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Species Diversity of Terrestrial Earthworms in Nan Province

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ได้ทำการจัดจำแนกไส้เดือนดินในจังหวัดน่านโดยใช้ลักษณะสัณฐานวิทยาภายนอกและกายวิภาค พบไส้เดือนดินทั้งสิ้น 3 วงศ์ 4 สกุล 20 ชนิด และ 1 ชนิดย่อย วงศ์ Megascolecidae มี 18 ชนิด 1 ชนิดย่อย ได้แก่ *Amynthas alexandri*, *A. borealis, A. exiguus exiguus, A. longicauliculatus*, *A. phatubensis, A. srinan, A. tontong, Amynthas* sp. 1*, Amynthas* sp. 2*, Amynthas* sp. 3*, Amynthas* sp. 4, *Metaphire anomala, M. birmanica, M. grandipenes, M. houlleti*, *M. peguana, M. posthuma*, *Metaphire* sp. 1 และ *Metaphire* sp. 2 วงศ์ Glossoscolecidae มี 1 ชนิด ได้แก่ *Pontoscolex corethrurus* และวงศ์ Octochaetidae มี 1 ชนิด ได้แก่ *Dichogaster bolaui* และที่พบเป็นครั้งแรกในประเทศไทย คือ *A. borealis, A. phatubensis, A. srinan, A. tontong, M. birmanica* และ *M. grandipenes*

การศึกษาความผันแปรทางพันธุกรรมของไส้เดือนชนิด *Metaphire peguana* ซึ่งเป็นชนิดที่มีการกระจายตัวกว้างพบได้ทั่วไปนั้น ได้เก็บตัวอย่าง 274 ตัวอย่าง จาก 13 พื้นที่ของไทย และนำเนื้อเยื่อบริเวณไคลเทลลัมมาศึกษาด้วยวิธีทางอัลโลไซม์อิเล็กโตรโฟลีซีสเพื่อวิเคราะห์ความผันแปรทางพันธุกรรมและความถี่ของอัลลีลในประชากรไส้เดือน ผลการวิเคราะห์อัลโลไซม์ในประชากรไส้ดือนดิน ทั้งหมด 17 ตำแหน่ง พบว่า 14 ตำแหน่งมีความผันแปรทางพันธุกรรมในขณะที่ 3 ตำแหน่งไม่มีความผันแปรทางพันธุกรรม มีค่า heterozygosity (0.059 - 0.147, ค่าเฉลี่ย 0.092 ± 0.022) ค่อนข้างต่ำเมื่อเปรียบเทียบกับไส้เดือนชนิดอื่นๆ มีค่า *F*st (0.318) ค่อนข้างสูงทั้งนี้อาจเป็นผลเนื่องมาจากการแบ่งแยกกันของถิ่นที่อยู่อาศัยที่จำเพาะในแต่ละพื้นที่พร้อมกับการเกิดกระบวนการทางวิวัฒนาการ เมื่อวิเคราะห์ค่าระยะห่างทางพันธุกรรมระหว่างกลุ่มประชากรพบว่ามีค่าระยะห่างทางพันธุกรรมค่อนข้างต่ำ (D = 0.002 - 0.171) แสดงว่าประชากรไส้เดือน *M. peguana* ในประเทศไทยยังมีการแลกเปลี่ยนพันธุกรรมกันอยู่ ทั้งนี้อาจเนื่องมาจากการที่ไข่ (cocoon) ของไส้เดือนดินติดไปกับดินหรือกิ่งพันธุ์พืชในระหว่างกระบวนการขยายพันธุ์ของเกษตรกร

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สาขาวิชา...สัตววิทยา............................ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..................
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UEANGFA BANTAOWONG : SPECIES DIVERSITY OF TERRESTRIAL EARTHWORMS IN NAN PROVINCE. ADVISOR : PROF. SOMSAK PANHA, Ph.D., CO-ADVISOR : PONGPUN PRASANKOK, Ph.D., 149 pp.

The terrestrial earthworms in Nan province were identified using external and internal morphological characters. A total of twenty species and one subspecies from 4 genera of terrestrial earthworms were recorded. There are eighteen species and 1 subspecies of Megascolecidae: *Amynthas alexandri*, *A. borealis*, *A. exiguus exiguus*, *A. longicauliculatus*, *A. phatubensis*, *A. srinan*, *A. tontong*, *Amynthas* sp. 1, *Amynthas* sp. 2, *Amynthas* sp. 3, *Amynthas* sp. 4, *Metaphire anomala*, *M. birmanica*, *M. grandipenes*, *M. houlleti*, *M. peguana*, *M. posthuma*, *Metaphire* sp. 1 and *Metaphire* sp. 2, a species of Glossoscolecidae (*Pontoscolex corethrurus*), and one species of Octochaetidae (*Dichogaster bolaui*). There are six species; *A. borealis*, *A. phatubensis*, *A. srinan*, *A. tontong*, *M. birmanica*, and *M. grandipenes* recorded in Thailand for the first time.

The genetic variation of the *Metaphire peguana*, which is a common species in Thailand was examined. A total of 274 individuals were collected from 13 localities in Thailand. Using horizontal starch gel electrophoresis, 17 presumptive allozyme loci were screened across all the samples and 14 of these presumed loci, from 11 enzyme systems, were found to be polymorphic. The expected heterozygosity (Hexp) was moderate to high ranging from 0.059 - 0.147 with an overall mean of 0.092 ± 0.02 which relative lower than other earthworms. Genetic heterogeneity among samples showing relatively high (Fst=0.318), may have been involved in an extensive local fragmentation under various selection forces. Genetic differentiation among the samples was low, with a low genetic distance (D = 0.002 - 0.171, mean = 0.048 ± 0.040 indicating the frequent gene flows among populations in Thailand. This is probably cocoons of *M. peguana* contaminated with soil or scions during propagation processes.

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Student's Signature
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CHAPTER I

INTRODUCTION

Earthworms are detritivorous invertebrates that play important roles as macro-decomposers in tropical forest ecosystem. They are dwelling in mostly the top soil, and their activities can improve soil fertility because the worms digest waste organic materials with assistance of microorganisms both in the organic materials and the worm guts. The worms release nutrients into the soil, manage optimal soil aeration and nutrient cycling, which eventually can increase crop yields. Thus, earthworms provide benefits to ecological services and for agriculture. To date earthworms are used by humans for fish bait, local medicines, decomposing garbage, vermicomposting, and as bioindicators for environmental impact. Furthermore, some local people, especially in northeast Thailand, consume worms as food and making dried earthworm products exported to China and Taiwan. The Chinese believe that earthworm consumption will help preventing or curing human illness such as stones in the bladder, jaundice, pile, fever and small pox (Edwards and Bohlen, 1996; Satchell, 1983).

Terrestrial earthworms in Thailand have been rarely investigated. Only two principal surveys have been conducted in the twentieth century. Gates (1939b) published ‘Thai Earthworms’ listing only 24 species, of which one was an endemic species: *Amynthas hupbonensis*. Later, Gates (1972) reported earthworms in Burma and adjacent areas, and listed 27 species from Thailand. Sixty five years later, Blakemore (2006) updated a checklist of Thai earthworms with a of total 28 species. Following that some studies have occurred in many areas in Thailand. Kosavititkul (2005) reported thirteen species from Khao Yai National Park, 6 from the genus *Amynthas*, 3 from the genus *Metaphire*, one species each found from the following genera *Drawida*, *Perionyx*, *Pithemera* and *Pontoscolex*. Kosavititkul (2005) included more than three unknown species. Chantaravisoot (2007) reported 40 terrestrial earthworm species collected from various parts of Thailand and investigated some available specimens, 10 from the genus *Amynthas*, 28 from the genus *Metaphire* and 2 from the genus *Pithemera*, of which many are still unidentified, and Somniyam and Suwanwaree (2009) recorded 21 species from Nakhonratchasima province, 7 from the genus *Amynthas*, 5 from the genus *Metaphire*, 3 from the genus *Drawida*, 3 from the genus *Dichogaster*, and one each from the following genera *Pontoscolex* and *Gordiodrilus*, of which many are still unidentified. Recently, a total of 33 species of earthworms were reported from Thailand which composed of four families; Megascolecidae, Moniligastridae, Octochaetidae and Gossoscolecidae, and eight genera; *Amynthas*, *Dichogaster*, *Drawida*, *Lampito*, *Metaphire*, *Perionyx*, *Polypheretima* and *Pontoscolex*. The majority belongs to the genus *Amynthas* and *Metaphire*, of which there is one of a common species, *Metaphire peguana* (Rosa, 1890). Recently, some lumbricid species have been introduced for many purposes, especially *Eisenia fetida* and *E. andrei* which are popular commercial earthworms. It is possible they can invade natural habitats of native species.

Thailand is a hub for biodiversity because it contains flora and fauna from north, south, east and west and also the unique Thai fauna in the central areas. This is an attractive region for biogeographic studies. Because research on Thai earthworms quite limited the number of reported species is still an underestimate of the total, and the systematic works are still not yet updated. This is due to the small number of earthworm taxonomists and people who are interest to use earthworms as systematic material.

Thailand contains very diverse habitats from pine forest on the high mountains in northern and northeastern areas, deciduous forest, dipterocarp forest, dry evergreen forest, moist evergreen forest, rain forest to mangrove areas. These are the habitats of earthworms, so it is difficult to do a complete survey. Nan province is located in the northern part of Thailand and is surrounded by mountains covered with several kinds of forest, namely evergreen forest, dipterocarp forest, deciduous forest and limestone forest, including various ecosystems, waterfalls, caves, streams, paddy fields and orchards. Mountains of the Phi Pan Nam Range and the Luang Prabang Range are covered by moderate to dense natural forests. The highest mountain is Doi Phu Kha which reaches 2,079 meters. The northern and eastern parts are next to Laos’s border, where Hong (2008) discovered two new species of earthworms. Currently, Nan province has seven national parks and varieties of wild flora and fauna. A rare species of plant which has a very beautiful flower, ‘Chompoo Phuka’ *Bretschneidera sinensis*, occurs only at Doi Phu Kha National Park and some rare species of wild animals such as the bird Rufous-throated Fulvetta and Clamorous Reed-Warbler, are also recorded (DNP, 2004).

This study is one of an effort to survey and identify terrestrial earthworms in Thailand emphasizing northern regions, especially Nan province. The conventional method on morphological investigation is still a very useful technique for basic species justification. The common species *Metaphire peguana* (Rosa, 1890) is abundant throughout the country and also in Nan province. The initial survey on evolution by analyzing allozymes of this species will be a good start for the further systematic analysis. And the other purposes after this study are for utilizing the worms for organic farming as the nowadays trend of the world.

Objectives

1. To classify and identify terrestrial earthworms in Nan province and nearby areas using morphological characteristics.
2. To investigate the degree of genetic variation in population of *Metaphire peguana* (Rosa, 1890), a common species in Thailand, by allozyme electrophoresis.

Anticipated Benefit

The taxonomic clarification of earthworms in Thailand will be an important information for a great benefit of biodiversity management and future agricultural utilization. May be also use of earthworms as bioindicators in agriculture and forestry.

CHAPTER II

LITERATURE REVIEW

Earthworms play a major role in converting large pieces of organic matter (e.g. decayed leaves) into rich humus, and thus improving soil fertility. This is achieved by the worm's actions of pulling down below organic matter deposited on the soil, such as leaf fall or manure. Once in the burrow, the worm will shred the leaf and partially digest it, then mix it with the earth by saturating it with intestinal secretions. Worm casts may contain 40% more humus than the soil in which the worm is living. Earthworms also serve as one of the most important links in the food chain. They feed on microscopic matter but they are eaten by larger species (Lee 1985; Edwards and Bohlen, 1996).

2.1 General character of earthworms

Earthworms are bilaterally symmetric, segmented, coelomate invertebrates. The external segments mostly correspond with internal segmentation, but there no parapodia; setae are borne on all segments except the first segment, arranged singly in minute setal sacs in skin, head indistinct; no tentacles and eyes, and sexually mature individuals develop a girdle or saddle-like glandular swelling in the skin, called clitellum**,** around a small part of body at anterior region (Fig. 1) (Gates, 1972; Reynolds, 1974; Edwards and Bohlen, 1996).

2.1.1 External characters

External form and segmentation.—The earthworms are segmented worms of variable size. The grooves which separate the segments from each other are clearly defined. The numbers of body segments are from small number (40-60) to as many as 1000 segments. The number of segments is not fixed for the species (Beddard, 1900; Stephenson, 1923).

Prostomium.—It is the first segment of the body and overhangs the mouth (peristomium) on the dorsal side. It is important as a tactile organ. It varies in size, and in some worms it is so small that it is almost indistinguishable. The peristomium and prostomium differ between species and are useful as taxonomic character. The following terms are used according to the shapes: zygolobous, prolobous, epilobous or tanylobous, depending on the demarcation of the prostomium (Fig. 2) (Beddard, 1900; Stephenson, 1923; Edward and Bohlen, 1996).

Setae.—Setae are bristle-like structure, which are partly buried in the thickness of the body-wall. As the setae are used to grip the substrate, their principal function is an aid for earthworm locomotion. They are controlled by special muscles which enable them to be retreated or protracted and pulled forwards or backwards, progression is effected by their movements in coordination with the body wall musculature. The arrangement of the setae among the earthworms are varied, some having numerous setae per segment called perichaetine setae, other with eight setae per segment called lumbricine setae. The number of setae per segment has been considered to be an important taxonomic characteristic in the perichaetine species, especially number of ventral setae between the male pores (Beddard, 1900; Gates, 1932; Stephenson, 1923; Edwards and Bohlen, 1996).

Perichaetine setae are arranged in a single ring around the periphery of each segment. They are designated by the letters a, b, c, d-beginning with the most ventral one on each side, and z, y, x, w- beginning with the most dorsal one on each side, irrespective of how many there are in between. In lumbricine setae, the distance between the setae in each pair is very small, they are termed ‘closely paired’, if wider apart, they are termed ‘widely paired’ (Fig. 3) (Edward and Bohlen, 1996).

Dorsal pores.—Present in most species of earthworms, beginning some distance behind the anterior end. The location of the first dorsal pore has long been considered a specific characteristic. The function of these pores opening into the body cavity from the exterior undoubtedly suggested a nephridial organ, but no relations are apparent between the dorsal pores and the nephridia. The position of the first dorsal pore is used as a taxonomic character at the species level (Stephenson, 1923; Gates, 1932).

Clitellum.—It is in the most fully developed condition, when the worms are mature. It is either ring-shaped or saddle-shaped. Sometimes setae are present, and sometimes the intersegmental grooves are visible; the colour also often differs from the neighboring part of the body. The development of the clitellar gland at sexual maturity is associated with cocoon production. The position of the clitellum and the number of segments occupies differs considerably among earthworms. Megascolecidae have the clitellum further forward, beginning at or in front of segment 14, thus including the female pore, and posteriorly it can include the male pore. Some aquatic or semi-aquatic earthworms have a wing-clitellum (Gates, 1932; Edwards and Bohlen, 1996).

Spermathecal pores.—Connected with the external apertures of the spermathecae. Usually paired but they may be single or numerous. Additional they may be minute. Their number and position are often used for systematic analysis. (Gates, 1932; Sims and Easton, 1972).

Male pores.—Among the Megascolecidae, the common opening for the vasa deferentia and the prostatic ducts. The distances apart of the paired pores are variable. They are usually paired in the Megascolecidae and characters of male pores are usually used for classification at the generic level, such as in *Metaphire* the presence of copulatory pouches containing the male pores but male pores superficial in *Amynthas* (Sims and Easton, 1972).

Female pore.—Occur at clitellum region, for example, in the Megascolecidae where they are often a single median pore on segment xiv (Edwards and Bohlen, 1996).

Genital markings.—They mostly occur near the spermathecal pores and/or the male pores. Their number and position are used as taxonomic characters and in many cases used for species identification. Shapes are circular to oval, but some are associated with internal parietal glands; either sessile or having a long stalked form (Sims and Easton, 1972).

2.1.2 Internal morphology

Coelom.—The coelom is a large cavity, extending from one end of the body to the other, containing the coelomic fluid, and divided up into a series of consecutive chambers by the *septa*. These septa correspond to the grooves on the exterior of the body that mark the segments, and they differ in thickness, depending on their position in the body, those in the anterior of the body being markedly thickened and more muscular. Septa are constructed from muscle fibers, mostly derived from the longitudinal muscle layer, together with some circular muscles on the posterior face, with connecting tissue and blood vessels. The coelom is lined throughout the body by the peritoneal epithelium, which is connected to all the organs that lie within it; from its wall are developed the gonads. The coelom communicates with the exterior directly by means of the dorsal pores, and indirectly by means of the nephridia and the genital ducts. The coelomic fluid is a milky white liquid which is sometimes yellow. The consistency of the coelomic fluid production differs between different species of earthworms, and also depends upon the humidity of the environment in which the worms live. Many earthworms release coelomic fluid through the dorsal pores, in response to mechanical or chemical irritation, or when subjected to extremes of heat or cold conditions (Edwards and Bohlen, 1996; Jamieson, 1997).

Digestion system.—The alimentary canal or gut of earthworms is basically a tube extending from the mouth to the anus, begins at the mouth, which is situated at the anterior end of the first segment (peristomium). The buccal cavity is protrusible in many or all earthworm, and during the ingestion of food, the upper and lower walls of the pharynx are protruded so as to form an upper and a lower transverse cylinder, the food particle is included between them, and the cylinders are rolled backwards into mouth, carrying with them the included particle. The *pharynx* is a thicker portion of the alimentary tube, not very definitely separated from the buccal cavity in front, but more distinctly defined behind where it is continued into the narrow oesophagus, which is posteriorly widened to become the intestine (Stephenson, 1972).

In many earthworms a dilated thin walled portion of the alimentary canal is distinguishable as a crop. In the Lumbricidae such a region occupies segments xv-xvi or close to these segments, immediately in front of the gizzard; in many of the Megascolecidae, where the gizzard is farther forward (in segments v, vi, vii or viii), the crop is also farther forward. The gizzard is a very firm and thick-walled muscular region of the alimentary tube, its function is the trituration of food-matters by the help of the mineral particles of the soil which are also taken in. This organ(s) may occupy a position in the oesophagus, or may be situated behind the oesophagus at the beginning of the intestine. The number of gizzards may be any number from two to ten; for examples, six are found in the genus *Drawida*, seven to ten in species in the genus *Desmogaster* (Gates, 1972; Stephenson, 1972), a single in *Metaphire* and *Amynthas* (Bantaowong *et al*., 2011a, b).

The intestine is the longest and widest portion of the alimentary tract. The intestinal caecum may be present, as usually in the genus *Pheretima* present from the segments xxvi or xxvii and extending forwards through three or four segments. There are various types of intestinal caecae, such as simple or manicate (glove-like), which are significant for identification at species level. The dorsal longitudinal fold which constitutes the typhlosole has been investigated in a number of species of earthworm. The typhlosole begins usually at the beginning of the intestine, and ceases some distance in front of the anus. The function of the typhlosole, is generally believed to be to increase the absorptive surface of the intestine (Stephenson, 1972).

Vascular system.—Earthworms have a closed vascular system. There are three principal blood vessels, one dorsal and two ventral. The paired commissural vessels pass round the body in several head segments from the dorsal vessel to the ventral vessel. Some of the anterior commissures are enlarged, contractile, and with valves termed ‘heart’ or ‘pseudo-hearts’. The position and number of pseudo-hearts differs in among worms (Fig. 4). The blood of all earthworms is red, the color being due to haemoglobin dissolved in the plasma (Stephenson, 1972; Edwards and Bohlen, 1996).

Respiration system.—The worms have no special respiratory organ. The respiratory exchange is through the skin, so a moist condition of the surface is essential. Earthworms perish if faced with dry conditions; thus the secretion of the mucous gland of the epidermis has its importance for respiration as well as lubrication of the surface. This method of gaseous exchange depends on a network of small blood vessels buried in the body wall of terrestrial earthworms, so that oxygen dissolved in the surface moisture film can be permeated through the cuticle and the epidermis to the thin walls of these vessels, where it is taken up by the hemoglobin in the blood and passed around the body. The hemoglobin in earthworms has a very high affinity for oxygen (Edwards and Bohlen, 1996).

Reproductive system.—Earthworms are hermaphrodites containing both male and female genital organs opening to the exterior, and possess a complicated series of organs related to the reproductive function. The essential organs are the ovaries and testes—the gonads; there are ducts which convey the sexual products to the exterior; the sexual organs are important for systematic purposes (Stephenson, 1923).

The female organs comprise the ovaries, female funnel and oviduct or ovisacs. The ovaries are situated in segment 13 of Megascolecidae, in a position corresponding to that of the testes. Each ovary is a small and always lie behind the testes in segment 13 (Stephenson, 1972). They are located in segment 13 of *Metaphire grandipenes* (Bantaowong *et al.*, 2011a) and in all earthworms.

The *spermathecae* are connected with spermathecal pores and are important reproductive organs; the function is for the reception of sperms during copulation, and storage of them untill the time of oviposition. Usually earthworms have one or more pairs of spermathecal pores which are usually intersegmental and are most often situated in the ventral or latero-ventral position. In each organ there may usually be distinguished the ampulla, spherical, ovoid, or of many other diverse shapes, and its duct, the canal which communicates to the exterior. In the Megascolecidae and Acanthodrilidae there is usually a diverticulum. The point at which the diverticulum passes into the duct should be noted; its may form also be of value in some species (Gates, 1932).

The *testes* may be either one or two pairs and they vary in position also. In the majority of earthworms there are two pairs, in segments x, xi, called holandry. If it is only the anterior pair of testes which is retained the worm are said to be proandric, if the posterior pair alone is present, they are metandric (Edward and Bohlen, 1996).

The *seminal vesicles* are the storage of the ripened spermatozoa which communicate to the testes, or the testis sac. Their position, size, and lobulation or its absence are to be noted (Sims and Easton, 1972).

The *prostates* or *prostatic glands* are large glands associated with the male pores. Their function is to produce a fluid in which sperm cells can be transferred between worms during copulation. In the Megascolecidae they are of two types, tubular or racemose (as in *Pheretima*) where the glandular tissue is compact with branching canals opening into the associated ducts. Other families have prostates in the form of muscular finger-like processes, or convoluted tubes. Prostate glands are rare in the family Lumbricidae, but there are other glands producing fluids used during mating.

The *genital marking glands* are of various kinds and presumably function in connection with reproduction. They are usually associated with genital markings but the genital markings are not always visible from the exterior (Gates, 1932; 1955).

However, the number, position, and arrangement of these several reproductive organs are very greatly variable from family to family and from genus to genus, often (e.g. spermathecae, genital markings) from species to species. Hence the reproductive system is important for systematic purposes (Sims and Easton, 1972). Gates (1972) thought that reproductive organ were less important.

Excretory system.—The principal excretory organs in earthworms are the nephridia, which extract waste materials from the coelomic fluid, and excrete them to the exterior, as urine consisting mainly of ammonia and urea, through the nephridiopores. There are at least one pair per segment, or they may be more numerous, two or more, often indeed a very large number, on each side of each segment. The large organs, one pair to a segment, are called meganephridia or holonephridia, and nephridia present in larger numbers are called meronephridia or micronephridia. This type occurs in *Pheretima* (Megascolecidae). In general the difference is clear between mega- and micronephridia (meronephridia), meganephridia are of relatively large size and are one pair per segment, while micronephridia are smaller, sometime minute, and are multiple in each segment. The nephridia obviously act as differential filters, because there is much more urea and ammonia, but less of protein, in the urine they produce than in the coelomic fluid (Stephenson, 1972; Edwards and Bohlen, 1996).

Nervous system and sense organs.—The nervous system is different in each family for examples, in the family Megascolecidae, each typical body segment is supplied by three pairs of nerves, whereas in *Lumbricus* there are four pairs of lateral nerves except the first two segments. The first segment is supplied by a pair of nerves originating in the lateral portions of the subpharyngeal connectives, which branch into two, shortly after they leave these nerves, and ramify through the first segment without forming nerve rings. A small nerve comes from the most ventral of these two branches to supply the ventral surface of the buccal cavity (Edwards and Bohlen, 1996).

The sense organs have two main types, photoreceptor organs and epithelial organs. The photoreceptor organs occurred at the ends of the nerves to the skin. Earthworms are in general negatively phototactic, it is that they retreat into their burrows during the day, and emerge at night. The epithelial sense organs are groups of

35-45 elongated cells, broader at their bases, with distal ends terminating in sensory hairs.

2.2 Earthworm biology

The Earthworms are amongst the commonest animals in nature, numbers are to be found in moist soils in all parts of the world, thus in the rainy season, earthworms come up from the deeper burrows where they have spent the dry season. They first begin to appear in early rainy season. At night, the earthworms come to the surface and excrete their waste products called ‘cast’, because it is a night animal or nocturnal.

A system of classifying earthworms according to their habitat divides them into three categories (Bouché, 1972):

Epigeic: species lives in the surface litter above the mineral soil or the top 2 or 3 cm of soil and make no permanent burrows. They feed on surface litter, digesting it and the fungi and microorganisms found there. They are reddish brown or dark in color and small in size, usually less than 7.5 cm long when mature.

Endogeic: species make extensive branching burrow systems in the top 50 cm of the soil. They feed by ingesting large amounts of soil and digest the soil organic matter and fungi and other microorganisms occurring in surrounding environments. They are easily separated from epigeic and anecic species by their color: endogeics have little or no skin pigmentation, but rather light grey, sometimes with an albino pink head. Adults have range in size 3-100 cm long.

Anecic: species make vertical burrows up to 2 meters deep in the soil, but they feed on fresh surface litters. The common night crawler, *Lumbricus terrestris* is an example of anecic species. They are reddish brown in color and larger than the other two groups. Adults are usually 12.5–20 cm long, but some are over 1 meter.

Earthworms are hermaphrodite having both male and female reproductive organs in the same individual. Cross fertilization and copulation are observed in earthworms. The earthworm hatches from an egg which is laid in the soil, along with many other eggs, in an egg case, or cocoon, whose outer wall is secreted by the clitellum. Two worms mate by coming together in a side-by-side and head-to-tail position. The two worms separate after they have exchanged the male sex cells or sperms. The worm forms a thick ring of slime around its body from the several glandular cell layers of the *clitellum* (Wilmoth, 1967).

The cocoon or egg case contains a milk-white sticky substance which both protects and nourishes the embryos. While there are always several eggs and more than enough sperms within each egg case, only one or two worms usually hatch. The time it takes for the embryo to develop seems to depend both on the temperature and the amount of moisture in the soil. The egg case shrinks slightly before hatching and turns a darker yellow-red of tiny worms bodies before the earthworms hatch out. Development time of cocoons was short in the topsoil endogeic worms, *Lampito mauritii* (15 days), *Drawida nepalensis* (25 days) and *Pontoscolex corethrurus* (29 days) and long (50 days) in the subsoil endogeic earthworm such as *Polypheretima elongata*. Prolonged development time (110 days) was observed in the cocoon of the subsoil anecic worm *Eutyphoeus gammiei* (Bhattacharjee and Chaudhuri, 2002). The total length of time from egg laying until hatching may run from 15-100 days depending on species and weather (Edwards and Bohlen, 1996).

The newly hatched worms must grow for a few months to several years before reaching maturity. Earthworms have been kept in captivity for nearly 7 years and some species are known to live for 10 years (Edwards and Bohlen, 1996).

2.3 Earthworm diversity and geographic distribution

Earthworms are classified within the phylum Annelida and the class Oligochaeta, which consists of as many as 36 families worldwide. About two-thirds of oligochaete families comprise aquatic or semi-aquatic worms, and the remaining families comprise mostly or exclusively terrestrial worms or earthworms. There are over 5000 known earthworm species and it is estimated that the global total may be twice that number. Distinct taxonomic groups have arisen on every continent except Antarctica, and some groups are now distributed throughout the world (Bohlen, 2002).

It is possible that the Polychaeta are ancestral to the Oligochaeta, or they may both be derived from a common aquatic ancestor. Struck *et al*. (2011) suggested that earthworms evolved from the Sedentaria division of Polychaetes rather than Errantia. The Sedentaria have a sedentary life style, as more or less sessile organisms that live below stones, or tube builders, or burrowers by means of peristalsis such as earthworms, while Errantia show a more mobile and active life strategy than Sedentaria. The molecular analysis of Struck *et al*. (2011) is consistent with this life style difference.

Fossil records of oligochates show that there is little paleontological information concerning the history and development of the order. There were fossil segmented worms without setae or appendages found during the Upper Ordovician period in Kentucky, USA, which is described as the genus *Protoscolex*. Later, Bather (1920) described *Protoscolex latus* from the Upper Silurian period in Herefordshire, England, which apparently found papillae in one or two rows on each segment (Edwards and Bohlen, 1996).

However, the early oligochaetes probably lived in mud rather water, becoming transiently terrestrial when the mud dried up periodically. They could have separated gradually into two groups, one purely terrestrial, the other aquatic (in fresh water). Stephenson (1930) considered that the Moniligastridae is the most primitive of the oligochaete families, because their possession of a single-layer clitellum is more similar to that of the aquatic worms than that of any of the other terrestrial earthworm families. The Megascolecidae and Eudrilidae have more advanced characteristics, but they still retain one primitive feature in the position of the spermathecae and male or prostatic pores which come into contact during mating (Edwards and Bohlen, 1996).

2.4 Systematic: classification of the earthworms

Beddard (1895) recognized five families of microdriles and seven of megadriles Oligochaeta. Michaelsen (1921) classified earthworms into 21 families, and Stephenson (1930) simplified this arrangement into 14 families of which seven families; Aeolosomatidae, Naididae, Tubificidae, Phreodrilidae, Enchytraeidae, Lumbriculidae and Branchiobdellidae were placed in the Microdrili group and the remaining seven families were in the Megadrili. Stephenson’s classifications of the megadrile families (1930) are as follows:

1. Family Alluroididae
2. Family Haplotaxidae

3. Family Moniligastridae
    Subfamilies Syngenodrilinae
        Moniligastrinae
4. Family Megascolecidae
    Subfamilies Acanthodrilinae
        Megascolecinae
        Octochaetinae
        Ocnerodrilinae
5. Family Eudrilidae
    Subfamilies Pareudrilinae
        Eudrilinae
6. Family Glossoscolecidae
    Subfamilies Glossoscolecinae
        Sparganophilinae
        Microchaetinae
        Homogastrinae
        Criodrilinae
7. Family Lumbricidae

Latter, Gates (1959) considered the structures of the prostatic glands and excretory system, and position of the califerous glands as important characters and raised up all the main groups to family status. Sims (1967) partially agreed with the definitions of the groupings proposed by Gates (1959) by recognizing only two families, the Megascolecidae and the Acanthodrilidae. Gates (1959) classified the majority of oligochaetes as follows:

1. Family Moniligastridae
2. Family Megascolecidae
3. Family Ocnerodrilidae
4. Family Acanthodrilidae
5. Family Octochaetidae
6. Family Eudrilidae
    Subfamily Pareudrilinae
        Eudrilinae

7. Family Glossoscolecidae
8. Family Sparganophilidae
9. Family Microchaetidae
10. Family Hormogastridae
11. Family Criodrilidae
12. Family Lumbricidae

Jamieson (1988) reviewed the overall phylogeny and higher classification of the oligochaeta based on a cladistic analysis. He placed all the megadrile families that were predominantly or wholly terrestrial into a new cohort. Cohort: Terrimegadrili was divided into 4 superfamilies, Ocnerodriloidae (family Ocnerodrilidae), Eudriloidae (family Eudrilidae), Lumbricoidae (family Kynotidae, Komarekionidae, Ailoscolecidae, Microchaetidae, Hormogastridae, Glossoscolecidae, and Lumbricidae) and Megascolecoidea (family Megascolecidae).

Sims and Easton (1972) reviewed the genus *Pheretima* (family Megascolecidae) and divided it into eight genera: *Archipheretima*, *Pithemera*, *Ephemitra*, *Metapheretima*, *Planaphertima*, *Amynthas*, *Metaphire*, and *Pheretima*. Later Easton (1979) reexamined the specimens and revised some species without caecum in the *Pheretima* group. In his study, the genus *Ephemitra* was combined into *Metapheretima*, the genus *Polypheretima* was divided from *Metapheretima*, and the genus *Pleionogaster* with intestinal gizzards was considered to be a member of the *Pheretima* group. In addition, *Begemius* was separated from *Amynthas* by Easton (1982) (Fig. 7). The subdivision of the *Pheretima* group is based on body shape, caecum, gizzard, male pore, clitellum, spermathecal diverticula, and spermathecal duct (Sims and Easton, 1972; Easton, 1979, 1982). For the key to the genera of the *Pheretima* group refer to Easton (1979, 1982).

The latest classification of terrestrial species by Reynolds and Cook (1993) showing the following arrangement:

Order Moniligastrida
    1. Family Moniligastridae
Order Haplotaxida:

2. Family Acanthodrilidae
3. Family Ailoscolecidae
4. Family Alluroididae
5. Family Almidae
6. Family Biwadrilidae
7. Family Criodrilidae
8. Family Eudrilidae
9. Family Glossoscolecidae
10. Family Hormogastridae
11. Family Komarekionidae
12. Family Kynotidae
13. Family Lumbricidae
14. Family Lutodrilidae
15. Family Megascolecidae
16. Family Microchaetidae
17. Family Ocnerodrilidae
18. Family Octochaetidae
19. Family Sparganophilidae

For a field of science as limited as oligochaetology, there are books that combined all the description citations and type depositions of earthworms: the *Nomenclatura Oligochaetologica* and its three supplements (Reynolds and Cook, 1981, 1989, 1993). The third supplement (*Supplementum Tertium*), which recorded new taxa found up to December 31, 1992, suggested that 739 earthworm genera, 40 subgenera, and 7,254 species have been described. A fourth supplement concluded the new description of 1,048 species (Reynolds and Wetzel, 2004).

2.5 Earthworm zoogeography

Stephenson (1972) simplified information of biogeography of Oligochaeta from many papers and recognizing the small importance of some groups of Oligochaeta for zoogeographical considerations:

1. Holarctic Region.—The characteristic family of the Palaearetic division of the great Holarctic region is that of the Lumbricidae. The exact limits of the Lumbricid

territory in Asia are hardly known, there are a few endemic lumbricids in the Himalayas and the south and east of Calcutta. There are endemic Lumbricidae in the United States, but most Lumbricidae there are European.

2. Neotropic region.—Roughly from Mexico southwards to the Antarctic, is the Glossoscolecidae. This group extends northwards into Central America (Costa Rica) and the West Indies to Trinidad and Tobago.

3. The African region.—Two great groups characterized this area, the family Eudrilidae,the genus *Dichogaster* (family Octochaetidae). The cape region, composing the southern portion of the continent, is distinguished by the presence of two groups, the Microchaetidae and the Acanthodrilidae. In Madagascar with the immediately neighboring islands, the genus which is particularly characteristic of this area is Kynetidae, Acanthodrilidae is also recorded; there is an endemic species of *Gordiodrilus madagascariensis* (Ocnerodrilidae).

4. The Oriental and Australasian Region.—The large and important region now to be considered is distinguished by the preponderance of the great family of Megascolecidae; and though it is divisible into several sub-regions. The Indian division can again be subdivided into a number of fairly distinct territories (Stephenson, 1923), some of which are characterized by particular genera. Thus while the north-west territory is partically occurred without endemic earthworms, *Drawida* is the characteristic of South India and Burma while *Pheretima* is for Burma, *Eutyphoeus* for the west Himalayan region, *Perionyx* for the North-East Frontier. The Ceylon division, it is characterized by the overwhelming preponderance of *Megascolex*, by a number of endemic species of *Notoscolex*.

The Indo-Malayan division is again impossible of accurate delimitation; it joins the Indian division on the west, and includes south-east Asia, China, and Japan for the most part, together with the Malay Archipelago as far to the east. The great characteristic earthworm is *Pheretima*, of the Megascolecinae, and a few species of Moniligastridae. The Australian division and New Zealand division, the endemic earthworm fauna is again Megascolecid in character.

The current checklist of 505 earthworms from India, Sri Lunka and adjacent regions. The Indian earthworm fauna is predominantly composed of native species, which constitute about 89% of total earthworm diversity in the country (Stephenson, 1923; Julka, 1988).

In China, about 300 species of terrestrial earthworms have been recorded. Five families of earthworms in China i.e. Moniligastridae, Lumbricidae, Megascolecidae, Octochaetidae and Ocnerodrilidae were reported by many research works. (Chen, 1930, 1931, 1933, 1935, 1936, 1938; Chen *et al*., 1975; Gates, 1935, 1939a; Tsai *et al*., 2000).

In Japan, the current revision provisionally lists 77 valid earthworm taxa in seven families, Moniligastridae, Lumbricidae, Biwadrilidae, Megascolecidae, Acanthodrilidae, Octochaetidae and Ocnerodrilidae (Blakemore, 2003; Easton, 1980; Hatai, 1930a; Hatai and Ohfuchi, 1936, 1937; Kobayashi, 1941; Ohfuchi, 1935, 1937, 1938, 1941; Tsai *et al*., 2000).

In Korea, There are currently 118 species of earthworms recorded in Korea placed in three families and 10 genera (Kobayashi 1934, 1936, 1937, 1938a; Hong *et al*. 2001; Hong and Kim 2002; Hong, 2007; Hong and James, 2001, 2009).

In Taiwan, the total number of 76 nominal species of terrestrial earthworms (Tsai *et al*. 2009) and of oligochaetes (aquatic and terrestrial combined) to 106 (Tsai *et al*. 2009; Ohtaka and Chen, 2010).

In Singapore, there is a total 19 terrestrial earthworms that are now known from the island. The pheretimoid earthworm made up about half of the total number of the species (Shen and Yao, 2005).

Philippine earthworms have never been collected in an organized manner. the number of indigenous species in the Philippines could be very large, perhaps as many as 250, compared to the 24 species known up to now (James, 2004; Hong and James, 2010).

In Vietnam, there is a total 40 species distributed in Moniligastridae, Megascolecidae, Lumbricidae and Octochaetidae (Thai, 1982).

In Laos, there is a total 28 species distributed in Megascolecidae (Thai and Samphon, 1990; Hong, 2008), and many more undescribed species in collections.

In Myanmar, Gates (1972) published the “Burmese Earthworms and Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia” which recorded 231 earthworm species belonging to Moniligastridae, Lumbricidae, Glossoscolecidae, Microchaetidae, Megascolecidae, Acanthodrilidae, Ocnerodrilidae and Octochaetidae.

