII–XIX. Prostate duct C-shaped, no internally visible copulatory sacs.

Ovaries in XIII. Two pairs spermathecae in VII–VIII, long stalked genital marking gland attached near spermathecal duct. Ampulla oval sac with strength duct, diverticulum tubular slightly dilated in tip.

Remarks. The single type specimen examined is semi-clitellate, and so the internal sexual organs are not well developed and it is missing one of the six spermathecae, but some important characters are quite clear, especially the spermathecae oval ampulla with tubular diverticulum beside which is a long stalked genital marking gland.

Metaphire planata is superficially similar to *M. saxicalcis* in the position and number of spermathecae and simple intestinal caeca, but differs from *M. saxicalcis* by the smaller body and presence of stalk genital marking glands on each spermathecae.

Metaphire surinensis **Bantaowng & Panha, sp. n.**

Fig. 4.13

Type material. Holotype: clitellate (CUMZ 3395), Thailand, Surin, Sangkha, Pasonnongkoo Forest Park, 14° 41’ 12.0” N, 103° 44’ 47.9” E, 165 m amsl, 16 October 2012, leg. U. Bantaowong, C. Sutcharit, R. Chanabun, J. Taptimon & W. Siriwut. Paratypes: 2 adults (CUMZ 3396), 1 adult (ZMH), 1 adult (NHMUK), same data as holotype.

Etymology. The specific is named after Surin, the province where it was collected.

Diagnosis. Large; length 208–300 mm. Male pores in a single deep transverse body wall invagination in segment XVIII, genital markings closely paired, protuberant in 18/19. Spermathecal pores closely paired in 6/7. Spermathecae elliptical ampulla, diverticulum long slender with sausage-like sperm chamber. Holandric, intestinal caeca simple, first dorsal pore in 11/12. Prostate glands large, posterior to copulatory sacs, ducts slender joining large circular copulatory sac near its center; large genital marking gland posterior to each copulatory sac.

Description of holotype. Dimensions: 275 mm by 8.8 mm at segment VII, 9.2 mm at segment XX, 9.0 mm at clitellum; body cylindrical with 190 segments. Setae regularly distributed around segmental equators, numbering 123 at VII, 128 at XX, no setae between male pores, setal formula AA:AB:ZZ:ZY = 1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI.

Male pores are in a single, deep, transverse body wall invagination, each pore sunken into the body wall invagination of XVIII, 0.15 circumference apart ventrally, distance between male pores 5 mm. Genital markings closely paired, protuberant in 18/19. One pair of spermathecal pores, very close paired, each on a tiny tubercle, in

furrow 6/7, ventral, distance between each pair about 0.05 body circumference ventrally apart, distance between spermathecal pores 1.5 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originate in XXVII, are simple and extend to XXIII. Typhlosole a simple fold, one-fourth the lumen diameter, beginning in XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles two pairs in XI–XII, large but not meeting mid-dorsally with one another, nearly equal in size and in shape. Prostate glands well developed, large, extending anteriorly to segment XX, posteriorly to XXVII. Prostate duct very long, *l*-shape, copulatory pouch in segment XVIII. Genital marking glands, cushion-like glandular mass which may possibly be protrusible externally.

Ovaries in XIII. One pair spermathecae in VII. Ampulla elliptical. Diverticulum terminal chamber sausage-like, longer than ampulla, and is remarkable in being actually slightly larger than the ampulla.

Variation. Holotype measures 275 mm body length with 190 segments. Paratypes range in size from 208–330 mm (272 ± 50.1) body length with 133–169 segments. One paratype has a pair of genital markings on 17/18, with prostate glands situated on segments XVIII–XXI. One paratype shows long prostate glands in segments XVIII–XXXIII.

Distribution. This species is known only from the type locality. They live in the soil at about 20–30 cm depth, in a forest park.

Remarks. The only bithecal species of the genus *Metaphire* known to date that has the spermathecal pores at 6/7 is *M. ladjangensis* (Ude, 1932). Differences between *M. surinensis* and *M. ladjangensis* are primarily in the male field and the location of genital markings. The secondary male pores of *M. ladjangensis* are small, visible from the surface and have 14 setae between them, but in *M. surinensis* they are in a deep invagination of the body wall with no setae between them. Moreover, in *M.*

ladjangensis the genital markings are situated on segment VIII and intersegments 17/18–18/19, and male pores are 0.30 body circumference ventrally apart, while in *M. surinensis* the genital markings are situated on intersegment 18/19 in holotype and the distance between male pores is about 0.15 circumference.

Discussion

The seven new species ranged in size, with respect to other *Metaphire* members, from moderate to large, of which *M. saxicalcis*, *M. surinensis*, *M. songkhlaensis* and *M. doiphamon* are the largest (255–377 mm), while the remaining three new species are medium sized (155–220 mm). *Metaphire saxicalcis* is the largest, and *M. trangensis* is the smallest species. All of these new species live in the top soil at a depth of 10–30 cm from the soil surface. *Metaphire doiphamon* were observed to emerge from the soil in October 2008 and November 2012, where they almost covered the road, but all of them were in the juvenile stage. This is similar to the report in Gates (1961) about a mass migration of juvenile worms from the genus *Perionyx* beginning in October to November (the cold season) in Burma.

However, newly discovered worms were found in various habitats. Two species, *M. songkhlaensis* and *M. trangensis*, were found in botanical gardens, while *M. orientalis* and *M. surinensis* were found in dipterocarp forest. *M. saxicalcis* was found in the top soil of a limestone forest area and *M. khaoluangensis* was found in modified deciduous forest areas used for integrated farming. The soil pH was close to 7 in all habitat types.

The newly collected material was compared with type and other reference material at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH) and the Natural History Museum (NHMUK), London. This critical re investigation led to new findings, illustrations and redescriptions as given above. The critical morphological investigation with type specimens finally confirmed the three separated species. However, microhabitat analysis and molecular phylogenetic studies are needed to clarify their systematic position and evolution of the genus *Metaphire*.

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Table 4.1 Comparison of morphological characters within *Metaphire* (*M.*) species of the *pulauensis* group. The comma is used to separate body length and width, and setal counts in two segments. Presence and absence of an organ is indicated by + or –.

Characters	*M. songkhlaensis*	*M. trangensis*	*M. pulauensis*	*M. baruana*
Body length, width (mm)	300, 9.0	155, 7.3	165, 6.0	132, 6.0
Segment number	116	100	110	127
First dorsal pore	12/13	11/12	12/13	10/11, 11/12
Setae vii, xx	71, 74	70, 88	44, 63	42, 58
Male pore setae	20	20	11	17
Male pore spacing	0.22	0.27	0.25	0.25
Genital marking	8, 9, 19, 20, 21, 22	–	7, 8, 9, 18, 19	18
Spermathecae	Elongated	Oval	Elongated	Pear-shaped
Diverticulum	Rod shape	Ellipsoidal	Twisted curve	Ovoid knob
Prostate gland	17–19	17–21	17–20	16–20
Genital marking gland	Sessile	–	Sessile	Sessile
Copulatory sac	–	+	–	–
Intestina caeca	27–24	27–21	27–24	27–24
Type locality	Thailand	Thailand	Malaysia	Malaysia

Table 4.2 Comparison of *Metaphire* (*M.*) species of the *houlleti* group. The comma is used to separate body length and width, and setal counts in two segments. Presence and absence of an organ is indicated by + or –. (*) indicated the morphological characters from Gates (1972), and (**) from Stephenson (1932). Missing data are shown by a question mark (?).

Characters	*M. orientalis*	*M. khaoluangensis*	*M. perichaeta*	*M. houlleti**	*M. virgo***
Body length, width (mm)	166, 6	220, 10	160, 5	92–200, 4–7	152–157, 5
Segment number	118	119	118	92-140	129
First dorsal pore	12/13	12/13	12/13	11/12	11/12
Setae vii, xx	34, 60	36, 51	36, 46	? , 48-62	?
Male pore setae	–	14	12	11–12	12
Male pore spacing	0.30	0.28	0.28	0.30	0.33
Genital marking	–	–	–	–	–
Spermathecae	Elliptic	Elongate	Pear shape	Large sac	Small
Diverticulum	Slender	Slender	Zigzag	Looped	Tubular
Prostate gland	17–23	16–19	17–19	16–21	17–18
Genital marking gland	–	–	–	Stalk	–
Copulatory sac	–	+	+	+	+
Intestinal caeca	27–23	27–21	27–24	27–22	27–25
Type locality	Thailand	Thailand	Thailand	India	Thailand

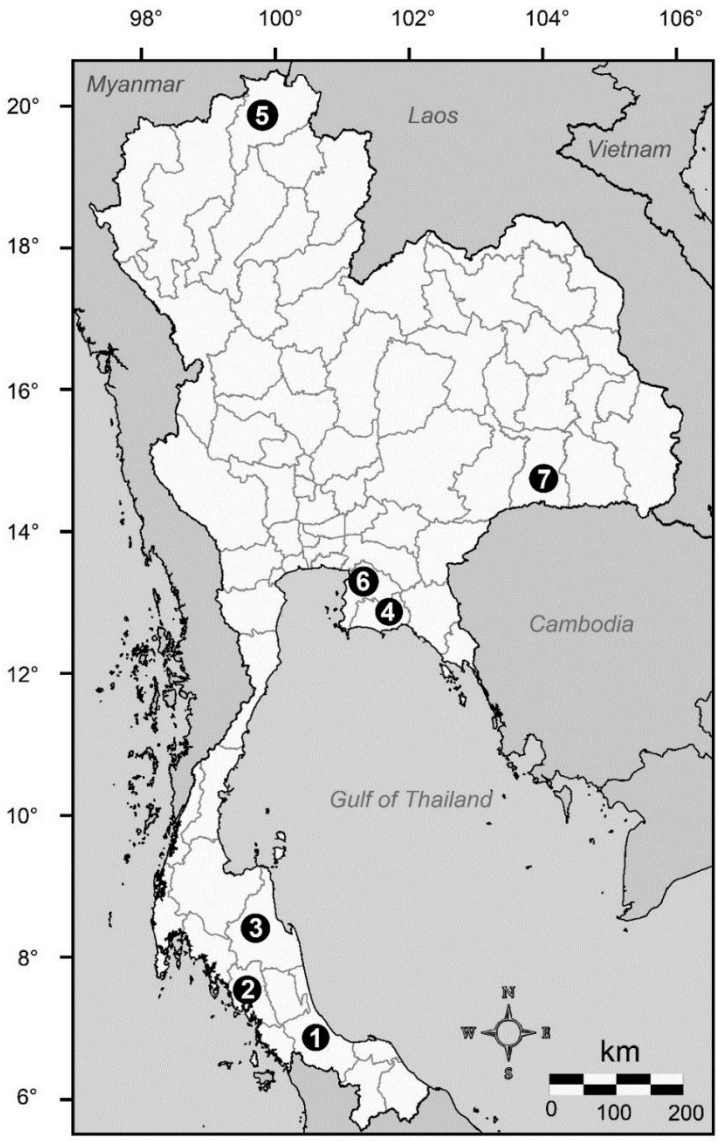

Figure 4.1 Map of type localities of *Metaphire songkhlaensis* sp. n. from Literary Botanical Garden, Hat Yai, Songkhla (1), *Metaphire trangensis* sp. n. from Peninsula Botanical Garden, Na Yong, Trang (2), *Metaphire khaoluangensis* sp. n. from Ban Khlong Phod, Nopphitam, Nakorn Si Thammarat (3), *Metaphire orientalis* sp. n. from Wat Ma Dua, Khaochamou, Rayong (4), *Metaphire doiphamon* sp. n. from Phu Chee Fah, Thoeng, Chiang Rai (5), *Metaphire saxicalcis* sp. n. from Khao Cha Ang On, Bo Thong, Chonburi (6), and *Metaphire surinensis* sp. n. from Pasonnongkoo Forest Park, Sangkha, Surin (7).

