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**Taxonomy and phylogeny of pheretimoid earthworms
(Clitellata: Megascolecidae) from Mindanao and
associated islands, Philippines**

(フィリピン・ミンダナオ島及び周辺島嶼産フトミミズ類 (環帯類：フトミミズ科) の分類と系統)

A Dissertation

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Department of Natural History Sciences,
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ABSTRACT

This dissertation presents a total of 39 new pheretimoid species of the family Megascolecidae, from Mindanao and associated islands, in the Philippines. Among these, 29 are in the genus *Pheretima*, characterized by having nephridia on the spermathecal ducts, having prominent dome-shaped copulatory bursae, and having a pair of caeca originating in xxvii. Of these, 27 are in the subgenus *Pheretima*, while two species are the first records of the subgenus *Parapheretima* in the Philippines. *Parapheretima* is characterized by having secretory diverticula projecting from the copulatory bursae, in contrast to members in the subgenus *Pheretima*, which do not possess such organ. Also, three species are in *Pithemera*, characterized by having a pair of caeca originating in or near xxii, three are in *Polypheretima*, characterized by having no caeca, and three are in *Amyntas*, characterized by having no copulatory bursae. The three latter genera also do not possess nephridia on the spermathecal ducts, in contrast to *Pheretima*. With the new species described, there are now 80 known *Pheretima* s. str. species in the Philippines, comprising 76% of the world's *Pheretima*, and there are now 14 species of *Pithemera*, comprising 47% of the world's *Pithemera*. These figures suggest that the Philippine archipelago may be the center of species radiation for these groups. Also, there are now 16 species of *Amyntas* in the Philippines representing less than 1% of the world's *Amyntas* and there are now 10 species of *Polypheretima* in the Philippines representing 15% of the world's *Polypheretima*. The high diversity of the two latter genera in mainland Asia and

Indonesia, respectively, strongly suggests that Indochina may be the center of species radiation for these two genera. The known ranges of the Philippine species are restricted to areas around the type localities. This pattern indicates a remarkable degree of endemism, both among local areas, among islands in the Philippines, and in the Philippines as a whole, and suggesting that many species remain to be detected in the Philippines.

A molecular phylogenetic study was done in attempt to infer phylogenetic relationships among the pheretimoid species in Mindanao and associated islands. Gene markers used include the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA, and the nuclear 28S rRNA and protein-coding histone H3 genes. Despite having limited taxa and limited genes included in the analyses, the combined data set generated a phylogeny more or less consistent with morphology-based expectation. Results show that taxonomic assignment of the genus *Amyntas* and the subgenus *Parapheretima* do not reflect phylogeny. The species grouping in *Pheretima* based on the location of spermathecae is partially reflected in the pheretimoid phylogeny. Also, results show that loss of spermatheca or fusion of two spermathecae into one can occur in pheretimoid evolution. In general, several of the nodes of the tree based on combined data set have support values that are very weak and have formed polytomies, which is most likely due to insufficient data. The results could have improved if more data were available. Further molecular work including more taxa is needed to be able to establish a more robust system of classification of the pheretimoid species and come up with a better-resolved phylogeny.

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“Worthy are you, our Lord and God, to receive glory and honor and power, for you created all things, and by your will they existed and were created.”

-Revelation 4:11

CHAPTER 1

General Introduction

1.1 Biodiversity in the Philippines under threat

The Philippines is considered to be one of the megadiverse countries in the world, being a home to one of the widest arrays of species in the world. With around 7,100 islands, the country hosts more than 52,177 described floral and faunal species of which more than half is found nowhere else in the world (CI *et al.* 2006). However, the country is also identified as one of the biodiversity hotspots in the world having its rich ecosystems being under serious threat. Species are being lost at an incredible rate of up to 10,000 a year due to human activities (Mallari *et al.* 2001). In the early 1900's, huge tracts of primary rainforests covered most of the Philippine landscape. Terrestrial ecosystems flourished, with large stands of primary forests, and timberlands dominating the land. Sadly, extremely high population growth has demanded increasingly damaging and extractive techniques in order to support human needs. The threats to natural habitats include illegal logging, mining, quarrying, conversion of forest ecosystems to agricultural lands, urban development, wildlife poaching, and illegal trade. Today, only a fraction of the original landscapes that blanketed the country remains. As the loss of natural habitats is at a very alarming rate, there is an urgent need to assess the biological resources, of which the information is essential in formulating conservation and management strategies, before it is too late. This study is

primarily undertaken to provide significant information on the diversity of earthworms in Mindanao and associated islands.