2.6 Thailand earthworm

Terrestrial earthworms in Thailand have been rarely investigated. The first scientifically named species from Thailand were *Perichaeta (=Metaphire) peguana* (Rosa, 1890) and *Perionyx excavatus* Perrier, 1872 from Chanthaburi and Bangkok, respectively (Beddard, 1900). In the first study of earthworms from Thailand, Gates (1939b) published 'Thai earthworms' a summary of information then known about the 24 species. Later, Gates (1972) surveyed earthworms in Burma and adjacent areas, he recorded 25 Thai earthworms which have been reported in the Burmese earthworms surveys.

These earthworms have been placed in Glossoscolecidae (*Pontoscolex corethrurus*), 23 species in Megascolecidae [*Lampito* (*L. mauritii*), *Perionyx* (*P. excavatus*), 12 species of *Amynthas* (*A. alexandri*, *A. compta*, *A. longicauliculatus*, *A. exiguu*s, *A. austrinus*, *A. decorosa*, *A. gracilis*, *A. hupbonensis*, *A. manicatus*, *A. mekongianus*, *A. morrisi*, *A. papulosa*,), 8 species of *Metaphire* (*M. anomala*, *M. bipora*, *M. houlleti*, *M. malayana*. *M. peguana*, *M. planata*, *M. posthuma*, *M. virgo*), one species of *Polypheretima* (*P. elongata*)], and 1 species in Octochaetidae (*Dichogaster affinis*) (Gates, 1939b; 1972). Sixty five years later, Blakemore (2006) updated checklist of Thai earthworms to be 28 species.

The study of earthworms by Thai taxonomist officially started by Kosavititkul in 2005, recording 13 earthworm species belonging to three families in Khao Yai National Park: there are Glossocsolecidae (*Pontoscolex corethrurus*), Megascolecidae (*Amynthas alexandri*, *A. fucosus*, *A. longicauliculatus*, *Metaphire peguana*, *M. houlleti*, *Perionyx excavates*, and *Pithemera bicincta*), and Moniligastridae (*Drawida beddardi*), which included an unknown species. Later, Chantaravisoot (2007) reported five species of *Amynthas* and four species of *Metaphire* from various areas in Thailand. Somniyam and Suwanwaree (2009) studied diversity and distribution of terrestrial earthworms in Sakaerat Environmental Research Station and adjacent areas, Nakhon Ratchasima province, and reported twenty one earthworm species in five families; these are Megascolecidae (*Amynthas alexandri*, *A. defecta*, *Metaphire bahli*, *M. houlleti*, *M. peguana*, *M. planata*, *M. posthuma*, and *Polypheretima elongata),* Moniligastridae (*Drawida beddardi*), Octochaetidae (*Dichogaster affinis*, *D. bolaui*, and *D. modiglianii*), Glossoscolecidae (*Pontoscolex corethrurus*) and Ocnerodrilidae (*Gordiodrilus elegans*). Accordingly, a total of 34 species of earthworms were reported from Thailand (Table 2), but only one Thai native species *Amynthas hupbonensis* was included.

2.7 Earthworm Utilization

Recently earthworms have been increasingly investigated by people for wide application as ecologically clean technologies for utilization of wastes. There are many species of earthworms now in use and looking very promising such as the two introduced species *Eisenia fetida* and *E. andrei*. At the same time, other earthworm species can be used in vermiculture, for instance, soil litter worms *Lumbricus rubellus*, *Perionyx excavatus*, etc. Earthworms help decomposing organic matters, and thus may be used in vermicomposting (Dash and Senapati, 1986; Reynolds and Eggen, 1993). They aerate the soil, help water penetrating vertically and horizontally, and neutralize acidic soil. Charles Darwin was the first to appreciate the key-role of earthworms for the soil sciences (Reynolds, 1994).

Since 1935 earthworm farms have become a much more important source of dissemination than the greenhouse industry. Three of the commonly cultured forms are lumbricid worms and are established throughout much of the US and Canada. The fourth group which was raised for a time is a tropical species of the African family Eudrilidae. Although purchased primarily by anglers who are likely to scatter them in all places, the earthworms are also sold to organic gardeners and farmers to improve soil fertility (Stephenson, 1972).

Gates (1926a) describes some of the medical uses of earthworms in Burma. Their ashes are used as a tooth-powder, alone or with roasted tamarind seed and betel nuts, in a condition the description of which suggests pyorrhea. The ashes are also used in a disease characterized by white spots on the tongue and mucous membrane of the mouth with slight fever.

Some species of earthworms play an important role in the metabolism of the soil nutrients and prove to be highly suitable as environmental indicators because of their species richness and abundance (Graefe, 1993). The ability of many species of earthworms to accumulate heavy metals and various pesticides into their tissues offers opportunities to trace the movement of these materials in the soil. One aquatic microdrile species, *Tubifex tubifex* has been used for decades as a biological indicator in polluted waters. *Eisenia andrei* is used in a standardized terrestrial assay using artificial soil to assess the toxicity of pesticides and other chemicals (Edwards and Bohlen, 1996). Research is needed on the toxicities uptake mechanisms, distribution, and concentration of chemicals in various types of earthworm tissues. In particular, interpretation of the findings on chemicals and earthworms as they relate to human daily lives (Reynolds, 2004).

2.8 Allozyme electrophoresis

The basic principle of allozyme electrophoresis is to run samples, using an electric current, through a medium (gel) that causes proteins to travel different distances through the gel, depending on size, shape and charge. These gels are then stained for a particular enzyme such that the location of the forms (alleles) of the enzyme are indicated by colored bands in the gel (Richardson *et al*., 1986; Murphy *et al*., 1996). Enzyme that differ in electrophoretic mobility as a result of allelic differences at a single locus are called allozyme. These are opposed to isozymes which are enzymes that perform the same function, but which are coded by genes located at different loci. The other data set consists of allozymes, a subset of isozymes, which are variants of polypeptides representing different allelic alternatives of the same gene locus. Both forms of data are important in molecular systematic, and both involve proteins that can be separated on the basis of net charge and size. The horizontal starch gel methods are in widespread use and are efficient (Murphy *et al*., 1996).

2.8.1 Allozyme electrophoresis study

Electrophoresis of enzymes has been applied to the study of earthworm species since the early 1980s but such genetic studies have mainly focused on local populations, whereas only a few studies have covered a wider geographic area (Jaenike *et al*., 1980; Jaenike and Selander, 1983).

Øien and Stenersen (1984) use polyacrylamide gel electrophoresis of esterase to clarified breeding and grafting of *Eisenia fetida* by following an esterase electromorph character. In 2001, McElroy and Diehl used allozymes to evaluate two related species of popular commercial earthworms (*Eisenia fetida* and *E. andrei*), and found that eight polymorphic allozyme loci in the parent and offspring generations of two species can be used to evaluate possible mechanisms of allozyme-associate heterosis for grown rate.

Peles *et al.* (2003), studied the genetic structure of *Lumbricus rubellus* populations from reference sites and contaminate sites. Their result suggested that the allele and genotype frequencies at certain enzymatic loci in earthworm have the potential to serve as useful bioindicators of environmental impact in terrestrial system. Like, Simonsen and Fordsmand (2004) analyzed the genetic variation of the enzyme esterase in the earthworm *L. rubellus*, in metal contaminated areas in three successive years. The result revealed that mainly Cu and Zn had an impact on the *Est* genotypes.

Mezhzherin *et al.* (2008) investigated the genetic structure of earthworms *Aporrectodea caliginosa* complex by allozyme electrophoresis. Genetic variation in the earthworm *Dendrobaena octaedra* populations was measured by allozyme; the results suggested that genetic diversity of their populations is only weakly affected by pollution with heavy metals (Simonsen *et al*., 2008). At the same time, Shepeleva *et al.* (2008) compared the assessment of genetic diversity in *Lumbricus rubellus* populations inhabiting different regions of the species ranges. This study indicated that *L. rubellus* from the Moscow and Kiev region formed two genetically distinct spatial groups.

2.8.2 Allozyme statistical analysis

The first assignment before analyzing data is that a genotype should be interpreted from observed phenotype. It may be appropriate to provide some variation of allele nomenclature. Allozyme alleles are often named using alphabetical characters following alphabetical order with A being the fastest moving allele. Therefore, the genotype at a gene locus coding for an enzyme can be inferred for each individual in the sample from the number and position of the spots observed on the gels.

Test for the Hardy-Weinberg

The Hardy-Weinberg equation is a key concept in population genetics that describes the relationship between gene and genotype frequencies. It states that “in the absence of migration, mutation and natural selection, gene frequencies and genotypic frequencies remain constant in a large, randomly mating population”. Such a population may called “in Hardy-Weinberg equilibrium”, i.e. the frequency of genotypes is dependent on the frequency of genes, and these are both constant over time. Thus, the Hardy-Weinberg equation is a statement of the null hypothesis that no evolutionary forces are acting on a large, randomly breeding population. If we sample a population and discover that genotype frequencies are not in Hardy-Weinberg equilibrium then we can conclude that one or more external forces are at work.

The Hardy-Weinberg principle serves as a kind of a null hypothesis, i.e. there is random mating, no selection, no mutation and no migration occurring in the population studied. It tells us what to expect if all of the seven stated conditions are true in the population. If we sample a population and find that the genotype frequencies are different from Hardy-Weinberg expectations, then we can conclude that one or more of these assumptions is violated, or at least one evolutionary process is operating, or sometimes variations are not properly scored. This motivates us to study the population in more detail.

The usual way to compare a set of observed values to a set of expected values (based on some null hypothesis) is to use a goodness of fit test. The most commonly used goodness of fit test for Hardy-Weinberg conditions is the Chi-square (χ^2) test. The χ^2 statistic is:

$$\chi^2 = \sum \frac{(\text{observed-expected})^2}{\text{expected}}$$

If to be compare statistic to values from the χ^2 distribution. But first we need to know how much information, or the degrees of freedom (commonly abbreviated as df), was used to estimate the χ^2 statistic. In general, degrees of freedom are based on the number of categories of data: df = no. of classes compared – no. of parameters estimated – 1 for the χ^2 test itself. Check the χ^2 value, if it will have P value >0.05 we accept the null hypothesis of Hardy-Weinberg equilibrium in the population.

***Genetic distance* (*D*)**

Many genetic distance measures have been proposed (Nei, 1978; Reynolds 1981). One useful measure when dealing with genetic data was developed by Nei (1972), called standard genetic distance (*D*), which used estimated genetic differentiation among populations and to compare local population within a species to estimate the amount of gene flow and genetic drift between populations. The values of *D* have range from 0 to infinity.

The fixation index **(*F*st)**

The fixation index was extend to measure the pattern of population structure. It can be expresses the amount of variation detect among subpopulations as a fraction of that expected if the same set of genetic markers were randomly distributed. *F*st ranges from 0.0 (no differentiation) to 1.0 (complete differentiation - subpopulations are fixed for different alleles). For example, the value *F*st = 1 was estimated for two subpopulations. This means that there is absolute differentiation between the two subpopulations, with each fixed for a different allele. Simply speaking, this can be interpreted as 100% of the total genetic variation is between subpopulations, with zero variation present within subpopulations.

Although *F*st has a theoretical range of 0 to 1, the observed maximum is usually less than 1. Wright (1978) suggests the following general guidelines for the interpretation of *F*st based on allozyme loci:

*F*st = 0.00-0.05 may be considered as indicating little genetic differentiation
*F*st = 0.05-0.15 indicates moderate genetic differentiation
*F*st = 0.15-0.25 indicates very large genetic differentiation
*F*st = > 0.25 indicates extensive genetic differentiation

Furthermore, the quantify the genetic variation of sample from each locality, the population genetic variability was determined using mean expected heterozygosity (*H*exp), which represents the adaptive potential of the species and is an extremely powerful evolutionary force, mean number of alleles per locus (*A*), and percentage of polymorphic loci (*P*).

Table 1. The regional occurence of the 10 recognized major families of terrestrial earthworms (Hendrix and Bohlen, 2002)

Family	**Geographical region of origin**
Ailoscolecidae	Europe
Eudrilidae	Africa
Glossoscolecidae	Central America, South America
Hormogastridae	Mediterranean
Komarekionidae	North America
Kynotidae	Madagascar
Lumbricidae	Europe, North America
Megascolecidae	Africa, Central America, North America, South America, Asia, Madagascar, Oceania
Microchaetidae	Africa
Ocnerodrilidae	Africa, Central America, South America, Asia, Madagascar

Table 2. List species of the terrestrial earthworms have been recorded in Thailand. Presence and absence of each species is indicated by + and – respectively.

Species	Gates, 1939b	Gates, 1972	Kosavititkul, 2005	Blakemore, 2006	Somniyam, 2009
Family Glossoscolecidae					
Pontoscolex corethrurus	+	+	+	+	+
Family Moniligastridae					
Drawida vulgaris	+	+	-	+	-
Drawida bedardi	-	+	+	-	+
Family Megascolecidae					
Lampito mauritii	+	+	-	+	-
Amynthas alexandri	+	+	+	+	+
Amynthas compta	+	+	-	+	-
Amynthas corticis	-	-	-	-	+
Amynthas evansi	+	+	-	+	-
Amynthas exiguus exiguus	+	+	-	+	-
Amynthas exiguus austrinus	+	+	-	+	-
Amynthas fucosus	-	-	+	-	-
Amynthas gracilis	+	+	-	+	-
Amynthas hupbonensis	+	+	-	+	-
Amynthas longicauliculatus	+	+	+	+	-
Amynthas mekongianus	+	+	-	+	-
Amynthas manicatus	+	+	-	+	-
Amynthas morrisi	+	+	-	+	-
Amynthas papulosus	+	+	-	+	-
Metaphire anomala	+	+	-	+	-
Metaphire bipora	+	+	-	+	-
Metaphire houlleti	+	+	-	+	-
Metaphire peguana	+	+	+	+	+
Metaphire virgo	+	+	-	+	-
Metaphire planata	+	+	-	+	+
Metaphire posthuma	+	+	-	+	+
Metaphire bahli	-	-	-	+	+
Perionyx excavatus	+	+	+	+	-
Pithemera bicincta	-	-	+	-	-
Polypheretima elongata	+	+	-	+	+
Family Octochaetidae					
Dichogaster affinis	+	+	-	+	+
Dichohaster modiglianii	-	-	-	-	+
Dichogaster bolaui	-	-	-	-	+
Family Ocnerodrilidae					
Gordiodrilus elegans	-	-	-	-	+
Family Lumbricidae					
Eisenia fetida	-	-	-	+	-

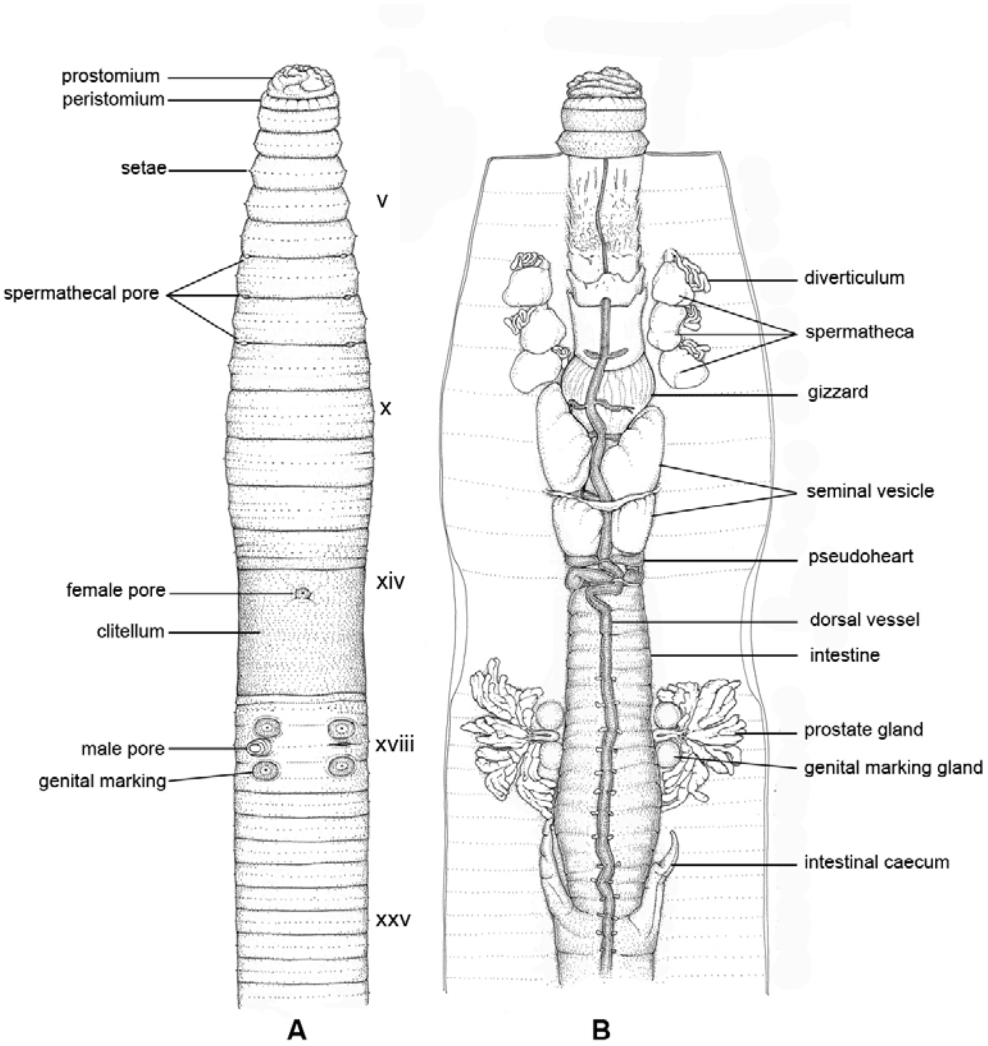

Figure 1. External (A) and internal character (B) of an earthworm in the family Megascolecidae, *Metaphire peguana* (Rosa, 1890).

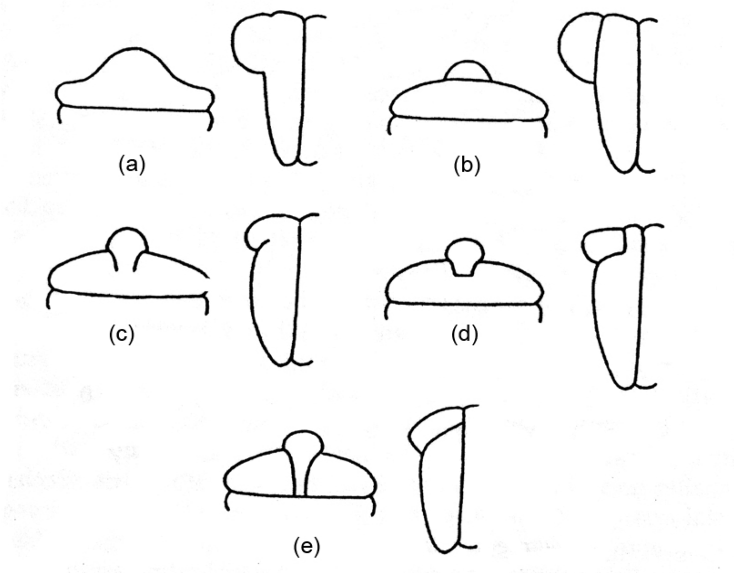

Figure 2. Various forms of prostomium; (A): zygolobous; (B): prolobous; (C), (D): epilobous; and (E): tanylobous (after Edwards and Bohlen, 1996).

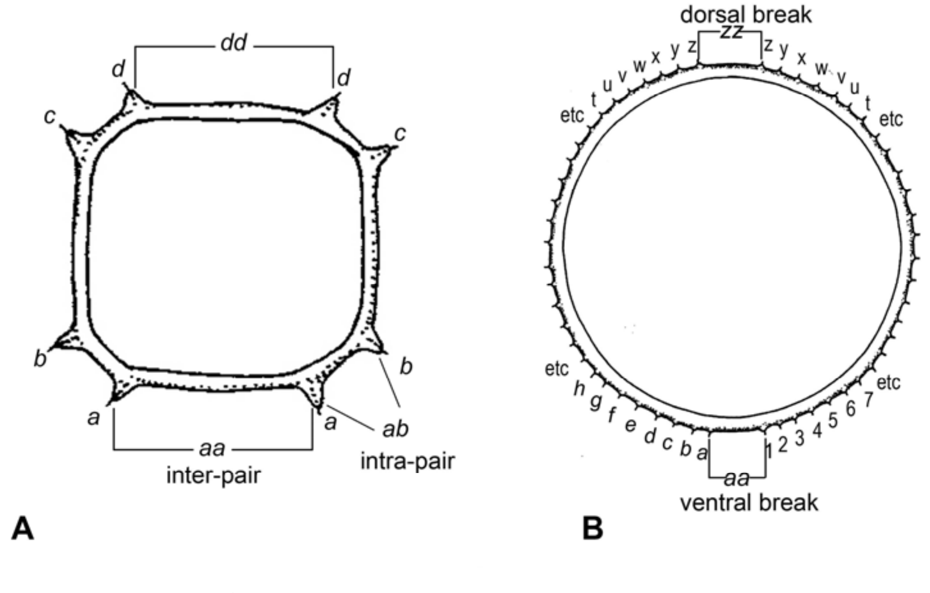

Figure 3. Arrangement of earthworm setae (A) Lumbricine setae arrangement, (B) Perichaetine setae arrangement (after Edwards and Bohlen, 1996).

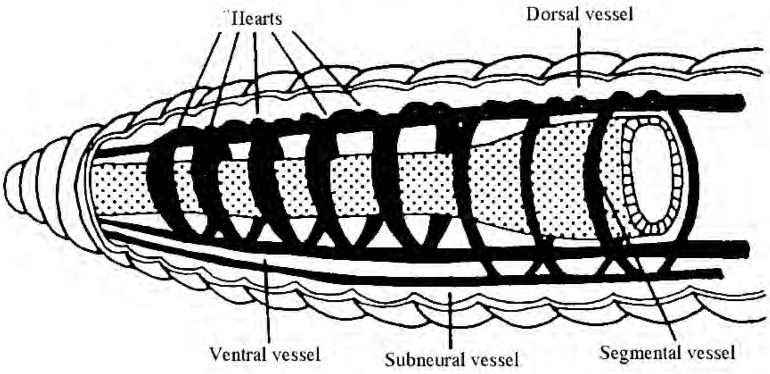

Figure 4. The vascular system of a lumbricid earthworm (after Wallwork, 1983).

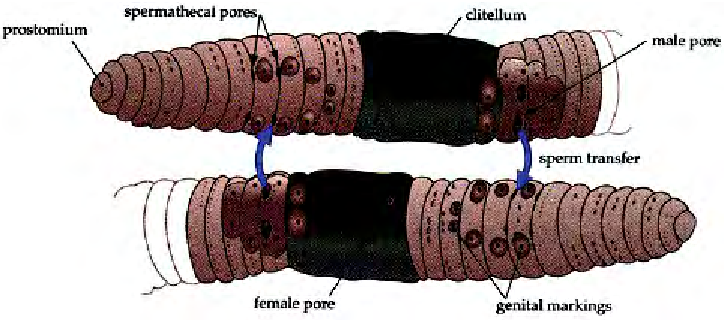

Figure 5. *Lumbricus* sp. in copulation; the segment are indicated. The worms are actually closely applied in copulation (after Jamieson, 2001).

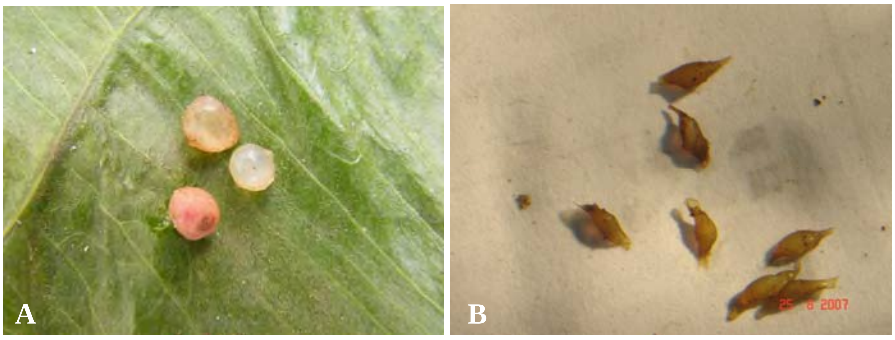

Figure 6. Cocoons of earthworms, (A) *Metaphire posthuma* (length about 2 mm) and (B) *Perionyx excavatus* (length about 3 mm).

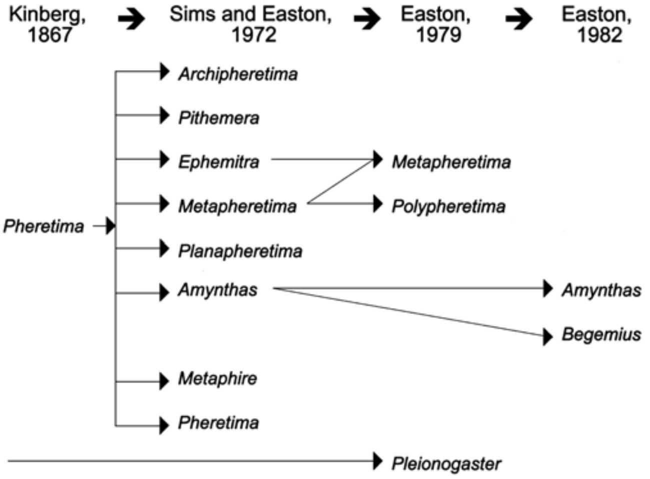

Figure 7. The taxonomic status changed in the *Pheretima* group (after Shih *et al*., 1999).

CHAPTER III

MATERIALS AND METHODS

3.1 Study site

This study was conducted at Nan province, which is located in the north of Thailand, at 18°00′45″ to 19°37′05″ N and 100°24′03″ to 101°06′29″ E (Fig. 8). The northern and eastern parts are connected to Laos's border whereas the southern and the western parts are connected to Uttaradit, Phrae, and Payao provinces, respectively. The central valleys of Nan province are surrounded by mountains covered with several types of forest. Luang Phrabang and Phi Pan Nam are parallele mountain ranges oriented north to south, becoming natural barriers between Thailand and Laos, and covered by moderate to dense natural forests. The highest mountain peak of 2,079 is Doi Phu Kha, which is Doi Phu Kha national park. There are many important rivers running within Nan province and the main Nan River is the principal are, receiving water from small rivers such as Sa river, Wa river, and Pua river and later running down to the south (DNP, 2004).

The sampling areas have been systematically selected as from both political boundary and also ecological characteristics showed in the map of Figure 8. The political related areas determined in order to cover all microhabitats but the zonings indicated in the map are determined from the ecological condition as follows; A) limestones and deciduous forest with Nan River running along B) granitic waterfalls and modified agricultural farms C) river valleys D) mainly anthropogenic areas and orchard plantations and paddy fields.

Nan has seven national parks (Fig. 8) and consisted of many types of forests, namely evergreen forest, dipterocarp forest, deciduous forest and limestone forest, including various ecosystem, waterfalls, caves, streams, paddy field and orchard.

3.2 Earthworms sampling and preservation

Earthworms were collected from 2009 to 2010. The samples were taken by encounter surveys in various ecosystem types from highly degraded anthropogenic places to native forests in Nan province as determined and showed in the map (Fig. 8). The worms were collected from various microhabitats by digging the top soil under new casts, under rocks, in rotten woods, wastewater saturated soil from household, ridges of paddy fields, etc.

Collections have been done by simple digging and hand-sorting soil. GPS readings were taken at many of the collection localities. Each specimen of earthworms was washed with water and killed in 30% ethanol then preserved in 4% formalin solution for 18-24 hours. Later specimens were kept in 70% ethanol for morphological study. For allozyme electrophoresis study in *Metaphire peguana*, the samples were preserved in liquid nitrogen at -196°C. Earthworms were identified in laboratory of Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University. The specimens were identified under a binocular stereomicroscope, and their body length and body width were measured. The earthworms were examined by external and internal morphological characteristics, and internal anatomy was examined by dorsal dissection, and drawings. All materials are deposited at the Chulalongkorn University Museum of Zoology (CUMZ).

3.3 Earthworm Identification

Earthworms were identified based on external and internal morphology. Various morphological characteristics were recorded. The data obtained was then used for identification and classification of the earthworms by using the keys provided by Gates (1972), Sims and Easton (1972).

External characters

- Body length and width
- Number of segment
- Body color
- Prostomium
- Numbers and arrangement of setae
- Position of spermathecal pore
- Position and form of clitellum
- Position and appearance of male pores and female pore
- Distribution and appearance of genital marking (if present)

Internal characters

- spermathecae and diverticulum
- Location of gizzard
- Location of pseudohearts
- Location and appearance of septum
- Location and appearance of seminal vesicle
- The origin of intestine
- Location of testis sac
- Form and position of prostate gland and prostate duct
- Form and location of intestinal caeca
- Form and location of genital marking gland

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

3.4 Allozyme electrophoresis Study

A total of 274 individuals of *Metaphire peguana* was collected in 2009-2010 from thirteen localities throughout Thailand (Fig. 9 and Table 3). In the field, earthworms were removed free of soil particles by soaking in the water and immediately immersed and maintained in liquid nitrogen and later stored at -20°C until processing electrophoretic steps.

The populations of *M. peguana* will be compared with three outgroups; *Metaphire* sp., *M. posthuma* and *M. bahli* from Phrae, Pitsanulok and Kalasin, respectively (Fig. 9).

3.4.1 Starch Gel Electrophoresis

The individual was then cut through the clitellar region and two segments above the clitellum. Tissue samples were taken from pseudoheart from each specimen and homogenized. The supernatant was absorbed by Whatman No. 3 filter paper. Protein extract from the homogenates were subjected to horizontal starch gel electrophoresis following Murphy *et al*. (1996). Four buffer systems were used (Table 4). Before electrophoretic processing, bromophenol blue was added to the sample. Electrophoresis was run at 4°C continued running until the dye marker migrated to

100-120 mm anodally. The gels were sliced into seven or eight slabs (about 1 mm for a sliced gel) for staining.

Starch gel electrophoresis technique was performed following the methods described by Steiner and Joslyn (1979) and Murphy *et al*. (1996). The nomenclature for protein-coding loci and alleles followed the recommendations in a paper of Shaklee *et al*. (1990). The running buffers and staining procedures were used from a report by Richardson *et al*. (1986). The results of allozyme electrophoresis technique were interpreted and calculated the variability of individual and genetic variation and population structure. Fifteen enzyme loci were applied in this study.

After electrophoresis, each gel was stained for a difference allozyme locus, using specific chemical (Table 4) stains mixed with melted agar, which was prepared. The stain-covered gel was incubated at 37°C until colored bands appeared. Banding patterns were scored by photography and drawing observed patterns on paper. The patterns were transformed into zymogram data sheets for analysis.

3.4.2 Data Analysis

Electrophoresis data on single individual genotypes were loaded into the computer program BIOSYS-1 (Swofford and Selander, 1981). The program computes allele frequencies and genetic variation were to test deviation of genotype frequencies from Hardy-Weinberg expectation by exact probability test ($p \leq 0.05$) and the mean number of alleles per locus (A), and the percentage of polymorphic loci (P).

To estimate the heterogeneity amongst samples and between each population, Wright's (1965) *F*st was calculated for each locus. East *F*st value was tested for significant departure from zero following the method of Workman and Niswander (1970). Nei's (1978) unbiased genetic distance and Rogers' (1972) genetic distance were calculated for all pairwise comparisons of samples in order to estimate the extent of differentiation among populations. *F*is is the mean reduction in heterozygosity of an individual due to non-random mating within a subpopulation. *F*it is the mean reduction of heterozygosity of an individual relative to total population.

Isolation by distance for the entire set of 14 populations was tested using a Mantel's test (Mantel, 1967) from the comparisons of all pairwise *F*st/(1-*F*st) values with pairwise geographic distance (Rousset, 1997) using the program ISOLDE in GENEPOP version 3.4 (Raymond and Rousset, 1995).

Table 3. Localities, and sample sizes (N) of *Metaphire peguana* used in this study. The locality numbers correspond to those in Fig. 9.

Locality		**Latitude, Longitude**	**N**
Northern region	1. Tham Chiang Dao, Chiang Mai	98°55′45.3″ E, 19°23′41.1″ N	20
	2. Tadman waterfall, Chiang Klang, Nan	100°47′24.0″ E,19°17′10.5″N	25
	3. Bhumibol Dam, Sam Ngao, Tak	98°58′54.8″ E, 17°14′22.1″ N	21
	4. Sakunothayan waterfall, Wang Tong, Phitsanulok	100°31′55.2″E, 16°50′17.8″N	16
Central region	5. Watkiriwong, Mueang, Nakhon Sawan	100°07′29.9″E, 15°43′06.6″N	30
	6. Bang Ban, Ayutthaya	100°28′11.8″E, 14°27′16.3″N	24
	7.Chulalongkorn University, Bangkok	100°31′49.8″E, 13°44′18.9″N	20
Eastern region	8. Khaomaka, Sa Kaeo	101°56′52.2″E, 13°47′13.9″N	32
	9. Bang Sakaow, Laemsing, Chanthaburi	102°07′8.7″ E, 12°30′26.7″N	16
Southern region	10. Tham Rong, Phetchaburi	99°55′02.3″ E, 13°01′27.0″N	28
	11. Huai Yang waterfall, Bang Sapan, Phachuab Khiri Khan	99°36′54.3″ E, 11°37′30.5″N	22
	12. Somdejphrasrinakarin Park, Lungsuan, Chumphon	99°02′27.9″ E, 09°56′51.4″N	9
	13. Khao Sok National park, Phanom, Surat Thani	98°31′47.6″ E, 08°54′37.8″N	11

Table 4. Enzymes and presumptive loci with tissues and buffer systems used.

Enzyme	**E.C. Number**	**Locus**	**Buffer system**[*]
Aspartate aminotransferase	2.6.1.1	Aat-1,2	TBE8.7
Esterase	3.1.1-	Est	TC8
Glucose-6-phosphate isomerase	5.3.1.9	Gpi	TC8
3-Hydroxybutyrate dehydrogenase	1.1.1.30	Hbdh	TBE8.7
Peptidase (leucyl-glycyl-glycine)	3.4.11.-	Lgg-2,3,4	LioH
Malate dehydrogenase	1.1.1.37	Mdh-1,2	CAPM6
Isocitrate dehydrogenase	1.1.1.42	Idh-1,2	CAPM6
Mannose-6-phosphate isomerase	5.3.1.8	Mpi	CAPM6
Malate dehydrogenase (NADP+)	1.1.1.40	Mdhp	CAPM6
Phosphoglucomutase	2.7.5.1	Pgm	TC8
Phosphogluconate dehydrogenase	1.1.1.44	Pgd	CAPM6
Superoxide dismutase	1.15.1.1	Sod	CAPM6

[*] Buffer systems: CAPM6, Citrate-aminopropylmorpholine, pH 6.0 (Clayton and Tretiak, 1972); LioH, lithium hydroxide-boric acid, pH 8.1 (Ridgway *et al*., 1970); TC8, tris-citrate, pH 8.0 (Clayton and Tretiak, 1972); TBE8.7, tris-borate-EDTA, pH 8.7 (Boyer *et al*., 1963).

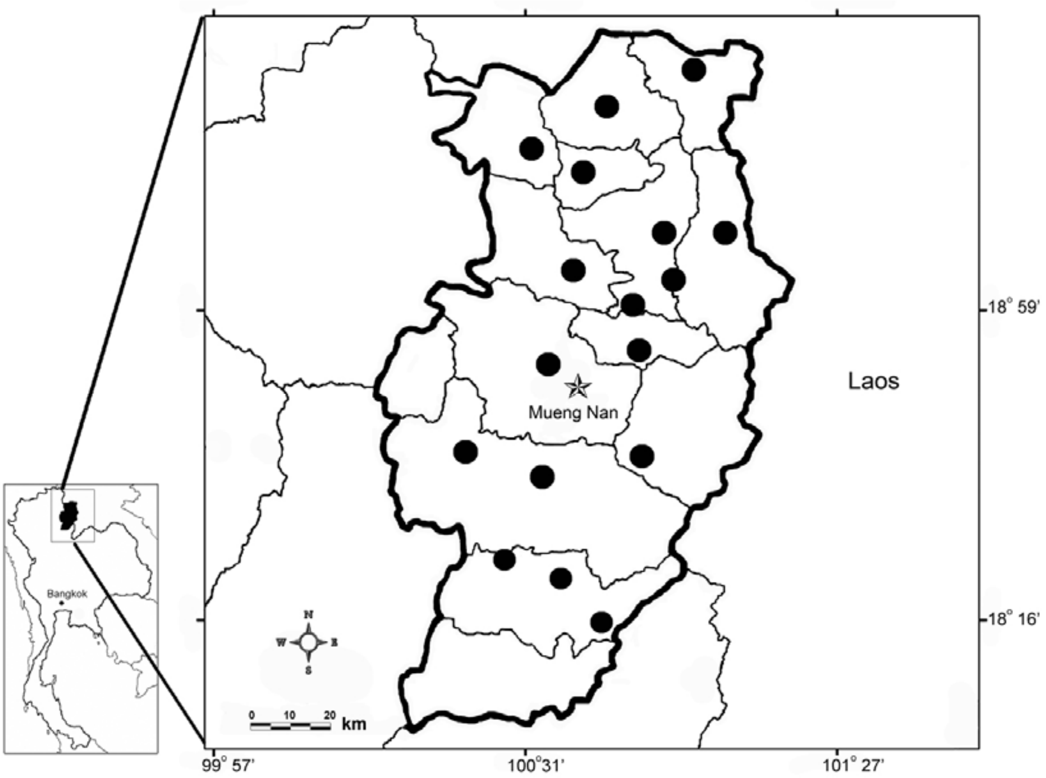

Figure 8. The map of Nan province, showing sampling localities (black circles).