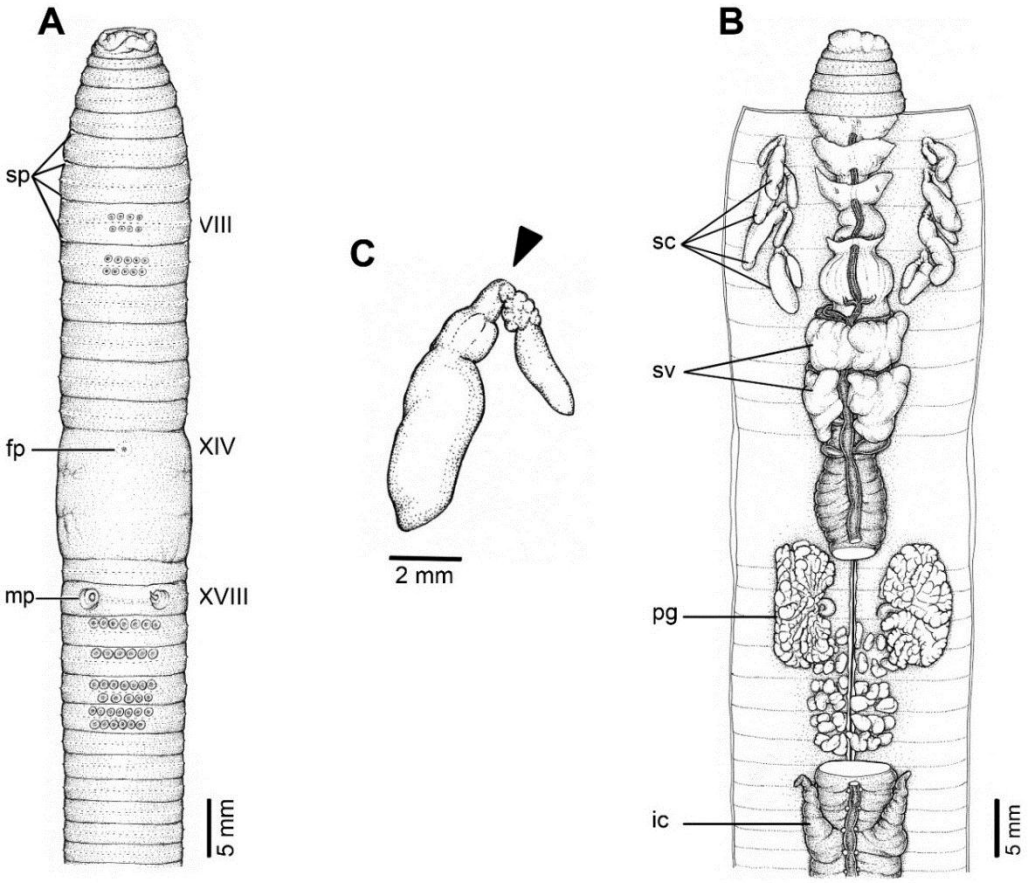

Figure 4.2 External and internal morphology of *Metaphire songkhlaensis* sp. n. holotype (CUMZ 3373): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

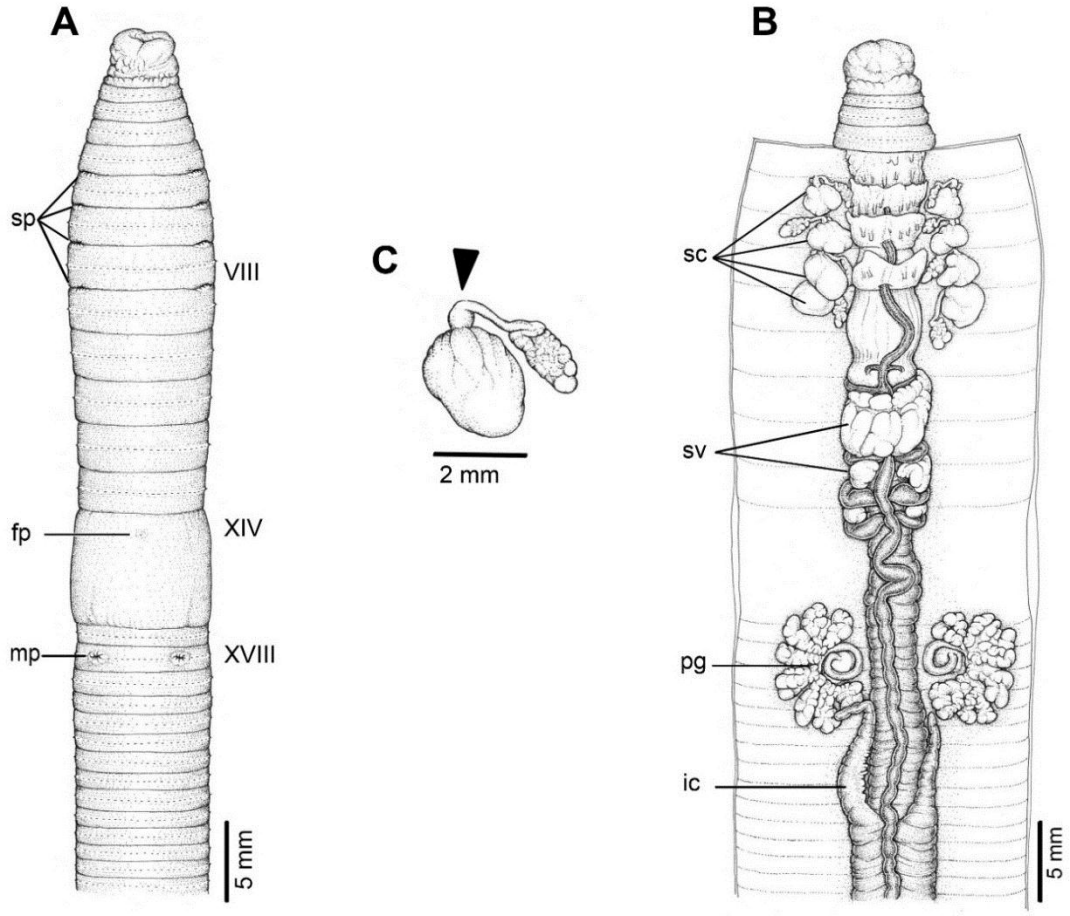

Figure 4.3 External and internal morphology of *Metaphire trangensis* sp. n. holotype (CUMZ 3375): A, external ventral view, B, internal dorsal view and C, spermathecae (Black arrow indicates the connection of the spermathecae and spermathecal pore).

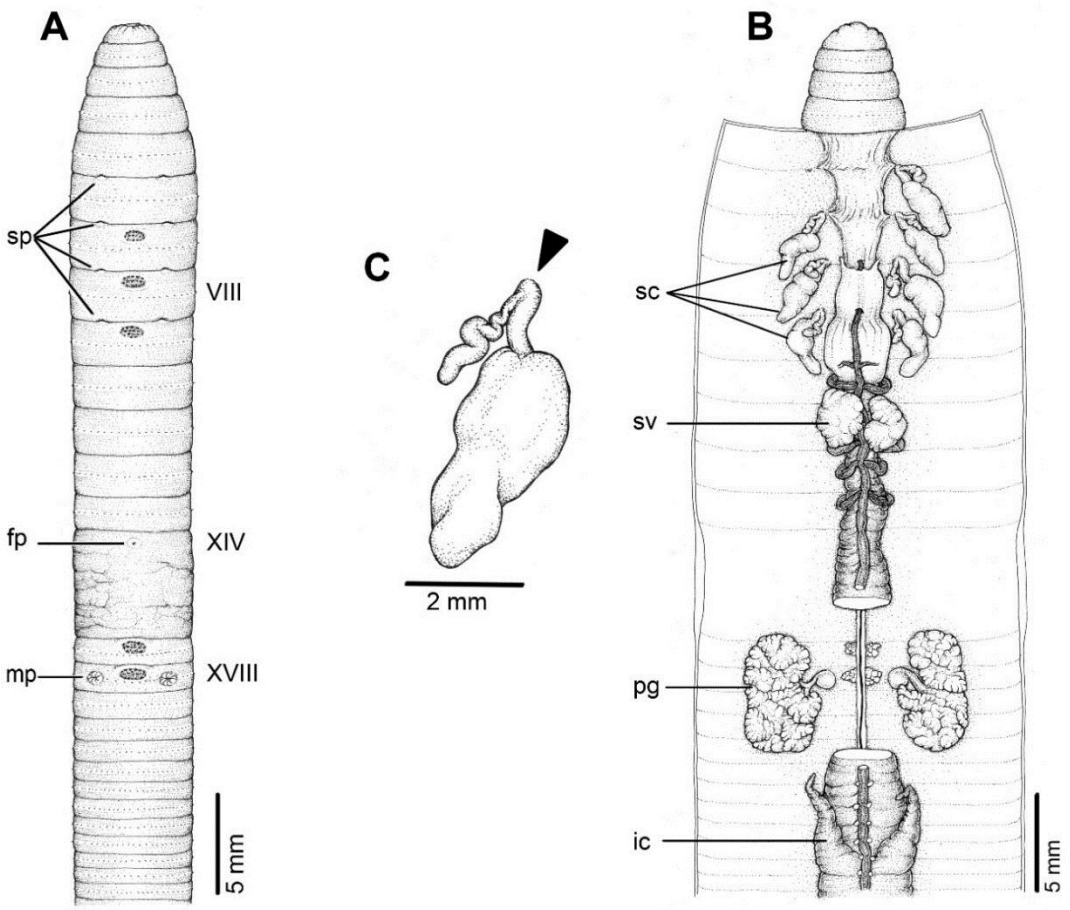

Figure 4.4 External and internal morphology of *Metaphire pulauensis* lectotype NHMUK 1924.3.1.214–215.1: A, external ventral view; B, internal dorsal view; C, spermathecae (Black arrow indicates the connection of the spermathecae and spermathecal pore).

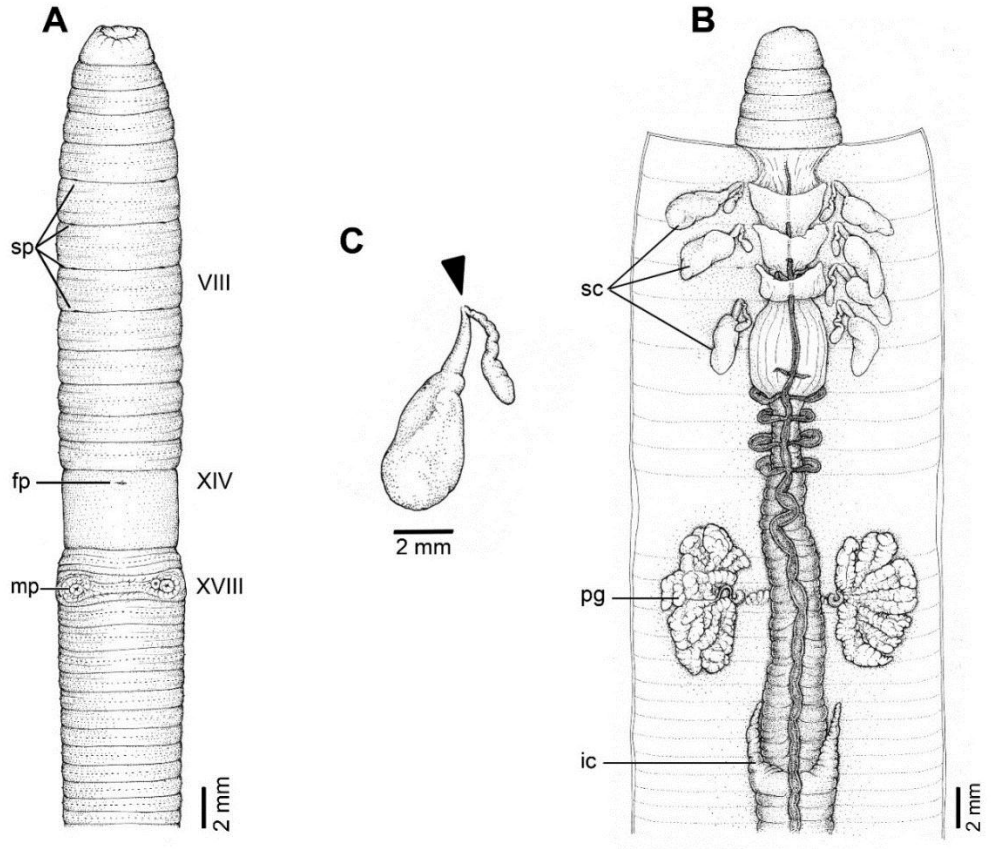

Figure 4.5 External and internal morphology of *Metaphire baruana* holotype NHMUK 1924.10.5.14.1: A, external ventral view; B, internal dorsal view; C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