1.2 General biology of earthworms

Earthworms are tube-shaped, segmented animals that have no internal skeleton or exoskeleton. They maintain their structure with fluid-filled coelom chambers that function as a hydrostatic skeleton. When observing an earthworm move, one will most likely see it move forward, with its mouth and prostomium in the anterior end.

Although it has no eyes, it possesses cells that are sensitive to light and it can also feel vibrations created by movements in its surroundings. As it works its way forward, successive peristaltic waves of thickening and thinning (7 to 10 per minute) pass down the body. At each place where the body bulges out at a given moment, the setae are extended and grip the burrow walls. Setae, which are bristles surrounding each segment, push against the ground with each contraction and help the animal move (Lorus & Milne 1992). Secretions from the mucous glands of the epidermis keeps the cuticle moist and lubricates the worm's body to ease passage through the burrows. The mucus-covered skin helps bind soil particles together and prevents the walls of the burrow from collapsing.

Earthworms are very sensitive to touch, and the pattern of their reaction vary with both the species and circumstances. If an earthworm is touched, it withdraws back into its burrow, sometimes very quickly, and does not emerge again for some time. If a worm is grasped while partly out of its burrow it will actively resist and attempt to pull its body out by extending its posterior setae into the burrow wall and

expanding its posterior segments, so as to grip the walls of the burrow and completely fill its exit. Other species react vigorously to tactile stimuli. Some, if pricked sharply or handled, produce a series of lashing movements from side to side. Many East Asian species exhibit serpentine motion, and may thrash violently to escape danger. Some often eject coelomic fluid from the dorsal pores when touched and other species can eject this fluid to a considerable height. The least common response is autotomy, or breaking off posterior segments to escape danger. Stimuli applied to the anterior end does not cause reaction in the same way (Edwards & Lofty 1977).

The earthworm's internal body plan is relatively simple. It has a central and a peripheral nervous system. The central nervous system consists of two ganglia above the mouth, one on either side, connected to a nerve cord running back along its length to motor neurons and sensory cells in each segment. Large numbers of chemoreceptors are concentrated near its mouth. Its digestive system is mostly composed of the intestine, which stretches along the body, starting from the first few segments of the anterior end of the body towards the anus. Earthworms have no specialized respiratory organs. Oxygen and carbon dioxide diffuse through the cuticle and epidermal tissues into the blood, which contains hemoglobin. It has a double transport system composed of coelomic fluid that moves within the fluid-filled coelom and a simple, closed blood circulatory system. The morphological characters used to discriminate earthworm species include color, size, the length and location of the clitellum, the presence or absence of genital markings, the number of setae per segment, the distance between spermathecal pores, the distance between male pores, presence or absence of dorsal or ventral setal gaps, the number, location, size and shape of spermathecae, the

arrangement of septa, the origin of gizzard, intestine and caeca, the size and shape of the prostate glands, the presence or absence of copulatory bursae, and the presence or absence of penes. Fig. 1.1 shows the external and internal morphology of an earthworm showing some of the diagnostic morphological characters.

Earthworms are hermaphrodites, having both male and female reproductive organs. Most species reproduce by cross-fertilization although many species can also produce cocoons parthenogenetically. However, most parthenogenetic species are only parthenogenetic because they lost the male organ functions (Gates 1972). Most species mate periodically throughout the year, except when conditions are unsuitable or they are aestivating. Methods of copulation are not identical for all species; in *Lumbricus terrestris*, two worms which are attracted to each other by thick and slimy glandular secretions, lie with the ventral parts of their bodies together, and their heads pointed in opposite directions. They come into close contact in the region of the spermathecal openings and where the clitellar region of one worm touches the surface of the other. Sperm cells are released from the male pores and are either transported towards the sperm receptacles or the spermathecal pores of the partner, or are directly inserted into the receptacles (Gates 1972). In *Pheretima* species that have three or four pairs of spermathecae, the male pores first come into contact with the hindmost pair of spermathecal apertures and discharge seminal fluid and prostatic fluid into them. Each worm then moves backwards, and the seminal fluid is discharged into the next pair of spermathecae, until all have been 'charged' (Tembe & Dubash 1961). While copulating, the worms do not respond readily to external stimuli such as touch and light. After copulation, which may take as long as an hour, the worms separate. When