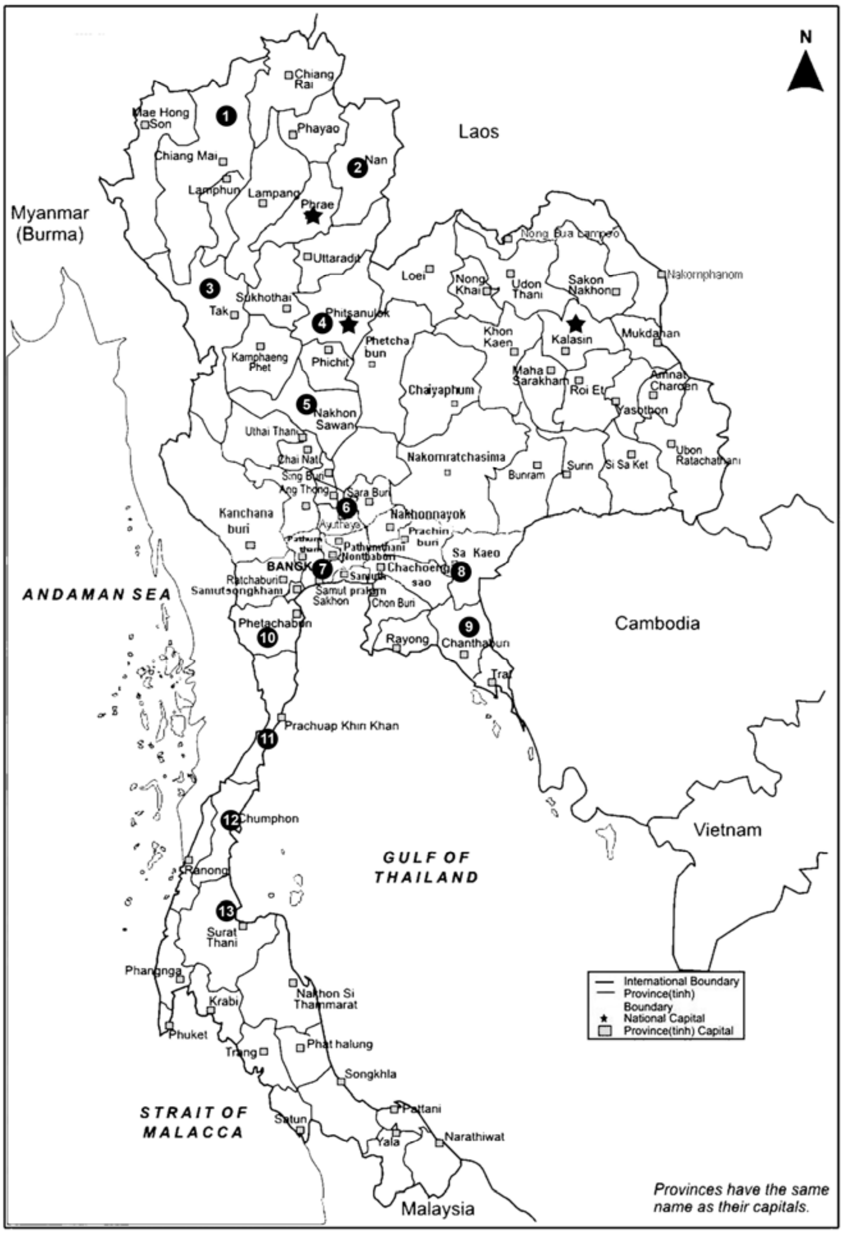

Figure 9. The map of Thailand, showing sampling localities of collected *Metaphire peguana* for allozyme electrophoresis (The locality numbers correspond to those in Table 3, and the black stars indicated outgroup).

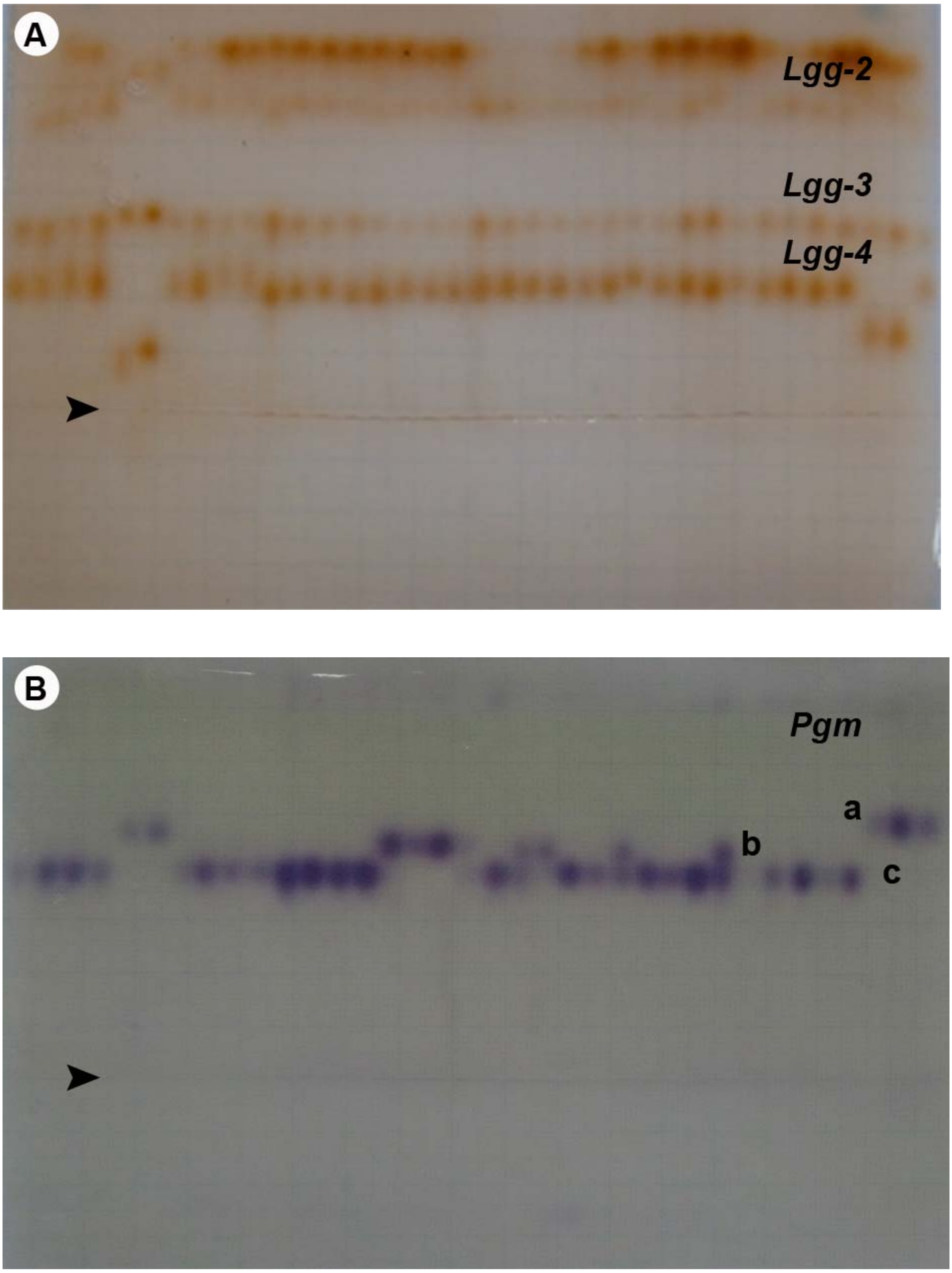

Figure 10. Allozyme electrophoresis running samples demonstrated the activity of enzymes (A) peptidase; luecyl-gylcyl-glycine, (B) phosphoglucomutase. Black arrow heads indicate the origin.

CHAPTER IV
RESULTS

4.1 Terrestrial earthworms in Nan province

Twenty species and a subspecies of earthworms are recorded from Nan province. These species can be classified into 4 genera: *Amynthas* Kinberg, 1867, *Metaphire* Sims and Easton, 1972, *Dichogaster* Beddard, 1888, and *Pontoscolex* Schmarda, 1861. Because of the great morphological similarity of the first two genera, they have been combined as the *Pheretima* group by male pores and prostatic ducts on segment xviii and spermathecal pores located on furrows 4/5 to 9/10, the racemose structure of the prostatic glands and the situation of the spermathecae anteriorly to the testes segment and present paired caeca (Sims and Easton, 1972). The *Pheretima* group (*Amynthas* and *Metaphire*), which is dominant for Nan localities, contains 18 species and 1 subspecies. *Amynthas* contains maximum diversity of 10 species and 1 subspecies, followed by the genus *Metaphire* with 8 species, while each genus of the rest contains only one species (Bantaowong *et al*., 2011a, b). A new record for Thailand, *Metaphire birmanica* (Rosa, 1888) has been reported (Bantaowong *et al*., 2011a). The exotic species *Pontoscolex corethrurus* (Müller, 1856) occurs almost everywhere in anthropogenic areas (Gates, 1972).

4.1.1 Classification of Nan earthworms

There are 20 species and 1 subspecies of terrestrial earthworms which containing 15 nominal species and six unidentied species of the genus *Amynthas* and *Metaphire* from Nan province. The classification was critically studied by using the following literatures; Reynolds and Cook (1993) for family, and to Gates (1972), Sims and Easton (1972) and Easton (1979) for generic levels, and Gates (1972); Bantaowong *et al*. (2011a, b) for species level.

Order Haplotaxida
  Suborder Lumbricina
    Superfamily Glossoscolecoidea
      Family Glossoscolecidae
        1. *Pontoscolex corethrurus* (Müller, 1856)

Superfamily Megascolecoidae

Family Megascolecidae

2. *Amynthas alexandri* Beddard, 1900
3. *Amynthas borealis* Panha and Bantaowong, 2011
4. *Amynthas exiguus exiguus* (Gates, 1930)
5. *Amynthas longicauliculatus* (Gates, 1931)
6. *Amynthas phatubensis* Panha and Bantaowong, 2011
7. *Amynthas srinan* Panha and Bantaowong, 2011
8. *Amynthas tontong* Panha and Bantaowong, 2011
9. *Amynthas* sp.1
10. *Amynthas* sp.2
11. *Amynthas* sp.3
12. *Amynthas* sp.4
13. *Metaphire anomala* (Michaelsen, 1907)
14. *Metaphire birmanica* (Rosa, 1888)
15. *Metaphire grandipenes* Bantaowong and Panha, 2011
16. *Metaphire houlleti* (Perrier, 1872)
17. *Metaphire peguana* (Rosa, 1890)
18. *Metaphire posthuma* (Vaillant, 1869)
19. *Metaphire* sp. 1
20. *Metaphire* sp. 2

Family Octochaetidae

21. *Dichogaster bolaui*

Tamonomy

Family Glossoscolecidae Michaelsen, 1900

Glossoscolecidae Michaelsen, 1900: 420.

Diagnosis: Male pores usually intraclitellar, usually in the anterior portion of the region; or in front of the clitellum, rarely behind it. Spermathecal pores rather present. Setae sigmoid, mostly single-pointed, rarely double-pronged, usually ornamented, with few exceptions 8 per segment. Dorsal pores absent. Clitellum usually beginning behind xiv. Usually one gizzard in vi, rarely several, in front of the testis segments;

often one rudimentary gizzard at the hinder end of the oesophagus, behind the ovarian segment. Calciferous gland present in segments vii-xiv. Ectal end of as vas deferens usually simple, rarely with prostatic glands. Penial setae absent; copulatory setae often present. Ovaries, in xiii.

Genus *Pontoscolex* Schmarda, 1861

Pontoscolex Schmarda, 1861: 11.

Diagnosis: Setae at the hinder end of the body usually arranged in quincunx. Male pores and copulatory papillae in the region of the clitellum. Digestive system, with three pairs of calciferous glands with paniculate tubular structure in vii-ix, a large laminar intestinal typhlosole but without caeca. Vascular system, with complete dorsal, ventral, and subneural trunks, the latter adherent to the parietes and the dorsal trunk markedly moniliform in several pre-intestinal segments, hearts of vi-ix lateral, of x, xi latero-esophageal. Nephridia, macroic, paired in intestinal segments, vesiculate, the transversely placed bladders elongately ocarina-shaped and opening to the exterior through short, thick walled and rather conical ducts from the ventral side, anteriorly bladder elongately sausage-shaped and opening to the exterior through terminal ducts. Nephropores, obvious. Pigment, none. Septa, all present at least from 5/6. Sexual apparatus metandric. Seminal vesicles very long, piercing the successive septa for long distance. Male pores in the clitellar region.

1. ***Pontoscolex corethrurus*** (Müller, 1856)

(Fig. 11)

Lumbricus corethrurus Müller, 1856: 26 (Type locality: Itajahy, Brazil).

Pontoscolex corethrurus—Stephenson, 1923: 488. Gates, 1930: 351. 1955: 92, 1972: 54. Bhattacharjee and Chaudhuri, 2002: 83.

Description: Body length 46-69 mm, width 3-5 mm, segment number 84-195. Prostomium absent. Setae, closely paired from iii, in the anterior part of the body closely paired, and exceptionally so throughout the body usually from about x or xii onwards the pairing is wider and in the hinder part of the body the setae are alternatively widely and closely paired with a quincunx arrangement (Fig. 1C). Setae in the hinder part of the body much enlarged, with straight distal end. Clitellum, saddle-shaped, xv or xvi –xxi or xxii (usually seven segments). Dorsal pores absent. Spermathecal pores three pair, minute and superficial, in 6/7-8/9. Live specimens without pigment, head portion pink, clitellum yellowish.

Septa, 5/6-10/11 thickened. Gizzard in vi. Intestinal origin in xiv or xv. Intestinal caecum absent. Typhlosole, beginning xxi. Calciferous glands 3 pairs in viii-x. Hearts greatly enlarged in x and xi. Metandric, passing posteriorly through 8-10 segments, seminal vesicle large in xii. No prostate glands. Ovaries small, in xiii. Spermathecae, 3 pairs in vii, viii and ix, very slenderly club-shape, no diverticulum.

Diagnosis: Clitellum saddle shaped, in xv or xvi – xxi or xxii (usually seven segments). Dorsal pore absent. Spermathecal pores three pair, in 6/7-8/9, minute and superficial. Setae, closely paired, quincunx arrangement. Calciferous glands 3 pairs in vii-ix. No intestinal caecum. Spermathecae, 3 pairs in vii, viii and ix, no diverticulum.

Distribution: Pua district, Santi Suk district.

Habitat: This species was found only in anthropogenic areas.

Family Megascolecidae Rosa, 1891

Megascolecidae Rosa, 1891: 379. Stephenson, 1923: 162. Gates, 1959: 130, 1972: 130.

Diagnosis: Setae simple or sigmoid, four pairs per segment or numerous, in latter case setal ring may be interrupted dorsally and ventrally. Clitellum beginning with or in front of xv. Male pores one pair, usually on xviii combined with prostatic pores. Female pores one pair or single on xiv. Dorsal pores, present. Oesophagus usually with one or several gizzards in front of testis segments. Two pairs testes and funnels in x and xi, or one pair in x or xi. One or two pairs of prostates, rarely more. One pair of ovaries in xiii. Spermathecae in front of gonadal segments.

Genus *Amynthas* Kinberg, 1867

Amynthas Kinberg, 1867: 97. Sims and Easton, 1972: 211.

Diagnosis: Setae numerous, more or less regularly arranged around each segment. Clitellum annular, xiv-xvi. Combind prostatic and male pores paired, discharging directly onto the surface of xviii. Female pore single, rarely paired, xiv. Spermathecal pores small or large, usually paired (bithecal) but occasionally numerous (polythecal) or single (monothecal) between 4/5 and 8/9. Gizzard between septa 7/8 and 9/10. Oesophageal pouches absent. Intestinal caeca present originating in xxvii. Testes holandric, metandric or prolandric. Prostatic glands racemose. Copulatory pouches absent. Ovaries paired in xiii. Spermathecae usually paired, rarely multiple or single. Meronephridial, nephridial layer rarely present on spermathecal ducts.

2. *Amynthas alexandri* Beddard, 1900

(Fig. 12)

Amynthas alexandri Beddard, 1900: 998 (Type locality: Calcutta).

Pheretima lignicola—Stephenson, 1914: 399 (Type locality: Dibrugarh), 1923: 305, 1925: 61.

Pheretima alexandri—Gates, 1925: 567, 1939: 80, 1955: 80, 1961: 62, 1972: 155.

Amynthas alexandri—Sims and Easton, 1972: 234.

Description: Dark dorsal pigment, almost black. Body length 111-187 mm, width 4-7 mm, segment number 87-134. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, annular, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segment; 22-26 at vii, 62-65 at xx, 9-17 between male pores.

Male pores paired in xviii, minute, each in small disc, 0.28 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 4 pairs at 5/6-8/9, minute, superficial, 0.33 circumference apart. Genital markings absent.

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened. Gizzard large within ix-x. Intestinal origin at xv. Intestinal caeca paired at xxvii extending forward to xxiii, simple with smooth margin. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles large, paired at xi, xii. Prostate glands racemose, paired in xviii, large expansion from xvii-xxii. Prostatic ducts fairly slim, U-shaped.

Ovaries paired at xiii. Spermathecae 4 pairs in vi, vii, viii, ix. Ampulla oval, rather large, duct stout, with a swollen basal portion. The diverticulum long, narrow, and twisted (Fig. 12C).

Diagnosis: Four pairs ventrolateral spermathecal pores at 5/6-8/9. Female pore medio-ventral at xiv. Male pores paired at xviii. No genital markings. Spermathecae, oval, diverticulum long, narrow, and twisted. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired at xvii-xxii.

Distribution: Tham Pha Tub Arboretum, Tadman waterfall, Sapan waterfall, Santi Suk district, Wiang Sa district, Thawang Pa district, Tung Chang district.

Habitat: This species can be found in both natural forest and anthropogenic areas. It is a South and South-East Asian peregrine species. We have observed it crawling on gravel roadsides and pavement in mid-day sun, without any apparent distress. This suggests it is very resistant to dryness and high temperatures.

3. *Amynthas borealis* Panha and Bantaowong, 2011

(Fig. 13)

Amynthas borealis P & B in Bantaowong *et al*., 2011: 35 (Type locality: Nan province).

Description: Body length 42-54 mm, width 2.7 mm, 77-89 segments. Prostomium epilobic. First dorsal pore at 5/6. Clitellum annular at xiv-xvi with no setae. Setae regularly distributed around segmental equators, numbering 40-54 at vii, 45-52 at xx, No setae between male pores.

Male pores pocket-like structures indistinctly occur in xviii, porophores small, lip-like and surrounded by an elevated skin fold at medial pores, and there is a long ridge with a sharp posterior boundary traversing the body in front of the male pores, 0.10 circumference apart ventrally. Female pore single, midventral at xiv. Spermathecal pores 1 pair at 7/8, each pore minute. Genital markings absent.

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent, 10/11–13/14 thin. Gizzard large within viii-x. Intestinal origin in xv. Intestinal cecae originated from xxvii extending forward to xxv, simple. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles are small, at xi, xii. Prostates glands racemose, paired in xvii-xx; prostatic ducts long slender bent in U-shape. Genital marking glands absent (Fig. 13B).

Ovaries at xiii. Spermathecae one pair in viii; ampulla large sac-shape, flattened by gizzard, narrow duct shorter than ampulla. Diverticulum with elongated tubular shape, stalk attached to duct near body wall (Fig. 13C).

Diagnosis: A pair of spermathecal pores at 7/8. Female pore midventral at xiv. Male pores, a transverse ridge anterior to the male pores in xviii, and no genital markings. Spermathecae consists of a large sac-shaped ampulla and elongated tubular shaped diverticulum.

Distribution. Chaloemprakiet District.

Habitat: This species found in the topsoil layer covered with leaf litter of deciduous limestone forest, mostly disturbed.

4. *Amynthas exiguus exiguus* (Gates, 1930)

(Fig. 14)

Pheretima exiguus exiguus Gates, 1930: 310 (Type locality: Lashio). Gates, 1932: 513, 1939b: 89, 1972: 184.

Amynthas exiguus exiguus—Sims and Easton, 1972: 234.

Description: Body length 54 mm, width 3 mm, segment number 69. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segment; 34 at vii, 38 at xx, 12 between male pores.

Male pores discs are transversely oval, paired in xviii. Female pore single, midventral location at xiv. Spermathecal pores 4 pairs at 5/6-8/9 each pore a minute opening on a tiny. Genital markings are small, always presetal, median pair on viii. Genital markings on xix, the paired lateral markings are separated from midventral making by a distance about equal to 6 intersetal intervals (Fig. 14A).

Septa, 6/7-7/8 slightly muscular, 8/9-9/10 aborted. The gizzard is in ix-x. Intestinal origin at xv. Intestinal caeca paired at xxvii extending forward to xxv, simple with smooth margin. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles are small, at xi, xii. No prostate glands, but ducts are present (Fig. 14B).

Ovaries paired at xiii. Spermathecae, duct about equal to ampulla, narrowed in parietes ampulla not much larger than duct, diverticulum long unlooped stalk, narrow seminal chamber.

Diagnosis: Four pairs ventrolateral spermathecal pores at 5/6-8/9. Female pore midventral at xiv. Male pores paired at xviii. Genital markings on viii and xix. Spermathecae narrowed, diverticulum long, unlooped. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. No prostates glands.

Distribution: Tadman waterfall.

Habitat: This species was found in the top soil layer of natural forest.

5. *Amynthas longicauliculatus* (Gates, 1931)

(Fig. 15)

Pheretima longicauliculata Gates, 1931: 395 (Type locality: Tolo Senca). Gates, 1936: 423, 1939b: 95, 1972: 158.

Amynthas longicauliculatus—Sims and Easton, 1972: 235.

Description: Body length 208-342 mm, width 7 mm, segment number 136-137. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, annular, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segments; 89 at vii, 96 at xx, 18-24 between male pores.

Male pores paired in xviii, minute, each in small disc, 0.33 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 4 pairs at 5/6-8/9, each pore a minute opening on a tiny, whitish area in the intersegmental furrow, 0.33 circumference apart. Genital markings two or three pairs, transversely oval, slightly raised area, one pair on each of 18/19, 19/20 and 20/21 (Fig. 15A).

Septa, 5/6-7/8, 10/11-11/12 thickened, 8/9-9/10 are lacking. The gizzard is elongate, narrowed anterior, posterior and enlarged and flange-shape within ix-x. Intestinal origin at xv. Intestinal caeca paired at xxvii extending forward to xxiv, simple with smooth margin. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles large, paired at xi, xii. Prostate glands racemose, small confined in xviii. Prostatic ducts fairly slim. U shape (Fig. 15B).

Ovaries paired at xiii. Spermathecae 4 pairs in vi, vii, viii, ix. Ampulla roughly spherical, duct short and stout. The diverticulum much longer than the length of duct and ampulla (Fig. 15C). Genital marking glands, sessile, at 18/19, 19/20 and 20/21.

Diagnosis: Four pairs ventrolateral spermathecal pores at 5/6-8/9. Female pore medventral at xiv. Male pores paired at xviii. Genital markings three pairs intersegment 18/19-20/21. Spermathecae spherical, diverticulum long, narrow, and zigzag. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands small, confined in xviii.

Distribution: Silapet waterfall, Tham Phatub Arboretum, Srinan Nation park.
Habitat: This species was found in the natural forest only.

6. *Amynthas phatubensis* Panha and Bantaowong, 2011

(Fig. 16)

Amynthas phatubensis P & B in Bantaowong *et al*., 2011b: 35 (Type locality: Nan province).

Description: Body length 80-148 mm, width 4.3 mm, 85-114 segments. Prostomium epilobic. First dorsal pore at 5/6. Clitellum annular at xiv-xvi with no setae. Setae regularly distributed around segmental equators, numbering 51-64 at vii, 60-67 at xx, 9-15 between male pores.

Male pores, porophores, papilla-like structures in xviii, 0.33 circumference apart. Each male pores surrounded by six flat, circular genital markings almost the same diameter as male pores. Female pore single, midventral at xiv. Spermathecal pores 1 pair at 7/8, each pore minute. Genital markings, rounded, flat, located close to spermathecal pores and male pores (Fig. 16A).

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent, 10/11–13/14 thin. Gizzard large within viii-x. Intestinal origin in XV. Intestinal caeca, simple, originate from xxvii extending forward to xxiii. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles are small, at xi, xii. Prostates gland racemose, paired in xvii-xx; prostatic ducts U-shape (Fig. 16B).

Ovaries in xiii. Spermathecae one pair in viii; ampulla large ovate sac, duct stout, short; long stalked diverticulum, convoluted kinks enclosed within membrane, spherical knob terminal. A large sessile genital marking gland in vii and viii.

Diagnosis: A pair of spermathecal pores at 7/8. Female pore midventral at xiv. Male pores surrounded by six genital papillae on segment xviii. Genital marking nearly the spermathecal pores, and the spermathecal characters of the large ovate ampulla, stalked diverticulum whose folds are membrane-bound, and spherical knob terminal diverticulum sac.

Distribution. Tham Phatub Arboretum and Tontong waterfall.

Habitat: This species found in the topsoil layer covered with leaf litter of natural forests.

7. *Amynthas srinan* Panha and Bantaowong, 2011

(Fig. 17)

Amynthas srinan P & B in Bantaowong *et al.*, 2011b: 35 (Type locality: Nan province).

Description: Body length 35-47 mm, width 2.3 mm, 70-78 segments. Prostomium epilobic. First dorsal pore at 4/5 or 5/6. Clitellum annular at xiv-xvi with no setae. Setae regularly distributed around segmental equators, numbering 36-43 at vii, 40-47 at xx, No setae between male pores.

Male pores on circular porophores in xviii, 0.30 circumference apart ventrally. Female pore single, midventral at xiv. Spermathecal pores 1 pair at 7/8, each pore minute. Genital markings small, postsetal, closely paired near mid ventral of vii, viii, xvii and xviii (Fig. 17A).

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent, 10/11–13/14 thin. Gizzard globular within viii-x. Intestinal origin in xv. Intestinal cecae, simple, originated from xxvii extending forward to xxiii. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles are small, at xi, xii. Prostate glands racemose, paired in xvii-xx, prostatic ducts tightly folded twice. Genital marking glands paired in xvii and xviii corresponding to external genital papillae, each consisting of a stalk with terminal multi-lobed glandular part (Fig. 17B).

Ovaries at xiii. Spermathecae one pair in viii; ; ampulla oval to kidney-shaped, with stout duct shorter than ampulla. Diverticulum with oval bulb terminal, stalk attached to duct near body wall. Genital markings stalked, corresponding to external genital papillae; each gland small consisting of a stalk with terminal multi-lobed glandular part (Fig. 17C).

Diagnosis: A pair of spermathecal pores at 7/8. Female pore midventral at xiv. Male pores on distinct round porophores, genital markings paired near mid ventral of vii, viii, xvii and xviii; each with genital marking glands. Each spermathecae consists of a kidney-shaped ampulla and an oval shaped diverticulum.

Distribution. Srinan National park.

Habitat: This species found in the topsoil layer covered with leaf litter of deciduous forest.

8. *Amynthas tontong* Panha and Bantaowong, 2011

(Fig. 18)

Amynthas tontong P & B in Bantaowong *et al*., 2011b: 35 (Type locality: Nan province).

Description: Body length 39-53 mm, width 2.7 mm, 71-80 segments. Prostomium epilobic. First dorsal pore at 5/6. Clitellum annular at xiv-xvi with no setae. Setae regularly distributed around segmental equators, numbering 41-46 at vii, 52-55 at xx, No setae between male pores.

Male pores, round disc in xviii, 0.19 mm circumference apart ventrally. Female pore single, midventral at xiv. Spermathecal pores 1 pair at 7/8, each pore minute. Genital markings closely paired located medial to male pore level in intersegment 18/19 (Fig. 18A).

Septa 5/6 and 6/7 thick, 7/8 thin, 8/9 and 9/10 absent 10/11–13/14 thin. Gizzard large within viii-x. Intestinal origin in xv. Intestinal caeca originated from xxvii extending forward to xxv, simple. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles, at xi, xii. Prostates glands racemose, paired in xvii-xx; prostatic ducts long slender with U-shape (Fig. 18B).

Ovaries in xiii. Spermathecae one pair in viii; ampulla thumb shape, duct stout, shorter than ampulla. Diverticulum slender stalk with spherical knob terminal (Fig. 18C).

Diagnosis: A pair of spermathecal pores at 7/8. Female pore midventral at xiv. Male pores with a pair of genital markings in intersegment 18/19. Spermathecae consists of a thumb shaped ampulla and a spherical terminal knob shaped diverticulum. Genital marking glands absent.

Distribution. Tontong waterfall.

Habitat: This species found in the topsoil layer covered with leaf litter.

9. *Amynthas* sp. 1

(Fig. 19)

Description: Body length 65 mm, width 3.5 mm, segment number 76. Prostomium epilobous. First dorsal pore at 11/12. Clitellum annular at xiv-xvi, annular, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segments; 43 at vii, 47 at xx, 7 between male pores.

Male pores paired in xviii, minute, 0.10 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores two paired groups located on 5/6 and 6/7, each group consisting of 2 pores which are separated from each other by 2 or 3 setae, each pore a minute opening on a tiny. Genital markings absent (Fig. 19A).

Septa, 5/6-7/8, 10/11-11/12 thickened, 8/9-9/10 are lacking. The gizzard is within ix-x. Intestinal origin at xv. Three pairs of manicate intestinal caeca in xxvii, xxviii and xxix, largest in xxvii. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles large, paired in xi, xii. Prostate glands racemose in xvii-xxii. Prostatic ducts fairly slim. U shape (Fig. 19B).

Ovaries paired in xiii. Spermathecae, groups paired of two on each side, 4 per segment in vi, vii; each ampulla ellipsoidal, duct shorter than ampulla, diverticulum stalk coiled, ovate knob terminal chamber (Fig. 19C).

Diagnosis: Spermathecal pores two paired groups located on 5/6 and 6/7, each group consisting 2 pores. Female pore medio-ventral at xiv. Male pores paired at xviii. Genital markings absent. Spermathecae, ampulla ellipsoidal, duct shorter than ampulla, diverticulum stalk coiled ovate terminal knob. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands in xvii-xx.

Distribution. Tham Phatub Arboretum.

Habitat: This species found in the topsoil layer covered with leaf litter of limestone forest.

10. *Amynthas* sp. 2

(Fig. 20)

Description: Body length 65 mm, width 3 mm, segment number 79. Prostomium epilobous. First dorsal pore at 5/6. Clitellum annular at xiv-xvi. Setae regularly arranged around each segment, usually absent from the clitellar segments; 56 at vii, 72 at xx, 9 between male pores.

Male pores paired in xviii, minute. 0.33 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores minutes, two paired groups located on 5/6 and 6/7, first group consisting of 2 pores at 5/6 and another group consisting of 3 by 3 pores at 6/7, 0.33 circumference apart. Genital markings, larger than male pore on xvii (Fig. 20A).

Septa, 5/6-7/8, 10/11-11/12 thickened, 8/9-9/10 are lacking. The gizzard in ix-x. Intestinal origin at xv. Three pairs of manicate intestinal caeca, segments xxvii, xxviii and xxix. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles large, paired in xi, xii. Prostate glands racemose, in xvii-xxi. Prostatic ducts short and stout. U shape (Fig. 20B).

Ovaries paired at xiii. Spermathecae, groups paired of two on each side, 4 per segment in vi, vii; each ampulla ellipsoidal, duct shorter than ampulla, diverticulum stalk coiled and ovat knob terminal chamber (Fig. 20C). Genital marking glands, sessile, in segment xvii.

Diagnosis: Spermathecal pores two paired groups located at 5/6-6/7, each group consisting 2 pores. Female pore medio-ventral at xiv. Male pores paired at xviii. Genital markings large on xvii. Spermathecae, ampulla ellipsoidal, duct shorter than ampulla, diverticulum stalk coiled ovate terminal knob. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands in xvii-xxi. Genital marking glands sessile in xvii.

Distribution: Tham Phatub Arboretum.

Habitat: This species found in the topsoil layer covered with leaf litter.

Remarks: There are no other known *Amynthas* species with 4 spermathecae per segment in segments vi and vii, and 3 pairs of intestinal caeca. These are new to science.

11. *Amynthas* sp. 3

(Fig. 21)

Description: Body length >139 mm, width 3.7 mm, segment number >61. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segment; 48 at vii, 53 at xx, no setae between male pores.

Male pores paired in xviii, minute, each in small disc on thickened are lateral to a large plate of genital markings between the male pores. 0.33 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 3 pairs at 5/6-7/8, each pore a minute opening on a tiny (Fig. 21A).

Septa, 5/6-7/8, 10/11-11/12 thickened, 8/9-9/10 are lacking. The gizzard in ix-x. Intestinal origin at xv. Intestinal caeca, manicate at xxvii-xxv. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles large, paired at xi, xii. Prostate glands racemose, in xvii-xxi. Genital marking glands large sessile mass located between prostatic ducts (Fig. 21B).

Ovaries paired at xiii. Spermathecae 3 pairs in vi, vii, viii. Ampulla rather large ovate, duct shorter than ampulla. The diverticulum long zigzag and smooth knob terminal (Fig. 21C).

Diagnosis: Three pairs ventrolateral spermathecal pores at 5/6-7/8. Female pore medio-ventral at xiv. Male pores paired at xviii. Genital markings large between male pores. Spermathecae ovate, diverticulum long zigzag stalk and smooth terminal knob. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands in xvii-xx.

Distribution: Tontong waterfall.
Habitat: This species was found in the natural forest.

12. *Amynthas* sp. 4

(Fig. 22)

Description: Body length 88-93 mm, width 4 mm, number of segment 104-114. Prostomium epilobous. First dorsal pore 12/13. Clitellum xiv-xvi, annular, without setae. Setae arrange regular, 52-55 in vii, 56-62 in xx. No setae between male pors.

Male pores ventrally on xviii, each pore minute, on a quadrilateral pad wider laterally, surrounded by a thickened ring encroaching on 17/18, 18/19, position of the male pores about 0.33 circumference apart. Female pore single, on xiv midventrally. Spermathecal pores minute, two paired groups of 2 located on 5/6 and 6/7 (Fig. 22A).

Septum 5/6-7/8 thickened, 8/9-9/10 absent, 10/11-12/13 thickened. Gizzard in vii-x, moderate sized, barrel-shaped and anteriorly slight narrower. Intestinal beginning in xv. Intestinal caeca, manicate, originating in xxvii, extending as far anteriorly as about xxiv; second smaller pair of manicate caeca in xxviii. Last heart in xiii. Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Prostate glands racemose, paired in xviii, extending from xvii-xxiii, ducts stout, with a V-shaped loop, its passing into a flattened indistinct glandular mass (from the male pore disc) (Fig. 22B).

Ovaries paired in xiii. Spermathecae, group and transversely arranged in two by two; each ampulla somewhat ellipsoidal, duct shorter than ampulla, diverticulum stalk kinked, slender; multi-lobed terminal chamber (Fig. 22C).

Diagnosis: Two pairs spermathecal pores at 5/6-6/7, two grouped pairs, minute. Female pore medio-ventral at xiv. Male pores paired at xviii, each pore minute, on a quadrilateral pad wider laterally, surrounded by a thickened ring encroaching on 17/18, 18/19. Spermathecae, group and transversely arranged in two by two; each ampulla ellipsoidal. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired in xvii-xxii. Intestinal caeca manicate.

Distribution: Doi Phuka National Park.

Habitat: This species found in the topsoil layer covered with leaf litter of natural forest.

Genus *Metaphire* Sims and Easton, 1972

Perichaeta Beddard, 1895: 388.
Pheretima—Michaelsen, 1900: 234, 1934: 15.
Metaphire—Sims and Easton, 1972: 215.

Diagnosis: Setae numerous, regularly arranged around each segment. Clitellum annular, xiv-xvi. Male pores paired within copulatory pouches on xviii, rarely xix or xx. Female pores single, rarely paired. Spermathecal pores usually large transverse slits, seldom small, paired, occasionally single or multiple, between 4/5 and 9/10. Gizzard between septa 7/8 and 9/10. Oesophageal pouches absent. Intestinal caeca present, originating in or near xxvii. Testes holandric, rarely proandric or metandric. Prostatic glands racemose. Copulatory pouches present, often with stalked glands, secretory diverticula absent. Ovaries paired xiii. Spermathecae paired, rarely single or numerous. Meronephridial, nephridia absent from the spermathecal ducts.

13. *Metaphire anomala* (Michaelsen, 1907)

(Fig. 23)

Pheretima anomala Michaelsen, 1907: 167, fig. 14. (Type locality: Botanical Gardens, Sibpur, Calcutta). Stephenson, 1923: 294. Gates, 1926a: 151, 161, 1930: 307, 1931: 372, 1932: 387, 1933: 496, 1936: 396, 1939b: 82, 1972: 166.

Metaphire anomala—Sims and Easton, 1972: 239. Bantaowong *et al*., 2011a: 55.

Description: Body length 134-154 mm, diameter 4.6-6.6 mm at clitellum. Segments 116-126. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, smooth with no setae. Setae regularly arranged around each segment, usually present on ventral side of xvi; 84-96 at vii, 58-67 on at xx, 12-20 between male pores.

Male pores within paired copulatory pouches opening onto the surface through tumescent lips at xx, 0.33 circumference apart. Female pore single, midventral at xiv. Spermathecal pores 3 pairs at 5/6-7/8, slit-like; 0.30 circumference apart. Genital markings present at 17-19, or 17-23, or absent (Figs 23A, D, E).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened. Gizzard large within ix-x. Intestinal origin in xv. Intestinal caeca paired in xxvii extending forward to xxiii, simple. Last hearts in xiii. Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Prostate glands racemose, paired in xx, large extending from xvii-xxii. Prostatic ducts long, hairpin shaped (Fig. 23B).

Ovaries paired in xiii. Spermathecae 3 pairs in vi, vii, viii. Ampulla slender oval, duct shorter than ampulla. Diverticulum with slender multiply kinked stalk, coiled terminal chamber (Fig. 23C). Genital marking glands, sessile corresponding to external genital markings.

Diagnosis: Three pairs spermathecal pores at 5/6-7/8, slit-like. Female pore medio-ventral at xiv. Male pores paired at xx, copulatory pouch present. Spermathecal diverticulum with coiled terminal chamber. Genital markings at xvii-xix. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired in xvii-xxii.

Distribution: Tham Phatub Arboretum, Tadman waterfall, Chaloem Phra Kiat district, Santi Suk district, Wiang Sa district, Song Khwae district.

Habitat: This species can be found in both natural forest and anthropogenic areas.

Remarks: Individuals of a hermaphroditic morph (H morph after Gates (1955)) having a full hermaphroditic complement of reproductive organs usually have spermathecal pores at 5/6-7/8, and male pores at xx, with three pairs genital markings on xvii, xviii, xix, and spermathecae as well as prostate glands (seven specimens) (Figs 23A, B, C). R morphs are distinguished by absence of male terminalia as well as of all genital marking glands of xvii-xix. Spermathecal pores are occasionally found in intersegments 5/6-7/8, and with them usually spermathecae (nine specimens) (Fig. 23D). Reproduction in this morph must often be uniparental (Gates, 1955). An A morph (Gates, 1955) is distinguished from the H by the presence of three pairs of genital markings and GM glands behind xx and by absence of six spermathecae (Fig. 23E). Male terminalia are present and apparently normal (prostates large) but seminal vesicles usually are small (six specimens). Reproduction in this morph must be not only uniparental but also parthenogenetic (Gates, 1955).