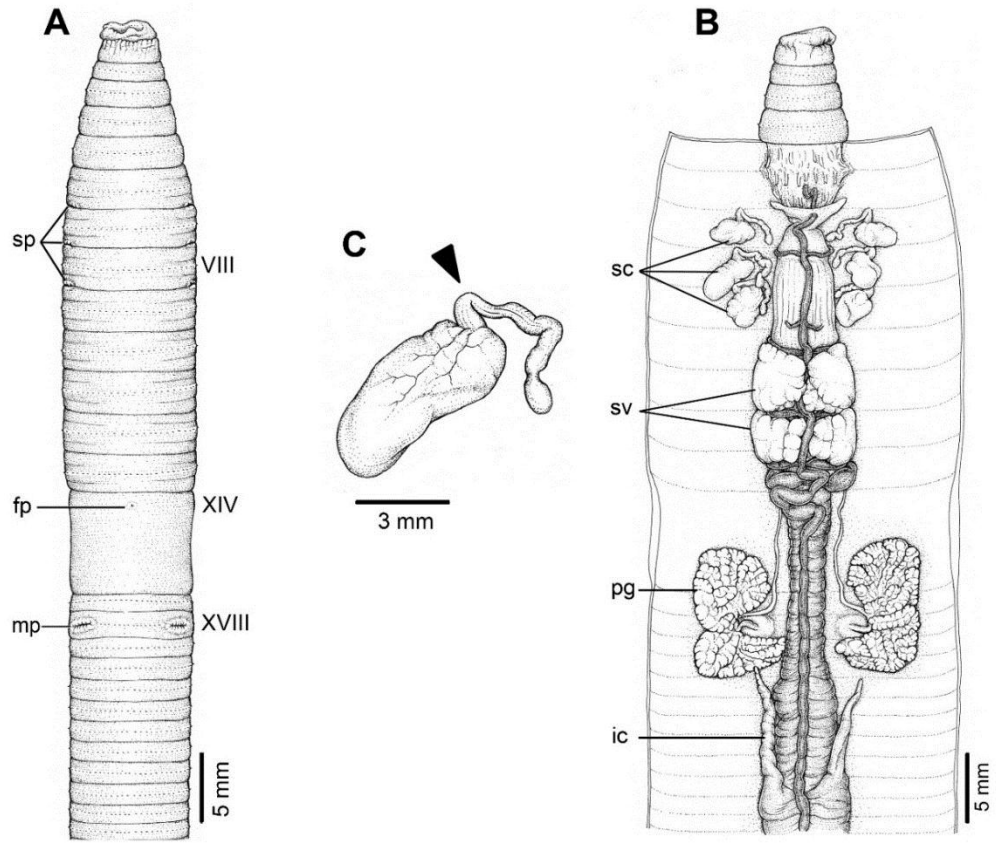

Figure 4.6 External and internal morphology of *Metaphire khaoluangensis* sp. n. holotype (CUMZ 3377): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

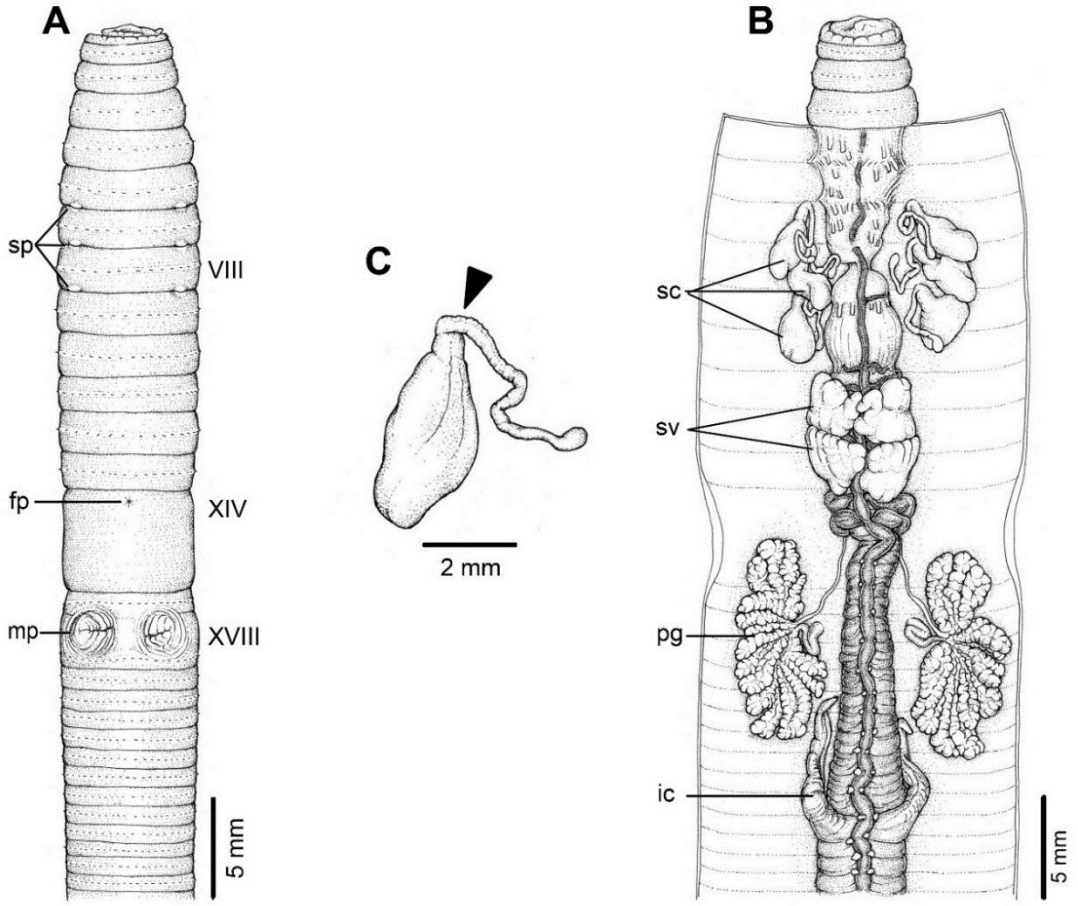

Figure 4.7 External and internal morphology of *Metaphire orientalis* sp. n. holotype (CUMZ 3382): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

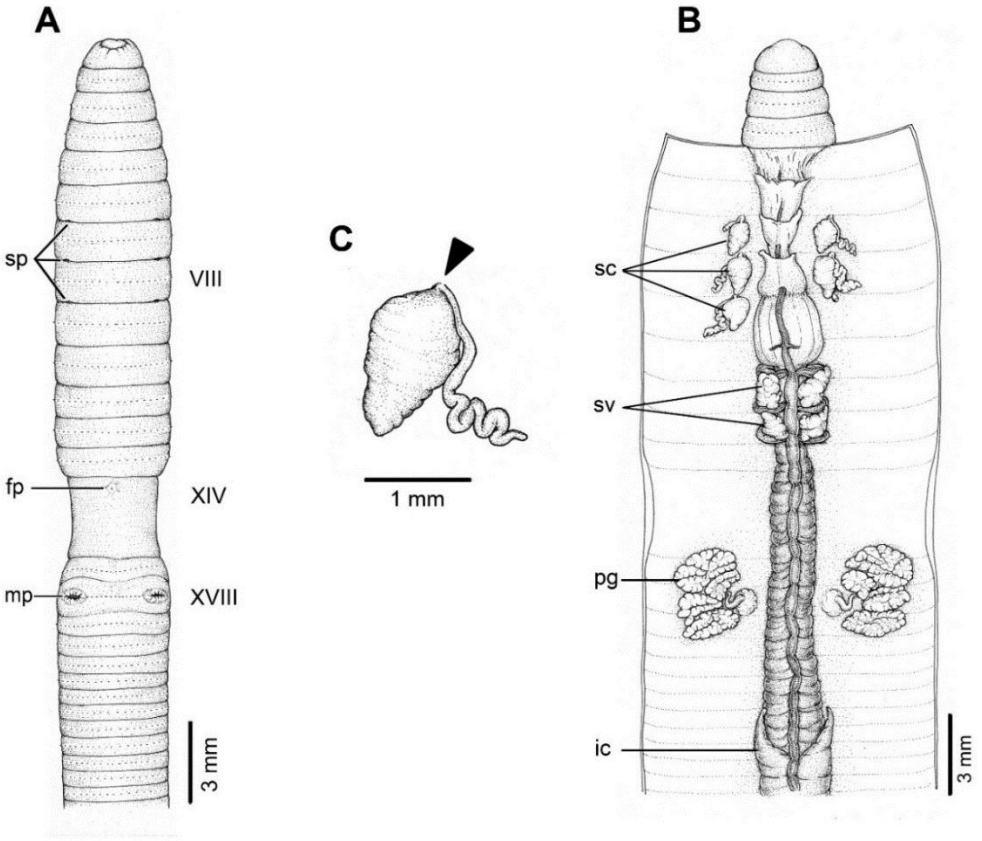

Figure 4.8 External and internal morphology of *Metaphire perichaeta* holotype (NHMUK 1924.3.1.231): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

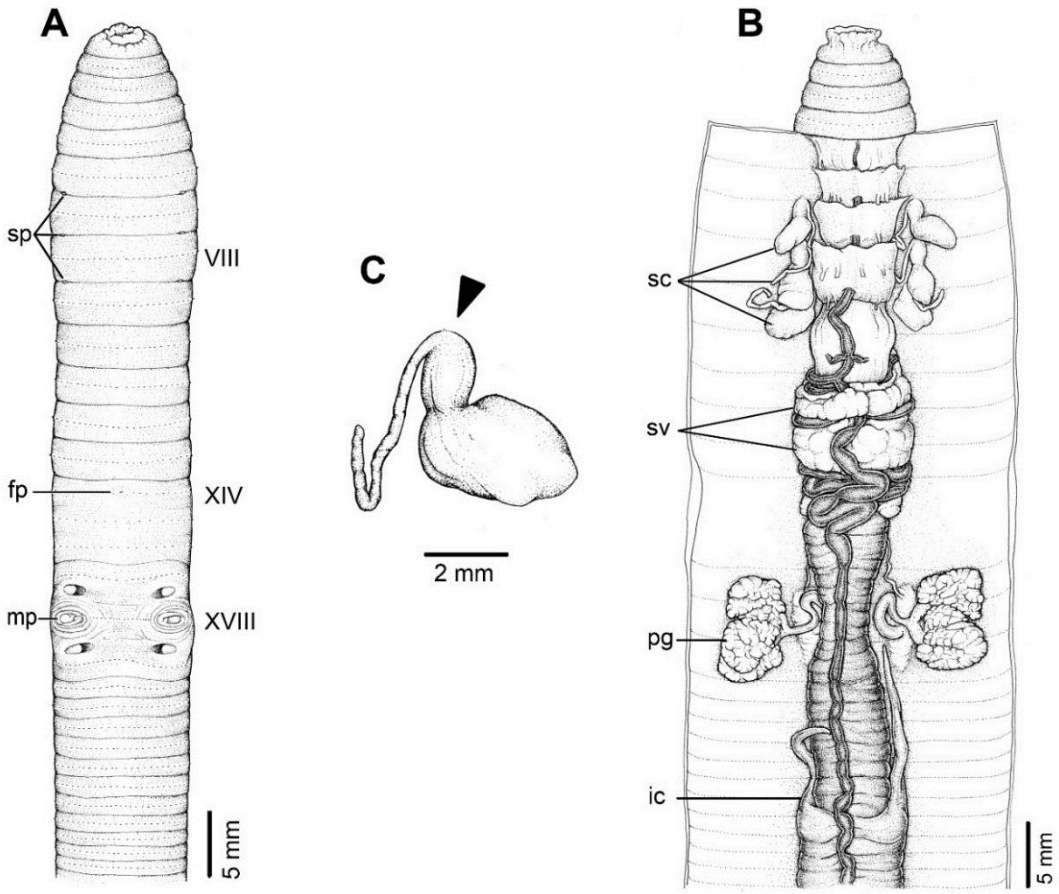

Figure 4.9 External and internal morphology of *Metaphire doiphamon* sp. n. holotype (CUMZ 3390): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