the worms already have parted ways, a mucous ring is secreted throughout the clitellum slips off the front of the worm, closing at both ends to form the cocoon which is roughly lemon-shaped. The cocoon contains a nutritive albuminous fluid, produced by the gland cells of the clitellum, the ova, and the spermatozoa. Cocoons continue to be formed until all the stored seminal fluid has been used up. Fertilization is external in the cocoon. One or two worms hatch from this cocoon, in two or three weeks. Worms that have been mated may form new egg capsules every few days (Lorus & Milne 1992).

1.3 Ecology of earthworms

Earthworms are naturally found living in soil, feeding on live and dead organic matter. However, they may also be arboreal and can be found inside tree barks, ferns, mosses, and the insides of rotten logs several meters above ground. Although they are most numerous in the top 15 cm, some species work in the subsoil, bringing mineral-rich soil from below to the surface. The leaf litter they digest contain nutrients such as calcium, nitrogen, potassium and phosphorus. They also consume organic minerals and nutrients from dead animals and animal feces. Their excrement, called castings, is deposited both on the surface and within the soil and is rich in nutrients, providing food for other animals and microorganisms. This organic material is then further broken down by microorganisms of the soil, releasing nutrients in a form available for absorption by plants. Earthworms may be able to acquire parasites such as protozoa, platyhelminthes and nematodes through ingestion and these parasites can be found in

many parts of the earthworms' bodies like the blood system, testes, in the inner surface of the epidermis, or in the cocoons (Lee 1985).

Earthworms are perhaps the most important soil organisms in terms of their influence on organic matter breakdown, soil structure development, and nutrient cycling. Their tunneling and burrowing aerates the soil, which not only help bring oxygen down into the soil, but their tunnels also allow rainwater carrying organic and inorganic nutrients down deep into the soil where the roots lie. The roots then take up the water and the minerals and recycle them back to the herbaceous plants and woody trees. The tunneling of the earthworms also provides an access to deeper soil levels for the numerous smaller organisms that contribute to the health of the soil (Lee 1985). Also, as the body of the earthworm is composed of 70% protein, it is preyed upon by birds and by burrowing animals like moles, forest rats, pigs, and shrews. In addition, researchers have found that bacteria living in the guts of worms detoxify many hazardous chemicals such as hexachlorocyclohexane (Applehof 2000). Because of these significant features, earthworms are being utilized for their ecological and economic importance, through vermiculture (Dominguez & Edwards 2010).

There are three primary ecological categories of earthworms: endogeic, anecic, and epigeic. Endogeic worms live in the upper layers of soil but very rarely come to the surface. Their complex horizontal burrow systems are not permanent but they are very important for aerating soil and allowing moisture and nutrients to move through the soil. Many endogeic worms live in the rhizosphere, the area immediately around plant roots, and they help with the exchange of nutrients there. These worms are medium-sized and pale in color. Anecic worms build permanent, deep vertical

burrows in the soil, which can be up to 2 m deep, and rise to the surface at night to search for food. Anecic worms often leave little mounds of castings alongside or atop the opening of their burrows. They have long lives, which can be up to six years, and they mature and reproduce slowly. Anecic worms are usually large, ranging from 15 cm up to 1 m, and have dark color on the anterior end. Epigeic worms are surface dwellers and feed only on decaying organic material, not soil. They do not burrow very often but rather live in loose organic litter or very loose topsoil rich in organic matter. They may not survive in most garden soils unless there is a good layer of organic matter on top. They are usually small and are reddish brown in color, but they may also be striped. They are better able to withstand temperature and moisture fluctuations than other worms as they commonly inhabit the soil surface. In ideal environmental conditions, they can reproduce at a high rate and create castings that are many times higher in nutrients than the material they originally consumed. Because of these, they are ideal for vermiculture and vermicomposting (Stewart 2004; Dominguez & Edwards 2010). These ecological categories make it possible to judge earthworm ecological functions by simple visual cues. All three may be present in a habitat, or one or two may be missing. For example, epigeic and anecic worms can be missing from grasslands or agricultural soils where there is very little surface litter (Edwards & Lofty 1977).