14. *Metaphire birmanica* (Rosa, 1888)

(Fig. 24)

Perichaeta birmanica Rosa, 1888: 164 (Type locality: Bhamò (upper Irawaddi River)).

Pheretima birmanica—Stephensen, 1923: 295. Gates, 1926a: 152, 1930: 307, 1931: 372, 1932: 428, 1933: 510, 1972: 207.

Metaphire birmanica—Sims and Easton, 1972: 239. Bantaowong *et al.*, 2011a: 55.

Description: Body Length 85-210 mm, width 5.8-6.7 mm at clitellum. Segments 105-111. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular on xiv-xvi, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segments; 65-70 at vii, 63-69 at xx, 12-17 between male pores.

Male pores discharged into paired copulatory pouches opening onto the surface through tumescent lips at xviii, 0.30 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 3 pairs at 5/6-7/8, visible. Genital markings absent (Fig. 24A).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened. Gizzard large within ix-x. Intestinal origin in xv. Intestinal caeca paired in xxvii extending forward to xxv or xxiv, manicate, dorsalmost of 3-6 secondary caeca the longest. Last hearts in xiii. Male system holandric, paired testis sacs in x, xi. Small seminal vesicle paired in xi, xii. Prostate glands racemose, paired in xviii, large extending from xvii-xxi. Prostatic ducts U-shaped (Fig. 24B).

Ovaries paired at xiii. Spermathecae 3 pairs in vi, vii, viii. Ampulla large sac, duct short. Diverticulum stalk coiled, no terminal chamber (Fig. 24C).

Diagnosis: Three pairs ventrolateral spermathecal pores at 5/6-7/8. Female pore midventral at xiv. Male porophores paired at xviii, each in copulatory pouch, tumescent lips. Spermathecae irregular sac-shaped, diverticulum coiled, no terminal chamber. Genital markings absent. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired at xvii-xxi.

Distribution: Chaloem Phra Kiat district, Silapet waterfall.

Habitat: This worm is generally found on the hills or mountains, and lives in the deep clay substrates.

15. *Metaphire grandipenes* Bantaowong and Panha, 2011

(Fig. 25)

Metaphire grandipenes B & P in Bantaowong *et al.*, 2011a: 55 (Type locality: Nan province).

Description: Body Length of head fragment 40 mm, diameter 4.5 mm at clitellum. Number of segments 71. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, with no setae. Setae regularly arranged around each segment, except in x-xiii, and xvii- xxvii where they are ventrally scattered; 81 at vii, 86 on at xx, 42 between male pores.

Male pores paired, on large protruding alate porophores on segment xviii, 0.5 circumference apart, distance between male pores 5 mm. Female pore single, midventral xiv. Spermathecal pores 3 pairs at 5/6-7/8, slit-like, 0.4 circumference apart, distance between spermathecal pores 4.5 mm. Genital marking very large, oval sucker-like, one pair on intersegmental boundary 19/20 (Fig. 25A).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened; 10/11-12/13 membranous. Gizzard large within ix-x. Intestinal origin in xv. Intestinal caeca paired at xxvii extending forward to xxiii, simple. Last heart in xiii. Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Pseudovesicles in xiii. Prostate glands racemose, paired xviii, large extending from xvii-xx. Prostatic ducts U-shaped (Fig. 25B).

Ovaries paired in xiii. Spermathecae 3 pairs in vi, vii, viii. Ampulla ovate, duct shorter than ampulla. Diverticulum with slender straight stalk, terminal chamber with five zig-zags pattern (Fig. 25C). Genital marking glands in xix-xx very large, sessile corresponding to each external genital marking.

Diagnosis: Three pairs spermathecal pores at 5/6-7/8, slit-like. Female pore mid-ventral on xiv. Male porophores paired, extremely large, alate on xviii. Spermathecae large ovate ampulla, diverticulum with straight stalk and long zig-zag terminal chamber. Genital markings extremely large, two sucker-like rings at 19/20, each with sessile genital marking glands.

Distribution: Community forest of Ban Bun Rueang, Lainan subdistrict, Wiang Sa district.

Habitat: Found in the top soil at about 10 cm depth, the soil surface was covered with leaf litter in a deciduous forest. Most surrounding areas have been modified to agricultural fields.

16. *Metaphire houlleti* (Perrier, 1872)

(Fig. 26)

Perichaeta houlleti Perrier, 1872: 99, pl. 2, figs 37-39, 41-44; pl. 3, figs 45-47, 50-55, 60-62. (Type locality: Calcutta).

Perichaeta campanulata—Rosa, 1890:115 (Type locality: Palon).

Amyntas houlleti—Beddard, 1900: 613.

Pheretima houlleti—Stephenson, 1923: 304. Gates, 1958: 16, 1972: 190.

Pheretima houlleti var. *tortuosa*—Gates, 1926a: 157. Type locality: Rangoon, 1926b: 454.

Pheretima campanulata—Gates, 1930: 307, 1931: 373.

Metaphire houlleti—Sims and Easton, 1972: 238, Shen, 2005: 13, fig. 2, Bantaowong *et al*., 2011: 55.

Description: Body length 82-193 mm, diameter 3.3-5.4 mm. Segments 89-138. Prostomium epilobic. First dorsal pore at 11/12 or 12/13. Clitellum annular at xiv-xvi, annular, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segment; 35-48 at vii, 48-60 at xx, 8-17 between male pores.

Male pores discharged into paired copulatory pouches opening onto the surface through tumescent lips at xviii, 0.30 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 3 pairs at 6/7-8/9, slit-like, 0.33 circumference apart. Genital markings absent (Fig. 26A).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened. Gizzard large within ix-x. Intestinal origin at xv. Intestinal caeca paired at xxvii extending forward to xxv, simple with smooth margin. Last hearts in xiii. Male sexual system holandric, paired testis sacs in x, xi. Seminal vesicles small, paired at xi, xii. Prostate glands racemose, paired in xviii, large expansion from xvii-xxi. Prostatic ducts fairly slim, U-shaped (Fig. 26B).

Ovaries paired at xiii. Spermathecae 3 pairs in vii, viii, ix. Ampulla oval, large, duct stout, with a swollen basal portion. Diverticulum originates below swelling, enlarged and greatly coiled toward distal end. Genital marking glands, long stalked, with a round or slightly lobed head, near the spermathecae (Fig. 26C).

Diagnosis: Three pairs ventrolateral slit-like spermathecal pores at 6/7-8/9. Female pore midventral at xiv. Male porophores paired at xviii, each in copulatory pouch with C-shaped opening (slit), tumescent lips. Spermathecal diverticulum enlarged and greatly coiled toward distal end. Genital marking glands stalked. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired in xvii-xxi.

Distribution: Sapan waterfall, Silapet waterfall, Tham Phatub Arboretum, Srinan National Park, Chiang Klang district, Sunti Suk district, Wiang Sa district, Song Khwae district. Tha Wang Pha district, Bo Kluea district.

Habitat: This worm was found in rotten leaves in anthropogenic areas as well as in jungle.

17. *Metaphire peguana* (Rosa, 1890)

(Fig. 27)

Perichaeta peguana Rosa, 1890: 113 (Type locality: Rangoon).

Amynthas peguana—Beddard, 1900: 628.

Pheretima peguana—Stephenson, 1923: 308. Gates, 1926a: 152, 1929: 14, 1930: 318, 1931: 404, 1932: 481, 1933: 540, 1936: 444, 1937: 327, 1939b: 102, 1972: 207.

Metaphire peguana—Sims and Easton, 1972: 239. Bantaowong *et al.*, 2011a: 55.

Desription: Body Length 95-220 mm, width 5-7 mm. Segments 77-118. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular at xiv-xvi, smooth with no setae. Setae regularly arranged around each segment, usually absent from the clitellar segments; 42-52 at vii, 54-66 at xx, 8-13 between male pores.

Male pores paired at xviii, ventro-lateral, each in circular porophore, invaginated within transversely slit-like structure, 0.30 circumference apart. Female pore single, midventral at xiv. Spermathecal pores 3 pairs at 6/7-8/9, minute, superficial, 0.28 circumference apart. Genital markings two pairs at 17/18 and 18/19 (Fig. 27A).

Septa, 8/9-10/11 aborted; 5/6-7/8, 11/12-12/13 thickened. Gizzard large within ix-x. Intestinal origin in xv. Intestinal caeca paired at xxvii extending forward to xxiii, simple with smooth margin. Last hearts xiii. Male system holandric, ventral testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Prostate glands racemose, paired in xviii, large extending from xvii-xx. Prostatic ducts short (Fig. 27B).

Ovaries paired at xiii. Spermathecae 3 pairs in vii, viii, ix. Ampulla oval, large, duct narrow. Diverticulum with slender stalk, tightly folded, leading to oval terminal chamber (Fig. 27C). Genital marking glands, nearly spheroidal, slightly protuberant at 17/18 and 18/19.

Diagnosis: Three pairs ventrolateral spermathecal pores at 6/7-8/9. Male pores within copulatory pouches at xviii. Two large genital markings paired at 17/18 and 18/19. Intestinal caeca simple. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands large, paired in xvii-xx. Spermathecae oval and diverticulum with slender stalk, tightly folded, leading to oval terminal chamber.

Distribution: Silapet waterfall, Maejarim National Park, Tadman waterfall, Mueang Nan, Chiang Klang district, Santi Suk district, Wiang Sa district, Song Khwae district, Tha Wang Pha district, Bo Kluea district, Chaloem Phra Kiat district, Thung Chang district.

Habitat: This worm was found widely throughout Nan Province in dipterocarp forest, deciduous forest, and anthropogenic areas, even in wastewater saturated soil from households.

18. *Metaphire posthuma* (Vaillant, 1869)

(Fig. 28)

Perichaeta posthuma Vaillant, 1868: 228, pl. 1, figs 1-8. Type locality: Java.

Pheretima posthuma—Stephenson, 1923: 309; Gates, 1930: 321, 1931: 405, 1932: 487, 1936: 391, 1937: 363, 1939: 104, 1972: 212.

Metaphire posthuma—Sims and Easton, 1972: 217. Bantaowong *et al.*, 2011: 55.

Description: Body length 64-126 mm, diameter 3-6 mm. Segments 90-126. Prostomium epilobous. First dorsal pore at 12/13. Clitellum annular xiv-xvi, setae present. Setae regularly arranged around each segment, retained at ventral of clitellum; 85-99 on vii, 58-67 on xx, 17-20 between male pores.

Male pores paired in xviii, minute, each in small disc, 0.30 circumference apart. Female pore single, midventral location at xiv. Spermathecal pores 4 pairs at 5/6-8/9, minute, superficial, 0.33 circumference apart. Genital markings paired intrasegmental at xvii, xix (Fig. 28A).

Septa, 5/6-8/9 thick, 9/10 aborted. Gizzard large within ix-x. Intestine origin at xv. Intestinal caeca paired at xxvii extending forward to xxv, simple with smooth margin. Last hearts at xiii. Male sexual system holandric, testis sacs paired and ventral at x, xi. Seminal vesicles paired, rather small at xi, xii. Prostate glands racemose, paired in xviii, two main branches extending from xvii-xx. Prostatic ducts each a U-shaped loop (Fig. 28B).

Ovaries paired at xiii. Spermathecae four pairs in vi-ix. Ampulla small oval, duct stout. Diverticulum with short stalk, convoluted terminal section (Fig. 28C). Genital marking glands, nearly spheroidal, sessile at xvii and xix.

Diagnosis: Spermathecal pores minute and superficial, four pairs in 5/6-8/9. Male pores minute in xviii, each in a small disc. Genital markings two rather large pairs in xvii and xix. Intestinal caeca simple in xxvii-xxv. Testis sacs paired in x and xi. Seminal vesicles paired in xi and xii. Prostate glands paired in xvii-xx, each has two main branches.

Distribution: Pua district, Santi Suk district, Thung Chang district, Bo Kluea district.
Habitat: This species is generally found near anthropogenic habitats, for example paddy fields, ridges of paddy fields, and in wastewater saturated soil from households. These worms are very active, and twist away when touched.

19. *Metaphire* sp. 1

(Fig. 29)

Description: Body length 61-65 mm, width 3.7 mm, 96-113 segments. Prostomium epilobous, First dorsal pore 12/13. Clitellum xiv-xvi, annular, setae present. Setae regularly arranged around each segment, 2 setae retained ventrally in xiv, 4 in xv, 4 in xvi; 49-51 on vii, 52-56 on xx, 4 between male pores; these 4 more widely spaced than ventral setae of 17, 19.

Male pores superficial on small poropores surrounded by an epidermal ring separated from the poropore by a shallow circular trench on xviii. Glandular widely U-shaped ridge open anteriorly, medial and posterior to male pores, and spanning a greater width than the 4 setae between the male pores. Male pores. 0.30 circumference apart. Female pore single, midventral at xiv. Spermathecal pores 3 pairs at 6/7-8/9, slit-like (Fig. 29A).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened, 10/11-12/13 membranous. Gizzard within ix-x. Intestinal origin in xv. Intestinal caeca, simple at xxvii-xxv. Last heart in xiii. Male system holandric, paired testis sacs in x, xi. Seminal vesicle in xi, xii. Prostate glands racemose, paired xvii-xxii, duct U-shape passing into a flattened sessile multi-glandular mass (Fig. 29B).

Ovaries paired in xiii. Spermathecae 3 pairs in vii, viii and ix. Ampulla ovate, duct shorter than ampulla, diverticulum long, no marked widening at its terminal (Fig. 29C).

Diagnosis: Three pairs spermathecal pores at 6/7-8/9, slit-like. Female pore mid-ventral on xiv. Male pores superficial on small poropores surrounded by an epidermal ring separated from the poropore by a shallow circular trench on xviii. Spermathecae ovate ampulla, diverticulum, long, no marked widening at its terminal. Large sessile glandular mass in xviii.

Distribution: Tham Phatub Arboretum.

Habitat: They can be found in the top soil layer covered with leaf litter in limestone forest.

20. *Metaphire* sp. 2

(Fig. 30)

Description: Body length 164 mm, width 6.5 mm, 118 segments. Prostomium epilobous, First dorsal pore 12/13. Clitellum xiv-xvi, annular. Setae regularly arranged around each segment; 43 on vii, 46 on xx, 11 between male pores.

Male pores within paired copulatory pouches opening onto the surface through tumescent lips at xviii. 0.33 circumference apart. Female pore single, mid-ventral at xiv. Spermathecal pores 3 pairs at 6/7-8/9, slit-like; Genital markings absent (Fig. 30A).

Septa, 8/9-9/10 aborted, 5/6-7/8 thickened, 10/11-12/13 membranous. Gizzard within ix-x. Intestinal origin in xv. Intestinal caeca, simple at xxvii-xxiv. Last heart in xiii. Male system holandric, paired testis sacs in x, xi. Seminal vesicle in xi, xii. Prostate glands racemose, paired xvii-xxii (Fig. 30B).

Ovaries paired in xiii. Spermathecae 3 pairs in vii, viii and ix. Ampulla ovate, largest in ix, duct shorter than ampulla, diverticulum long and rather tightly folded (Fig. 30C).

Diagnosis: Three pairs spermathecal pores at 6/7-8/9. Female pore midventral on xiv. Male pores paired within copulatory pouch opening onto the surface at xviii. Spermathecae ovate, the largest in ix, duct shorter ampulla, diverticulum rather tightly folded.

Distribution: Huay Samsop conservation Unit.

Habitat: They can be found in the top soil layer covered with leaf litter.

Family Octochaetidae

Genus *Dichogaster* Beddard, 1888

Dichigaster Beddard, 1888: 251, 1895: 476. Michaelsen, 1900: 334.

Diagnosis: Lumbricine. Male pores paired in seminal grooves, on xviii or 17/18. Prostatic pores 2 pairs at the end of seminal grooves, on xvii and xix, or one pair on xvii. Oesophagus with two gizzards anterior to septum 8/9 and one pair of discrete extramural calciferous glands, each gland trilobed in segments xv-xvii. Micronephridia tufts in ii-iv; several on body wall.

Type species : *Dichogaster damonis* Beddard, 1888

21. *Dichogaster bolaui* (Michaelsen, 1891)

(Fig. 31)

Benhamia bolaui Michaelsen, 1891: 9.

Dichogaster bolaui—Michaelsen, 1900: 340. Stephenson, 1923: 475. Righi *et al*., 1978: 38. Julka, 1988: 103.

Description: Body Length 32 mm, diameter 1 mm, 99 segments. Prostomium epilobous. First dorsal pore 5/6. Clitellum xiii-xxi, saddle-shape. Setae closely paired, 8 setae per segment. Male pores minute, in xvii, in seminal grooves, between prostatic pores in xvii and xix, seminal grooves slightly concave on segment xvii and xix. Female pore single, median, presetal. Spermathecal pores rarely to see (Fig. 31A).

Septa missing or reduced anteriorly. Gizzards two in vi and vii. Calciferous glands in xv-xvii. Last pair of hearts in xii. Testes and male funnels in unpaired sacs, in x and xi. Intestinal caeca absent. Seminal vesicles, vestigial, in xi and xii. Prostate glands paired in xvii and xix. Spermathecae two pairs in viii and ix (Fig. 31B). Ovaries in xiii.

Distribution: Srinan National Park.

Habitat: They can be found in the top soil layer covered with leaf litter.

Key to species of terrestrial earthworms reported from Nan province

1.	Setae 8 per segment	2
−	Setae numerous, more than 10 per segment	3
2.	Setae of a posterior part of the body in the “quincunx” arrangement	*P. corethrurus*
−	Caudal setae in regular rows	*D. bolaui*
3	Male pores, superficial	4
−	Male pores, invaginate	13
4	Spermathecal pores, four pairs	5
−	Spermathecal pores, less than four pairs	7
5	Genital marking, absent	*A. alexandri*
−	Genital markings, present	6
6	Genital markings, at xix	*A. exiguus exiguus*
−	Genital markings, at 18/19, 19/20 and 20/21	*A. longicauliculatus*
7	Spermathecal pores, three pairs	*Amynthas* sp. 3
−	Spermathecal pores, less than three pairs	8
8	Spermathecal pore, two pairs	9
−	Spermathecal pores, one pairs	11
9.	Spermathecal pores, two paired group and genital marking absent	10
−	Spermathecal pores, two paired group and genital marking present	*Amynthas* sp. 2
10.	No setae between male pores	*Amynthas* sp. 4
−	Seven setae between male pores	*Amynthas* sp. 1
11.	Genital markings, absent	*A. borealis*
−	Genital markings, present	11
12.	Genital marking glands, absent	*A. tontong*
−	Genital marking glands, sessile	*A. phatubensis*
−	Genital marking glands, stalked	*A. srinan*
13.	Spermathecal pores, four pairs	*M. posthuma*
−	Spermathecal pores, less than four pairs	14
14.	First spermathecal pores at 5/6	15
−	First spermathecal pores after 5/6	17

15.	Male pores at xx…………………………………………	*M. anomala*
−	Male pores at xviii……………………………………..	16
16	Genital markings, absent………………………………….	*M. birmanica*
−	Genital markings, present…………………………………	*M. grandipenes*
17	Genital markings, present at 17/18, 18/19………………..	*M. peguana*
−	Genital markings, absent………………………………….	18
18	Genital marking glands, absent…………………………...	*Metaphire* sp. 2
−	Genital marking glands, stalked at spermathecae…………	*M. houlleti*
−	Genital marking glands, sessile at prostatic duct………….	*Metaphire* sp. 1

4.2 The genetic variation of the *Metaphire peguana*

The twelve enzymes studied produced distinct and reliable zymograms for the statistical analysis. The zymograms for Pep-*lgg* were divided into four zones, of which only three were scored, namely *Pep*-*lgg*-2, *Pep*-*lgg*-3, and *Pep*-*lgg*-4. *Mdh*, *Aat* and *Idh* were divided into two zones designated *Mdh*-1, *Mdh*-2, *Aat*-1, *Aat*-2, *Idh*-1, *Idh*-2, respectively. Only one zone was seen in the zymograms for other enzymes.

17 allozyme loci of 12 enzyme systems were screened for *M. peguana*. Of these, 14 were polymorphic (Tables 5). Three loci, *Aat*-2, *Lgg*-3 and *Sod* were monomorphic, with all individuals from thirteen populations scored possessing a single enzyme band with an identical mobility band for each locus.

Genetic variability in *M. peguana* was quantified using standard measures of genetic variation in term of mean number of alleles per locus, level of polymorphism and heterozygosity. Genetic variability (Table 6) in *M. peguana* differed among populations. *Metaphire peguana* samples showed moderate *A* and *P* values (1.2-1.6 and 23.5 - 47.1, respectively). The observed (*H*obs) and expected heterozygosity (*H*exp) were moderate to high (0.049 - 0.119; mean 0.079 ± 0.019 and 0.059 - 0.147; mean 0.092 ± 0.022 respectively).

The *F*-statistics are presented in table 7. The mean *F*is value among all the *M. peguana* samples was low (0.107). In contrast, the mean *F*it value at all loci was relatively high (0.390). Moreover, the *F*it displayed positive values for all loci with the exception of locus *Pep*-*lgg*-2, *Pep*-*lgg*-4, *Idh*-1, *Idh*-2, *Mdh*-1 and *Gpi*. Likewise, the mean *F*st value for the whole *M. peguana* was relatively high (0.318) with the significant higher than zero for 11 polymorphic loci (Table 7).

Matrices of Nei’s (1978) distance (D) and Roger’s (1972) distances among sample are presented in Table 8. The D values obtained within the *M. peguana* samples were relatively small (D = 0.002 - 0.170, mean 0.048 ± 0.040). Small genetic distances (Nei’s D) were observed between eastern and southern Thailand samples (average 0.067 ± 0.058; range, 0.002 - 0.130),between central and eastern Thailand samples (average 0.063 ± 0.052; range, 0.008 - 0.121), between eastern and northern Thailand samples (average 0.056 ± 0.033; range, 0.009 - 0.101), between northern and central Thailand samples (average 0.054 ± 0.048; range, 0.008 - 0.170), between southern and northern Thailand samples (average 0.054 ± 0.041; range, 0.012 - 0.152), between southern and central Thailand samples (average 0.029 ± 0.022; range, 0.002 - 0.084). Likewise, small genetic distances (Nei’s) were obtained between samples within northern (average 0.036 ± 0.022; range 0.011 - 0.069), central (average 0.028 ± 0.016; range 0.010 - 0.042), southern (average 0.027 ± 0.022; range, 0.010 - 0.062) and eastern Thailand sample (0.098).

Genetic similarities (S) between the samples were calculated from allelic frequencies using Rogers' coefficient of genetic similarity (Rogers, 1972). The overall S among samples in *M. peguana* was relatively high (S = 0.785 - 0.967, mean 0.897 ± 0.043). High genetic similarities (Roger’s S) were observed between eastern and southern Thailand samples (average 0.882 ± 0.067; range, 0.801 - 0.967), between central and eastern Thailand samples (average 0.885 ± 0.052; range, 0.823 - 0.946), between eastern and northern Thailand samples (average 0.885 ± 0.037; range, 0.842 - 0.947), between northern and central Thailand samples (average 0.894 ± 0.050; range, 0.785 - 0.966), between southern and northern Thailand samples (average 0.889 ± 0.042; range, 0.793 - 0.928), between southern and central Thailand samples (average 0.918 ± 0.023; range, 0.864 - 0.949). Likewise, Large genetic similarities (Roger’s) were obtained between samples within northern (average 0.906 ± 0.021; range 0.879 - 0.933), central (average 0.927 ± 0.020; range 0.906 - 0.945), southern (average 0.922 ± 0.026; range, 0.886 - 0.948) and eastern Thailand sample (0.842).

Isolation by distance (IBD) was test for the entire 13 *Metaphire peguana* populations sample from the graph of *F*st/(1- *F*st) versus geographic distance. Mantel tests did not detect significant IBD pattern for the 13 populations across the entire 1480 km study area (*P*-value>0.05) (Fig. 32).

Nei’s genetic distance values were used to construct an UPGMA dendrogram (Fig. 33). There are six major clusters; one of which consisted of northern (Chiang Mai (CM) and Tak (TK)) populations; the second cluster consisted of Nan (NA), Nakhon Sawan (NS), Bangkok (BK), Sa Kaeo (SK), Chumphon (CP), Phetchaburi (PH) and Prachuab Khiri Khan (PC); the third consisted of Surat Thani (SR); the fourth cluster consisted of Ayutthaya (AY); the fifth and the last cluster consisted of Phitsanulok (PN) and Chanthaburi (CH), respectively. However, an UPGMA dendrogram did not show concordance with geographic area.

Table 5. Allele frequencies at polymorphic loci of *Metaphire peguana*. Locality numbers correspond to those in Fig. 9 except 14, 15 and 16 represents the outgroup species *Metaphire* sp., *M. posthuma* and *M. bahli* respectively. Notations of alleles are alphabetical in order of anodal mobilities.

Locus	Locality															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Aat-1																
a				0.031										0.208		0.075
b	0.975	1	0.950	0.969	1	1	1	1	1	0.696	0.929	1	1	0.417		0.925
c	0.025		0.050							0.304	0.071			0.375		
d															1	
Aat-2																
a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Est																
a															0.364	
b	0.575	0.520	0.950		0.883	1	0.925	0.717	0.050	0.964	0.976	0.778	0.333		0.455	0.175
c	0.425	0.480	0.050	1	0.117		0.075	0.283	0.950	0.036	0.024	0.222	0.667	0.333	0.182	0.825
d														0.667		
Gpi																
a																
b														0.731	1	0.825
c														0.269		0.175
d							0.050									
e	1	1	1	1	1	1	0.950	1	1	1	1	1	1			
Hbdh																
a															1	
b												0.125				
c	0.975	1	0.806	1	1	1	0.921	0.707	1	0.625	0.524	0.813	0.864	1		1
d	0.025		0.194				0.079	0.103		0.250	0.476	0.063	0.136			
e								0.190		0.125						

Table 5. cont.

Locus	Locality															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Me																
a														0.385		
b	0.725	1	0.900	1	0.900	0.833	0.775	0.952	0.219	0.786	0.881	0.944	0.955	0.615	1	
c	0.275		0.100		0.100	0.167	0.225	0.032	0.781	0.214	0.119	0.056	0.045			0.550
d								0.016								0.450
Mpi																
a	0.500	0.275	0.711	0.900	0.271	0.050	0.133	0.80	0.667	0.038	0.056					
b	0.500	0.725	0.289	0.100	0.729	0.950	0.867	0.920	0.333	0.962	0.944	1	1			
c															0.708	
d															0.292	0.050
e																0.500
f																0.450
g														0.077		
h														0.923		
Pep-lgg-2																
a														1	0.200	
b										0.018	0.024				0.800	
c	1	1	1	1	1	1	1	1	1	0.982	0.976	1	1			1
Pep-lgg-3																
a														1		
b	1	1	1	1	1	1	1	1	1	1	1	1	1		0.813	1
c															0.188	
Pep-lgg-4																
a		0.020		0.125												
b	1	0.980	1	0.875	1	1	1	1	1	1	1	1	1		0.069	0.575
c															0.933	0.425
d														0.962		
e														0.038		

Table 5. cont.

Locus	Locality															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Idh-1																
a							0.079									
b	1	1	1	1	1	1	0.921	1	1	1	1	1	1			0.975
c														1	1	0.025
Idh-2																
a			0.025		0.034	0.045										0.050
b	1	1	0.975	1	0.966	0.955	1	1	1	1	1	1	1	1	1	0.950
Mdh-1																
a				0.125												
b	1	1	1	0.875	1	1	1	1	0.938	1	1	1	1			
c									0.063							1
d														1		
e															1	
Mdh-2																
a	0.200			0.156											0.882	
b	0.800	1	1	0.844	1	1	1	1	1	1	1	1	1		0.118	1
c														1		
Pgm																
a																1
b	0.075				0.017	0.674	0.025	0.129	0.250	0.018	0.100	0.111	0.045	0.545	1	
c														0.455		
d	0.925	1	1	1	0.983	0.326	0.975	0.871	0.719	0.982	0.875	0.889	0.955			
e									0.031		0.025					
Pgd																
a																
b	0.075				0.017	0.674	0.025	0.129		0.296		0.444	0.682	0.545	1	0.500
c									1	0.685	0.921	0.556	0.273	0.455		0.900
d	0.925	1	1	1	0.983	0.326	0.975	0.871		0.019	0.079					0.050
Sod																
a	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1
b															1	

Table 6. Localities, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), observed heterozygosity (*H*obs), and expected heterozygosity (*H*exp) in sample of *M. peguana*. Standard error of *H*exp is indicated in parentheses.

Locality		***A***	***P***	***H*obs**	***H*exp**
Northern region	1. Doi Chiang Dao, Chiang Mai	1.5	47.1	0.119	0.147 (0.051)
	2. Tadman waterfall, Nan	1.2	23.5	0.059	0.075 (0.040)
	3. Bhumibol Dam, Tak	1.4	41.2	0.073	0.097 (0.039)
	4. Sakunotayan waterfall, Phitsanulok	1.4	35.3	0.089	0.087 (0.036)
Central region	5. Watkiriwong, Nakhon Sawan	1.4	35.3	0.055	0.068 (0.029)
	6. Bangban, Ayutthaya	1.3	29.4	0.049	0.059 (0.030)
	7. Chulalongkorn University, Bangkok	1.5	47.1	0.097	0.100 (0.036)
Eastern region	8. Khaomaka, Sa Kaeo	1.5	35.3	0.088	0.101 (0.040)
	9. Laemsing, Chanthaburi	1.4	29.4	0.089	0.087 (0.040)
Southern region	10. Thamrong, Phetchaburi	1.6	47.1	0.074	0.117 (0.046)
	11. Huai Yang waterfall, Phachuab Khiri Khan	1.5	47.1	0.092	0.085 (0.033)
	12. Somdejphrasrinakarin Park, Chumphon	1.4	29.4	0.081	0.091 (0.040)
	13. Khao Sok National park, Surat Thani	1.4	29.4	0.072	0.081 (0.039)

Table 7. Summary of *F*-statistics at all loci of *Metaphire peguana* from Thailand.

[a] Significant at the level of $p<0.05$.

Locus	*F*is	*F*it	*F*st
Mpi	0.027	0.450	0.435 [a]
Lgg-4	-0.124	-0.011	0.100 [a]
Lgg-2	-0.022	-0.030	0.018
Me	0.134	0.379	0.283 [a]
Aat-1	0.203	0.348	0.182 [a]
Est	0.100	0.558	0.509 [a]
Idh-1	-0.086	-0.060	0.073 [a]
Idh-2	-0.038	-0.008	0.029
Mdh-1	-0.116	-0.011	0.094 [a]
Mdh-2	0.122	0.260	0.158 [a]
Gpi	-0.053	-0.004	0.046
Pgd	0.191	0.356	0.204 [a]
Pgm	0.111	0.384	0.307 [a]
Hbdh	0.083	0.258	0.191 [a]
Mean	0.107	0.390	0.318

Table 8. Matrix of genetic distances among 13 samples across all loci of *Metaphire peguana* from Thailand. Below diagonal: Nei (1978) unbiased genetic distance. Above diagonal: Rogers (1972) genetic similarity.

Population	Locality												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Chiang Mai	-	0.933	0.923	0.895	0.922	0.867	0.922	0.912	0.875	0.889	0.873	0.920	0.905
2. Nan	0.011	-	0.915	0.888	0.966	0.897	0.920	0.947	0.872	0.900	0.902	0.933	0.928
3. Tak	0.016	0.028	-	0.879	0.941	0.880	0.928	0.916	0.847	0.921	0.911	0.923	0.889
4. Phitsanulok	0.038	0.052	0.069	-	0.854	0.785	0.847	0.842	0.866	0.804	0.793	0.851	0.878
5. Nakhon Sawan	0.016	0.008	0.017	0.090	-	0.931	0.945	0.946	0.858	0.925	0.932	0.941	0.904
6. Ayuttaya	0.059	0.049	0.067	0.170	0.031	-	0.906	0.913	0.839	0.899	0.922	0.908	0.864
7. Bangkok	0.018	0.021	0.023	0.104	0.010	0.042	-	0.931	0.823	0.936	0.915	0.949	0.924
8. Sa Kaeo	0.023	0.009	0.032	0.093	0.008	0.031	0.012	-	0.842	0.936	0.940	0.967	0.929
9. Chanthaburi	0.051	0.070	0.101	0.068	0.091	0.121	0.114	0.098	-	0.801	0.820	0.827	0.835
10.Phetchaburi	0.038	0.032	0.035	0.139	0.017	0.044	0.012	0.012	0.130	-	0.945	0.932	0.894
11.Prachuab Khiri Khan	0.049	0.033	0.040	0.152	0.018	0.034	0.026	0.013	0.130	0.010	-	0.929	0.886
12.Chumphon	0.022	0.012	0.034	0.099	0.010	0.033	0.002	0.002	0.115	0.011	0.019	-	0.948
13.Surat Thani	0.028	0.023	0.063	0.065	0.043	0.084	0.026	0.023	0.107	0.045	0.062	0.013	-

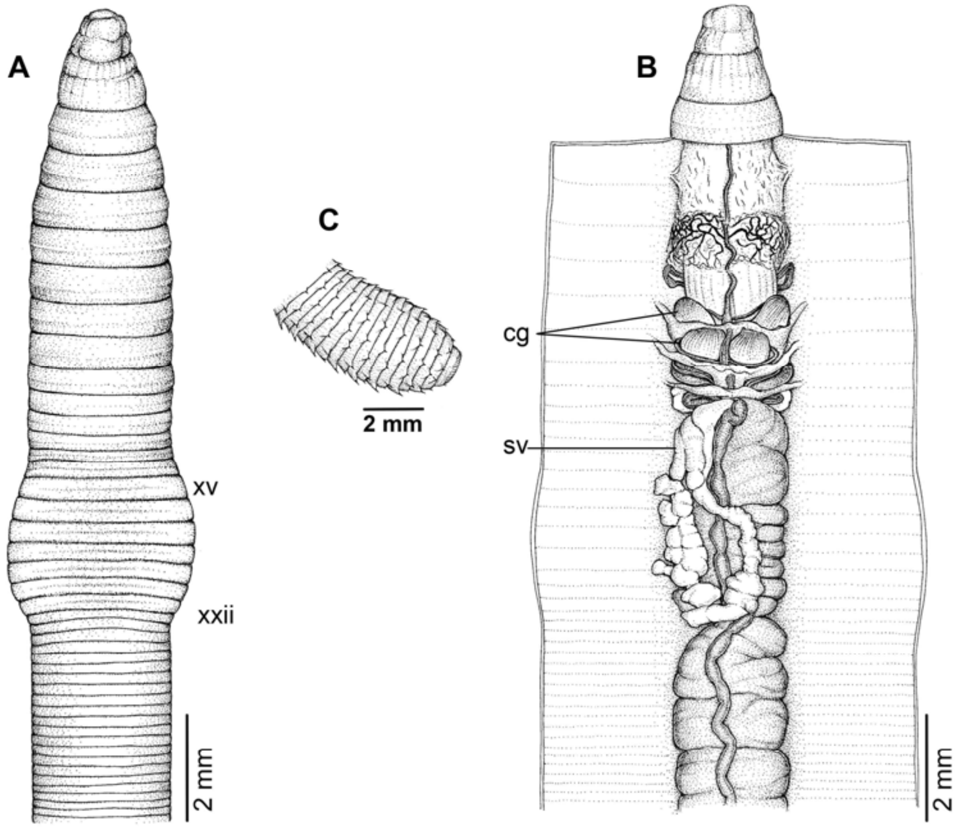

Figure 11. External and internal morphology of *Pontoscolex corethrurus* (A) External ventral view, (B) internal dorsal view and (C) Setae of a posterior part of the body of the “quincunx” arrangement.

Anatomical abbreviations: cg, calciferous gland; sv, seminal vesicles.