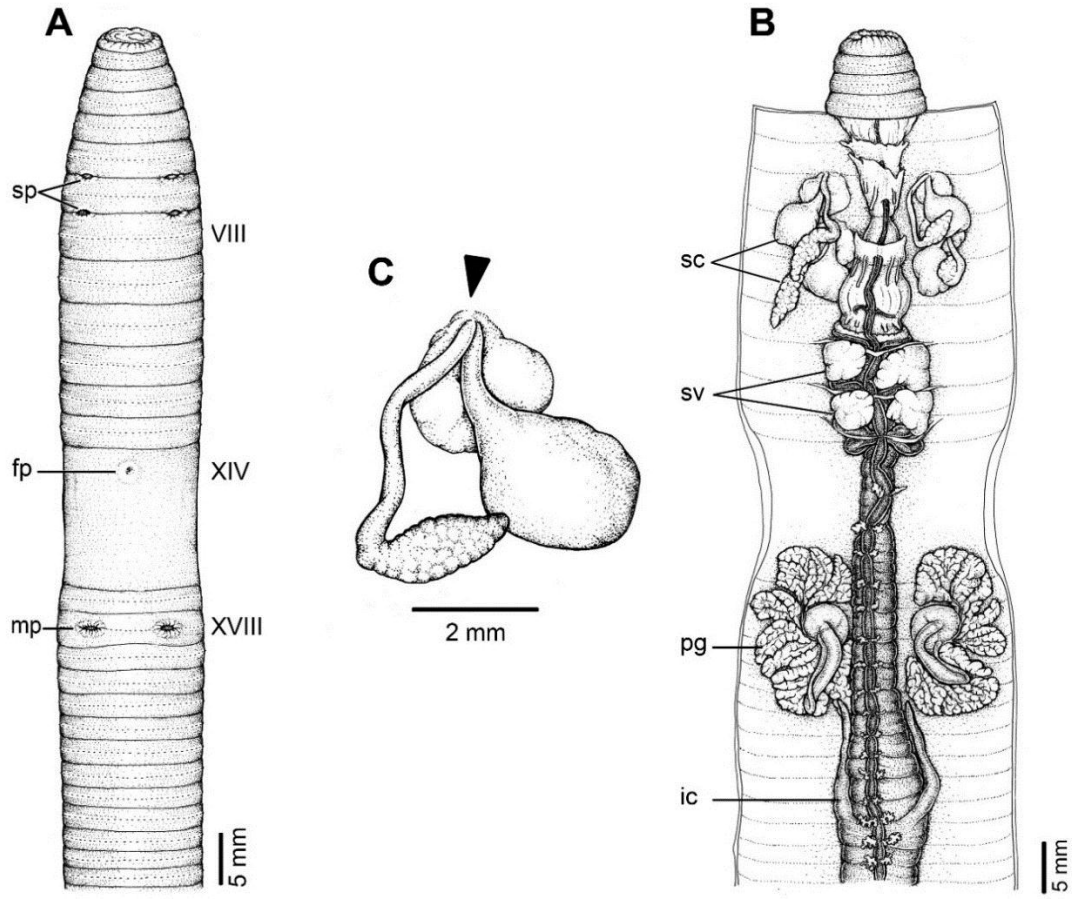

Figure 4.10 External and internal morphology of *Metaphire saxicalcis* sp. n. holotype (CUMZ3392): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

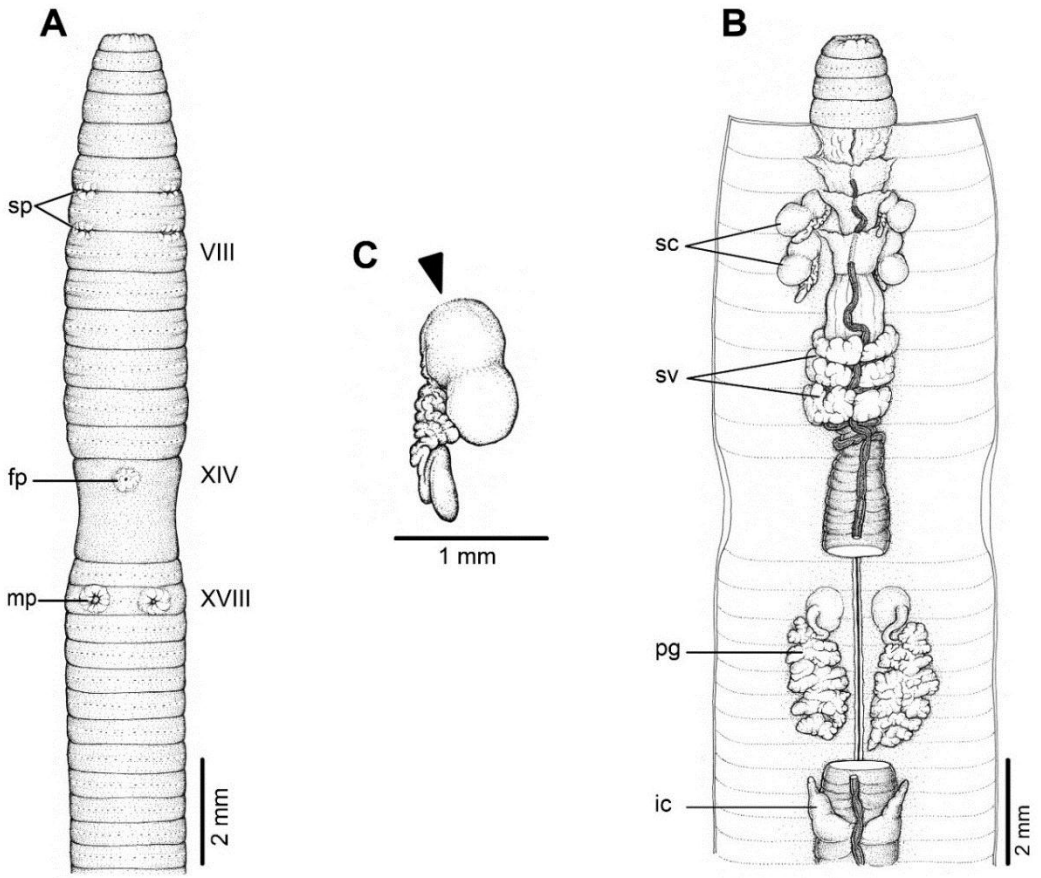

Figure 4.11 External and internal morphology of *Metaphire dunckeri* lectotype ZMH V5876.1: A, external ventral view; B, internal dorsal view; C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

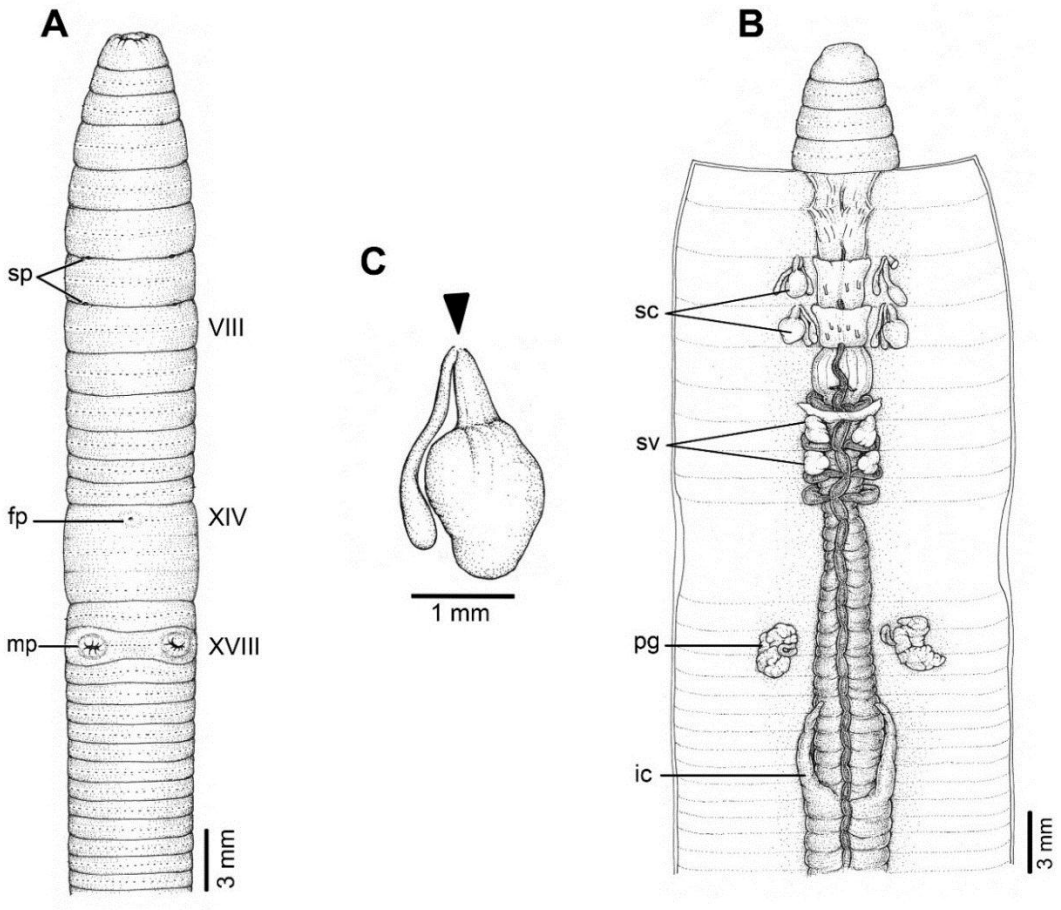

Figure 4.12 External and internal morphology of *Metaphire planata* type NHMUK 1928.4.2.1: A, external ventral view; B, internal dorsal view; C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

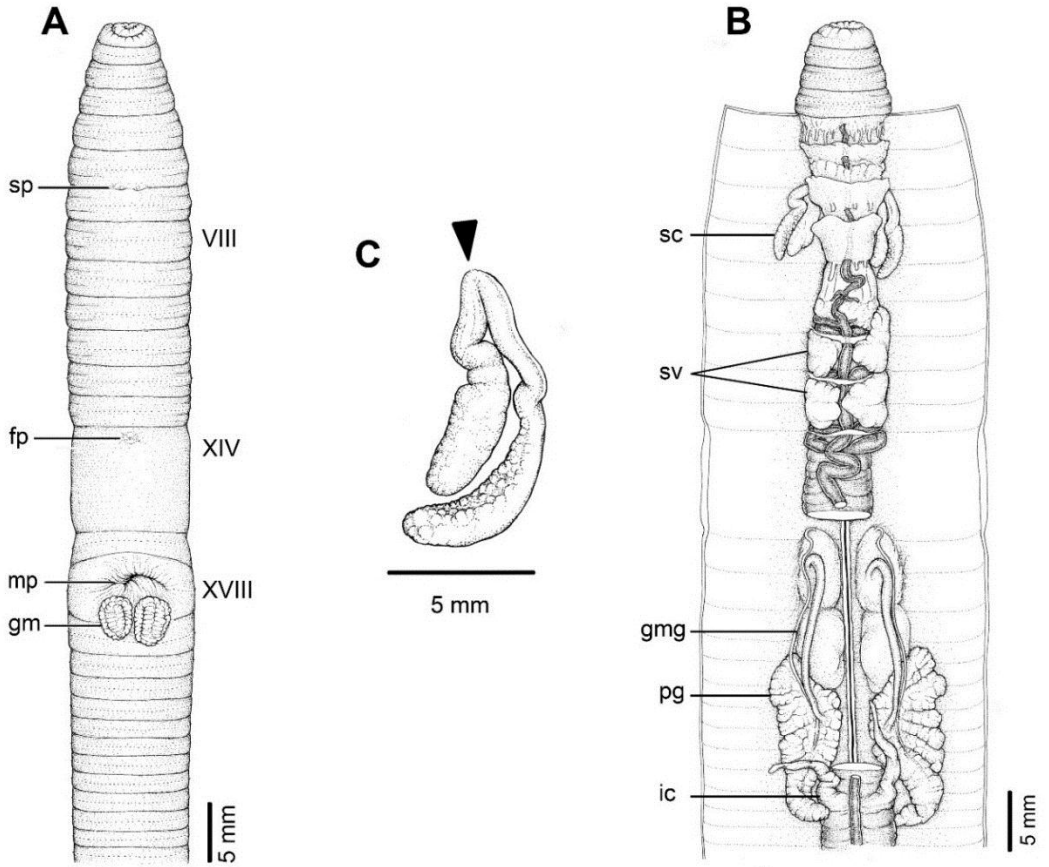

Figure 4.13 External and internal morphology of *Metaphire surinensis* sp. n. holotype (CUMZ 3395): A, external ventral view, B, internal dorsal view and C, spermathecae. (Black arrow indicates the connection of the spermathecae and spermathecal pore).

CHAPTER 5
Molecular Phylogeny

Introduction

The terrestrial earthworms genera *Amynthas* Kinberg, 1867 and *Metaphire* Sims and Easton, 1972 are dominated and the most diverse in species composition as recorded in Thailand, and other countries in Southeast and East Asia (Blakemore, 2007). The generic anatomical and morphological features of the two genera are almost similar, except for the male pore area which is represented as a distinct copulatory pouch in *Metaphire*, but absent in *Amynthas* (Sims and Easton, 1972). However, the definition of the true copulatory pouch is still clearly undefined, and various organ descriptions of some organs have been gradually proposed (Blakemore, 2012; Chang et al., 2009b). Some authors considered only the invaginations of body walls as copulatory pouches, but others also included the intramural chambers, and even the shallow indentations into the description scope of the organ (James, 2005a; James et al., 2005; Tsai et al., 2009). This confusion has led into many synonymy and mis-identification and mis-leading to systematic interpretation between members of the two genera. For examples, *A. chaishanensis* has been stated as a junior synonym of *M. formosae*, *A. kaopingensis* of *M. paiwanna,* and *A. huangi* of *M. houlleti* (Blakemore, 2012; Michaelsen, 1922; Perrier, 1872). In addition, following to the description of copulatory pouches, *A. chaishanensis*, *A. hengchunensis*, *A. kaopingensis*, *A. ailioensis* and *A. huangi* should be included in the genus *Metaphire*.

In present study, taxonomic status of the Thai *Amynthas* and *Metaphire* has been revised by DNA barcoding based on COI sequences, and the hypothesis of the phylogenetic relationship of the *Amynthas* and *Metaphire* species in Thailand are provided by using multilocus approaches (COI and ITS2).

Materials and Methods

Adults earthworms were collected from 70 localities throughout Thailand during 2009-2014, representing of 15 and 13 described species of *Amynthas* and *Metaphire*, respectively (Table 5.1). Three closely related species from the same family Megascolecidae were used as outgroup, namely *Polypheretima elongata*, *Lampito mauritii*, and *Pontodrilus* sp. Samples were anesthetized in 30% ethanol; small pieces of muscle tissue from behind the clitellum region were isolated and preserved in 70% (v/v) or 95% (v/v) ethanol solution for DNA extraction. The residual earthworm sample were fixed in 10% (v/v) formalin and then preserved in 70% (v/v) ethanol solution for voucher and deposited at the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ).

DNA extraction, gene amplification and sequencing

Total genomic DNA was extracted from ventral integument tissue samples using the DNAeasy Tissue Kit (QIAGEN) with two consecutive steps of elution (70 µl of buffer each). The mitochondrial cytochrome oxidase I gene (COI) was then PCR-amplified using the universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), (Folmer et al., 1994). The fragments of the nuclear ribosomal internal transcribed spacer (ITS2) gene were amplified using the forward primer E5.8s-F (5'ATCACTGGGTTCGTGCGT) and the reverse primer E28s-2 (3'CCKCTTCACTCGCCGTTA). For COI, all samples were initially denatured for 2 min at 94 °C with 35 cycles of 94 °C for 30s denaturation, 46 °C for 60s annealing and 72 °C for 60s extension, followed by final extension at 72 °C for 5 min. For ITS2, amplification process included initial denaturation time of 5 min at 94°C, followed by 36 cycles of 94 °C for 30s denaturation, 63°C for 45s annealing and 72°C for 60s extension; the last step and a final extension at 72°C for 10 min. All PCR products were checked by 1.5% agarose gel electrophoresis. The PCR products were then purified by the PEG precipitation method. All sequencing was done by commercial services at

MacroGen and Bioneer, Korea. The sequences were compared with known earthworm sequences in GenBank using BLAST search algorithm (Altschul et al., 1997).

In addition, the 19 outputs COI sequence of *Amynthas* and *Metaphire* (5 *Amynthas* spp. and 12 *Metaphire* spp.) were retrieved from GenBank (Table 5.1) and used in the phylogenetic analysis.

Phylogenetic analysis

Sequences of each individual gene were aligned using the default setting of ClustalW multiple alignment program (Thompson et al., 1994) as implemented in MEGA 6 (Tamura et al., 2013), followed by manual adjustment. The alignments were checked for saturation and phylogenetic signal using DAMBE v. 5.3.74 (Xia, 2013). Plots of uncorrected pairwise transition and transversion distances against total corrected distances were evaluated in order to suggest the possibility for saturation and identify taxa responsible.

Both maximum likelihood (ML) and Bayesian inference (BI) methods of phylogenetic inference were applied to both the COI dataset alone and all the gene combined (COI+ITS2). In the latter dataset, each gene was set as partition with the specific evolutionary model for each partition separately. Best-fit models of nucleotide substitution were estimated with Kakusan4 (Tanabe, 2007), as judged by the Akaike information criterion (AIC: Akaike, 1974) implemented for ML and the Bayesian information (BIC: Schwarz, 1978) for BI.

The ML analysis was performed in the program Treefinder (Jobb et al., 2004) using the likelihood-ratchet method with 1000 bootstraps for branch confidence values estimation. BI analyses were performed by using MrBayes v 3.2.5 (Ronquist et al., 2012), with the number of Markov chain Monte Carlo algorithm (MCMC) generations of six million (heating parameter=0.5), and saving trees and parameters every 1000th generation. The run was set to stop if topological convergence was reached between the two runs, which was determined by the presence of a standard deviation in split frequencies that was lower than 0.01. Consensus topology was estimated after

discarding the first 20% samples as burn-in. Statistical supports and branch length for the resultant BI trees were determined with bipartition posterior probability (Huelsenbeck and Ronquist, 2001). Tree branches with bootstrap values of 70% or greater for ML and/or with bipartition posterior probability of 0.95 or greater were regarded as sufficiently resolved (Huelsenbeck and Hillis, 1993; Larget and Simon, 1999).

Kimura 2-Parameters (K2P) substitution model distance (Kimura, 1980) were also calculated using MEGA 6.06 in order to access percentages of sequence divergence in COI gene for both inter- and intraspecific pairwise comparisons.

Results

Sequence characteristics

For COI gene, the final length of the alignments 620 base pair (bp) was obtained. The full dataset contained 287 variable site, 263 of which were parsimony informative. An average nucleotide base composition was A = 28.6%, C = 22.7 %, G = 19.3 %, and T = 29.4%, GC content was 28%. For ITS2 gene, the final length of the alignments 486 base pair (bp) was obtained. The full dataset contained 243 variable sites, 139 of which were parsimony informative. An average nucleotide base composition was A = 20.6%, C = 28.8 %, G = 30.4 %, and T = 20.3 %, GC content was 62%. A total length of the alignment of combine dataset (COI and ITS2) was 1146 base pair (bp) and contained 534 variable site, 398 parsimony informative.

Phylogenetic analyses

In the analyses using the 5' fragment of COI, phylogenetic tree based on BI analysis under GTR+G+I model is shown (Fig. 5.1). The posterior probabilities from the Bayesian analysis and the bootstrap value from the ML analysis were shown on the BI tree (Fig. 5.1). The COI gene tree revealed 51 monophyletic groups, corresponding to 51 candidate species. There were 20 species of *Amynthas* and 27 species of *Metaphire*. These monophyletic groups were largely congruent with morphological species

previously identified. However, *Metaphire houlleti* was clustered as three lineages with deep genetic divergences (Fig. 5.1). Although the COI gene tree proved to be useful in species identification, it contained a poor resolution for interspecific relationships interpretation when compared to those of combined dataset which provided stronger support.

In the combined dataset of COI and ITS2, the best-fit substitution models were chosen as GTR+G+I for both COI and ITS2 genes for AIC criterions, and GTR+G for COI and K80+G for ITS2 models for BIC criterions. The estimated trees from ML and BI showed minor topological differences (Fig. 5.2), but no inconsistency was detect between them. One of the outgroup taxon used in this study, *Polypheretima elongata*, was nested with the ingroups, while another, *Polypheretima elongata,* was placed at the basal position of the tree together with *Lampito mauritii.* Within the ingroups, each of nominal species were placed in isolate position. Species identified as *Amynthas* and *Metaphire* fail to form monophyletic clade, but distributed mixing throughout the tree, and formed a large monophyletic group with quite strongly supported of a ML bootstrap value of 84.1% and a BI bipartition posterior probability of 0.99 against the *Polypheretima elongata* and *Lampito mauritii* clade.

Among the remaining ingroup species, seven major clades can be distinguished (Fig. 5.2). Clade A was placed at the basal part of the phylogenetic tree, consisted of three lineages of *Metaphire houlleti,* and with strongly supported of 100% ML bootstrap value and 1 of BI bipartition posterior probability. This clade was formed as polytomy together with *Polypheretima elongata, Metaphire surinensis* and the remaining clades.

The remaining clades were clustered with relatively supported of 68.2 % ML bootstrap value and 0.89 of BI bipartition posterior probability. There were Clade B, Clade C and a strong supported polytomy clade of clade D, E, F, G, H, and two ungrouped taxa, *M. anomala* and *A. morrisi*. This major clade was supported by 68.2% ML bootstrap value and 0.99 of BI bipartition posterior probability. Clade B composed

of *A. papulosus* and *M. trangensis*, while clade C consisted of *A. cortices* and *M. khaoluangensis*.

Clade D comprised of four species of *Metaphire*, namely *M. peguana, M. bahli, M. saxicalcis*, and *Metaphire* sp.A, with strongly supported by ML bootstrap value of 84.1% and a BI bipartition posterior probability of 0.99. *Metaphire* sp.A was placed at the basal part, followed by *M. saxicalcis* and a strongly supported clade of *M. peguana* and *M. bahli*, respectively.

Clade E comprised of *Amynthas thakhantho* and *A. turris*, with strongly supported of a ML bootstrap value of 99.4% and a BI bipartition posterior probability of 1.

Clade F was weakly supported with a ML bootstrap value of 68.2%, but relatively high BI bipartition posterior probability of 0.98. It contained of two species of the genus *Metaphire*: *M. grandipenes* and *M. birmanica*; and three species of the genus *Amynthas*: *A. andersoni*, *A. longicauliculatus* and *A. comptus*. *Amynthas comptus* and *A. andersoni* were placed as a sister group closely to *A. longicauliculatus* with a ML bootstrap value of 95.3% and a BI bipartition posterior probability of 0.91; while *M. grandipenes* and *M. birmanica* were place at the basal part of the clade.