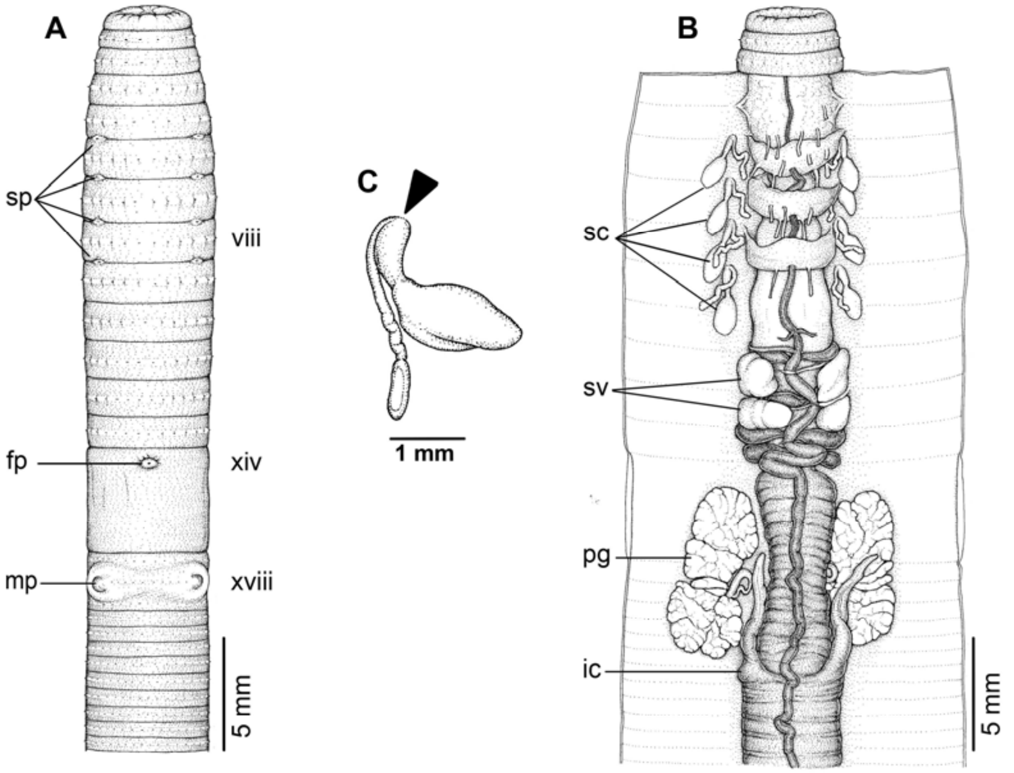

Figure 12. External and internal morphology of *Amynthas alexandri* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

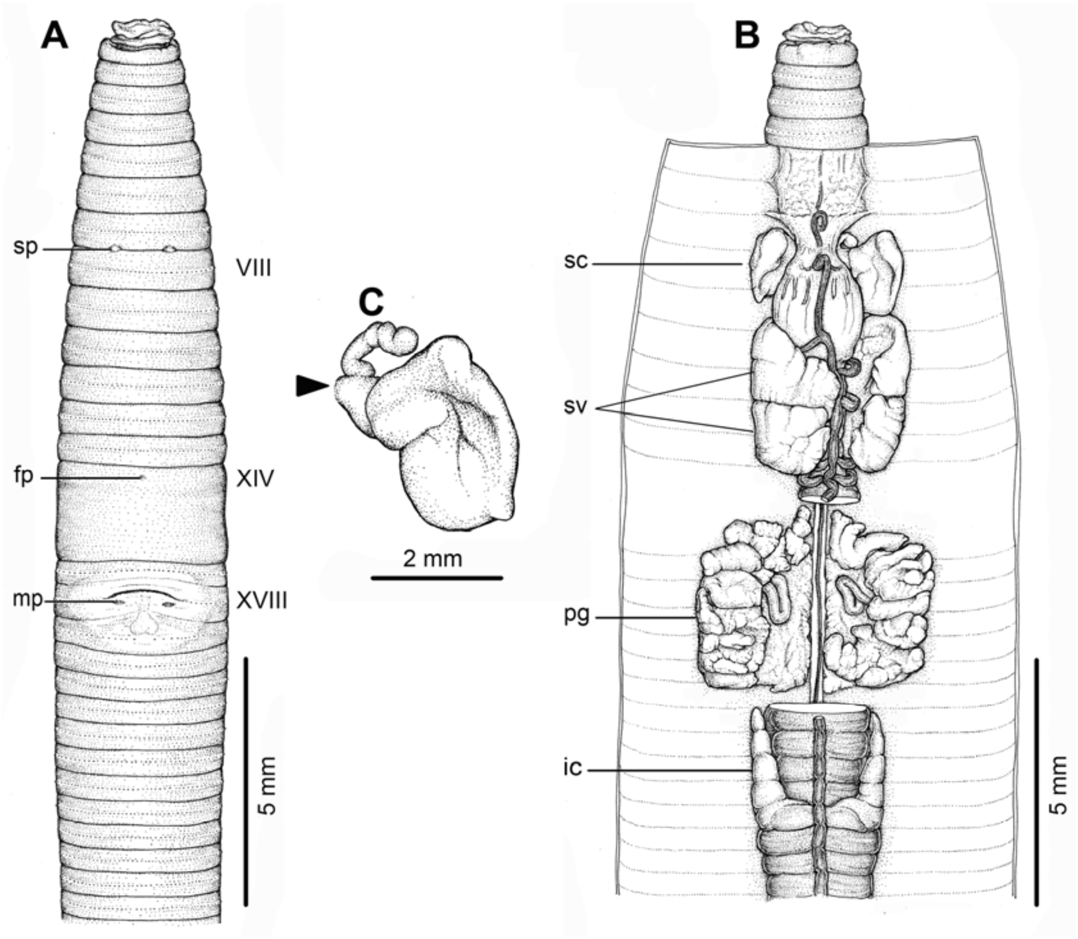

Figure 13. External and internal morphology of *Amynthas borealis* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

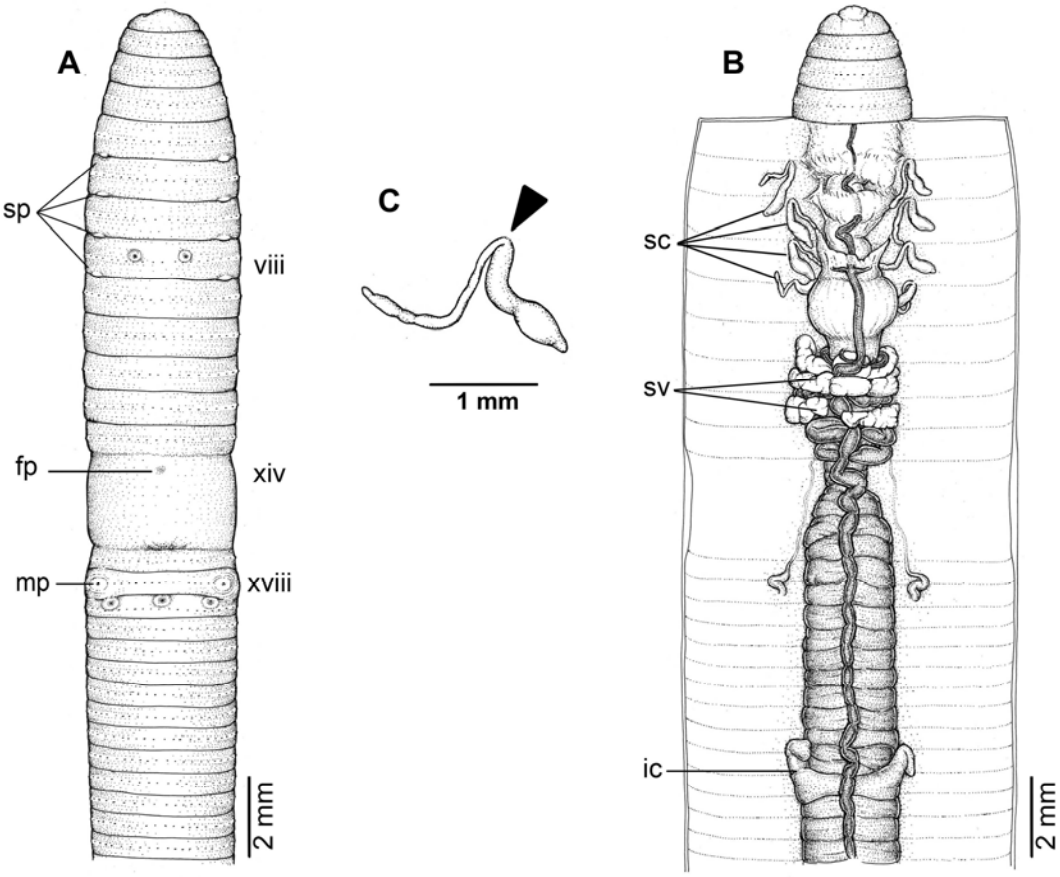

Figure 14. External and internal morphology of *Amynthas exiguus exiguus* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

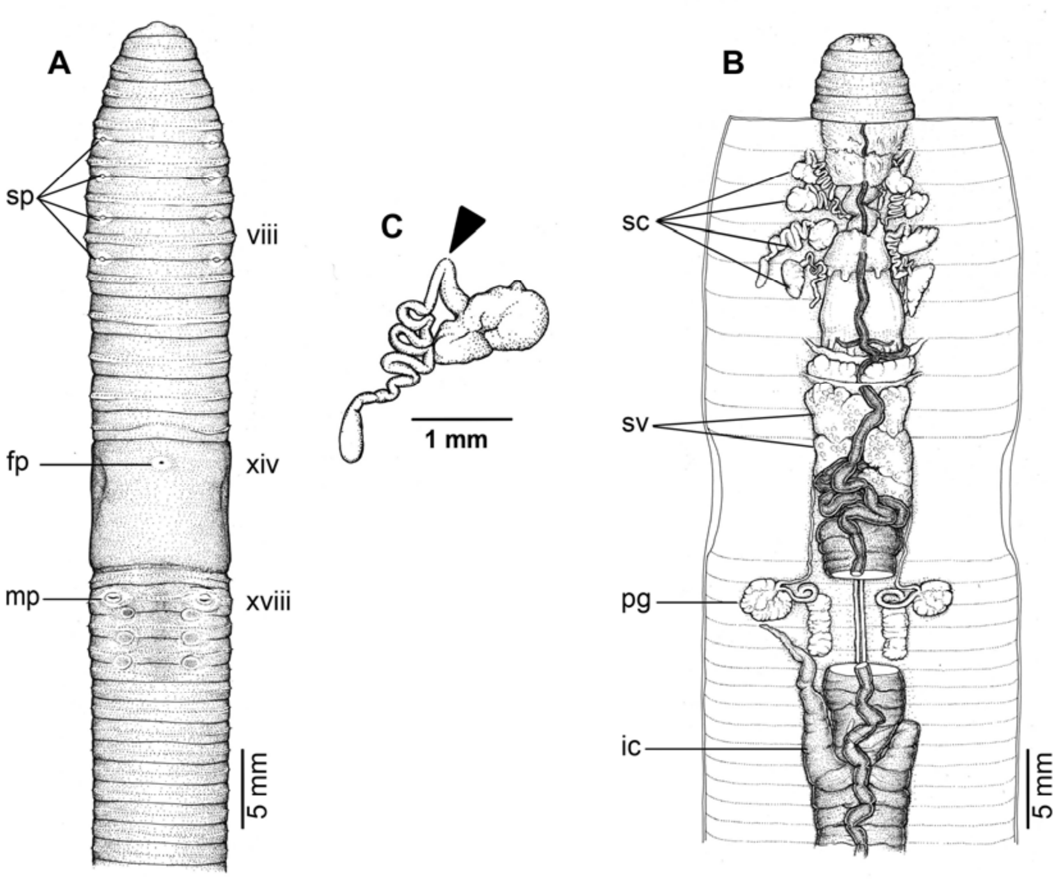

Figure 15. External and internal morphology of *Amynthas longicauliculatus* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

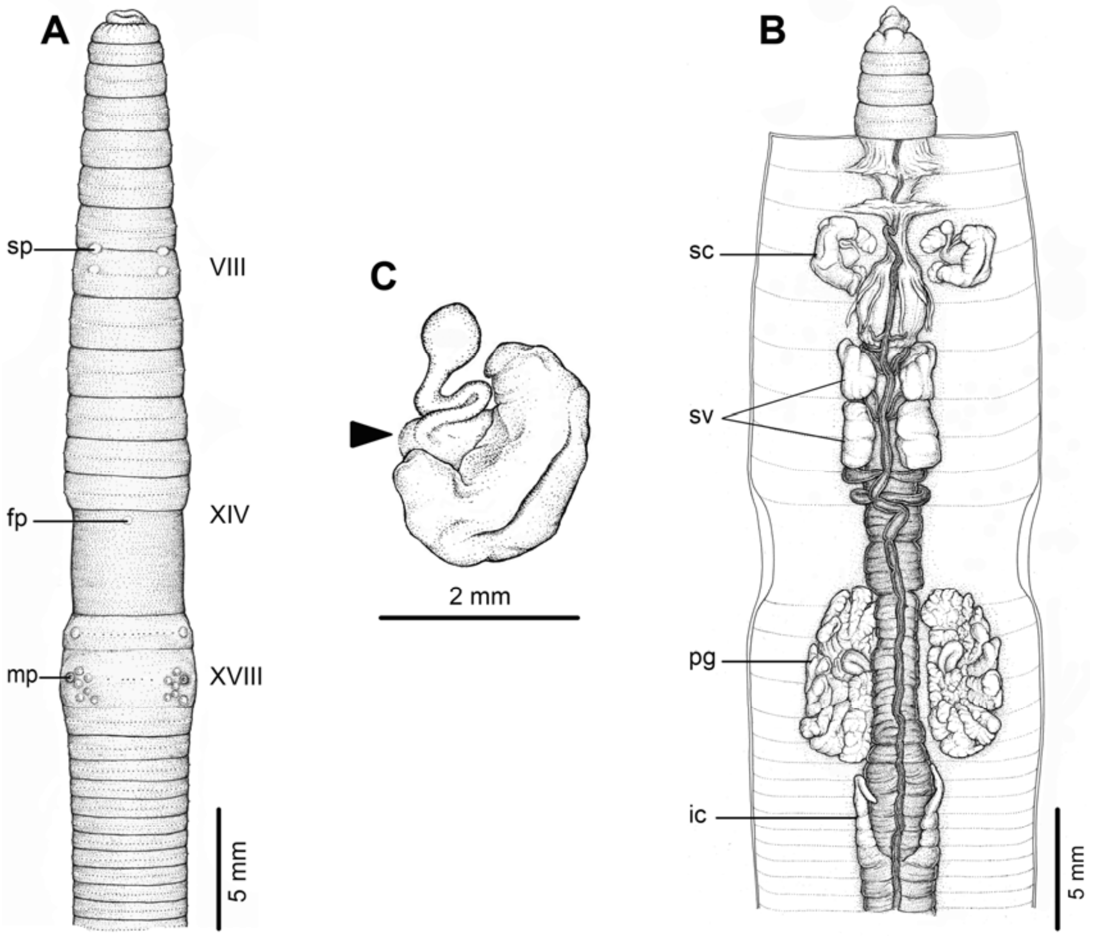

Figure 16. External and internal morphology of *Amynthas phatubensis* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

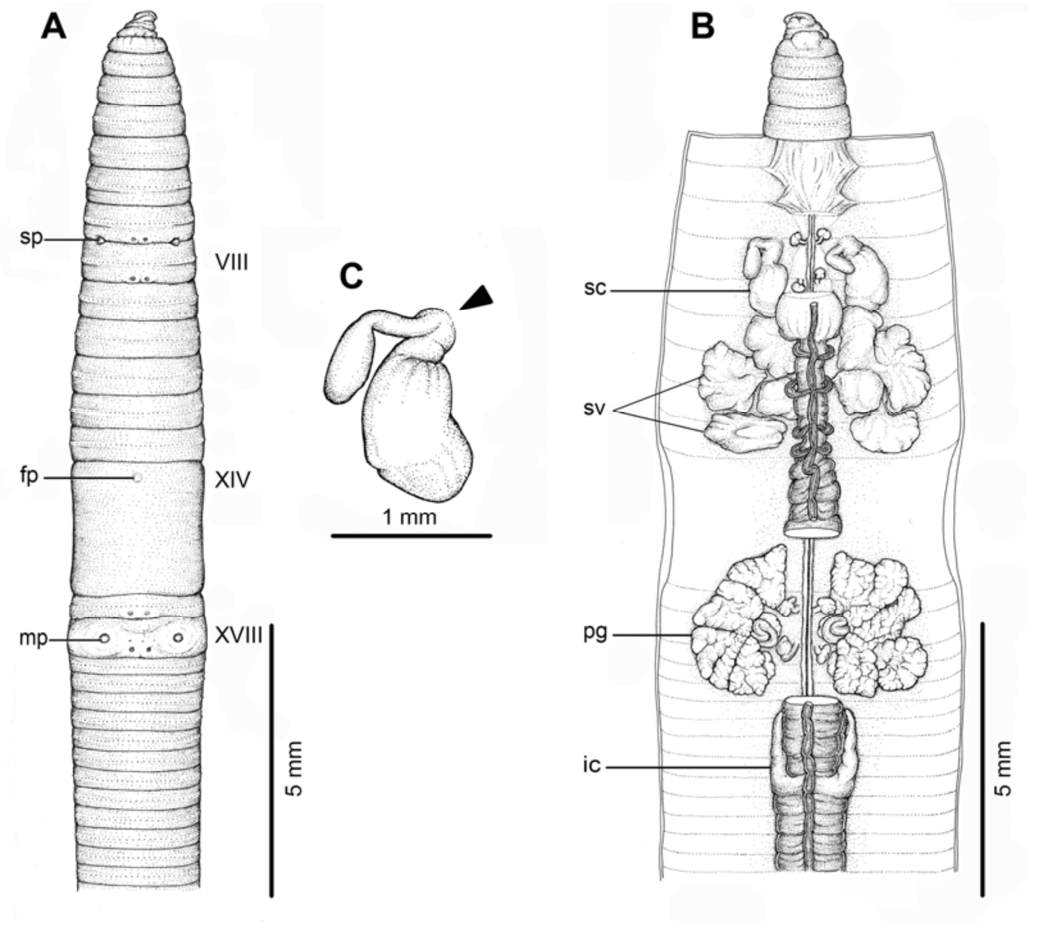

Figure 17. External and internal morphology of *Amynthas srinan* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

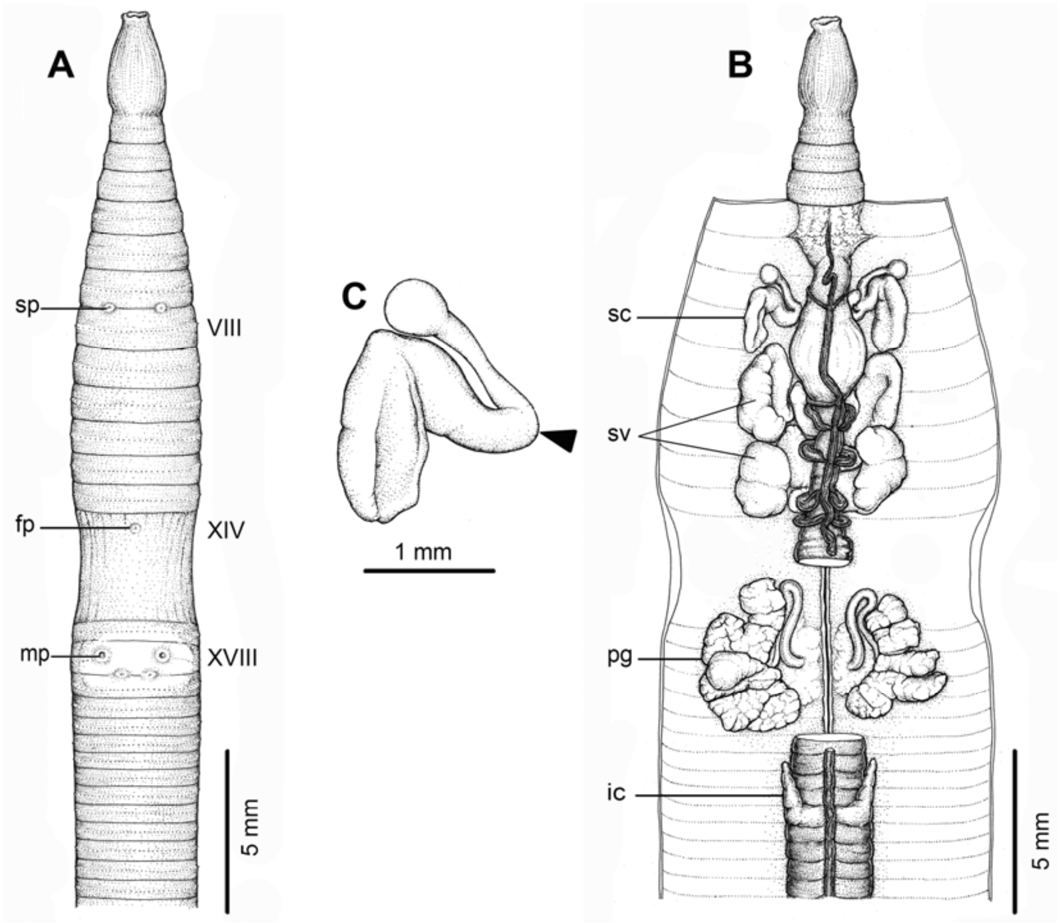

Figure 18. External and internal morphology of *Amynthas tontong* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

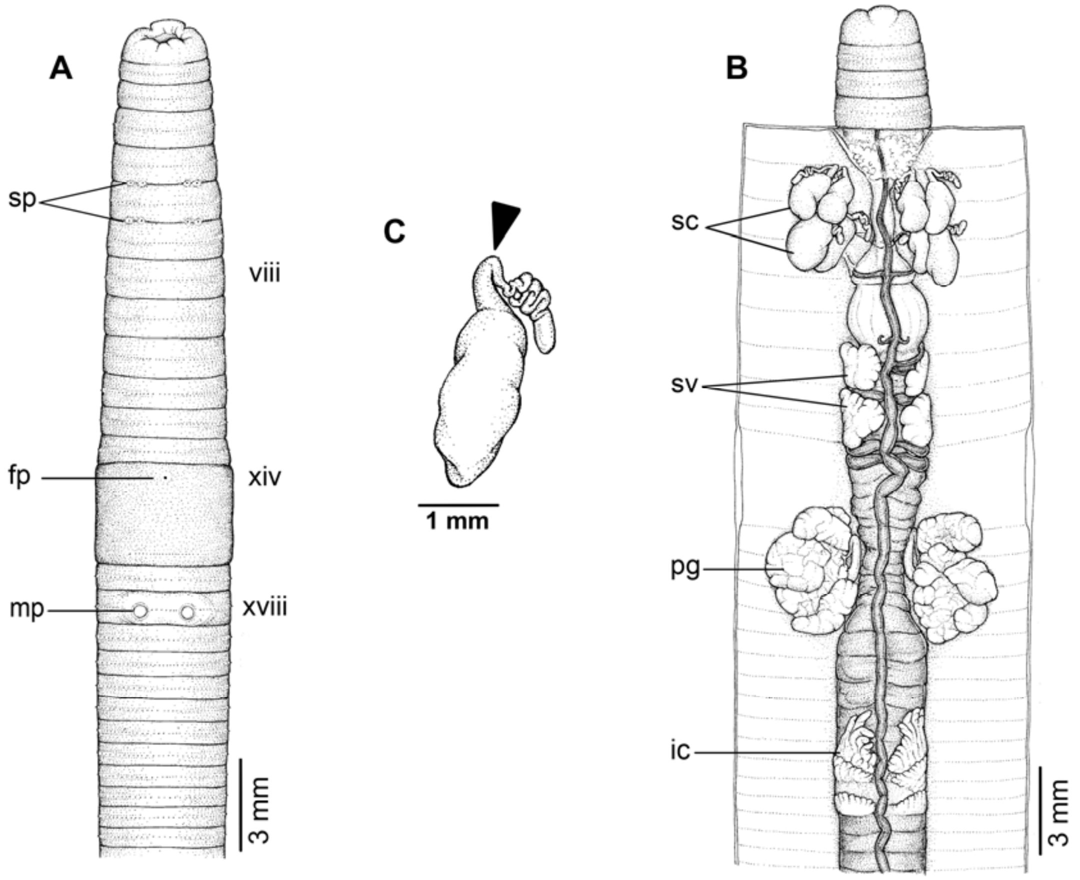

Figure 19. External and internal morphology of *Amynthas* sp.1 (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

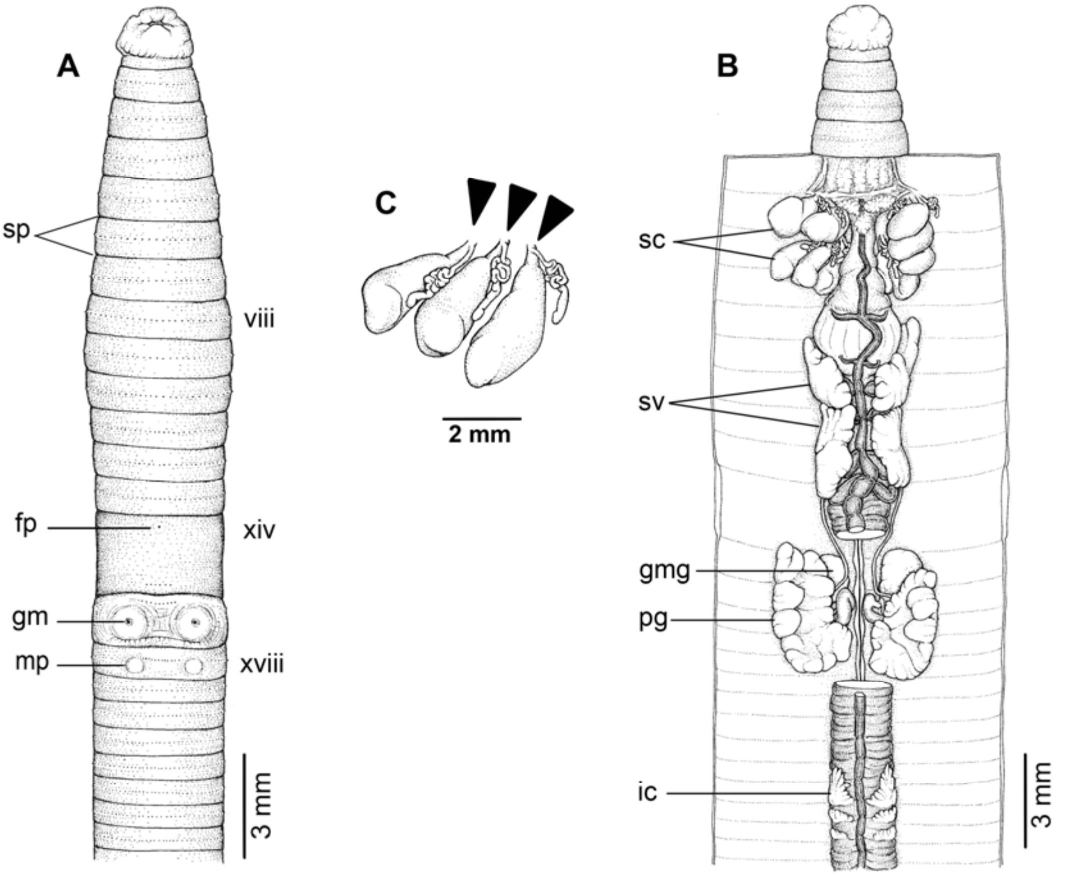

Figure 20. External and internal morphology of *Amynthas* sp.2 (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

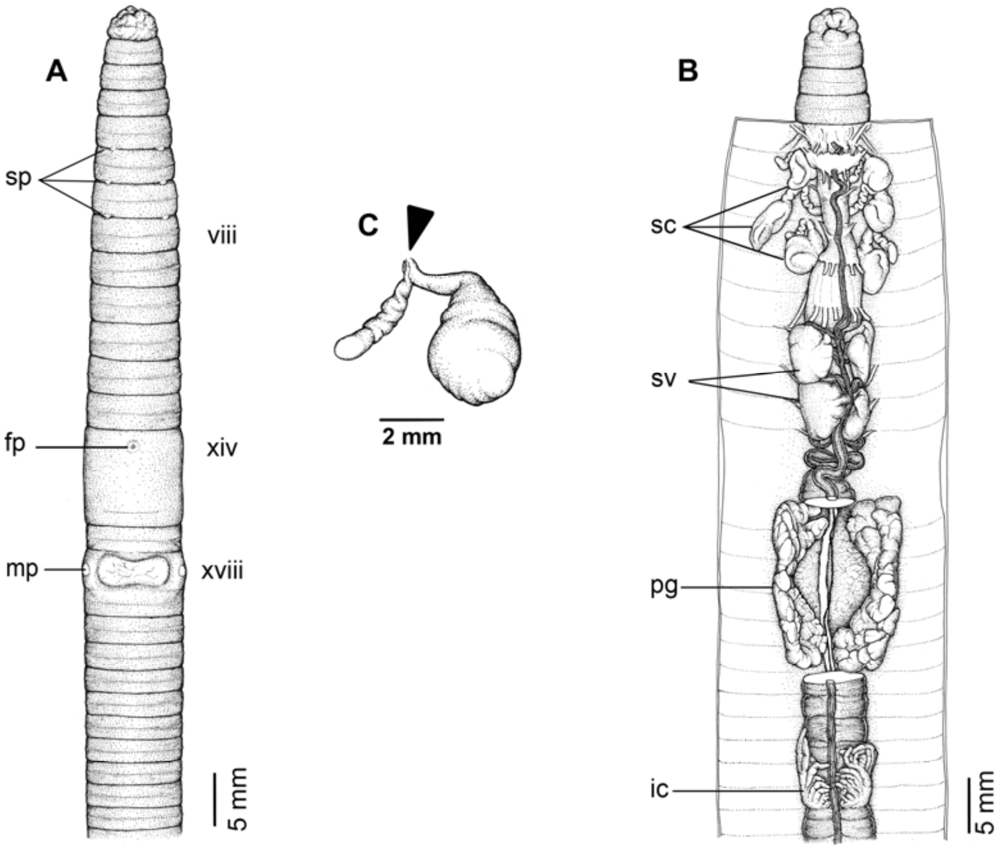

Figure 21. External and internal morphology of *Amynthas* sp.3 (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

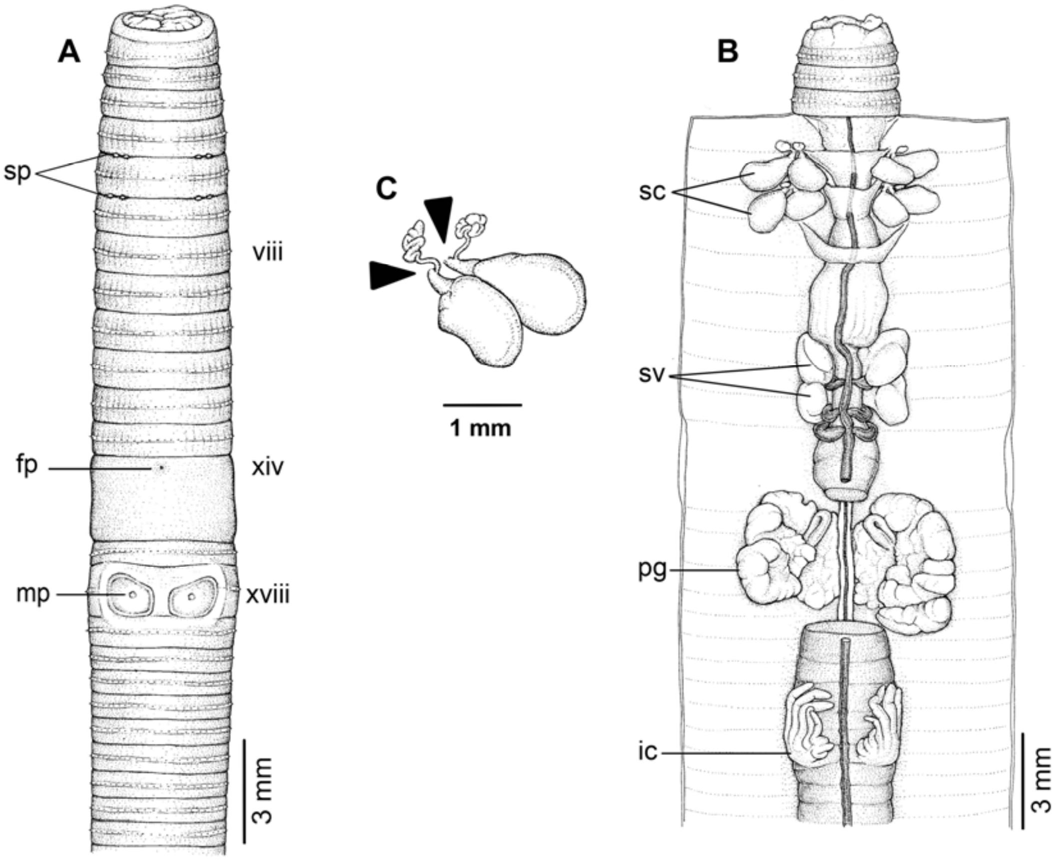

Figure 22. External and internal morphology of *Amynthas* sp. 4 (A) External ventral view of H morph, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

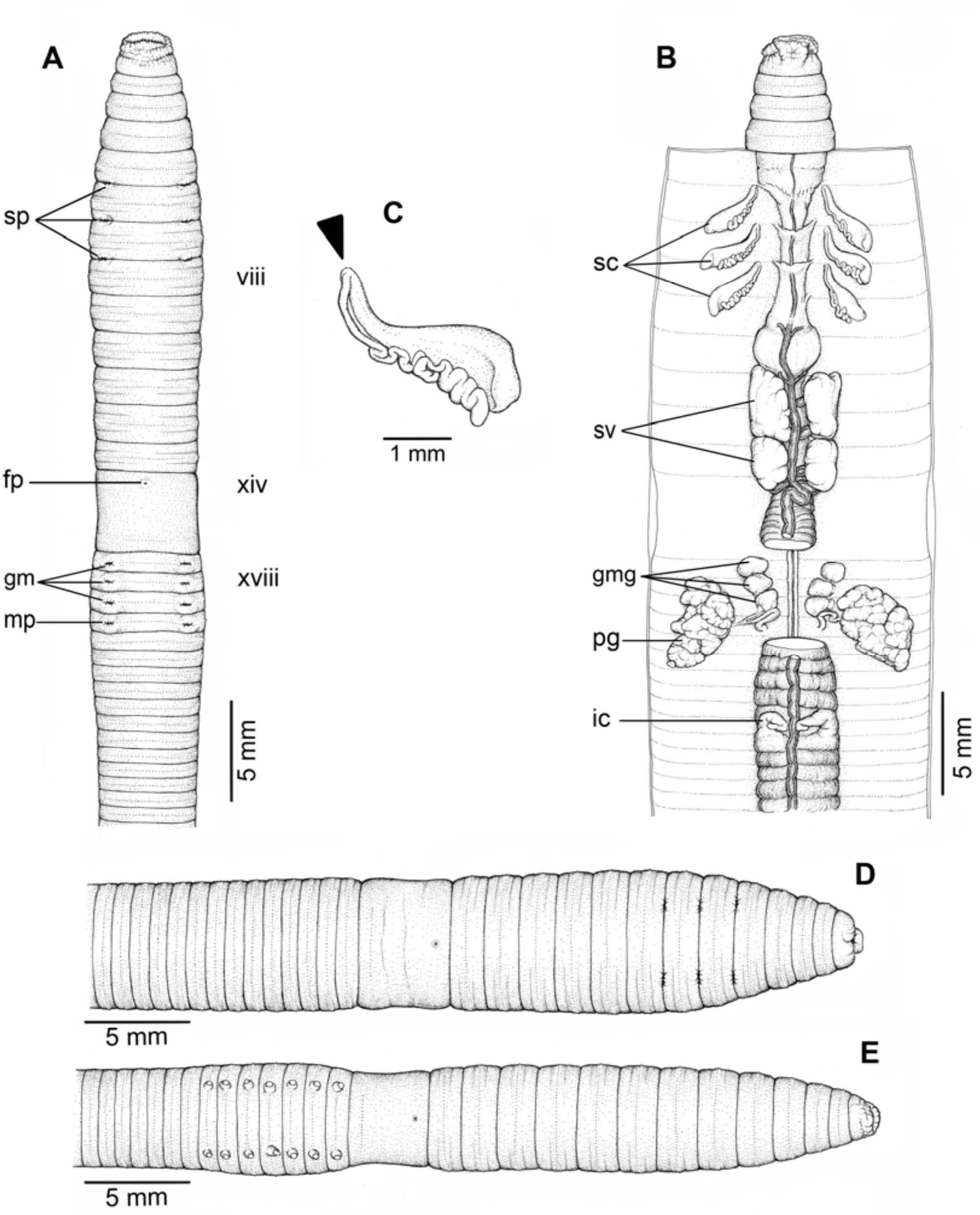

Figure 23. External and internal morphology of *Metaphire anomala* (A) External ventral view of H morph, (B) internal dorsal view of H morph and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore, (D) External ventral view of R morph, (E) External ventral view of A morph.