Clade G is the largest clade in this study and was not supported by ML bootstrap replicates, it showed a BI bipartition posterior probability of 0.86. This clade was divided into two subclades: subclade G-I (*A. mekongianus*, *M. posthuma* and *Amynthas* sp.A, with a ML bootstrap value of 68.9% and a BI bipartition posterior probability of 0.95), and subclade G-II (*A. alexandri*, *A. nangrongensis*, *A. longicaeca* and *Amynthas* sp.B with a ML bootstrap value of 100% and a BI bipartition posterior probability of 1), along with *A. srinan*.

K2P distances of COI

Total of 76 haplotypes were observed from 85 individuals of the *Amynthas* spp. and *Metaphire* spp. (Table 5.1). All haplotypes were 620 base pairs (bp) in length, and without insertion or deletions. Within the *Amynthas* species, the mean interspecific

sequence divergences ranged from 14.6% (*A. morrisi* vs *A. papulosus*) to 26.6% (*A. srinan* vs *A. nangrongensis*), while the mean interspecific sequence divergence of *Metaphire* species ranged from 14.2% (*M. soulensis* vs *M. hilgendorfii*) to 24.0% (*M. grandipenes* vs *M. soulensis*).

Discussion

Molecular phylogenetic analyses of earthworms have been widely used to test taxonomic, evolutionary and biogeographic hypotheses since the early 1990s, but with a little focus on the *Amynthas* and *Metaphire* (2008; Chang et al., 2007; 2009a; James, 2005b). According to the phylogenetic analyses from COI, all *Amynthas* and *Metaphire* are composed of at least 47 species, including 44 described species (18 *Amynthas*, 26 *Metaphire*) and three undescribed species (2 *Amynthas*, 1 *Metaphire*). In addition, the monophyly of the 47 species was strongly supported. This confirmed that a 658-bp fragment of the mitochondrial gene COI which had been widely used for most animal taxa is a useful DNA barcode marker in *Amynthas* and *Metaphire* earthworms, as previously suggested (Chang and Chen, 2005; 2008; Chang et al., 2007; 2009a; Hebert et al., 2003; Huang et al., 2007; James et al., 2010; Pérez-Losada et al., 2012; 2005; Rougerie et al., 2009; Voua Otomo et al., 2009).

Molecular phylogenetic analyses support the paraphyly or polyphyly of the *Amynthas* and *Metaphire*, including *Polypheretima elongata*. These three genera have been once grouped together as a *Pheretima* group (Sims and Easton, 1972). This unresolved phylogenetic relationship is similar to previous molecular phylogenetic studies that focusing on the *Pheretima* complex (Chang et al., 2008; James, 2005b). Multiple levels of rapid divergence may has occurred in this earthworms, and is one of the main reasons underlying the difficulties in reconstructing earthworm phylogeny (Chang et al., 2008; James, 2005b). However, the sample sizes used here are so small which are not able to represent the whole genera, therefore adding more taxa and more genes in analyses will be very useful to indicate phylogenetic accuracy.

Nevertheless, our finding can be an additional evidence in making the further discussions concerning the evolutionary history of the two earthworm genera.

The two species of the genus *Amynthas*, namely *A. thakhantho* and *A. turris*, were well supported as monophyly. They have a unique habit that can produce columnar or tower-like casts. Their anatomical and morphological features are quite similar, therefore, they have been placed in the *corticis* group (Sims and Easton, 1972). They have four pairs of spermathecal pores and presence of some pairs of genital marking at the male pore area; but with different number of genital marking, and also different shape of spermathecae. These characters can be used to distinguish them separately. Moreover, the genetic distances between *A. thakhantho* and *A. turris* is 16.4%. This is quite similar to other congerneric or intergeneric of *Amynthas* and *Metaphire*.

A subclade consisting of *M. peguana* and *M. bahli* received good supports from both ML and BI analyses. Two earthworm species have very similar in the morphological characters. They are medium-sized earthworm and share some characters such as the position of spermathecal pore in 6/7-8/9 and two pairs of genital markings in segment 17/18 and 18/19. Morphological phenetic analyses conducted about 40 years ago which were classified them in the *peguana* species group (Sims and Easton, 1972). Recently, Prasankok et al. (2013) reported genetic distance between *M. peguana* and *M. bahli* ranges from 12-14%. This divergence similar to this study which revealed 15.7% differentiated between them.

Our phylogenetic analyses also showed deep phylogenetic structure within *Metaphire houlleti*, where samples were clustered into three lineages. Given the range of morphological variation of this species, we've never found discriminatory morphological features in any case between the specimens from different lineages/OTUs, but the genetic divergence suggested that they may contain several of cryptic species. Recently, mitochondrial DNA (mtDNA) sequence analyses have uncovered evidence of substantial cryptic variation within a wide range of earthworm species (Andre et al., 2010; King et al., 2008; Novo et al., 2010), and some of them have

been confirmed as slower evolution nuclear DNA (Donnelly et al., 2013). Cryptic speciation is prevalent in other genera of annelids, e.g. in polychaetes which numerous examples are known (Nygern, 2014), but usually not occured sympatrically. However for earthworms, in some cases there was no clear geographical pattern to lineage distribution (King et al., 2008). Some crypsis are living together, and hence shared ecological niches. High genetic divergence in earthworms may be because of such soil-dwelling invertebrates have evolved to live in the specialized habitats and stabilizing selection may penalize forms that deviate from the morphological optimum (Bickford et al., 2007) .

Table 5.1 Sample used in the phylogenetic study.

No.	Species	Locality name	Lat/Long coordinate	COI GeneBank accession No.
1.	*A. alexandri*	Phetcharat Campus, Muaklek, Saraburi	14° 38' 4.6" N, 101^0 08' 43.5" E	-
		Kuiburi National Park, PrachuapKhiri Khan	12^0 03' 24.6" N, 99^0 37' 40.6" E	-
		Watthampla, Maesai, Chiangrai	20^0 19' 55.9" N, 99^0 51' 49.2" E	-
		Watnonsaothong, Mueang, Phetchabun	16^0 20' 38.6" N, 101^0 13' 26.4" E	-
		Thao to waterfall, Mueang, Nong Bua Lamphu	17^0 13' 46.8" N, 102^0 27' 45.8" E	-
2.	*A. andersoni*	Wattham Lijia, Sangkhlaburi, Kanchanaburi	15^0 04' 21.8" N, 98^0 34' 10.7"E	-
3.	*A. comptus*	Huayrong waterfall, Rongkwang, Phrae	18^0 26'32" N, 100^{0}27'0.2" E	-
		Tham Pha Thai Ntional Park, Lampang	18^0 06' 32 N, 100^0 27' 0.2" E	-
		Srinan National Park, Nan	18^0 21' 55.9" N, 100^0 50' 25" E	-
4.	*A. corticis*	Siriphum waterfall, Doi Inthanon, Mae Chaem, Chiangmai	18^0 32' 48.9" N, 98^0 30' 56.7" E	-
5.	*A. longicaeca*	Phulaenkha National Park, Nongbuadaeng, Chaiyaphum	16^0 00' 01.5" N, 101^0 52' 35.4" E	-
6.	*A. longicauliculatus*	Wattham Pha Ngao, Chiang Saen, Chiangrai	20^{0}14' 44.6" N, 100^0 06' 1.2" E	-
7.	*A. mekongianus*	Wat Phothisompon, Sanakam, Viangchan, Laos	17^0 54' 57.1" N, 101^0 41' 28.7" E	-
		Mekong River, Wiang Kaen, Chiangrai	20^0 11' 45.2" N, 100^0 27' 32.0" E	-
8.	*A. morrisi*	Gunung Datok Tambun Ipoh Perak Malaysia	4^{0}37' 44.5" N, 101^0 09' 20.2" E	-

No.	Species	Locality name	Lat/Long coordinate	COI GeneBank accession No.
9.	*A. nangrongensis*	Nangrong waterfall, Nakhon Nayok	14^0 19' 44.1" N, 101^0 19' 6.6" E	-
10.	*A. papulosus*	Khaosok National Park, Panom, Suratthani	8^0 54' 55.7" N, 98^0 31' 39.3" E	-
		Ban Mor, Huamueang, Huaphan, Laos	20^0 03' 23.4" N, 103^0 38'12.2" E	-
11.	*A. srinan*	Srinan National Park, Nanoi, Nan	18^0 21' 55.9" N, 100^0 50' 25" E	-
12.	*A. thakhantho*	Ban Danchang, Tha Khantho, Kalasin	16^0 50' 12.4" N, 103^0 16' 17.7" E	-
		Wattham Pra, Tha Khantho, Kalasin	16^0 51' 42.4" N, 103^0 14' 47.0" E	-
		Tham Nampok, Kranuan, Khonkaen	16^0 49' 16.3" N, $103^0$09' 0.2" E	-
13.	*A. turris*	Tham Pha Tha Pol, Nern Maprang, Phitsanulok	16^0 30' 19.4" N, 100^0 39' 39.0" E	-
14.	*Amynthas* sp.A	Watphathapwimutti, Mueang, Buengkan	18^0 22' 9.9" N, 103^0 39' 10.4" E	-
		Mekong River, Mueang, Buengkan	18^0 22' 21.9" N, 103^0 38' 57.9" E	-
		Wat Arhong, Mueang, Buengkan	18^0 25' 30" N, 103^0 28' 18" E	-
15.	*Amynthas* sp.B	Mekong River, Buengkan	18^0 22' 20.4" N, 103^0 38' 58" E	-
		Watsongkorn, Wanyai, Mukdahan	16^0 46' 33" N, 104^0 44' 20.4" E	-
16.	*M. anomala*	Km 175+200, Thong PhaPhum, Kanchanaburi	14^0 35' 17.1" N, 98^0 45' 12.4" E	-
		Watnonsaotong, Mueang, Phetchabun	16^0 20' 38.6" N, 101^0 13' 26.4" E	-
17.	*M. bahli*	PuKhao (Wat Phutthanimitr), Sahatsakhan, Kalasin	16^0 43' 40.9" N, 103^0 34' 8.2" E	-

No.	Species	Locality name	Lat/Long coordinate	COI GeneBank accession No.
		Tadton waterfall, Mueang, Chaiyaphum	15^0 58' 52.6" N, 102^0 02' 7.4" E	-
		Kaeng Tana National Park, Khong Chiam, Ubon Ratchathani	15^0 17' 58.7"N, 105^0 28' 38.0" E	-
		Watkingkaewthong, Mueang-Khong, Champasak, Laos	14^0 07' 13.3" N, 105^0 52' 24.9" E	-
		Nongbor, Borabue, Mahasarakham	16^0 01' 0.9" N, 103^0 06’ 58.4" E	-
18.	*M. birmanica*	Tadton waterfall, Mueang, Chaiyaphum	15^0 58' 52.6" N, 102^0 02' 7.4" E	-
		Watthathungnarittharam, Saiyok, Kanchanaburi	14^0 29' 48.5" N, 98^0 50' 19" E	-
19.	*M. grandipenes*	Lai Nan, Wiang Sa, Nan	18^0 34' 0.8" N, 100^0 44' 7.3" E	-
20.	*M. houlleti*	Khaohayod, Bo Thong, Chonburi	13^0 09' 46.4" N, 101^0 35’ 52.6" E	-
		Khaoyaida, Mueang, Rayong	13^0 09' 46.4" N, 101^0 35' 52.6" E	-
		Thamwararam, Phanom, Suratthani	8^0 53' 13.6" N, 98^0 39' 54.5" E	-
		Sekong-Attapeu, Laos	15^0 00' 27.2" N, 106^0 51' 14.6" E	-
		Km 175+200, Thong PhaPhum, Kanchanaburi	14^0 35' 17.1" N, 98^0 45' 12.4" E	-
		Khahayod, Bo Thong, Chonburi	13^0 09' 46.4" N, 101^0 35' 52.6" E	-
		Ban Ponebok, Sarawan, Laos	15^0 42’ 25.9" N, 106^0 26’ 11.3" E	-
21.	*M. khaoluangensis*	Ban Klongpod, Nopphitam, Nakhon Si Thammarat	8^0 48' 8.4" N, 99^0 35' 18.6" E	-
		Krungching waterfall, Nopphitam, Nakhon Si Thammarat	8^0 43' 34.8" N, 99^0 39' 58.4" E	-

No.	Species	Locality name	Lat/Long coordinate	COI GeneBank accession No.
22.	*M. pequana*	Tham Phadaeng, Phanom, Suratthani	8^0 53' 37.3" N, 98^0 33' 10.7" E	-
		Samlan waterfall, Mueang, Saraburi	14^0 26' 16.6" N, 100^0 57' 42.3" E	-
		Khaoyoi, Phetchaburi	13^0 14' 14.6" N, 99^0 49’ 24.2" E	-
		Wattham Pla, Maesai, Chiangrai	20^0 19’ 55.9" N, 99^0 51' 49.2" E	-
		Watkhaosompod, Chaibadan, Lopburi	15^0 09' 40.7" N, 101^0 16' 43.9" E	-
23.	*M. posthuma*	KhonePhapheng waterfall, MueangKhong, Champasak, Laos	13^0 57’ 31.0" N, 105^0 59' 26.9" E	-
		Songkram river, Tha Uthen, NakhonPhanom	17^0 39' 12" N, 104^0 27' 49.8" E	-
		Klong River, Khong Chiam, UbonRatchathani	15^0 19' 1.9" N, 105^0 30' 48.9" E	-
24.	*M. saxicalis*	Khaohayod, Bo Thong, Chonburi	13^0 09' 46.4" N, 101^0 35’ 52.6" E	-
		Khao Cha Ang On, Bo Thong, Chonburi	13^0 12' 0.6" N, 101^0 34' 56.0" E	-
25.	*M. surinensis*	Nong Khu Forest Park, Sang Kha, Surin	14^0 41' 12.0" N, 103^0 44' 47.9" E	-
26.	*M. songkhlaensis*	Literary Botanical Garden Botanical garden, Hat Yai, songkhla	7^0 01' 11.1" N, 100^0 17' 34.1" E	-
27.	*M. trangensis*	Khao Chang, Nayong, Trang	7^0 32' 57.1" N, 99^0 46' 58.3" E	-
		Thammalai, Mueang, Phatthalung	7^0 38' 07.6" N, 100^0 05' 4.4" E	-
		Tham Phutthakhodom, Srinakarin, Phatthalung	7^0 33' 38.5" N, 99^0 53' 5.6" E	-
28.	*Metaphire* sp.A	Khao Soi Dao Wildlife Sanctuary, Chanthaburi	12^0 55' 20.1" N, 102^0 14' 31.2" E	-

No.	Species	Locality name	Lat/Long coordinate	COI GeneBank accession No.
29.	*Lampito mauritii*	Lanta Islands, Krabi	7^0 28' 11.0" N, 99^0 06' 6.4" E	-
30.	*Polypheretima elongata*	Suranaree University, Nakornratchasima	14^0 52' 34.8" N 102^0 01' 15.7" E	-
31.	*Pontodrilus* sp.	Surin Islands, Khuraburi, Phang Nga	9^0 26' 3.5" N 97^0 52' 11.2" E	-
32.	*Perionyx* sp.	Surin Islands, Khuraburi, Phang Nga	9^0 26' 3.5" N 97^0 52' 11.2" E	-
33.	*A. incongruus*	China	-	KP030718
		China	-	EF077553
34.	*A. penpuensis*	Taiwan	-	KC897069
35.	*A. phaselus*	China	-	KP030707
36.	*A. taiwumontis*	Taiwan	-	KC897068
37.	*A. zhangi*	China	-	KP030726
38.	*M. acincta*	Japan	-	AB542596
39.	*M. agrestis*	Japan	-	AB542598
40.	*M. californica*	Japan	-	AB542619
		China	-	KP030722
41.	*M. communissima*	Japan	-	AB542628
42.	*M. hilgendorfi*	Japan	-	AB542639
43.	*M. megascolidioides*	Japan	-	AB536862
44.	*M. schmardae*	Japan	-	AB542651
45.	*M. servina*	Japan	-	AB542653
46.	*M. soulensis*	Japan	-	AB542665
47.	*M. tosaensis*	Japan	-	AB542683
48.	*M. tschiliensis*	China	-	KP030733
49.	*M. yamadai*	Japan	-	AB542696

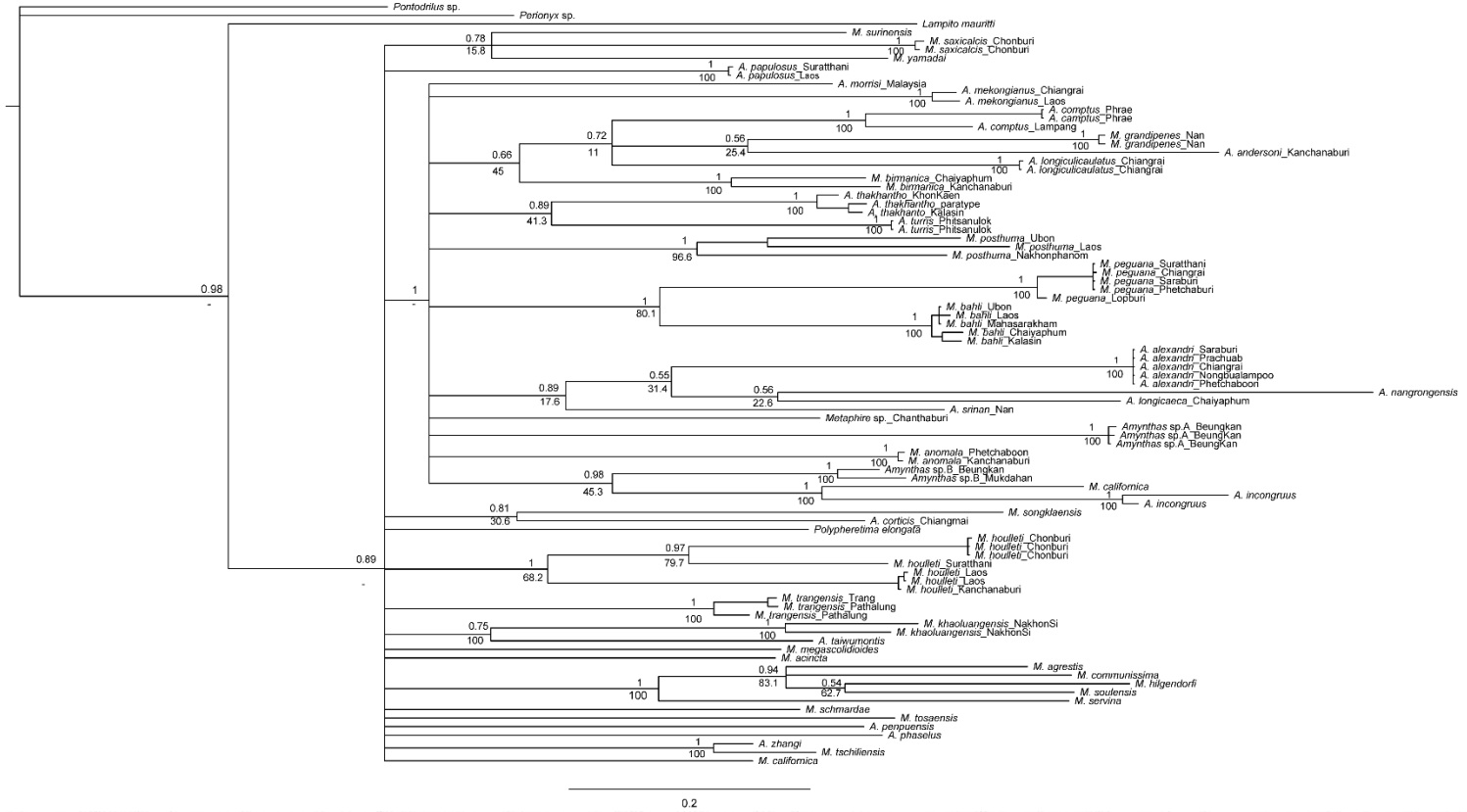

Figure 5.1 Phylogenetic analysis of the *Amynthas* and *Metaphire* with Bayesian analysis based on the cytochrome c oxidase subunit I (COI) gene. Number above branches are posterior probabilities recovered by the Bayesian analysis. Number below branches represent bootstrap value based on 1000 replicates in maximum likelihood analyses

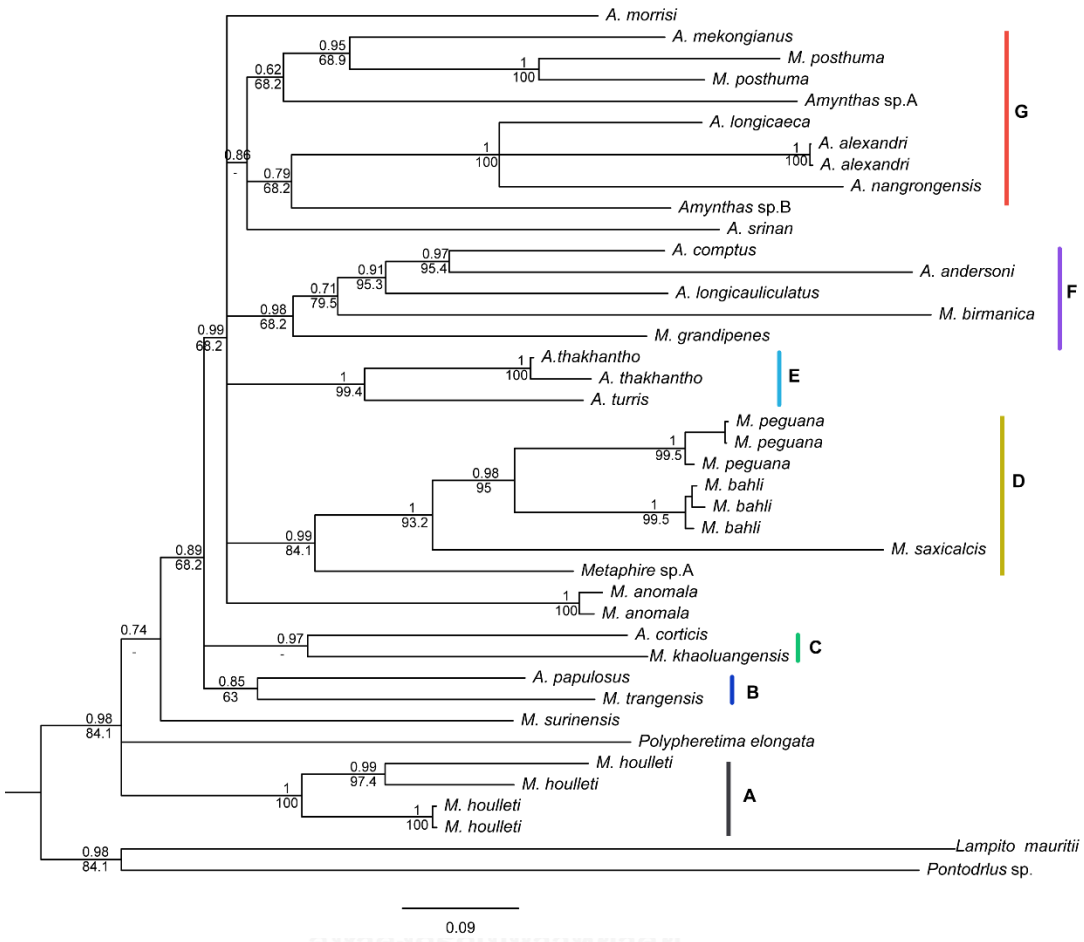

Figure 5.2 Bayesian inference tree based on the analysis of the concatenated data set (COI+ITS) in *Amynthas* and *Metaphire*. Posterior probability values are show above branches. Bootstrap values (1000 replicates) of MP are shown below branches.