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

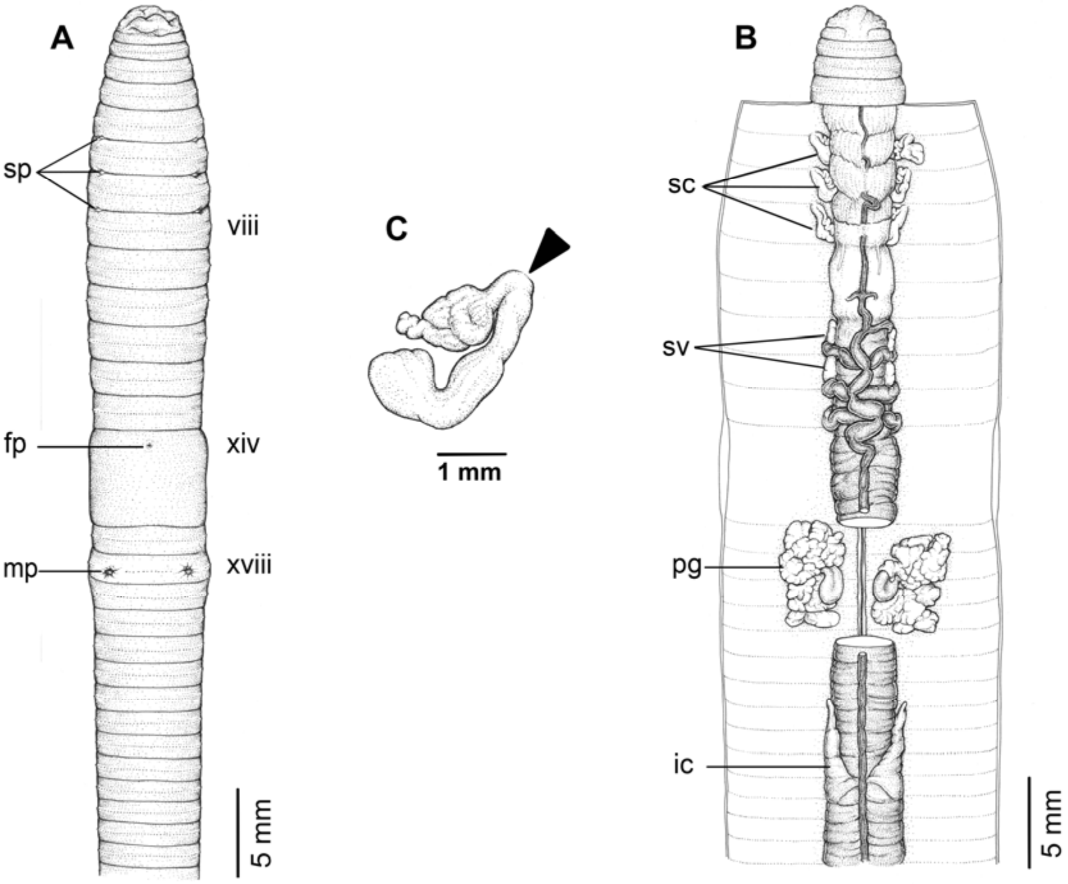

Figure 24. External and internal morphology of *Metaphire birmanica* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

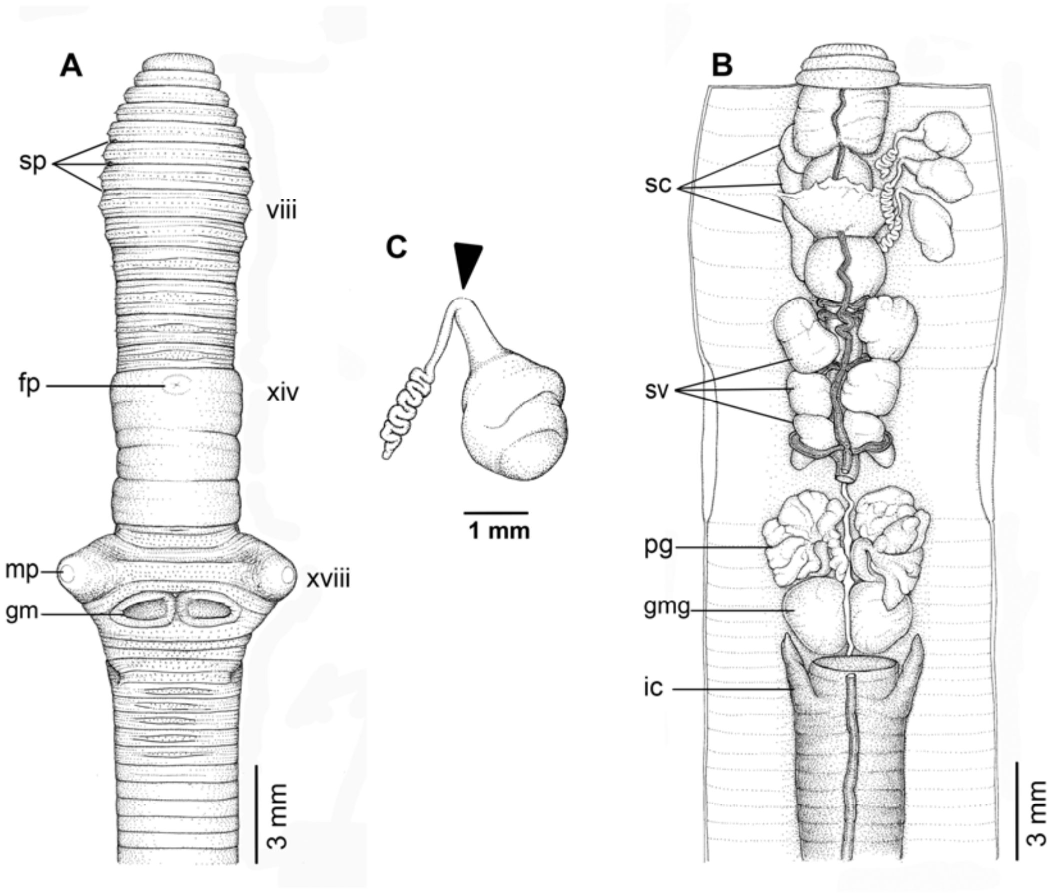

Figure 25. External and internal morphology of *Metaphire grandipenes* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

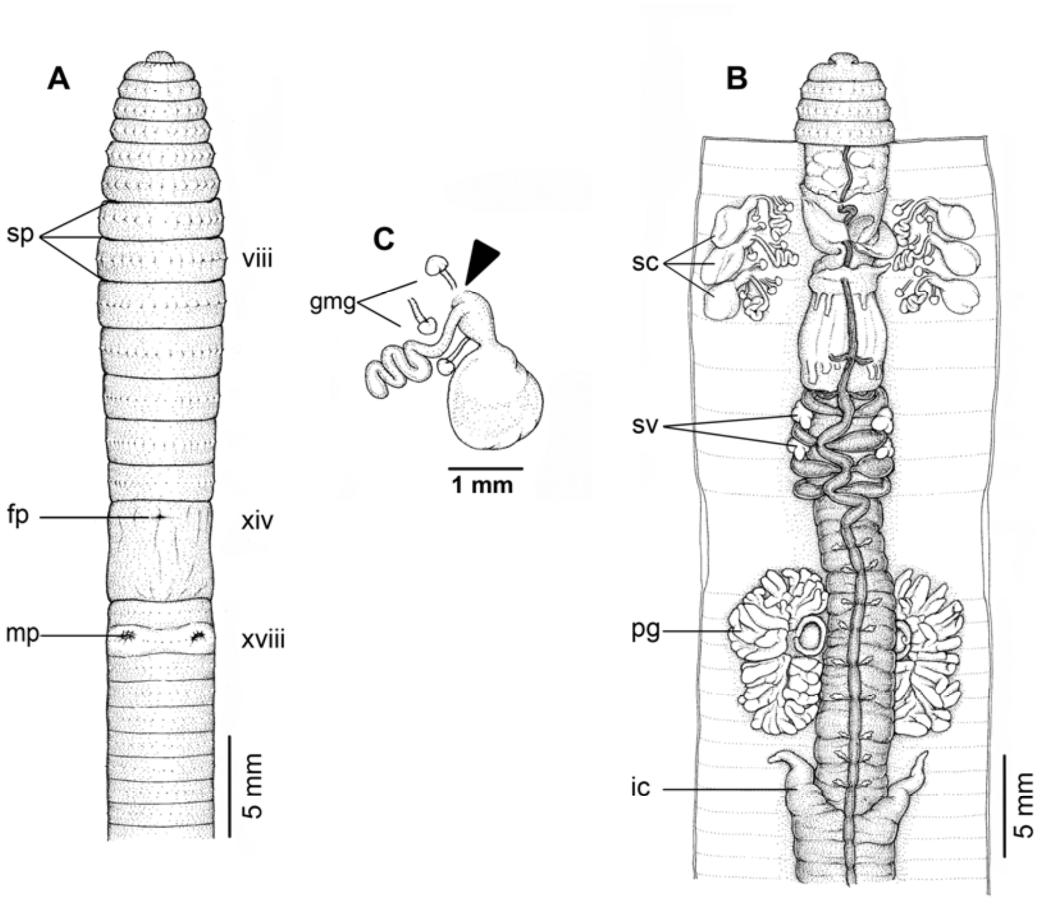

Figure 26. External and internal morphology of *Metaphire houlleti* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

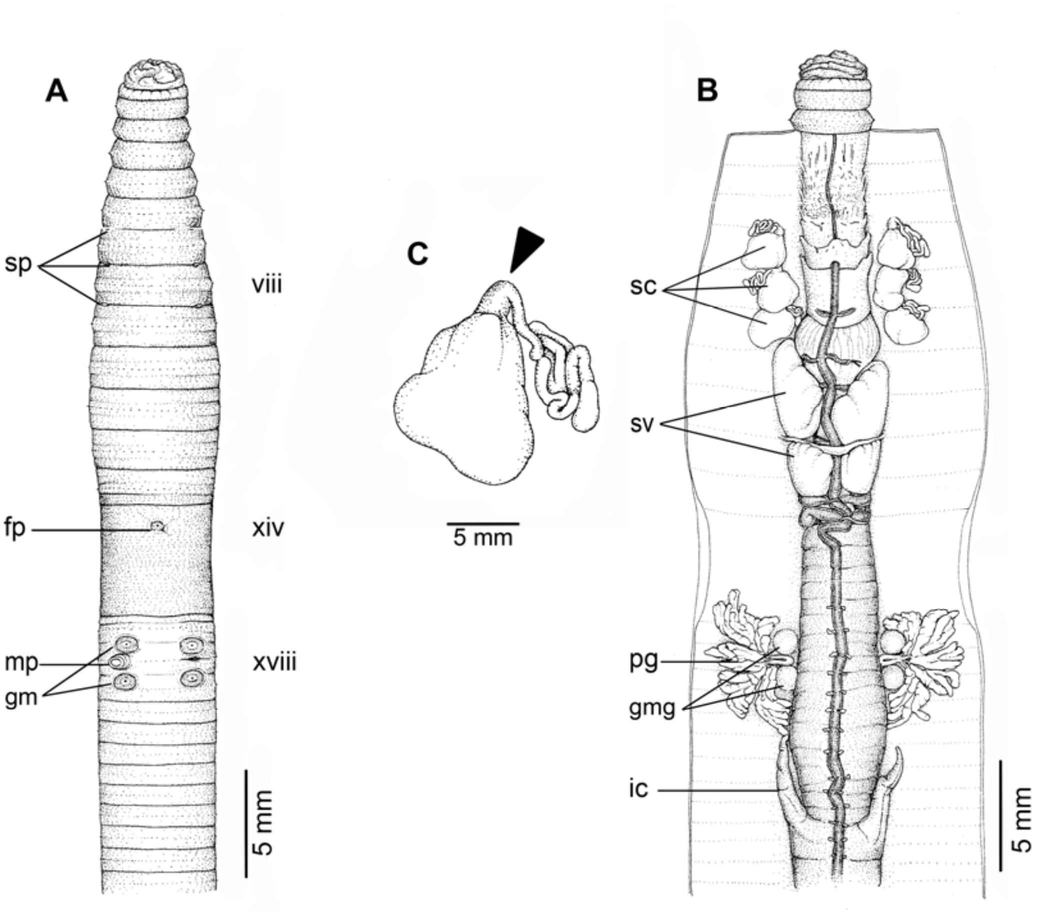

Figure 27. External and internal morphology of *Metaphire peguana* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

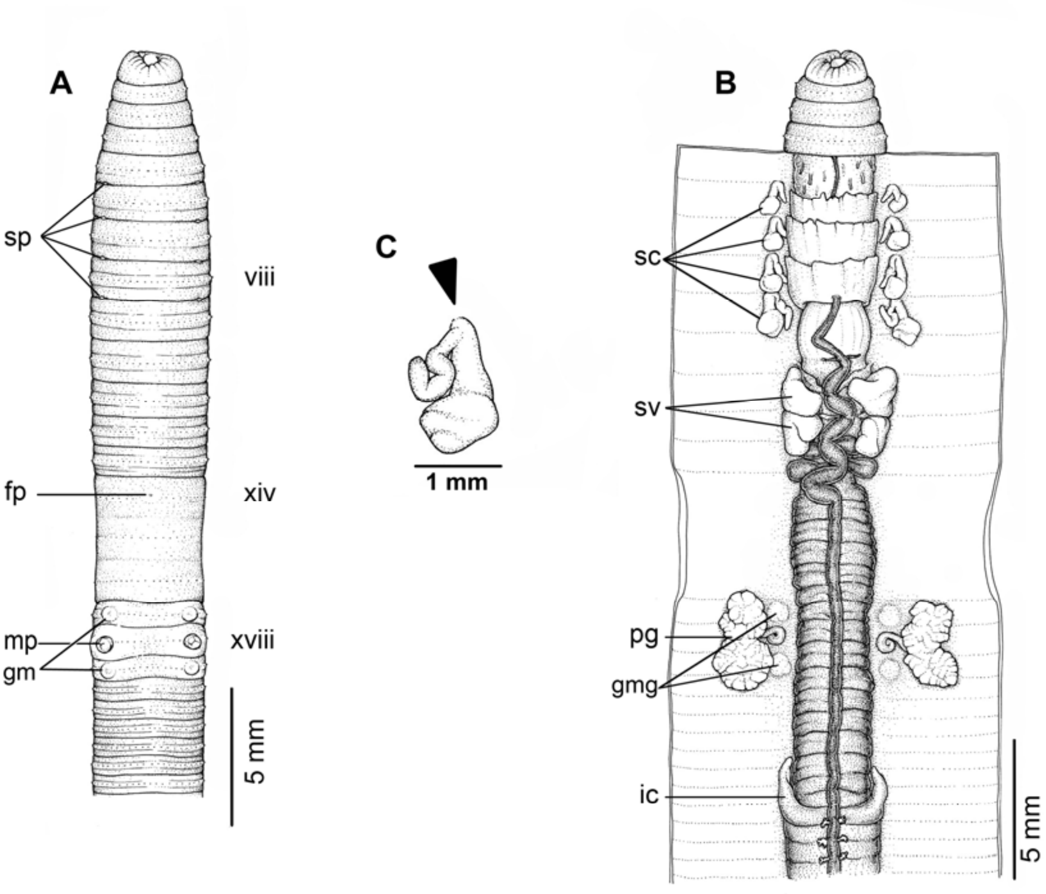

Figure 28. External and internal morphology of *Metaphire posthuma* (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

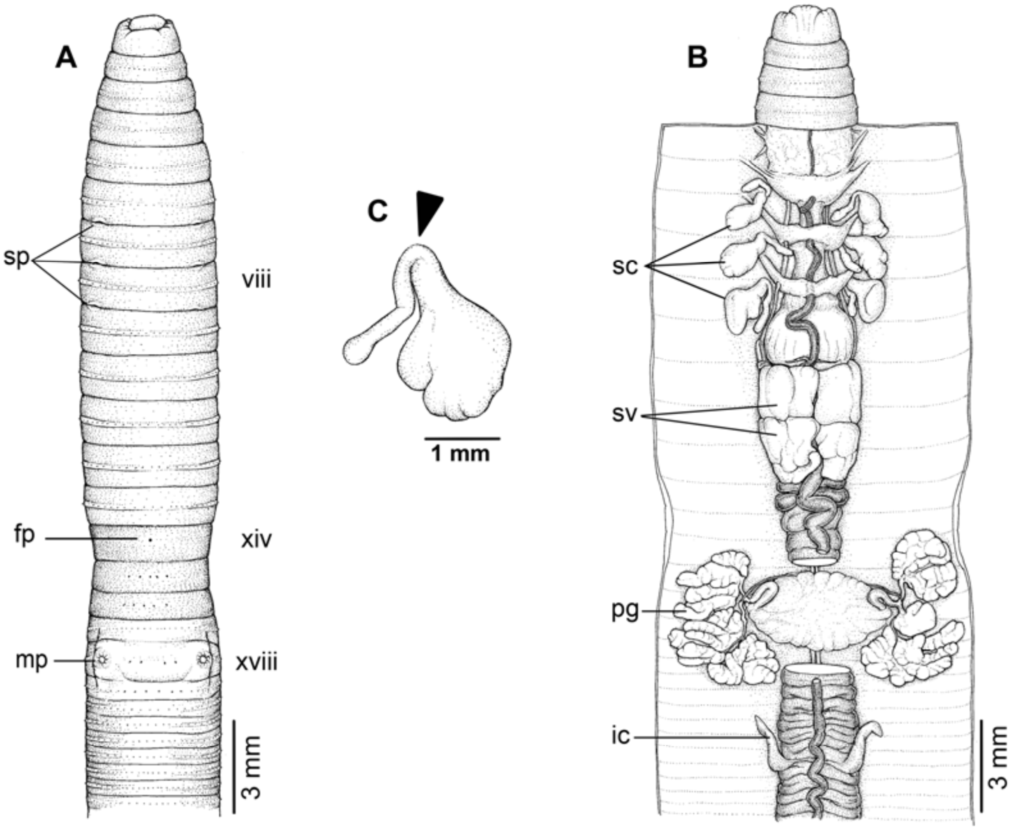

Figure 29. External and internal morphology of *Metaphire* sp.1 (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

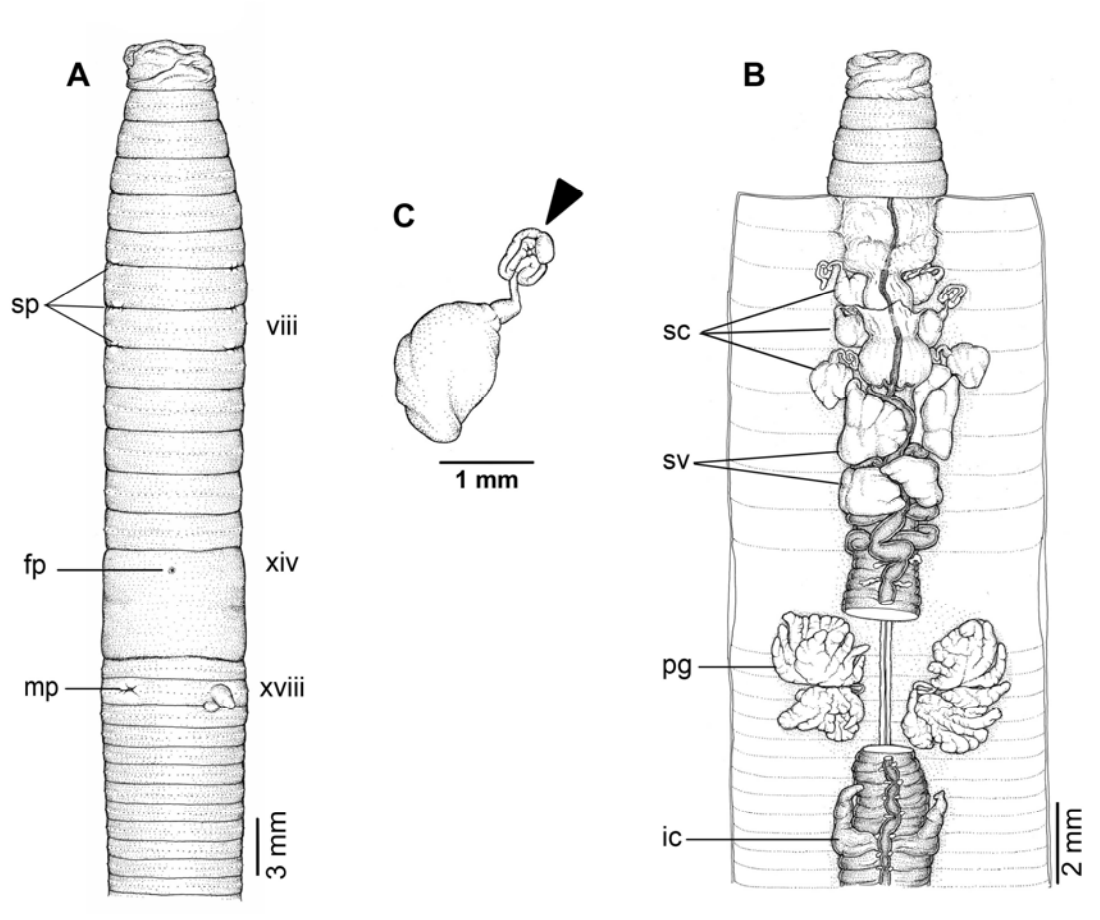

Figure 30. External and internal morphology of *Metaphire* sp.2 (A) External ventral view, (B) internal dorsal view and (C) spermathecae, and black arrow indicates the connection of the spermathecae and spermathecal pore.

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

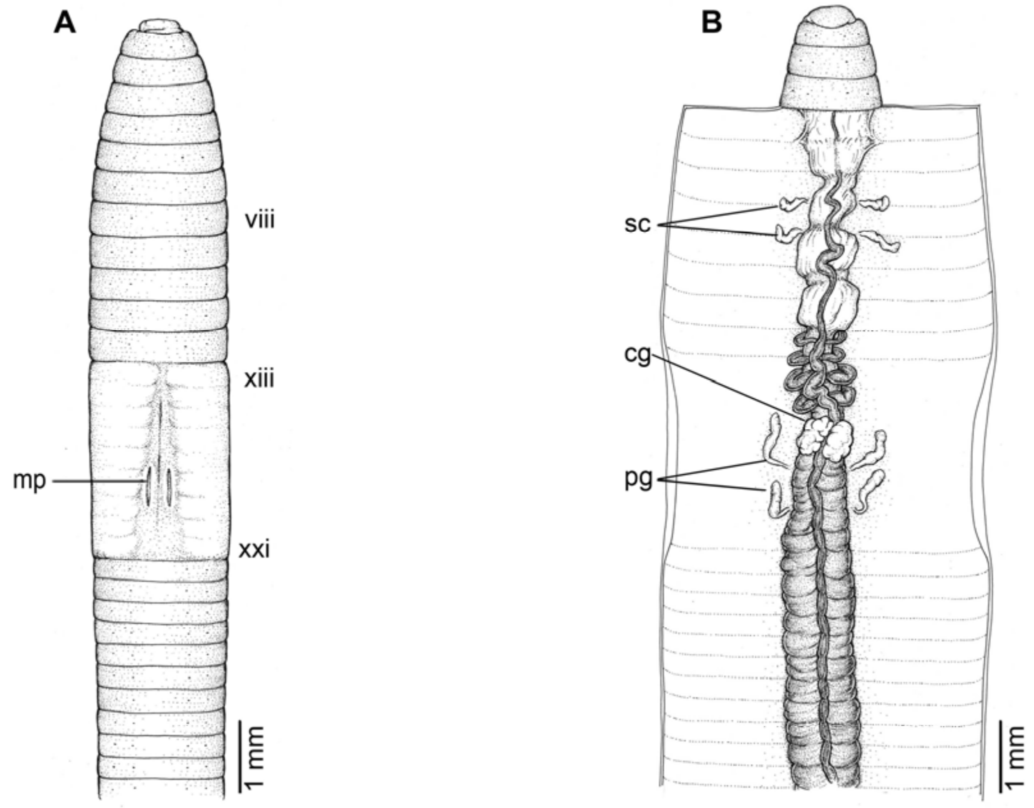

Figure 31. External and internal morphology of *Dichogaster bolaui* (A) External ventral view, (B) internal dorsal view.

Anatomical abbreviations: cg, calciferous gland; mp, male pores; pg, prostate gland; sc, spermathecae.

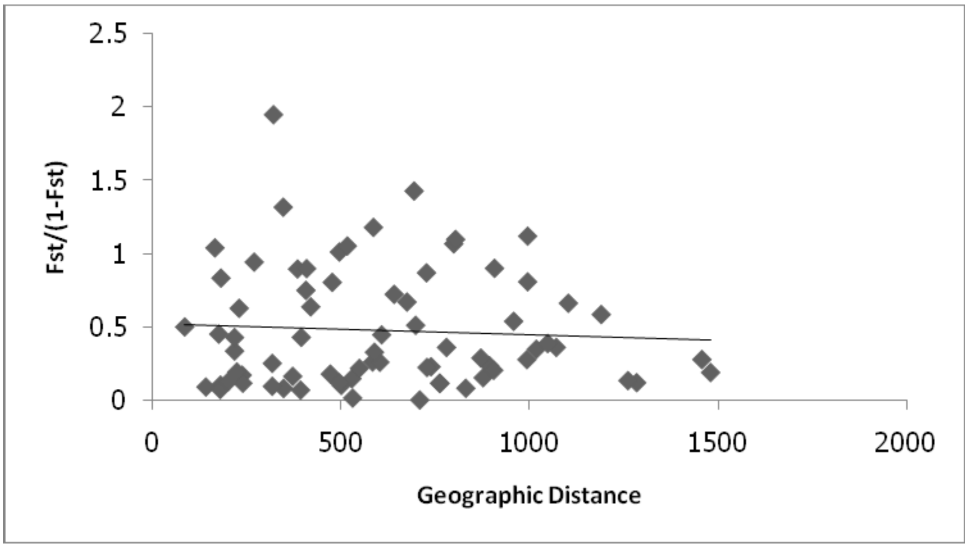

Figure 32. Isolation by distance in 13 *Metaphire peguana* populations. Comparison are pairwise between all populations.

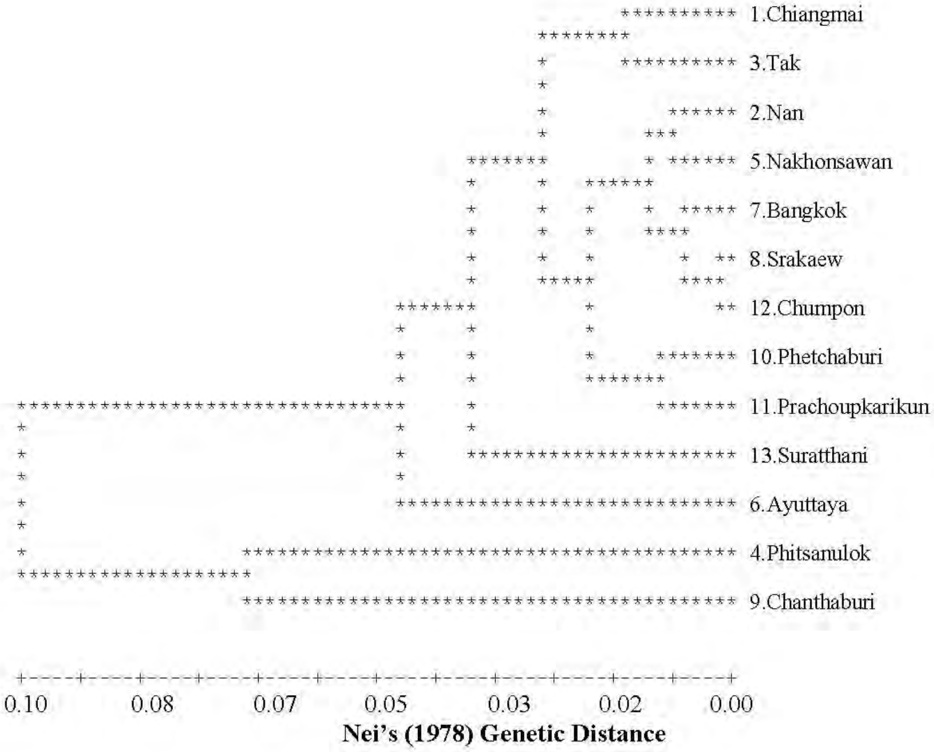

Figure 33. UPGMA tree for the sampled Thai populations of *Metaphire peguana* based on Nei's (1978) unbiased genetic distances. The population locality numbers correspond to those in Table 6.

CHAPTER V
DISCUSSION

The genus *Amynthas* and *Metaphire* of the family Megascolecidae contribute almost all of earthworm diversity in Nan areas, which confirmed that these two genera are the major taxon of the Southeast Asian tropical forests, as noted by several authors (Michaelson, 1907; Gates, 1972; Beddard, 1990; Omodeo, 2000). The genus *Amynthas* and *Metaphire* are the largest group of earthworms in Southeast Asia, consisting of more than 400 nominal species and subspecies (Sims and Easton, 1972). It is contains the majority of species found in Thailand. Gates (1939b) reported that *Pheretima* (=*Amynthas* or *Metaphire*) is a dominant genus in Thailand and has great value for zoogeographical research.

There are important external and internal characters used for classifying earthworm species, such as male pores, spermathecal pores, genital markings and the first dorsal pore, spermathecae and diverticulum, genital marking glands and intestinal caeca. These are also some characters which may vary within species for example body color, and body length. They just can be used together with other external and internal characteristics as mentioned before. In some species the genital marking glands are located near spermathecae and the prostatic duct region, and are important for identification. Genital marking glands are in different form unique forms such as sessile or stalked or may be absent (Bantaowong *et al*., 2011b).

Based on the literature, (Gates, 1939b, 1972; Sims and Easton, 1972, Blakemore, 2006) and the results of our studies in Nan areas, twenty six species are recorded before, 12 are newly reported and one new record for Thailand (Gates, 1939b; 1972; Bantaowong *et al*., 2011a, b) (Table 9).

Of the 16 nominal species from Nan province, there are five native species, *Amynthas phatubensis, A. tontong, A. borealis, A. srinan* and *Metaphire grandipenes*, and 4 the peregrine species, *M. peguana*, *M. posthuma*, *M. houlleti* and *P. corethrurus*. Based on the original home ranges suggested by Gates (1972), the peregrine species should be divided into the following two groups:

Group 1 consists of *Pontoscolex corethrurus*, which originated from the tropical America and *Dichogaster bolaui* from Africa. There is still no distinct record about the introduction period of these two species to Thailand. Group 2 consists of

Metaphire houlleti from Southeast Asia (probably occurs in eastern Burma); *M. peguana* and *M. posthuma* from Burma, Thailand, and Malay Peninsula (Gates, 1972).

Six megascolecids i.e. *Amynthas* sp.1, *Amynthas* sp.2, *Amynthas* sp.3, *Amynthas* sp. 4, *Metaphire* sp.1 and *Metaphire* sp. 2 are still hard to identify because they need to be investigated carefully with comparisons to type specimens. However *Amynthas* sp.1, *Amynthas* sp.2 and *Amynthas* sp.4 appear to be unlike any previously known species in that genus or *Metaphire* more up here.

In Table 9 indicated that *A. alexandri* and *M. peguana* are common species in Thailand, because they were recorded in various areas from 1939, 1972, 2005, 2009 and at the present study. (*Amynthas* sp.1, *Amynthas* sp. 2 and *Amynthas* sp. 4 contain two paired groups of spermathecae.)

Regarding habitat characteristics and distribution, the 3 recognized groups of earthworms occur in different habitat throughout Nan areas. It was found that Group 1) *P. corethrurus* and *M. posthuma* was found only in anthropogenic areas; 2) *A. alexandri, M. peguana, M. houlleti* and *M. anomala* were found in both natural forests and anthropogenic areas, while the other species were found only in natural forest. James (2004) reported that total earthworm diversity appeared quite high with approximately three to six species per site in the Philippine forest. Nan natural forest sites had 3 to 7 species per site.

The present study show that two genera of earthworm, *Amynthas* and *Metaphire*, occur sympatrically in various areas of Nan province. Body lengths of *Amynthas* and *Metaphire* species at Tham Phatub Arboretum varied from 60 to 300 mm are larger worms nainly in soil, smaller one in soil/litter interface. Both genera occupy various habitat types such as rotten wood, leaf litter, under barks and under rocks. Both genera are very common in Southeast Asia and found coexisting everywhere (Bantaowong *et al*., 2011a, b).

Endemic and introduced species

A literature on Thai earthworm contains only one endemic species *A. hopbonensis* which is known to be endemic to Thailand (Blakemore, 2006). Reliable example of introduced species appear to be the illustrated records of the Brazilian species *Pontoscolex corethrurus* and *Dichogaster bolaui* from Africa (Gates, 1972).

Many species of *Dichogaster* show an exceptional invasive power in warm countries or in warm microhabitats of boreal lands (Lee, 1985; Erseus *et al*., 1994). These introductions may be result from the cocoons that usually one deposited the soil surface. Plants and soil have been transported by humans, and this is an important cause of the spreading of earthworms. Over 100 exotic earthworms species have been reported as introductions throughout the world (Lee, 1987) and are generally found in disturbed habitats, close to human inhabitants. Stephenson (1930) emphasized that human activity is the main cause of earthworm passive dispersal.

Megascolecidae is a large family of earthworms which has native representatives in Australia, New Zealand, Southeast and East Asia, and North America. The genus *Amynthas* is the largest genus of the *Pheretima* group, consisting more than 400 nominal species, and *Metaphire* is the second largest group after *Amynthas*, which has reported 112 nominal species (Sims and Easton, 1972). The *Pheretima* group originated in the New Guinea/North Australia areas and invaded to Asia by way of the Indo-Australasian Archipelago during the Miocene or Oligocene, and it is endemic to Southeast Asia and the Malay Archipelago and goes up to Japan and Korae. Its western limit is in India. A number of species are highly peregrine and are established widely in the warmer regions of the global e.g. *A. gracilis, M. posthuma*, *M. houlleti*, *Polypheretima elongata* (Gates, 1972).

The genetic variation of *Metaphire peguana*

The genotypic and allelic variability in *M. peguana* appeared to show a similar number of different genotypes or alleles in the polymorphic loci to other worm species such as *Dendrobaena octaedra*, except in the case of the *Pgm* locus, which presented two alleles in *D. octaedra* while only one was detected in *M. peguana*. In contrast, *Idh* and *Mdh* found two alleles in *M. peguana*, but presented only one allele in *D. octaedra* (Hansen *et al*., 2006).

The percentage of polymorphic loci (*P*) in *M. peguana* displayed highly variable among samples (23.5%-47.1%). However, overall *M. peguana* appeared to show relatively lower than other annelids such as *P* values of 68.7% and 87.5% in *Esinea fetida* and *E. andrei*, (McElroy and Diehl, 2000), respectively. In comparison with polychaetes, *M. peguana* showed a similar *P* value to *Syllis gracilis*, 15.38% to

38.46% (Maltagliati *et al*., 2000), while exhibited relatively lower than *Riftia pachyptila*, 52.4% (Black *et al*., 1994),

The mean number of alleles per locus in *M. peguana* in this study varied from 1.2 to 1.6 which is lower than *Lumbricus rubellus*, 2.7 to 3.0 (Peles *et al*, 2003).

The levels of overall genetic variability in *M. peguana*, as indicated by the observed heterozygosity (*H*obs), was moderate to high among the different populations ranging from 0.059 to 0.119. However, the mean *H*obs value was somewhat similar to that of other European worms, *Esinea fetida* and *E. andrei* ranging from 0.094 to 0.262 and from 0.068 to 0.187 (McElroy and Diehl, 2000), respectively, Likewise, Peles *et al* (2003) also reported a similar heterozygosity value in *Lumbricus rubellus* ranging from 0.112 to 0.160. In comparison with other worm, *M. peguana* was relatively lower heterozygosity than *Riftia pachyptila* (0.095-0.125) (Black *et al*., 1994).

F statistics can also be used to describe genetic differentiation between populations. The weighted average value for all polymorphic loci of *F*is between all the *M. peguana* samples was 0.107 which is close to zero indicating a small amount of non-random mating, as suggested by Nei, 1986. In comparison with other annelids, *F*is value in *M. peguana* was relatively higher than *Riftia pachyptila* (0.056; Black *et al*., 1994). The mean *F*it value between all groups of *M. peguana* was relatively high (0.390) indicating amount of inbreeding due to population subdivision. Wright (1978) suggested the following guidelines for the interpretation of *F*st: the range from 0 to 0.05 indicates little genetic differentiation, 0.05 to 0.15 is indicative of moderate differentiation, 0.15 to 0.25 reflects great genetic differentiation, and very great genetic differences are present with values of more than 0.25. In this study, the mean *F*st value in *M. peguana* was relatively high (0.318) showing that 31.8% of the total genetic differentiation existed among populations. Therefore, the high *F*st value may have been involved in an extensive local fragmentation combined with the low dispersal distance approximately 10 m per year, (Marinissen and van den Borsch, 1992; Tiunov *et al*., 2006). In fact, *M. peguana* is a widely distributed and apparently broad-niched species (Blakemore, 2002).

On the basis of allozyme data, genetic distance among *M. peguana* samples was low as manifested by a low Nei's (1978) distance (*D*), and high Rogers (1972) similarity and the absence IBD. This suggests a high degree of gene flow/migration between populations across the geographic regions of Thailand (northern, central and southern). Moreover, An UPGMA (Sneath and Sokal, 1973) dendrogram based on the genetic distance matrix was constructed and showed no grouping of populations by geographic localities. In support of this evidence, earthworm can disperse or be transported-by animals, or man, or by inorganic. It is not always possible to distinguish between cases where a wide distribution is due to the powers of the worms themselves, which spread over a large region quite apart from human interference, and those where their introduction into widely remote localities is due to man. Therefore, the high genetic similarity among populations is commonly reported for conspecific.

Table 9. Terrestrial earthworms reported from Thailand. Presence and absence of each species is indicated by + and – respectively.

Species	**Gates, 1939b**	**Gates, 1972**	**Kosavititkul, 2005**	**Blakemore, 2006**	**Chantaravisoot, 2007**	**Somniyam, 2009**	**Present study**
Family Glossoscolecidae							
Pontoscolex corethrurus	+	+	+	+	-	+	+
Family Moniligastridae							
Drawida vulgaris	+	+	-	+	-	-	-
Drawida bedardi	-	+	+	-	-	+	-
Family Megascolecidae							
Lampito mauritii	+	+	-	+	-	-	-
Amynthas aeruginosus-group	-	-	-	-	+	-	-
Amynthas corticis-group	-	-	-	-	+	-	-
Amynthas minimus- group	-	-	-	-	+	-	-
Amynthas pomellus- group	-	-	-	-	+	-	-
Amynthas alexandri	+	+	+	+	-	+	+
Amynthas compta	+	+	-	+	-	-	-
Amynthas corticis	-	-	-	-	-	+	-
Amynthas evansi	+	+	-	+	-	-	-
Amynthas exiguus exiguus	+	+	-	+	-	-	+
Amynthas exiguus austrinus	+	+	-	+	-	-	-
Amynthas fucosus	-	-	+	-	-	-	-
Amynthas gracilis	+	+	-	+	-	-	-
Amynthas hupbonensis	+	+	-	+	-	-	-
Amynthas longicauliculatus	+	+	+	+	-	-	+
Amynthas mekongianus	+	+	-	+	-	-	-
Amynthas manicatus	+	+	-	+	-	-	-
Amynthas morrisi	+	+	-	+	-	-	-
Amynthas papulosus	+	+	-	+	-	-	-
Amynthas phatubensis	-	-	-	-	-	-	+
Amynthas tontong	-	-	-	-	-	-	+
Amynthas borealis	-	-	-	-	-	-	+
Amynthas srinan	-	-	-	-	-	-	+
Amynthas sp. 1	-	-	-	-	-	-	+
Amynthas sp. 2	-	-	-	-	-	-	+
Amynthas sp. 3	-	-	-	-	-	-	+
Amynthas sp. 4	-	-	-	-	-	-	+
Metaphire birmanica-group	-	-	-	-	+	-	-
Metaphire javanica-group	-	-	-	-	+	-	-
Metaphire ignobilis-group	-	-	-	-	+	-	-
Metaphire houlleti-group	-	-	-	-	+	-	-
Metaphire peguana-group	-	-	-	-	+	-	-
Metaphire sieboldi-group	-	-	-	-	+	-	-
Metaphire anomala	+	+	-	+	-	-	+
Metaphire bipora	+	+	-	+	-	-	-
Metaphire birmanica	-	-	-	-	-	-	+
Metaphire houlleti	+	+	-	+	-	-	+
Metaphire peguana	+	+	+	+	-	+	+
Metaphire virgo	+	+	-	+	-	-	-
Metaphire planate	+	+	-	+	-	+	-
Metaphire posthuma	+	+	-	+	-	+	+
Metaphire bahli	-	-	-	+	-	+	-
Metaphire grandipenes	-	-	-	-	-	-	+
Metaphire sp.1	-	-	-	-	-	-	+
Metaphire sp.2	-	-	-	-	-	-	+
Perionyx excavatus	+	+	+	+	-	-	-
Pithemera bicincta	-	-	+	-	-	-	-
Polypheretima elongate	+	+	-	+	-	+	-
Family Octochaetidae							
Dichogaster affinis	+	+	-	+	-	+	-
Dichohaster modiglianii	-	-	-	-	-	+	-
Dichogaster bolaui	-	-	-	-	-	+	+
Family Ocnerodrilidae							
Gordiodrilus elegans	-	-	-	-	-	+	-
Family Lumbricidae							
Eisenia fetida	-	-	-	+	-	-	-

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APPENDICES

APPENDIX A

genotype data set of *Metaphire peguana*

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
Chiengmai (CM)																	
CM01	AB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	AC	EE	CC	BB
CM02	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	AC	EE	CC	BB
CM03	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	AB	FF	0	EE	CC	BB
CM04	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM05	AB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AB	FF	BC	EE	CE	BB
CM06	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AB	FF	BB	EE	CC	BB
CM07	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM08	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM09	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
CM10	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AA	FF	CC	EE	CC	BB
CM11	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM12	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
CM13	AA	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM14	0	BB	BB	CC	DD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM15	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM16	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM17	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM18	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	EE	BB
CM19	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	AB	FF	BB	CE	EE	BB
CM20	AB	BB	BB	CC	CC	CD	AA	BB	BB	BB	BB	BB	FF	CC	CE	EE	BB
Nan (NA)																	
NA01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA02	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	AC	EE	CC	BB
NA03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	0	EE	CC	BB
NA04	0	BB	BB	CC	CC	CC	AA	CC	BB	0	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
NA05	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA06	0	BB	BB	CC	CC	CC	AA	DD	0	BB	BB	BB	FF	CC	EE	CC	BB
NA07	0	BB	BB	CC	CC	CC	AA	DD	DD	0	BB	BB	FF	CC	EE	CC	BB
NA08	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA09	0	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	EE	CC	BB
NA10	BB	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FF	CC	EE	CC	BB
NA11	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA12	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA13	BB	AB	BB	CC	CC	CC	AA	CC	BB	0	BB	BB	FF	CC	EE	CC	BB
NA14	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA15	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA16	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA17	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA18	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	AB	BB	FF	CC	EE	CC	BB
NA19	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	CC	EE	CC	BB
NA20	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AA	FF	CC	EE	CC	BB
NA21	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AB	FF	CC	EE	CC	BB
NA22	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	AB	FF	BC	EE	EE	BB
NA23	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	AB	FF	BC	EE	EE	BB
NA24	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	EE	BB
NA25	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	AB	BB	FF	BB	EE	EE	BB
Phrae (PR)																	
PR01	HH	CC	AA	BB	CC	AC	AA	DD	DD	BB	DD	CC	BB	DD	BB	DD	BB
PR02	HH	CC	AA	BB	AA	DD	AA	0	DD	AB	DD	CC	DD	DD	DD	DD	BB
PR03	HH	CC	AA	BB	AC	DD	AA	DE	DD	BB	DD	CC	BB	DD	DD	DD	BB
PR04	HH	DD	AA	BB	AC	CD	AA	DE	DD	BB	DD	CC	BD	EE	BD	CC	BB
PR05	HH	DD	AA	BB	AC	CC	AA	DE	DD	BB	DD	CC	BD	DE	BD	CC	BB
PR06	HH	DD	AA	BB	AC	CC	AA	DE	BB	AB	DD	CC	BB	EE	BD	CE	BB
PR07	HH	DD	AA	AA	CC	AC	AA	DE	DD	BB	DD	CC	BD	DD	DD	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
PR08	HH	DD	AA	AA	AA	DD	AA	EE	DD	AB	DD	CC	BB	0	BB	CC	BB
PR09	GH	DD	AA	AA	AC	CD	AA	DE	DD	BB	DD	CC	BD	EE	BD	CC	BB
PR10	GH	DE	AA	AA	CC	CD	AA	EE	BB	BB	DD	CC	BB	CD	BB	CC	BB
PR11	HH	DD	AA	AA	AC	AC	AA	EE	DD	BB	DD	CC	BB	DD	BB	CC	BB
PR12	HH	DD	AA	AA	AC	0	AA	EE	DD	AB	DD	CC	BD	0	0	CC	BB
PR13	HH	DD	AA	AA	CC	AC	AA	EE	DD	BB	DD	CC	BB	DD	0	CC	BB
Tak (TK)																	
TK01	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK02	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK04	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK05	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
TK07	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK08	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
TK09	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK10	CD	BB	AB	CC	CD	CD	AA	AA	0	BB	EE	BC	FF	DD	AA	BD	BB
TK11	AA	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK12	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK13	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK14	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK15	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	CC	EE	CC	BB
TK16	AA	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	AB	FF	CC	EE	EE	BB
TK17	AA	BB	BB	CC	DD	CC	AA	CC	BB	AB	BB	BB	FF	BB	EE	CC	BB
TK18	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
TK19	0	BB	BB	CC	CC	CD	AA	CC	BB	AB	BB	BB	FF	CC	EE	EE	BB
TK20	AA	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BB	EE	EE	BB
TK21	AB	BB	BB	CC	CC	CD	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
Pitsanulok (PN)																	

	MPI	*LGG-4*	*LGG-3*	*LGG-2*	*ME*	*AAAT-1*	*AAT-2*	*EST*	*IDH-1*	*IDH-2*	*MDH-1*	*MDH-2*	*GPI*	*PGD*	*PGM*	*HBDH*	*SOD*
PN01	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	CC	EE	0	BB
PN02	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	0	BB
PN03	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	CC	BB
PN04	AB	BB	BB	CC	CC	CC	AA	0	DD	BB	BB	AB	FF	BC	EE	CC	BB
PN05	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
PN06	AB	AB	BB	CC	CC	AC	AA	DD	BB	BB	AB	BB	FF	BB	EE	0	BB
PN07	AB	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	CC	BB
PN08	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AB	FF	BC	EE	CC	BB
PN09	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	AB	FF	BC	EE	0	BB
PN10	AB	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	0	BB
PN11	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
PN12	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
PN13	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	CC	EE	CC	BB
PN14	0	AB	BB	CC	CC	CC	AA	CC	BB	AB	AB	AB	FF	BB	EE	0	BB
PN15	AA	BB	BB	CC	CC	CC	AA	CC	0	BB	AB	AB	FF	CC	EE	CC	BB
PN16	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	AB	AB	FF	CC	EE	CC	BB
Nakhormsawan (NS)																	
NS01	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	AC	EE	CC	BB
NS02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS03	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS05	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS07	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS08	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS09	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS10	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS11	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS12	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
NS13	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS14	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS15	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	AA	BB	EE	CC	BB
NS16	BB	BB	BB	CC	CC	CC	AA	AA	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS17	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS18	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS19	AB	BB	BB	CC	CC	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS20	0	BB	BB	CC	CC	CC	AA	AB	0	0	BB	BB	FF	CC	EE	0	BB
NS21	BB	BB	BB	CC	CC	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	0	BB
NS22	AB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS23	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS24	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
NS25	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS26	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
NS27	0	BB	BB	CC	CC	CC	AA	BB	BB	AB	BB	BB	FF	CC	EE	EE	BB
NS28	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
NS29	BB	BB	BB	CC	CC	CC	AA	BB	BB	AB	BB	BB	FF	BB	EE	0	BB
NS30	0	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
Ayutthaya (AY)																	
AY01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
AY02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
AY03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CC	CC	BB
AY05	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY06	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY07	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	0	BB
AY08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY09	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY10	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB

	MPI	*LGG-4*	*LGG-3*	*LGG-2*	*ME*	*AAAT-1*	*AAT-2*	*EST*	*IDH-1*	*IDH-2*	*MDH-1*	*MDH-2*	*GPI*	*PGD*	*PGM*	*HBDH*	*SOD*
AY11	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY12	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
AY13	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY14	BB	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FG	CC	EE	0	BB
AY15	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY16	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	CC	CC	BB
AY17	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY18	AB	BB	BB	CC	CD	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY19	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY20	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	0	BB
AY21	0	BB	BB	CC	CC	CC	AA	0	0	0	BB	BB	FG	0	CC	0	BB
AY22	AA	BB	BB	CC	CC	CC	AA	BB	0	0	BB	BB	FF	CC	EE	0	BB
AY23	0	BB	BB	CC	CD	CC	AA	AB	BB	AB	BB	BB	FF	CC	EE	0	BB
AY24	BB	BB	BB	CC	CD	CC	AA	0	0	AB	BB	BB	FF	CC	CE	0	BB
Bangkok (BK)																	
BK01	BB	BB	BB	CC	CD	CC	AA	CC	0	BB	BB	BB	CF	BB	EE	CC	BB
BK02	BB	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK03	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK04	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
BK05	0	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK06	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK07	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK08	0	BB	BB	CC	CD	CC	AA	CC	BB	0	BB	BB	FF	BB	EE	CE	BB
BK09	0	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	EF	CC	EE	C	BB
BK10	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
BK11	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
BK12	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	C	BB
BK13	0	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FF	CC	EE	CC	BB
BK14	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	CE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
BK15	AA	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK16	BB	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	0	BB
BK17	BB	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	BC	EE	0	BB
BK18	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	EE	BB
BK19	AB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	EE	BB
BK20	BB	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	EE	BB
Sa Kaeo (SK)																	
SK01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
SK02	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	AC	EE	CE	BB
SK03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	E	BB
SK04	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK05	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
SK06	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CE	BB
SK07	BB	BB	BB	CC	CC	CC	AA	CD	AB	BB	BB	BB	FF	CC	EE	CC	BB
SK08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK09	0	BB	BB	CC	BC	CC	AA	CD	BB	BB	BB	BB	FF	CC	CE	CC	BB
SK10	AB	BB	BB	CC	CC	CC	AA	CD	0	BB	BB	BB	FF	BC	EE	CE	BB
SK11	BB	BB	BB	CC	BC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK12	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	FF	BB
SK13	BB	BB	BB	CC	CC	CC	AA	CD	AB	BB	BB	BB	FF	CC	CE	CC	BB
SK14	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	CC	CC	BB
SK15	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CD	EE	CC	BB
SK16	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK17	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK18	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	CC	BB
SK19	0	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK20	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	CF	CC	EE	CC	BB
SK21	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK22	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CE	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
SK23	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	FF	BB
SK24	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK25	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK26	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	EE	CC	BB
SK27	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	EE	EE	BB
SK28	AB	CC	BB	CC	EE	AA	AA	0	CC	AA	AB	BC	AA	CC	CE	0	BB
SK29	0	BB	BB	CC	EE	CC	AA	0	0	BB	BB	BB	FG	CC	EE	0	BB
SK30	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BC	CE	CC	BB
SK31	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	CE	CE	BB
SK32	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
Chanthaburi (CH)																	
CH01	BB	BB	BB	CC	DD	CC	AA	DD	0	BB	BB	BB	FF	CC	CC	CC	BB
CH02	BB	BB	BB	CC	CC	CC	AA	0	0	0	BB	BB	FF	CC	EE	CC	BB
CH03	BB	BB	BB	CC	DD	CC	AA	0	BB	AB	BB	BB	FF	CC	CE	CC	BB
CH04	BB	BB	BB	CC	DD	CC	AA	DD	0	BB	BB	BB	FF	CC	CC	CC	BB
CH05	AB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH06	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	CE	CC	BB
CH07	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BD	BB	FG	CC	EE	CC	BB
CH08	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	CE	CC	BB
CH09	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH10	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH11	AB	BB	BB	CC	DD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH12	BB	BB	BB	CC	DD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH13	0	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH14	AB	BB	BB	CC	CD	CC	AA	0	BB	BB	BB	BB	FF	CC	CE	0	BB
CH15	AB	BB	BB	CC	CD	CC	AA	0	BB	0	BD	BB	FF	CC	EF	CC	BB
CH16	BB	BB	BB	CC	CD	CC	AA	0	0	BB	BB	BB	FG	0	EE	0	BB
Phetchaburi (PH)																	
PH01	BB	BB	BB	CC	CC	AC	AA	CC	BB	0	BB	BB	FF	CC	EE	CE	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
PH02	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	AC	EE	EE	BB
PH03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH04	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
PH05	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PH07	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH08	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
PH09	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH10	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	BB	EE	EE	BB
PH11	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EF	CE	BB
PH12	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH13	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH14	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH15	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PH16	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	BB	EE	CE	BB
PH17	AB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CD	EE	CE	BB
PH18	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH19	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH20	BB	BB	BB	CC	DD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH21	BB	BB	BB	CC	CC	DD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH22	AB	BB	BB	CC	CC	DD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH23	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CF	BB
PH24	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	BC	EE	FF	BB
PH24	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CE	BB
PH25	BB	BB	BB	CC	CC	CC	AA	BC	0	0	BB	BB	FF	0	EE	0	BB
PH26	0	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH27	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	DD	BB
Prachuab Khiri Khan (PC)																	
PC01	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB

	MPI	*LGG-4*	*LGG-3*	*LGG-2*	*ME*	*AAAT-1*	*AAT-2*	*EST*	*IDH-1*	*IDH-2*	*MDH-1*	*MDH-2*	*GPI*	*PGD*	*PGM*	*HBDH*	*SOD*
PC02	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC03	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BD	EE	CC	BB
PC04	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CE	EE	CE	BB
PC05	DD	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CE	EE	BC	BB
PC06	DD	BB	BC	CC	CC	EE	AA	CC	0	AB	EE	BB	AA	0	CC	CC	BB
PC07	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	0	CE	CC	BB
PC08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC09	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	EF	BB
PC10	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FG	CC	0	CE	BB
PC11	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC12	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC13	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC14	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PC15	AB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	EF	CC	BB
PC16	00	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	FF	BB
PC17	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC18	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC19	BB	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC20	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	CE	CE	BB
PC21	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC22	BB	BB	BB	CC	CC	CC	AA	BB	0	AB	BB	BB	FF	0	EE	DD	BB
Chumphon (CP)																	
CP01	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	AA	EE	CE	BB
CP02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
CP03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
CP04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	BC	BB
CP05	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	CE	BC	0
CP06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CP07	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
CP08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BD	EE	0	BB
CP09	0	BB	BB	CC	CD	CC	AA	CC	BB	AB	BB	BB	FF	CC	CE	0	BB
Surat Thani (SR)																	
SR01	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CE	EE	CC	BB
SR02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
SR03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	0	BB
SR04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
SR05	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CE	BB
SR06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	CC	BB
SR07	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	BC	EE	CC	BB
SR08	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	BB	EE	CC	BB
SR09	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
SR10	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CE	BB
SR11	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
M. posthuma																	
MP01	0	BC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CE	0	CC	0	CC
MP02	0	CC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CC	0	CC	0	CC
MP03	0	BC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CE	0	CC	0	CC
MP04	CD	CC	BC	BB	CC	EE	AA	DD	DD	AB	EE	BB	CC	BB	CC	0	DD
MP05	DD	CC	BC	BB	CC	EE	AA	DD	0	AB	EE	BB	CC	0	CC	0	DD
MP06	CD	CC	BC	BB	CC	EE	AA	DD	0	AB	EE	BB	CC	0	CC	0	DD
MP07	CC	CC	AA	AA	CC	EE	AA	BB	DD	BB	EE	BB	0	AB	0	AA	AA
MP08	CC	BC	0	AA	CC	EE	AA	BB	DD	0	EE	BB	0	0	0	AA	AA
MP09	CC	CC	AC	AA	CC	EE	AA	DD	DD	BB	EE	BB	CC	AB	CC	AA	AA
MP10	BB	CC	BB	BB	CC	EE	AA	0	DD	BB	EE	BB	CC	AA	0	AA	CC
MP11	CC	CC	BB	BB	CC	EE	AA	DD	DD	BB	EE	BC	CC	AA	0	AA	CC
MP12	CC	CC	BB	BB	CC	EE	AA	DD	DD	BB	EE	BB	CC	AA	0	AA	CC
MP13	CC	CC	BB	BB	CC	EE	AA	BB	0	BB	EE	0	CC	BB	CC	EE	0
MP14	CC	CC	BB	BB	CC	EE	AA	BB	0	0	EE	0	CC	BB	C	EE	0

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
MP15	CC	CC	BB	BB	C	EE	AA	BB	BB	BB	EE	0	CC	BB	CC	EE	0
MP16	0	0	BB	0	CC	EE	AA	0	0	0	EE	0	0	0	0	CF	0
MP17	0	0	BB	0	CC	EE	AA	0	0	0	EE	0	CC	0	CC	CE	0
M. bahli																	
KS01	0	BB	BB	CC	FF	CC	AA	CC	BB	BB	CC	BB	CC	CC	AA	0	BB
KS02	FF	BC	BB	CC	EF	CC	AA	DD	BB	AB	CC	BB	CE	CC	AA	EE	BB
KS03	CD	BB	BB	CC	EE	BB	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS04	EF	BB	BB	CC	EF	CC	AA	CD		BB	CC	BB	CC	CC	AA	EE	BB
KS05	EE	BB	BB	CC	EF	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS06	EE	BB	BB	CC	EE	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS07	EF	CC	BB	CC	FG	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS08	FG	CC	BB	CC	FF	CC	AA	DD	BB	BB	CC	BB	CC	CD	AA	EE	BB
KS09	EF	BB	BB	CC	EF	BC	AA	DD	BB	BB	CC	BB	CE	CC	AA	EE	BB
KS10	GG	BC	BB	CC	EF	CC	AA	CD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS11	FG	BB	BB	CC	FG	CC	AA	DD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS12	EF	CC	BB	CC	GG	CC	AA	DD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS13	FG	CC	BB	CC	EE	CC	AA	DD	BB	BB	DD	BB	CE	CC	AA	EE	BB
KS14	FG	AA	BB	CC	FF	CC	AA	CC	BB	BB	DD	BB	CC	CD	AA	DD	BB
KS15	FG	AA	BB	CC	FF	CC	AA	CC	BB	AB	DD	BB	CC	CC	AA	DD	BB
KS16	FF	CC	BB	CC	FF	CC	AA	CC	BB	BB	DD	BB	CC	BC	AA	DD	BB
KS17	FG	AA	BB	CC	FF	CC	AA	BC	BB	BB	DD	BB	CC	BC	AA	DD	BB
KS18	EG	BC	BB	CC	EE	CC	AA	BC	BB	BB	CC	BB	CC	CC	AA	DD	BB
KS19	EF	BC	BB	CC	FG	CC	AA	CC	BB	AB	CC	BB	CC	CC	AA	DD	BB
KS20	GG	BB	BB	CC	EG	CC	AA	BC	BB	BB	CC	BB	CC	CC	AA	DD	BB

PPENDIX B

Table 10. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chiang Mai.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	5	4.500			
	A-B	8	9.000			
	B-B	5	4.500			
				.222	1	.637
ME						
	B-B	12	10.512			
	B-C	5	7.975			
	C-C	3	1.513			
				2.783	1	.095
AAT-1						
	B-B	19	19.013			
	B-C	1	.975			
	C-C	0	.013			
				.013	1	.909
EST						
	B-B	8	6.613			
	B-C	7	9.775			
	C-C	5	3.612			
				1.612	1	.204
MDH-2						
	A-A	2	.800			
	A-B	4	6.400			
	B-B	14	12.800			
				2.813	1	.094
PGD						
	B-B	3	3.368			
	B-C	10	9.263			
	C-C	6	6.368			
				.120	1	.729
PGM						
	B-B	0	.112			
	B-D	3	2.775			
	D-D	17	17.112			
				.131	1	.717
HBDH						
	C-C	19	19.013			
	C-D	1	.975			
	D-D	0	.013			
				.013	1	.909

Table 11. Chi-square test for deviation from Hardy-Weinberg equilibrium in Nan.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	2	1.513			
	A-B	7	7.975			
	B-B	11	10.512			
				.299	1	.585
LGG-4						
	A-A	0	.010			
	A-B	1	.980			
	B-B	24	24.010			
				.010	1	.919
EST						
	B-B	8	6.760			
	B-C	10	12.480			
	C-C	7	5.760			
				.987	1	.320
PGD						
	B-B	2	.844			
	B-C	5	7.313			
	C-C	17	15.844			
				2.400	1	.121

Table 12. Chi-square test for deviation from Hardy-Weinberg equilibrium in Tak.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	10	9.592			
	A-B	7	7.816			
	B-B	2	1.592			
				.207	1	.649
ME						
	B-B	17	16.200			
	B-C	2	3.600			
	C-C	1	.200			
				3.951	1	.047
AAT-1						
	B-B	18	18.050			
	B-C	2	1.900			
	C-C	0	.050			
				.055	1	.814
EST						
	B-B	18	18.050			
	B-C	2	1.900			
	C-C	0	.050			
				.055	1	.814
IDH-2						
	A-A	0	.013			
	A-B	1	.975			
	B-B	19	19.013			
				.013	1	.909
PGD						
	B-B	5	2.813			
	B-C	5	9.375			
	C-C	10	7.813			
				4.356	1	.037
HBDH						
	C-C	12	11.681			
	C-D	5	5.639			
	D-D	1	.681			
				.231	1	.631

Table 13. Chi-square test for deviation from Hardy-Weinberg equilibrium in Phitsanulok.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	12	12.150			
	A-B	3	2.700			
	B-B	0	.150			
				.185	1	.667
LGG-4						
	A-A	0	.250			
	A-B	4	3.500			
	B-B	12	12.250			
				.327	1	.568
AAT-1						
	A-A	0	.016			
	A-B	1	.969			
	B-B	15	15.016			
				.017	1	.897
MDH-1						
	A-A	0	.250			
	A-B	4	3.500			
	B-B	12	12.250			
				.327	1	.568
MDH-2						
	A-A	0	.391			
	A-B	5	4.219			
	B-B	11	11.391			
				.549	1	.459
PGD						
	B-B	5	4.516			
	B-C	7	7.969			
	C-C	4	3.516			
				.236	1	.627

Table 14. Chi-square test for deviation from Hardy-Weinberg equilibrium in Nakhon Sawan.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	3	1.760			
	A-B	7	9.479			
	B-B	14	12.760			
				1.642	1	.200
ME						
	B-B	25	24.300			
	B-C	4	5.400			
	C-C	1	.300			
				2.016	1	.156
EST						
	B-B	23	23.408			
	B-C	7	6.183			
	C-C	0	.408			
				.523	1	.469
IDH-2						
	A-A	0	.034			
	A-B	2	1.931			
	B-B	27	27.034			
				.037	1	.847
PGD						
	B-B	2	.675			
	B-C	5	7.650			
	C-C	23	21.675			
				3.600	1	.058
PGM						
	B-B	0	.008			
	B-D	1	.983			
	D-D	29	29.008			
				.009	1	.926

Table 15. Chi-square test for deviation from Hardy-Weinberg equilibrium in Ayutthaya.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	0	.050			
	A-B	2	1.900			
	B-B	18	18.050			
				.055	1	.814
ME						
	B-B	17	16.667			
	B-C	6	6.667			
	C-C	1	.667			
				.240	1	.624
IDH-2						
	A-A	0	.045			
	A-B	2	1.909			
	B-B	20	20.045			
				.050	1	.823
PGD						
	B-B	0	.043			
	B-C	2	1.913			
	C-C	21	21.043			
				.048	1	.827
PGM						
	B-B	12	10.446			
	B-D	7	10.109			
	D-D	4	2.446			
				2.175	1	.140

Table 16. Chi-square test for deviation from Hardy-Weinberg equilibrium in Bangkok.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	1	.267			
	A-B	2	3.467			
	B-B	12	11.267			
				2.685	1	.101
ME						
	B-B	11	12.012			
	B-C	9	6.975			
	C-C	0	1.013			
				1.686	1	.194
EST						
	B-B	17	17.112			
	B-C	3	2.775			
	C-C	0	.112			
				.131	1	.717
IDH-1						
	A-A	0	.118			
	A-B	3	2.763			
	B-B	16	16.118			
				.140	1	.709
GPI						
	D-D	0	.050			
	D-E	2	1.900			
	E-E	18	18.050			
				.055	1	.814
PGD						
	B-B	6	5.512			
	B-C	9	9.975			
	C-C	5	4.512			
				.191	1	.662
PGM						
	B-B	0	.013			
	B-D	1	.975			
	D-D	19	19.013			
				.013	1	.909
HBDH						
	C-C	16	16.118			
	C-D	3	2.763			
	D-D	0	.118			
				.140	1	.709

Table 17. Chi-square test for deviation from Hardy-Weinberg equilibrium in Sa Kaeo.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	0	.160			
	A-B	4	3.680			
	B-B	21	21.160			
				.189	1	.664
ME						
	B-B	29	28.073			
	B-C	1	1.903			
	B-D	0	.952			
	C-C	0	.032			
	C-D	1	.032			
	D-D	0	.008			
				30.483	3	.000
EST						
	B-B	15	15.408			
	B-C	13	12.183			
	C-C	2	2.408			
				.135	1	.714
PGD						
	B-B	2	1.565			
	B-C	9	9.870			
	C-C	16	15.565			
				.210	1	.647
PGM						
	B-B	1	.516			
	B-D	6	6.968			
	D-D	24	23.516			
				.598	1	.439
HBDH						
	C-C	16	14.491			
	C-D	4	4.241			
	C-E	5	7.776			
	D-D	1	.310			
	D-E	0	1.138			
	E-E	3	1.043			
				7.503	3	.057

Table 18. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chanthaburi.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	4	5.333			
	A-B	8	5.333			
	B-B	0	1.333			
				3.000	1	.083
ME						
	B-B	1	.766			
	B-C	5	5.469			
	C-C	10	9.766			
				.118	1	.732
EST						
	B-B	0	.025			
	B-C	1	.950			
	C-C	9	9.025			
				.028	1	.868
MDH-1						
	B-B	14	14.063			
	B-C	2	1.875			
	C-C	0	.063			
				.071	1	.790
PGM						
	B-B	2	1.000			
	B-D	4	5.750			
	B-E	0	.250			
	D-D	9	8.266			
	D-E	1	.719			
	E-E	0	.016			
				1.974	3	.578

Table 19. Chi-square test for deviation from Hardy-Weinberg equilibrium in Phetchaburi.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	0	.038			
	A-B	2	1.923			
	B-B	24	24.038			
				.042	1	.838
LGG-2						
	B-B	0	.009			
	B-C	1	.982			
	C-C	27	27.009			
				.009	1	.923
ME						
	B-B	19	17.286			
	B-C	6	9.429			
	C-C	3	1.286			
				3.702	1	.054
AAT-1						
	B-B	16	13.580			
	B-C	7	11.839			
	C-C	5	2.580			
				4.678	1	.031
EST						
	B-B	26	26.036			
	B-C	2	1.929			
	C-C	0	.036			
				.038	1	.845
PGD						
	B-B	5	2.370			
	B-C	6	10.963			
	B-D	0	.296			
	C-C	15	12.676			
	C-D	1	.685			
	D-D	0	.009			
				6.040	3	.110
PGM						
	B-B	0	.009			
	B-D	1	.982			
	D-D	27	27.009			
				.009	1	.923
HBDH						
	C-C	13	10.938			
	C-D	8	8.750			
	C-E	1	4.375			
	D-D	3	1.750			
	D-E	0	1.750			
	E-E	3	.438			
				20.709	3	.000

Table 20. Chi-square test for deviation from Hardy-Weinberg equilibrium in Prachuab Khiri Khan.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	0	.056			
	A-B	2	1.889			
	B-B	16	16.056			
				.062	1	.803
LGG-2						
	B-B	0	.012			
	B-C	1	.976			
	C-C	20	20.012			
				.012	1	.911
ME						
	B-B	16	16.298			
	B-C	5	4.405			
	C-C	0	.298			
				.383	1	.536
AAT-1						
	B-B	18	18.107			
	B-C	3	2.786			
	C-C	0	.107			
				.124	1	.724
EST						
	B-B	20	20.012			
	B-C	1	.976			
	C-C	0	.012			
				.012	1	.911
PGD						
	C-C	16	16.118			
	C-D	3	2.763			
	D-D	0	.118			
				.140	1	.709
PGM						
	B-B	0	.200			
	B-D	4	3.500			
	B-E	0	.100			
	D-D	15	15.313			
	D-E	1	.875			
	E-E	0	.013			
				.408	3	.939
HBDH						
	C-C	5	5.762			
	C-D	12	10.476			
	D-D	4	4.762			
				.444	1	.505

Table 21. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chumphon.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
ME						
	B-B	8	8.028			
	B-C	1	.944			
	C-C	0	.028			
				.031	1	.860
EST						
	B-B	5	5.444			
	B-C	4	3.111			
	C-C	0	.444			
				.735	1	.391
PGD						
	B-B	3	1.778			
	B-C	2	4.444			
	C-C	4	2.778			
				2.723	1	.099
PGM						
	B-B	0	.111			
	B-D	2	1.778			
	D-D	7	7.111			
				.141	1	.708
HBDH						
	B-B	0	.125			
	B-C	2	1.625			
	B-D	0	.125			
	C-C	5	5.281			
	C-D	1	.813			
	D-D	0	.031			
				.426	3	.935

Table 22. Chi-square test for deviation from Hardy-Weinberg equilibrium in Surat Thani.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
ME						
	B-B	10	10.023			
	B-C	1	.955			
	C-C	0	.023			
				.025	1	.875
EST						
	B-B	2	1.000			
	B-C	2	4.000			
	C-C	5	4.000			
				2.250	1	.134
PGD						
	B-B	5	5.114			
	B-C	5	4.091			
	B-D	0	.682			
	C-C	0	.818			
	C-D	1	.273			
	D-D	0	.023			
				3.667	3	.300
PGM						
	B-B	0	.023			
	B-D	1	.955			
	D-D	10	10.023			
				.025	1	.875
HBDH						
	C-C	8	8.205			
	C-D	3	2.591			
	D-D	0	.205			
				.274	1	.601

Table 23. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metaphire* sp.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	G-G	0	.077			
	G-H	2	1.846			
	H-H	11	11.077			
				.090	1	.764
LGG-4						
	D-D	12	12.019			
	D-E	1	.962			
	E-E	0	.019			
				.021	1	.885
ME						
	A-A	3	1.923			
	A-B	4	6.154			
	B-B	6	4.923			
				1.593	1	.207
AAT-1						
	A-A	1	.521			
	A-B	3	2.083			
	A-C	0	1.875			
	B-B	2	2.083			
	B-C	3	3.750			
	C-C	3	1.688			
				3.893	3	.273
EST						
	C-C	1	1.333			
	C-D	6	5.333			
	D-D	5	5.333			
				.188	1	.665
GPI						
	B-B	7	6.942			
	B-C	5	5.115			
	C-C	1	.942			
				.007	1	.935
PGM						
	B-B	4	3.273			
	B-C	4	5.455			
	C-C	3	2.273			
				.782	1	.376

Table 24. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metahire posthuma*.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	C-C	6	6.021			
	C-D	5	4.958			
	D-D	1	1.021			
				.001	1	.977
LGG-4						
	B-B	0	.067			
	B-C	2	1.867			
	C-C	13	13.067			
				.077	1	.782
LGG-3						
	B-B	10	10.563			
	B-C	6	4.875			
	C-C	0	.563			
				.852	1	.356
LGG-2						
	A-A	0	.600			
	A-B	6	4.800			
	B-B	9	9.600			
				.937	1	.333
EST						
	A-A	2	1.455			
	A-B	4	3.636			
	A-C	0	1.455			
	B-B	3	2.273			
	B-C	0	1.818			
	C-C	2	.364			
				11.110	3	.011
MDH-2						
	A-A	13	13.235			
	A-B	4	3.529			
	B-B	0	.235			
				.302	1	.582

Table 25. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metaphire bahli*.

Locus	Class	frequency	Observed frequency	Expected square	Chi- DF	P
MPI						
	D-D	0	.050			
	D-E	2	1.000			
	D-F	0	.900			
	E-E	5	5.000			
	E-F	8	9.000			
	F-F	5	4.050			
				2.284	3	.516
LGG-4						
	B-B	8	6.613			
	B-C	7	9.775			
	C-C	5	3.612			
				1.612	1	.204
ME						
	C-C	8	6.050			
	C-D	6	9.900			
	D-D	6	4.050			
				3.104	1	.078
AAT-1						
	A-A	1	.112			
	A-B	1	2.775			
	B-B	18	17.112			
				8.183	1	.004
EST						
	B-B	1	.613			
	B-C	5	5.775			
	C-C	14	13.613			
				.360	1	.548
IDH-1						
	B-B	19	19.013			
	B-C	1	.975			
	C-C	0	.013			
				.013	1	.909
IDH-2						
	A-A	0	.050			
	A-B	2	1.900			
	B-B	18	18.050			
				.055	1	.814
GPI						
	B-B	13	13.613			
	B-C	7	5.775			
	C-C	0	.613			
				.900	1	.343
PGD						
	B-B	0	.050			
	B-C	2	1.800			
	B-D	0	.100			
	C-C	16	16.200			
	C-D	2	1.800			
	D-D	0	.050			
				.247	3	.970

APPENDIX C

Table 26. Significance test using exact probabilities in Chiang Mai.

Locus	R1	R2	R3	P
MPI	5	8	5	0.654
ME	12	5	3	0.107
AAT-1	19	1	0	1.000
EST	8	7	5	0.204
MDH-2	14	4	2	0.129
PGD	6	10	3	1.000
PGM	17	3	0	1.000
HBDH	19	1	0	1.000

Table 27. Significance test using exact probabilities in Nan.

Locus	R1	R2	R3	P
MPI	11	7	2	0.591
LGG-4	24	1	0	1.000
EST	8	10	7	0.422
PGD	17	5	2	0.152

Table 28. Significance test using exact probabilities in Tak.

Locus	R1	R2	R3	P
MPI	10	7	2	0.607
ME	17	2	1	0.150
AAT-1	18	2	0	1.000
EST	18	2	0	1.000
IDH-2	19	1	0	1.000
PGD	10	5	5	0.053
HBDH	12	5	1	0.512

Table 29. Significance test using exact probabilities in Phitsanulok.

Locus	R1	R2	R3	P
MPI	12	3	0	1.000
LGG-4	12	4	0	1.000
AAT-1	15	1	0	1.000
MDH-1	12	4	0	1.000
MDH-2	11	5	0	1.000
PGD	5	7	4	0.638

Table 30. Significance test using exact probabilities in Nakhon Sawan.

Locus	R1	R2	R3	P
MPI	14	7	3	0.291
ME	25	4	1	0.241
EST	23	7	0	1.000
IDH-2	27	2	0	1.000
PGD	23	5	2	0.099
PGM	29	1	0	1.000

Table 31. Significance test using exact probabilities in Ayutthaya.

Locus	R1	R2	R3	P
MPI	12	2	1	0.199
ME	11	9	0	0.528
EST	17	3	0	1.000
IDH-1	16	3	0	1.000
GPI	18	2	0	1.000
PGD	6	9	5	0.670
PGM	19	1	0	1.000
HBDH	16	3	0	1.000

Table 32. Significance test using exact probabilities in Bangkok.

Locus	R1	R2	R3	P
MPI	18	2	0	1.000
ME	17	6	1	0.501
IDH-2	20	2	0	1.000
PGD	21	2	0	1.000
PGM	12	7	4	0.162

Table 33. Significance test using exact probabilities in Sa Kaeo.

Locus	R1	R2	R3	P
MPI	21	4	0	1.000
ME	29	1	1	0.049
EST	15	13	2	1.000
PGD	16	9	2	0.614
PGM	24	6	1	0.403
HBDH	16	9	4	0.190

Table 34. Significance test using exact probabilities in Chanthaburi.

Locus	R1	R2	R3	P
MPI	4	8	0	0.216
ME	10	5	1	1.000
EST	9	1	0	1.000
MDH-1	14	2	0	1.000
PGM	9	5	2	0.530

Table 35. Significance test using exact probabilities in Phetchaburi.

Locus	R1	R2	R3	P
MPI	24	2	0	1.000
LGG-2	27	1	0	1.000
ME	19	6	3	0.073
AAT-1	16	7	5	0.064
EST	26	2	0	1.000
PGD	15	7	5	0.065
PGM	27	1	0	1.000
HBDH	13	9	6	0.112

Table 36. Significance test using exact probabilities in Prachuab Khiri Khan.

Locus	R1	R2	R3	P
MPI	16	2	0	1.000
LGG-2	20	1	0	1.000
ME	16	5	0	1.000
AAT-1	18	3	0	1.000
EST	20	1	0	1.000
PGD	16	3	0	1.000
PGM	15	5	0	1.000
HBDH	5	12	4	0.675

Table 37. Significance test using exact probabilities in Chumphon.

Locus	R1	R2	R3	P
ME	8	1	0	1.000
EST	5	4	0	1.000
PGD	4	2	3	0.168
PGM	7	2	0	1.000
HBDH	5	3	0	1.000

Table 38. Significance test using exact probabilities in Surat Thani**.**

Locus	R1	R2	R3	P
ME	10	1	0	1.000
EST	5	2	2	0.163
PGD	5	5	1	1.000
PGM	10	1	0	1.000
HBDH	8	3	0	1.000

Table 39. Significance test using exact probabilities in *Metaphire* sp.

Locus	R1	R2	R3	P
MPI	11	2	0	1.000
LGG-4	12	1	0	1.000
ME	6	4	3	0.256
AAT-1	2	6	4	1.002
EST	5	6	1	1.000
GPI	7	5	1	1.000
PGM	4	4	3	0.543

Table 40. Significance test using exact probabilities in *M. posthuma*.

Locus	R1	R2	R3	P
MPI	6	5	1	1.000
LGG-4	13	2	0	1.000
LGG-3	10	6	0	1.000
LGG-2	9	6	0	1.000
EST	3	4	4	0.543
MDH-2	13	4	0	1.000

Table 41. Significance test using exact probabilities in *M. bahli*.