CHAPTER 6
Discussion

The two terrestrial earthworms genera*, Amynthas* Kinberg, 1867 and *Metaphire* Sims and Easton, 1972 are well represented in Thailand which occurring throughout the country. They occur in various types of habitat such as dry evergreen forest, moist evergreen forest, deciduous forest and limestone ecosystem, and also in the modified to very modified agricultural and anthropogenic areas (Blakemore, 2011; Bantaowong, 2011a; 2014). Based on the publications of Stephenson (1931); Gates (1939; 1972); Somniyam and Suwanwaree (2009); Blakemore (2011); Bantoawong et al. (2011a; 2011b), and the current results from the several surveys in various localities in Thailand, there are 44 nominal species of which 26 are *Amynthas* and 18 are *Metaphire*, and three are unidentified. Of the 44, two are new records to Thailand, *A. andersoni* and *A. corticis*, while three are peregrine species, *A. corticis*, *A. morrisi*, and *A. gracilis* (Hendrix et al., 2008). Fourteen new species were described: *Amynthas arenulus* Bantaowong & Panha, 2014, *A. thakhantho* Bantaowong & Panha, 2014, *A. phucheefah* Bantaowong & Panha, 2014, *A. longicaeca* Bantaowong & Panha, 2014, *A. turris* Bantaowong & Panha, 2015, *A. nangrongensis* Bantaowong & Panha, 2015, *A. khaohayod* Bantaowong & Panha, 2015, *Metaphire surinensis* Bantaowong & Panha, 2015, *M. songkhlaensis* Bantaowong & Panha, 2015, *M. trangensis* Bantaowong & Panha, 2015, *M. saxicalcis* Bantaowong & Panha, 2015, *M. orieantalis* Bantaowong & Panha, 2015, *M. khaoluangensis* Bantaowong & Panha, 2015, *M. doiphamon* Bantaowong & Panha, 2015 (Bantaowong et al., 2015a; 2015b; 2014). Although morpho-species have been used for new descriptions as above but the morphological classification of the genera *Amynthas* and *Metaphire* is still controversial which many times making confusion for systematic analysis. There are some recent attempts trying to solve the problem because the problem and existing materials are quite attractive. Prasankok et al. (2013) proved the low genetic diversity of the very common *Metaphire peguana*

(Rosa, 1890) collected throughout Thailand using allozyme and mitochondrial (mtDNA) cytochrome oxidase subunit 1 (COI) sequence analysis and suggested that genetic divergences among the sample were low with a low genetic distance indicating frequent gene flow among these populations in Thailand. This may be due to anthropogenic movement of *M. peguana* in soil or with plant scions throughout this region. Chang and Chen (2005) investigated synonyms of *Metaphire formosae* and *M. yuhsii* by using morphological comparison and molecular phylogeny and revealed the existence of two sibling species in the *Metaphire*. Chang et al. (2007) also discovered the cryptic species of *A. wulinensis* by using COI sequences and two separated species, *Amynthas lini* and *A. meishanensis*, were confirmed and described.

Those mentioned papers may not answer the questions perfectly because some reasons such as, some species are known only from single locality or from not many localities, so species boundary may be limited or the error in identification which neglected to consider the degree of morphological variation. And once some scientists have made species list and suggested through previous publications and type material investigations, it will create some more problem. The current list of 44 Thai species already represented an increasing number of species from the recent list by Blakemore (2006) who accounted for about 22 species of the *Amynthas* and *Metaphire* occur in some parts of Thailand. However, the same and close related species have been recorded in other countries such as Myanmar (Gates, 1972). We have made several museum visits at The Natural History Museum, London and Biozentrum Grindel und Zoologisches Museum, University of Hamburg for reference material studies and found that many of Gates's materials are ruin and some have been lost. However we have dissected a series of specimens and noticed some character variations from different localities for examples *Amynthas morrisi* from Malaysia has six genital markings near the anterior margin on segment 7 while *A. morrisi* from Chile has three genital markings on mid ventral near the presetal on segment 6, 7 and 8. There must be some wrong identified long time ago, or the judgments were controversial.

The type species of the genus *Amynthas* Kinberg, 1867 was re-investigated using paratypes of Biozentrum Grindel und Zoologisches Museum, University of Hamburg and Swedish Museum of Natural History in 2012 and 2013. Later in 2013, north Thailand specimens were collected and identified which three species were described with consideration mainly to previous reference material studies (Bantaowong et al., 2015a; 2015b; 2014), two species belonged to the genus *Amynthas*, namely, *A. phucheefah* Bantaowong & Panha, 2014 and *A. turris* Bantaowong & Panha, 2015, and the other species belonged to the genus *Metaphire*, *M. doiphamon* Bantaowong & Panha, 2015. However, *Amynthas phucheefah* is quite different from *A. turris* by the arrangement of genital markings at male pores area and spermathecae. *Amynthas turris* produces tower-liked cast, but there was no evidence of cast found in *A. phucheefah*. Furthermore, most huge numbers of juveniles of *Amynthas phucheefah* and *Metaphire doiphamon* were observed performing annually swarm and migrating when the dry season start in October 2008 and November 2012 at Phucheefah National Park area, Chiang Rai province. This is similar to the report by Reddy (1980) on mass migrations of *Amynthas alexandri* in India. However, There's still no any report suggested about the cause of this phenomenon, some suggested to drought conditions or chemical contamination are the triggers for their specific behaviours.

Four species of the genus *Amynthas* and *Metaphire* were described from northeast Thailand (Bantaowong et al., 2015b; 2014), three species belonged to the genus *Amynthas*, namely *A. arenulus* Bantaowong & Panha, 2014, and *A. longicaeca* Bantaowong & Panha, 2014, *A. thakhantho* Bantaowong & Panha, 2014, and the other species belonged to the genus *Metaphire*, namely *M. surinensis* Bantaowong & Panha, 2015. *Amynthas arenulus* and *A. thakhantho* live in sandy habitats of dry dipterocarp and deciduous forests respectively, and mostly found in the modified highland paddy systems, while the following two species, *A. longicaeca* and *M. surinensis* occur in deciduous forest reserve areas in clay condition. *Metaphire surinensis* is a bithecal

species that was recorded in Thailand for the first time. Genital markings are the main distinct characters together with ecological characters used for identification.

The specimens from the central Thailand were also described (Bantaowong et al., 2015a; 2015b), two species belonged to the genus *Amynthas*, namely *A. nangrongensis* Bantaowong & Panha, 2015 and A. *khaohayod* Bantaowong & Panha, 2015 and the other two species belonged to the genus *Metaphire*, namely *M. saxicalcis* Bantaowong & Panha, 2015 and *M. orientalis* Bantaowong & Panha, 2015. *Amynthas khaohayod* is one of the 14 bithecal members of the *minimus* species group (Sims and Easton, 1972). This group was also recorded in Thailand for the first time, but three species occur in Burma; *A. minimus* (Horst, 1892), *A. nugalis* (Gates, 1931) and *A. papilio* (Gates, 1930). The structure of male pore region, genital marking gland and spermathecae are the main characters to prove *A. khaohayod* different from the related species. Furthermore, *Metaphire saxicalcis* is only species in Thailand that present the saccular body at the spermathecal ducts, and this character involved with other characters such as spermathecae.

Three species of *Metaphire* were described from south Thailand (Bantaowong et al., 2015b), *M. trangensis*, *M. songkhlaensis* and *M. khaoluangensis*, the first two species belonged to the *puluaensis* species group, and the other species belong to *houlleti* species group. *Metaphire trangensis* and *M. songkhlaensis* found in Peninsular Botanical Garden and Literary Botanical Garden, respectively, while *M. khaoluangensis* was found in modified deciduous forest areas in so called “integrated farming”, the local wisdom practices. *Metaphire trangensis* shares similar external characters in body size, segment number, setae number, distance between male pores and has no genital markings at the invaginated male pores with *M. khaoluangensis*. But *Metaphire trangensis* usually has 4 pairs of spermathecal pores while *M. khaoluangensis* has 3 pairs. Moreover, the shape of spermathecae, prostate gland and prostate duct are distinct characters to distinguish the two species.

Amynthas and *Metaphire* can be found throughout Thailand occurring from the ground of the mean sea level to the higher attitude of 2,500 m above mean sea

level at Doi Inthanon National Park, Chiang Mai province. Both genera seem to prefer soil habitats of loamy texture either sandy or clay conditions with neutral or almost neutral conditions (Bantaowong et al., 2014; 2011a).

The species identification of this study were identified based on the following the classical and recent publications follows; Michaelsen (1900), Beddard (1900), Stephenson (1923), Gates (1939; 1972), Sims and Easton, (1972), and Bantaowong et al. (2011a; 2011b). The most important morphological characters are the male terminalia and the spermathecae, the intestinal caeca, the testis sac and the seminal vesicles and its allied and the glands of the genital markings. However there is still controversial in observing more details of some other additional structures such as size of genital apertures, spermathecal pore appearance primary male pores, the pattern and location of genital markings.

The molecular analysis revealed the paraphyly or polyphyly of the *Amynthas* and *Metaphire*. This is not so surprise. Non monophyletic in these two genera have been previously reported, such as Chang et al. (2008) found the non-monophyly of species from Taiwan. *Amynthas*, *Metaphire* and *Polypheretima* have been once grouped together as the genus *Pheretima* complex. It is the largest group of earthworms in the world, consisting of more than 700 nominal species and subspecies (Sims and Easton 1972). Sims and Easton (1972) divided the genus into 8 genera by phonetic analysis according to the greatest number of shared morphological attributes, however they still have several overlapping diagnostic characters and moreover various serious homoplasy (Blakemore, 2002; James, 2005b). Recently many researcher suggested the urgent need to revise the systematics of the *Pheretima* complex (Blakemore, 2002; Chang and James, 2011; Chang et al., 2008; James, 2005b; James et al., 2009). Phylogeny of this group is remain poorly resolved although many studies have tried to investigate it (2008; Chang et al., 2007; 2009a; James, 2005b). The distribution of polyphyly in two closely related genera *Amynthas* and *Metaphire* indicate that evolutionary relationships among these taxa are unresolved. According to the current evidence, more data are in essential need to resolve the relationships.

Because of only 27 species were analyzed here. This number cannot cover the whole *Amynthas* and *Metaphire* members that are more than 100 species in each genus (Sims and Easton, 1972). In addition, earthworms have been noted for having rapid genetic evolution. This is one of the main reasons underlying the difficulties in reconstructing earthworm phylogeny (Chang et al., 2008; James, 2005). Therefore, adding more genes or longer sequences, especially the slower evolve nuclear genes, may resolve the phylogenetic relationship in these genera.

The poor resolution of phylogenetic relationships among *Amynthas* and *Metaphire* was due to rapid divergence, and many authors suggested that using long sequences that are more than 2000 bp with a combination of three or more genes with different evolutionary rates to provide resolution for different taxonomic level (Chang et al., 2008; Novo et al., 2011; 2010; Pérez-Losada et al., 2009). In addition, increased taxon sampling is one of the most important ways to increase overall phylogenetic accuracy.

According the phylogenetic results may indicated that the morphology-based systematics in *Amynthas* and *Metaphire* is unreliable because they are separated by single character, the present or absent copulatory pouch, and showing a large degree of overlap in this characters, and therefore, the more exhaustive morphological study is necessary to help evaluated this situation.

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VITA

Ueangfa Bantaowong was born on the 28th of November 1976 in Chanthaburi province, Thailand. She graduated her Bachelor's Degree of Science in Agriculture from Kasetsart University in 1999, and Master's Degree of Science in Zoology from Chulalongkorn University in 2011. She was awarded the scholarship by the 60/40 Support for Tuition, and The 90th Anniversary of Chulalongkorn University Fund (Rachadaphiseksomphot Endowment Fund) for her study in Biological Sciences Program at Faculty of Science, Chulalongkorn University since 2011.