Locus	R1	R2	R3	P
MPI	5	10	5	1.000
LGG-4	8	7	5	0.204
ME	8	6	6	0.085
AAT-1	18	1	1	0.077
EST	14	5	1	0.468
IDH-1	19	1	0	1.000
IDH-2	18	2	0	1.000
GPI	13	7	0	1.000
PGD	16	4	0	1.000

APPENDICES

APPENDIX A

genotype data set of *Metaphire peguana*

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
Chiengmai (CM)																	
CM01	AB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	AC	EE	CC	BB
CM02	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	AC	EE	CC	BB
CM03	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	AB	FF	0	EE	CC	BB
CM04	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM05	AB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AB	FF	BC	EE	CE	BB
CM06	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AB	FF	BB	EE	CC	BB
CM07	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM08	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM09	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
CM10	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	AA	FF	CC	EE	CC	BB
CM11	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM12	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
CM13	AA	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM14	0	BB	BB	CC	DD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM15	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM16	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CM17	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CM18	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	EE	BB
CM19	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	AB	FF	BB	CE	EE	BB
CM20	AB	BB	BB	CC	CC	CD	AA	BB	BB	BB	BB	BB	FF	CC	CE	EE	BB
Nan (NA)																	
NA01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA02	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	AC	EE	CC	BB
NA03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	0	EE	CC	BB
NA04	0	BB	BB	CC	CC	CC	AA	CC	BB	0	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
NA05	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA06	0	BB	BB	CC	CC	CC	AA	DD	0	BB	BB	BB	FF	CC	EE	CC	BB
NA07	0	BB	BB	CC	CC	CC	AA	DD	DD	0	BB	BB	FF	CC	EE	CC	BB
NA08	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA09	0	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	EE	CC	BB
NA10	BB	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FF	CC	EE	CC	BB
NA11	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NA12	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA13	BB	AB	BB	CC	CC	CC	AA	CC	BB	0	BB	BB	FF	CC	EE	CC	BB
NA14	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA15	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA16	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA17	AB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NA18	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	AB	BB	FF	CC	EE	CC	BB
NA19	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	CC	EE	CC	BB
NA20	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AA	FF	CC	EE	CC	BB
NA21	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AB	FF	CC	EE	CC	BB
NA22	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	AB	FF	BC	EE	EE	BB
NA23	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	AB	FF	BC	EE	EE	BB
NA24	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	EE	BB
NA25	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	AB	BB	FF	BB	EE	EE	BB
Phrae (PR)																	
PR01	HH	CC	AA	BB	CC	AC	AA	DD	DD	BB	DD	CC	BB	DD	BB	DD	BB
PR02	HH	CC	AA	BB	AA	DD	AA	0	DD	AB	DD	CC	DD	DD	DD	DD	BB
PR03	HH	CC	AA	BB	AC	DD	AA	DE	DD	BB	DD	CC	BB	DD	DD	DD	BB
PR04	HH	DD	AA	BB	AC	CD	AA	DE	DD	BB	DD	CC	BD	EE	BD	CC	BB
PR05	HH	DD	AA	BB	AC	CC	AA	DE	DD	BB	DD	CC	BD	DE	BD	CC	BB
PR06	HH	DD	AA	BB	AC	CC	AA	DE	BB	AB	DD	CC	BB	EE	BD	CE	BB
PR07	HH	DD	AA	AA	CC	AC	AA	DE	DD	BB	DD	CC	BD	DD	DD	CC	BB

	MPI	*LGG-4*	*LGG-3*	*LGG-2*	*ME*	*AAAT-1*	*AAT-2*	*EST*	*IDH-1*	*IDH-2*	*MDH-1*	*MDH-2*	*GPI*	*PGD*	*PGM*	*HBDH*	*SOD*
PR08	HH	DD	AA	AA	AA	DD	AA	EE	DD	AB	DD	CC	BB	0	BB	CC	BB
PR09	GH	DD	AA	AA	AC	CD	AA	DE	DD	BB	DD	CC	BD	EE	BD	CC	BB
PR10	GH	DE	AA	AA	CC	CD	AA	EE	BB	BB	DD	CC	BB	CD	BB	CC	BB
PR11	HH	DD	AA	AA	AC	AC	AA	EE	DD	BB	DD	CC	BB	DD	BB	CC	BB
PR12	HH	DD	AA	AA	AC	0	AA	EE	DD	AB	DD	CC	BD	0	0	CC	BB
PR13	HH	DD	AA	AA	CC	AC	AA	EE	DD	BB	DD	CC	BB	DD	0	CC	BB
Tak (TK)																	
TK01	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK02	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK04	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK05	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
TK07	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
TK08	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
TK09	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK10	CD	BB	AB	CC	CD	CD	AA	AA	0	BB	EE	BC	FF	DD	AA	BD	BB
TK11	AA	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK12	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK13	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CC	BB
TK14	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
TK15	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	AA	FF	CC	EE	CC	BB
TK16	AA	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	AB	FF	CC	EE	EE	BB
TK17	AA	BB	BB	CC	DD	CC	AA	CC	BB	AB	BB	BB	FF	BB	EE	CC	BB
TK18	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
TK19	0	BB	BB	CC	CC	CD	AA	CC	BB	AB	BB	BB	FF	CC	EE	EE	BB
TK20	AA	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BB	EE	EE	BB
TK21	AB	BB	BB	CC	CC	CD	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
Pitsanulok (PN)																	

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
PN01	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	CC	EE	0	BB
PN02	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	0	BB
PN03	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	CC	BB
PN04	AB	BB	BB	CC	CC	CC	AA	0	DD	BB	BB	AB	FF	BC	EE	CC	BB
PN05	AB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
PN06	AB	AB	BB	CC	CC	AC	AA	DD	BB	BB	AB	BB	FF	BB	EE	0	BB
PN07	AB	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	CC	BB
PN08	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	BB	AB	FF	BC	EE	CC	BB
PN09	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	AB	FF	BC	EE	0	BB
PN10	AB	AB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	BC	EE	0	BB
PN11	AA	AB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
PN12	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CC	BB
PN13	AA	BB	BB	CC	CC	CC	AA	DD	BB	BB	AB	BB	FF	CC	EE	CC	BB
PN14	0	AB	BB	CC	CC	CC	AA	CC	BB	AB	AB	AB	FF	BB	EE	0	BB
PN15	AA	BB	BB	CC	CC	CC	AA	CC	0	BB	AB	AB	FF	CC	EE	CC	BB
PN16	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	AB	AB	FF	CC	EE	CC	BB
Nakhormsawan (NS)																	
NS01	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	AC	EE	CC	BB
NS02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS03	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS05	AA	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS07	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS08	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS09	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS10	AA	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS11	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS12	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
NS13	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS14	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
NS15	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	AA	BB	EE	CC	BB
NS16	BB	BB	BB	CC	CC	CC	AA	AA	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS17	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS18	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS19	AB	BB	BB	CC	CC	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS20	0	BB	BB	CC	CC	CC	AA	AB	0	0	BB	BB	FF	CC	EE	0	BB
NS21	BB	BB	BB	CC	CC	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	0	BB
NS22	AB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS23	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS24	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
NS25	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
NS26	0	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
NS27	0	BB	BB	CC	CC	CC	AA	BB	BB	AB	BB	BB	FF	CC	EE	EE	BB
NS28	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
NS29	BB	BB	BB	CC	CC	CC	AA	BB	BB	AB	BB	BB	FF	BB	EE	0	BB
NS30	0	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	0	BB
Ayutthaya (AY)																	
AY01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
AY02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
AY03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	CC	CC	BB
AY05	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY06	AB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY07	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	0	BB
AY08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY09	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY10	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
AY11	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY12	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
AY13	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY14	BB	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FG	CC	EE	0	BB
AY15	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY16	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	CC	CC	BB
AY17	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	CC	CC	CC	BB
AY18	AB	BB	BB	CC	CD	CC	AA	AB	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY19	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
AY20	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	0	BB
AY21	0	BB	BB	CC	CC	CC	AA	0	0	0	BB	BB	FG	0	CC	0	BB
AY22	AA	BB	BB	CC	CC	CC	AA	BB	0	0	BB	BB	FF	CC	EE	0	BB
AY23	0	BB	BB	CC	CD	CC	AA	AB	BB	AB	BB	BB	FF	CC	EE	0	BB
AY24	BB	BB	BB	CC	CD	CC	AA	0	0	AB	BB	BB	FF	CC	CE	0	BB
Bangkok (BK)																	
BK01	BB	BB	BB	CC	CD	CC	AA	CC	0	BB	BB	BB	CF	BB	EE	CC	BB
BK02	BB	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK03	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK04	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
BK05	0	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK06	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
BK07	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK08	0	BB	BB	CC	CD	CC	AA	CC	BB	0	BB	BB	FF	BB	EE	CE	BB
BK09	0	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	EF	CC	EE	C	BB
BK10	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
BK11	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
BK12	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	C	BB
BK13	0	BB	BB	CC	CC	CC	AA	CC	0	BB	BB	BB	FF	CC	EE	CC	BB
BK14	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	CE	CC	BB

	MPI	*LGG-4*	*LGG-3*	*LGG-2*	*ME*	*AAAT-1*	*AAT-2*	*EST*	*IDH-1*	*IDH-2*	*MDH-1*	*MDH-2*	*GPI*	*PGD*	*PGM*	*HBDH*	*SOD*
BK15	AA	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
BK16	BB	BB	BB	CC	DD	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	0	BB
BK17	BB	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	BC	EE	0	BB
BK18	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	EE	BB
BK19	AB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	EE	BB
BK20	BB	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	EE	BB
Sa Kaeo (SK)																	
SK01	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
SK02	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	AC	EE	CE	BB
SK03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	E	BB
SK04	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK05	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
SK06	0	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BC	EE	CE	BB
SK07	BB	BB	BB	CC	CC	CC	AA	CD	AB	BB	BB	BB	FF	CC	EE	CC	BB
SK08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK09	0	BB	BB	CC	BC	CC	AA	CD	BB	BB	BB	BB	FF	CC	CE	CC	BB
SK10	AB	BB	BB	CC	CC	CC	AA	CD	0	BB	BB	BB	FF	BC	EE	CE	BB
SK11	BB	BB	BB	CC	BC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK12	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	FF	BB
SK13	BB	BB	BB	CC	CC	CC	AA	CD	AB	BB	BB	BB	FF	CC	CE	CC	BB
SK14	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	CC	CC	BB
SK15	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CD	EE	CC	BB
SK16	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK17	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK18	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	CC	BB
SK19	0	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK20	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	CF	CC	EE	CC	BB
SK21	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK22	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	CE	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
SK23	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	FF	BB
SK24	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
SK25	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
SK26	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	EE	CC	BB
SK27	0	BB	BB	CC	CC	CC	AA	BB	0	BB	BB	BB	FF	CC	EE	EE	BB
SK28	AB	CC	BB	CC	EE	AA	AA	0	CC	AA	AB	BC	AA	CC	CE	0	BB
SK29	0	BB	BB	CC	EE	CC	AA	0	0	BB	BB	BB	FG	CC	EE	0	BB
SK30	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	BC	CE	CC	BB
SK31	BB	BB	BB	CC	CC	CC	AA	BC	BB	BB	BB	BB	FF	CC	CE	CE	BB
SK32	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
Chanthaburi (CH)																	
CH01	BB	BB	BB	CC	DD	CC	AA	DD	0	BB	BB	BB	FF	CC	CC	CC	BB
CH02	BB	BB	BB	CC	CC	CC	AA	0	0	0	BB	BB	FF	CC	EE	CC	BB
CH03	BB	BB	BB	CC	DD	CC	AA	0	BB	AB	BB	BB	FF	CC	CE	CC	BB
CH04	BB	BB	BB	CC	DD	CC	AA	DD	0	BB	BB	BB	FF	CC	CC	CC	BB
CH05	AB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH06	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	CE	CC	BB
CH07	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BD	BB	FG	CC	EE	CC	BB
CH08	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	CE	CC	BB
CH09	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH10	AA	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH11	AB	BB	BB	CC	DD	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH12	BB	BB	BB	CC	DD	CC	AA	DD	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH13	0	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	CC	EE	CC	BB
CH14	AB	BB	BB	CC	CD	CC	AA	0	BB	BB	BB	BB	FF	CC	CE	0	BB
CH15	AB	BB	BB	CC	CD	CC	AA	0	BB	0	BD	BB	FF	CC	EF	CC	BB
CH16	BB	BB	BB	CC	CD	CC	AA	0	0	BB	BB	BB	FG	0	EE	0	BB
Phetchaburi (PH)																	
PH01	BB	BB	BB	CC	CC	AC	AA	CC	BB	0	BB	BB	FF	CC	EE	CE	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
PH02	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	AC	EE	EE	BB
PH03	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH04	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB
PH05	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PH07	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH08	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
PH09	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH10	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	BB	EE	EE	BB
PH11	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EF	CE	BB
PH12	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH13	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH14	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH15	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PH16	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	BB	EE	CE	BB
PH17	AB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CD	EE	CE	BB
PH18	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH19	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH20	BB	BB	BB	CC	DD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH21	BB	BB	BB	CC	CC	DD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PH22	AB	BB	BB	CC	CC	DD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CC	BB
PH23	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CF	BB
PH24	BB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	BC	EE	FF	BB
PH24	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BB	EE	CE	BB
PH25	BB	BB	BB	CC	CC	CC	AA	BC	0	0	BB	BB	FF	0	EE	0	BB
PH26	0	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	CE	BB
PH27	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	BC	EE	DD	BB
Prachuab Khiri Khan (PC)																	
PC01	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	CE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
PC02	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC03	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BD	EE	CC	BB
PC04	AB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CE	EE	CE	BB
PC05	DD	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CE	EE	BC	BB
PC06	DD	BB	BC	CC	CC	EE	AA	CC	0	AB	EE	BB	AA	0	CC	CC	BB
PC07	0	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	0	CE	CC	BB
PC08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC09	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	CE	EF	BB
PC10	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FG	CC	0	CE	BB
PC11	BB	BB	BB	CC	CC	CD	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC12	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC13	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC14	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
PC15	AB	BB	BB	CC	CD	CD	AA	CC	BB	BB	BB	BB	FF	CC	EF	CC	BB
PC16	00	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	FF	BB
PC17	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC18	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	EE	BB
PC19	BB	BB	BB	CC	CD	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC20	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	CE	CE	BB
PC21	BB	BB	BB	CC	CC	CC	AA	BB	BB	BB	BB	BB	FF	CC	EE	CE	BB
PC22	BB	BB	BB	CC	CC	CC	AA	BB	0	AB	BB	BB	FF	0	EE	DD	BB
Chumphon (CP)																	
CP01	BB	BB	BB	CC	CD	CC	AA	CD	BB	BB	BB	BB	FF	AA	EE	CE	BB
CP02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	0	BB
CP03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	CC	BB
CP04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	CC	EE	BC	BB
CP05	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BB	CE	BC	0
CP06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
CP07	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	CC	EE	CC	BB

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
CP08	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BD	EE	0	BB
CP09	0	BB	BB	CC	CD	CC	AA	CC	BB	AB	BB	BB	FF	CC	CE	0	BB
Surat Thani (SR)																	
SR01	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	CE	EE	CC	BB
SR02	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CC	BB
SR03	BB	BB	BB	CC	CC	CC	AA	CD	BB	BB	BB	BB	FF	BC	EE	0	BB
SR04	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BC	EE	CE	BB
SR05	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CE	BB
SR06	BB	BB	BB	CC	CC	CC	AA	CC	BB	BB	BB	BB	FF	BB	EE	CC	BB
SR07	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	BC	EE	CC	BB
SR08	BB	BB	BB	CC	CC	CC	AA	0	BB	BB	BB	BB	FF	BB	EE	CC	BB
SR09	BB	BB	BB	CC	CD	CC	AA	CC	BB	BB	BB	BB	FF	BC	CE	CC	BB
SR10	BB	BB	BB	CC	CD	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CE	BB
SR11	BB	BB	BB	CC	CC	CC	AA	DD	BB	BB	BB	BB	FF	BB	EE	CC	BB
M. posthuma																	
MP01	0	BC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CE	0	CC	0	CC
MP02	0	CC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CC	0	CC	0	CC
MP03	0	BC	BC	BB	CC	EE	AA	0	0	0	EE	BB	CE	0	CC	0	CC
MP04	CD	CC	BC	BB	CC	EE	AA	DD	DD	AB	EE	BB	CC	BB	CC	0	DD
MP05	DD	CC	BC	BB	CC	EE	AA	DD	0	AB	EE	BB	CC	0	CC	0	DD
MP06	CD	CC	BC	BB	CC	EE	AA	DD	0	AB	EE	BB	CC	0	CC	0	DD
MP07	CC	CC	AA	AA	CC	EE	AA	BB	DD	BB	EE	BB	0	AB	0	AA	AA
MP08	CC	BC	0	AA	CC	EE	AA	BB	DD	0	EE	BB	0	0	0	AA	AA
MP09	CC	CC	AC	AA	CC	EE	AA	DD	DD	BB	EE	BB	CC	AB	CC	AA	AA
MP10	BB	CC	BB	BB	CC	EE	AA	0	DD	BB	EE	BB	CC	AA	0	AA	CC
MP11	CC	CC	BB	BB	CC	EE	AA	DD	DD	BB	EE	BC	CC	AA	0	AA	CC
MP12	CC	CC	BB	BB	CC	EE	AA	DD	DD	BB	EE	BB	CC	AA	0	AA	CC
MP13	CC	CC	BB	BB	CC	EE	AA	BB	0	BB	EE	0	CC	BB	CC	EE	0
MP14	CC	CC	BB	BB	CC	EE	AA	BB	0	0	EE	0	CC	BB	C	EE	0

	MPI	***LGG-4***	***LGG-3***	***LGG-2***	***ME***	***AAAT-1***	***AAT-2***	***EST***	***IDH-1***	***IDH-2***	***MDH-1***	***MDH-2***	***GPI***	***PGD***	***PGM***	***HBDH***	***SOD***
MP15	CC	CC	BB	BB	C	EE	AA	BB	BB	BB	EE	0	CC	BB	CC	EE	0
MP16	0	0	BB	0	CC	EE	AA	0	0	0	EE	0	0	0	0	CF	0
MP17	0	0	BB	0	CC	EE	AA	0	0	0	EE	0	CC	0	CC	CE	0
M. bahli																	
KS01	0	BB	BB	CC	FF	CC	AA	CC	BB	BB	CC	BB	CC	CC	AA	0	BB
KS02	FF	BC	BB	CC	EF	CC	AA	DD	BB	AB	CC	BB	CE	CC	AA	EE	BB
KS03	CD	BB	BB	CC	EE	BB	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS04	EF	BB	BB	CC	EF	CC	AA	CD		BB	CC	BB	CC	CC	AA	EE	BB
KS05	EE	BB	BB	CC	EF	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS06	EE	BB	BB	CC	EE	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS07	EF	CC	BB	CC	FG	CC	AA	DD	BB	BB	CC	BB	CC	CC	AA	EE	BB
KS08	FG	CC	BB	CC	FF	CC	AA	DD	BB	BB	CC	BB	CC	CD	AA	EE	BB
KS09	EF	BB	BB	CC	EF	BC	AA	DD	BB	BB	CC	BB	CE	CC	AA	EE	BB
KS10	GG	BC	BB	CC	EF	CC	AA	CD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS11	FG	BB	BB	CC	FG	CC	AA	DD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS12	EF	CC	BB	CC	GG	CC	AA	DD	BB	BB	DD	BB	CC	CC	AA	EE	BB
KS13	FG	CC	BB	CC	EE	CC	AA	DD	BB	BB	DD	BB	CE	CC	AA	EE	BB
KS14	FG	AA	BB	CC	FF	CC	AA	CC	BB	BB	DD	BB	CC	CD	AA	DD	BB
KS15	FG	AA	BB	CC	FF	CC	AA	CC	BB	AB	DD	BB	CC	CC	AA	DD	BB
KS16	FF	CC	BB	CC	FF	CC	AA	CC	BB	BB	DD	BB	CC	BC	AA	DD	BB
KS17	FG	AA	BB	CC	FF	CC	AA	BC	BB	BB	DD	BB	CC	BC	AA	DD	BB
KS18	EG	BC	BB	CC	EE	CC	AA	BC	BB	BB	CC	BB	CC	CC	AA	DD	BB
KS19	EF	BC	BB	CC	FG	CC	AA	CC	BB	AB	CC	BB	CC	CC	AA	DD	BB
KS20	GG	BB	BB	CC	EG	CC	AA	BC	BB	BB	CC	BB	CC	CC	AA	DD	BB

PPENDIX B

Table 10. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chiang Mai.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	5	4.500			
	A-B	8	9.000			
	B-B	5	4.500			
				.222	1	.637
ME						
	B-B	12	10.512			
	B-C	5	7.975			
	C-C	3	1.513			
				2.783	1	.095
AAT-1						
	B-B	19	19.013			
	B-C	1	.975			
	C-C	0	.013			
				.013	1	.909
EST						
	B-B	8	6.613			
	B-C	7	9.775			
	C-C	5	3.612			
				1.612	1	.204
MDH-2						
	A-A	2	.800			
	A-B	4	6.400			
	B-B	14	12.800			
				2.813	1	.094
PGD						
	B-B	3	3.368			
	B-C	10	9.263			
	C-C	6	6.368			
				.120	1	.729
PGM						
	B-B	0	.112			
	B-D	3	2.775			
	D-D	17	17.112			
				.131	1	.717
HBDH						
	C-C	19	19.013			
	C-D	1	.975			
	D-D	0	.013			
				.013	1	.909

Table 11. Chi-square test for deviation from Hardy-Weinberg equilibrium in Nan.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	2	1.513			
	A-B	7	7.975			
	B-B	11	10.512			
				.299	1	.585
LGG-4						
	A-A	0	.010			
	A-B	1	.980			
	B-B	24	24.010			
				.010	1	.919
EST						
	B-B	8	6.760			
	B-C	10	12.480			
	C-C	7	5.760			
				.987	1	.320
PGD						
	B-B	2	.844			
	B-C	5	7.313			
	C-C	17	15.844			
				2.400	1	.121

Table 12. Chi-square test for deviation from Hardy-Weinberg equilibrium in Tak.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**
MPI						
	A-A	10	9.592			
	A-B	7	7.816			
	B-B	2	1.592			
				.207	1	.649
ME						
	B-B	17	16.200			
	B-C	2	3.600			
	C-C	1	.200			
				3.951	1	.047
AAT-1						
	B-B	18	18.050			
	B-C	2	1.900			
	C-C	0	.050			
				.055	1	.814
EST						
	B-B	18	18.050			
	B-C	2	1.900			
	C-C	0	.050			
				.055	1	.814
IDH-2						
	A-A	0	.013			
	A-B	1	.975			
	B-B	19	19.013			
				.013	1	.909
PGD						
	B-B	5	2.813			
	B-C	5	9.375			
	C-C	10	7.813			
				4.356	1	.037
HBDH						
	C-C	12	11.681			
	C-D	5	5.639			
	D-D	1	.681			
				.231	1	.631

Table 13. Chi-square test for deviation from Hardy-Weinberg equilibrium in Phitsanulok.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	12	12.150			
	A-B	3	2.700			
	B-B	0	.150			
				.185	1	.667
LGG-4						
	A-A	0	.250			
	A-B	4	3.500			
	B-B	12	12.250			
				.327	1	.568
AAT-1						
	A-A	0	.016			
	A-B	1	.969			
	B-B	15	15.016			
				.017	1	.897
MDH-1						
	A-A	0	.250			
	A-B	4	3.500			
	B-B	12	12.250			
				.327	1	.568
MDH-2						
	A-A	0	.391			
	A-B	5	4.219			
	B-B	11	11.391			
				.549	1	.459
PGD						
	B-B	5	4.516			
	B-C	7	7.969			
	C-C	4	3.516			
				.236	1	.627

Table 14. Chi-square test for deviation from Hardy-Weinberg equilibrium in Nakhon Sawan.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	3	1.760			
	A-B	7	9.479			
	B-B	14	12.760			
				1.642	1	.200
ME						
	B-B	25	24.300			
	B-C	4	5.400			
	C-C	1	.300			
				2.016	1	.156
EST						
	B-B	23	23.408			
	B-C	7	6.183			
	C-C	0	.408			
				.523	1	.469
IDH-2						
	A-A	0	.034			
	A-B	2	1.931			
	B-B	27	27.034			
				.037	1	.847
PGD						
	B-B	2	.675			
	B-C	5	7.650			
	C-C	23	21.675			
				3.600	1	.058
PGM						
	B-B	0	.008			
	B-D	1	.983			
	D-D	29	29.008			
				.009	1	.926

Table 15. Chi-square test for deviation from Hardy-Weinberg equilibrium in Ayutthaya.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	A-A	0	.050			
	A-B	2	1.900			
	B-B	18	18.050			
				.055	1	.814
ME						
	B-B	17	16.667			
	B-C	6	6.667			
	C-C	1	.667			
				.240	1	.624
IDH-2						
	A-A	0	.045			
	A-B	2	1.909			
	B-B	20	20.045			
				.050	1	.823
PGD						
	B-B	0	.043			
	B-C	2	1.913			
	C-C	21	21.043			
				.048	1	.827
PGM						
	B-B	12	10.446			
	B-D	7	10.109			
	D-D	4	2.446			
				2.175	1	.140

Table 16. Chi-square test for deviation from Hardy-Weinberg equilibrium in Bangkok.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P

MPI						
	A-A	1	.267			
	A-B	2	3.467			
	B-B	12	11.267			
				2.685	1	.101
ME						
	B-B	11	12.012			
	B-C	9	6.975			
	C-C	0	1.013			
				1.686	1	.194
EST						
	B-B	17	17.112			
	B-C	3	2.775			
	C-C	0	.112			
				.131	1	.717
IDH-1						
	A-A	0	.118			
	A-B	3	2.763			
	B-B	16	16.118			
				.140	1	.709
GPI						
	D-D	0	.050			
	D-E	2	1.900			
	E-E	18	18.050			
				.055	1	.814
PGD						
	B-B	6	5.512			
	B-C	9	9.975			
	C-C	5	4.512			
				.191	1	.662
PGM						
	B-B	0	.013			
	B-D	1	.975			
	D-D	19	19.013			
				.013	1	.909
HBDH						
	C-C	16	16.118			
	C-D	3	2.763			
	D-D	0	.118			
				.140	1	.709

Table 17. Chi-square test for deviation from Hardy-Weinberg equilibrium in Sa Kaeo.

		Observed	Expected	Chi-

Locus	Class	frequency	frequency	square	DF	P
MPI						
	A-A	0	.160			
	A-B	4	3.680			
	B-B	21	21.160			
				.189	1	.664
ME						
	B-B	29	28.073			
	B-C	1	1.903			
	B-D	0	.952			
	C-C	0	.032			
	C-D	1	.032			
	D-D	0	.008			
				30.483	3	.000
EST						
	B-B	15	15.408			
	B-C	13	12.183			
	C-C	2	2.408			
				.135	1	.714
PGD						
	B-B	2	1.565			
	B-C	9	9.870			
	C-C	16	15.565			
				.210	1	.647
PGM						
	B-B	1	.516			
	B-D	6	6.968			
	D-D	24	23.516			
				.598	1	.439
HBDH						
	C-C	16	14.491			
	C-D	4	4.241			
	C-E	5	7.776			
	D-D	1	.310			
	D-E	0	1.138			
	E-E	3	1.043			
				7.503	3	.057

Table 18. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chanthaburi.

		Observed	Expected	Chi-		

Locus	Class	frequency	frequency	square	DF	P
MPI						
	A-A	4	5.333			
	A-B	8	5.333			
	B-B	0	1.333			
				3.000	1	.083
ME						
	B-B	1	.766			
	B-C	5	5.469			
	C-C	10	9.766			
				.118	1	.732
EST						
	B-B	0	.025			
	B-C	1	.950			
	C-C	9	9.025			
				.028	1	.868
MDH-1						
	B-B	14	14.063			
	B-C	2	1.875			
	C-C	0	.063			
				.071	1	.790
PGM						
	B-B	2	1.000			
	B-D	4	5.750			
	B-E	0	.250			
	D-D	9	8.266			
	D-E	1	.719			
	E-E	0	.016			
				1.974	3	.578

Table 19. Chi-square test for deviation from Hardy-Weinberg equilibrium in Phetchaburi.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P

MPI						
	A-A	0	.038			
	A-B	2	1.923			
	B-B	24	24.038			
				.042	1	.838
LGG-2						
	B-B	0	.009			
	B-C	1	.982			
	C-C	27	27.009			
				.009	1	.923
ME						
	B-B	19	17.286			
	B-C	6	9.429			
	C-C	3	1.286			
				3.702	1	.054
AAT-1						
	B-B	16	13.580			
	B-C	7	11.839			
	C-C	5	2.580			
				4.678	1	.031
EST						
	B-B	26	26.036			
	B-C	2	1.929			
	C-C	0	.036			
				.038	1	.845
PGD						
	B-B	5	2.370			
	B-C	6	10.963			
	B-D	0	.296			
	C-C	15	12.676			
	C-D	1	.685			
	D-D	0	.009			
				6.040	3	.110
PGM						
	B-B	0	.009			
	B-D	1	.982			
	D-D	27	27.009			
				.009	1	.923
HBDH						
	C-C	13	10.938			
	C-D	8	8.750			
	C-E	1	4.375			
	D-D	3	1.750			
	D-E	0	1.750			
	E-E	3	.438			
				20.709	3	.000

Table 20. Chi-square test for deviation from Hardy-Weinberg equilibrium in Prachuab Khiri Khan.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**

MPI						
	A-A	0	.056			
	A-B	2	1.889			
	B-B	16	16.056			
				.062	1	.803
LGG-2						
	B-B	0	.012			
	B-C	1	.976			
	C-C	20	20.012			
				.012	1	.911
ME						
	B-B	16	16.298			
	B-C	5	4.405			
	C-C	0	.298			
				.383	1	.536
AAT-1						
	B-B	18	18.107			
	B-C	3	2.786			
	C-C	0	.107			
				.124	1	.724
EST						
	B-B	20	20.012			
	B-C	1	.976			
	C-C	0	.012			
				.012	1	.911
PGD						
	C-C	16	16.118			
	C-D	3	2.763			
	D-D	0	.118			
				.140	1	.709
PGM						
	B-B	0	.200			
	B-D	4	3.500			
	B-E	0	.100			
	D-D	15	15.313			
	D-E	1	.875			
	E-E	0	.013			
				.408	3	.939
HBDH						
	C-C	5	5.762			
	C-D	12	10.476			
	D-D	4	4.762			
				.444	1	.505

Table 21. Chi-square test for deviation from Hardy-Weinberg equilibrium in Chumphon.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**

ME						
	B-B	8	8.028			
	B-C	1	.944			
	C-C	0	.028			
				.031	1	.860
EST						
	B-B	5	5.444			
	B-C	4	3.111			
	C-C	0	.444			
				.735	1	.391
PGD						
	B-B	3	1.778			
	B-C	2	4.444			
	C-C	4	2.778			
				2.723	1	.099
PGM						
	B-B	0	.111			
	B-D	2	1.778			
	D-D	7	7.111			
				.141	1	.708
HBDH						
	B-B	0	.125			
	B-C	2	1.625			
	B-D	0	.125			
	C-C	5	5.281			
	C-D	1	.813			
	D-D	0	.031			
				.426	3	.935

Table 22. Chi-square test for deviation from Hardy-Weinberg equilibrium in Surat Thani.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**

ME						
	B-B	10	10.023			
	B-C	1	.955			
	C-C	0	.023			
				.025	1	.875
EST						
	B-B	2	1.000			
	B-C	2	4.000			
	C-C	5	4.000			
				2.250	1	.134
PGD						
	B-B	5	5.114			
	B-C	5	4.091			
	B-D	0	.682			
	C-C	0	.818			
	C-D	1	.273			
	D-D	0	.023			
				3.667	3	.300
PGM						
	B-B	0	.023			
	B-D	1	.955			
	D-D	10	10.023			
				.025	1	.875
HBDH						
	C-C	8	8.205			
	C-D	3	2.591			
	D-D	0	.205			
				.274	1	.601

Table 23. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metaphire* sp.

Locus	**Class**	**Observed frequency**	**Expected frequency**	**Chi-square**	**DF**	**P**

MPI						
	G-G	0	.077			
	G-H	2	1.846			
	H-H	11	11.077			
				.090	1	.764
LGG-4						
	D-D	12	12.019			
	D-E	1	.962			
	E-E	0	.019			
				.021	1	.885
ME						
	A-A	3	1.923			
	A-B	4	6.154			
	B-B	6	4.923			
				1.593	1	.207
AAT-1						
	A-A	1	.521			
	A-B	3	2.083			
	A-C	0	1.875			
	B-B	2	2.083			
	B-C	3	3.750			
	C-C	3	1.688			
				3.893	3	.273
EST						
	C-C	1	1.333			
	C-D	6	5.333			
	D-D	5	5.333			
				.188	1	.665
GPI						
	B-B	7	6.942			
	B-C	5	5.115			
	C-C	1	.942			
				.007	1	.935
PGM						
	B-B	4	3.273			
	B-C	4	5.455			
	C-C	3	2.273			
				.782	1	.376

Table 24. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metahire posthuma*.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MPI						
	C-C	6	6.021			
	C-D	5	4.958			
	D-D	1	1.021			
				.001	1	.977
LGG-4						
	B-B	0	.067			
	B-C	2	1.867			
	C-C	13	13.067			
				.077	1	.782
LGG-3						
	B-B	10	10.563			
	B-C	6	4.875			
	C-C	0	.563			
				.852	1	.356
LGG-2						
	A-A	0	.600			
	A-B	6	4.800			
	B-B	9	9.600			
				.937	1	.333
EST						
	A-A	2	1.455			
	A-B	4	3.636			
	A-C	0	1.455			
	B-B	3	2.273			
	B-C	0	1.818			
	C-C	2	.364			
				11.110	3	.011
MDH-2						
	A-A	13	13.235			
	A-B	4	3.529			
	B-B	0	.235			
				.302	1	.582

Table 25. Chi-square test for deviation from Hardy-Weinberg equilibrium in *Metaphire bahli*.

Locus	Class	frequency	Observed frequency	Expected square	Chi- DF	P
MPI						
	D-D	0	.050			
	D-E	2	1.000			
	D-F	0	.900			
	E-E	5	5.000			
	E-F	8	9.000			
	F-F	5	4.050			
				2.284	3	.516
LGG-4						
	B-B	8	6.613			
	B-C	7	9.775			
	C-C	5	3.612			
				1.612	1	.204
ME						
	C-C	8	6.050			
	C-D	6	9.900			
	D-D	6	4.050			
				3.104	1	.078
AAT-1						
	A-A	1	.112			
	A-B	1	2.775			
	B-B	18	17.112			
				8.183	1	.004
EST						
	B-B	1	.613			
	B-C	5	5.775			
	C-C	14	13.613			
				.360	1	.548
IDH-1						
	B-B	19	19.013			
	B-C	1	.975			
	C-C	0	.013			
				.013	1	.909
IDH-2						
	A-A	0	.050			
	A-B	2	1.900			
	B-B	18	18.050			
				.055	1	.814
GPI						
	B-B	13	13.613			
	B-C	7	5.775			
	C-C	0	.613			
				.900	1	.343
PGD						
	B-B	0	.050			
	B-C	2	1.800			
	B-D	0	.100			
	C-C	16	16.200			
	C-D	2	1.800			
	D-D	0	.050			
				.247	3	.970

APPENDIX C

Table 26. Significance test using exact probabilities in Chiang Mai.

Locus	R1	R2	R3	P
MPI	5	8	5	0.654
ME	12	5	3	0.107
AAT-1	19	1	0	1.000
EST	8	7	5	0.204
MDH-2	14	4	2	0.129
PGD	6	10	3	1.000
PGM	17	3	0	1.000
HBDH	19	1	0	1.000

Table 27 Significance test using exact probabilities in Nan.

Locus	R1	R2	R3	P
MPI	11	7	2	0.591
LGG-4	24	1	0	1.000
EST	8	10	7	0.422
PGD	17	5	2	0.152

Table 28. Significance test using exact probabilities in Tak.

Locus	R1	R2	R3	P
MPI	10	7	2	0.607
ME	17	2	1	0.150
AAT-1	18	2	0	1.000
EST	18	2	0	1.000
IDH-2	19	1	0	1.000
PGD	10	5	5	0.053
HBDH	12	5	1	0.512

Table 29. Significance test using exact probabilities in Phitsanulok.

Locus	R1	R2	R3	P
MPI	12	3	0	1.000
LGG-4	12	4	0	1.000
AAT-1	15	1	0	1.000
MDH-1	12	4	0	1.000
MDH-2	11	5	0	1.000
PGD	5	7	4	0.638

Table 30. Significance test using exact probabilities in Nakhon Sawan.

Locus	R1	R2	R3	P
MPI	14	7	3	0.291
ME	25	4	1	0.241
EST	23	7	0	1.000
IDH-2	27	2	0	1.000
PGD	23	5	2	0.099
PGM	29	1	0	1.000

Table 31. Significance test using exact probabilities in Ayutthaya.

Locus	R1	R2	R3	P
MPI	12	2	1	0.199
ME	11	9	0	0.528
EST	17	3	0	1.000
IDH-1	16	3	0	1.000
GPI	18	2	0	1.000
PGD	6	9	5	0.670
PGM	19	1	0	1.000
HBDH	16	3	0	1.000

Table 32. Significance test using exact probabilities in Bangkok.

Locus	R1	R2	R3	P
MPI	18	2	0	1.000
ME	17	6	1	0.501
IDH-2	20	2	0	1.000
PGD	21	2	0	1.000
PGM	12	7	4	0.162

Table 33. Significance test using exact probabilities in Sa Kaeo.

Locus	R1	R2	R3	P
MPI	21	4	0	1.000
ME	29	1	1	0.049
EST	15	13	2	1.000
PGD	16	9	2	0.614
PGM	24	6	1	0.403
HBDH	16	9	4	0.190

Table 34. Significance test using exact probabilities in Chanthaburi.

Locus	R1	R2	R3	P
MPI	4	8	0	0.216
ME	10	5	1	1.000
EST	9	1	0	1.000
MDH-1	14	2	0	1.000
PGM	9	5	2	0.530

Table 35. Significance test using exact probabilities in Phetchaburi.

Locus	R1	R2	R3	P
MPI	24	2	0	1.000
LGG-2	27	1	0	1.000
ME	19	6	3	0.073
AAT-1	16	7	5	0.064
EST	26	2	0	1.000
PGD	15	7	5	0.065
PGM	27	1	0	1.000
HBDH	13	9	6	0.112

Table 36. Significance test using exact probabilities in Prachuab Khiri Khan.

Locus	R1	R2	R3	P
MPI	16	2	0	1.000
LGG-2	20	1	0	1.000
ME	16	5	0	1.000
AAT-1	18	3	0	1.000
EST	20	1	0	1.000
PGD	16	3	0	1.000
PGM	15	5	0	1.000
HBDH	5	12	4	0.675

Table 37. Significance test using exact probabilities in Chumphon.

Locus	R1	R2	R3	P
ME	8	1	0	1.000
EST	5	4	0	1.000
PGD	4	2	3	0.168
PGM	7	2	0	1.000
HBDH	5	3	0	1.000

Table 38. Significance test using exact probabilities in Surat Thani**.**

Locus	R1	R2	R3	P
ME	10	1	0	1.000
EST	5	2	2	0.163
PGD	5	5	1	1.000
PGM	10	1	0	1.000
HBDH	8	3	0	1.000

Table 39. Significance test using exact probabilities in *Metaphire* sp.

Locus	R1	R2	R3	P
MPI	11	2	0	1.000
LGG-4	12	1	0	1.000
ME	6	4	3	0.256
AAT-1	2	6	4	1.002
EST	5	6	1	1.000
GPI	7	5	1	1.000
PGM	4	4	3	0.543

Table 40. Significance test using exact probabilities in *M. posthuma*.

Locus	R1	R2	R3	P
MPI	6	5	1	1.000
LGG-4	13	2	0	1.000
LGG-3	10	6	0	1.000
LGG-2	9	6	0	1.000
EST	3	4	4	0.543
MDH-2	13	4	0	1.000

Table 41. Significance test using exact probabilities in *M. bahli*.

Locus	R1	R2	R3	P
MPI	5	10	5	1.000
LGG-4	8	7	5	0.204
ME	8	6	6	0.085
AAT-1	18	1	1	0.077
EST	14	5	1	0.468
IDH-1	19	1	0	1.000
IDH-2	18	2	0	1.000
GPI	13	7	0	1.000
PGD	16	4	0	1.000

BIOGRAPHY

Miss Ueangfa Bantaowong was born in Chanthaburi, Thailand, on the 28th of November 1976. She graduated Bachelor Degree of Science (Agriculture), Major in Horticulture from Department of Agriculture, Faculty of Agriculture, Kasetsart University in 1999. Until 2003 to 2008, she started working on Biodiversity Research and Training Program (BRT program), after that she studied Master Degree in Zoology at Department of Biology, Faculty of Science, Chulalongkorn University.