Most earthworms favor neutral pH but other earthworms are also either acid tolerant or acid intolerant. Edwards & Lofty (1977) studied populations of earthworms in plots that had a range of pH from 3.7 to 7.5. They reported that the species *Lumbricus terrestris* became increasingly numerous as the pH increased but most of

the other species present tended to have an optimum pH range of 5.0 to 6.0. Earthworms can help change acid or alkaline soils toward a more neutral pH by utilizing and excreting excess calcium carbonate. In terms of soil temperature, Bhattacharjee & Chaudhuri (1999) studied the cocoon production, morphology, hatching pattern and fecundity of tropical earthworm species in temperature ranging within 28 to 32°C under laboratory conditions. Their results show that there is high rate of cocoon production, short development time with high hatching success, as well as continuous breeding strategies in the epigeic species *Perionyx excavatus*, *Dichogaster modiglianii* and the top soil endogeic species, *Pontoscolex corethrurus*, as temperature is increased. However, there are some other species that prefer to inhabit environments with lower temperature.

Soils that are poor in organic matter do not usually support large numbers of earthworms. Decaying leaves in the forests are a great source of organic matter that usually favors earthworm multiplication. Li *et al.* (1999) studied the responses of soil carbon and nitrogen and crop yield to earthworm activity. It was concluded that earthworm was very important in promoting nitrogen recycling of crop residues and plant productivity, and in keeping the balance of soil carbon pool as well.

Earthworms, having a body weight that constitutes 75 to 90% water, live in habitats where moisture is favorable. They cannot tolerate heat and sun and so endogeic worms come up to the surface only at night. Nevertheless, they have considerable ability to survive adverse moisture conditions, either by moving to a more suitable area or by aestivating. If they cannot avoid dry soil they can survive the loss of a large part of the total water content of their bodies. *Lumbricus terrestris* can

lose 70% of its total body water and still survive (Grant 1955). A few small species of earthworms can survive in deserts and semi-deserts. It seems that although good quality soils are favorable for most of the earthworms rather than poor ones, they can survive in many different kinds of soils provided, there is adequate food and moisture (Kubienna 1955).

1.4 Systematic accounts of the pheretimoid earthworms

It is a common knowledge that earthworms belong to Phylum Annelida, the segmented worms. However, the lower level taxa are in a state of flux as different taxonomists proposed various taxonomic classifications giving weight to different sets of morphological characters. The debates on taxonomy are sometimes in an evolutionary context, but rarely with any explicit analysis of character data, resulting in intuitive conclusions (James & Davidson 2012). For example, some taxonomists consider earthworms to be of Class Oligochaeta while others would consider it to be of Class Clitellata and consider Oligochaeta as its Order. Likewise, some taxonomists create additional taxonomic levels such as “Superclass” or “Subclass” to accommodate taxonomic reassignment but these may not be acknowledged by others. Also, different authors recognize different numbers of families in earthworms: 15 by Jamieson (1988), 21 by Reynolds & Cook (1993), and 18 by Blakemore (2000). DNA sequences, which provide characters independent of morphology, have become widely used to reconstruct relationships among earthworms and test the reliability of morphological characters used in taxonomy. Phylogenetic analyses based on molecular data may either support or refute taxonomic classifications based on

morphological data. In the latter case, the morphological character/s a taxonomist used to assign such taxonomic groupings may be found to be homoplasious and not homologous. Only monophyletic grouping, which is based on homologous characters, is therefore considered to infer true phylogeny. For the time being, due to the state of flux in different taxonomic levels in earthworms, taxonomists using molecular data to infer phylogenetic relationships prefer to brush aside the hierarchical system of classification (Linnean system). Below is the summary of the systematic accounts leading to the pheretimoid species (*Pheretima* s. lat.) of Megascolecidae, which is the focus of this dissertation.

Members of Clitellata Michaelsen, 1919 are defined by the clitellum, which is located partly behind the female pores and secretes a cocoon in which the eggs are laid are annelids. These include the oligochaetes (earthworms and their allies), branchiobdellids (ectoparasites of freshwater crayfish) and leeches. Clitellata was confirmed to be monophyletic based on molecular analyses (Martin *et al.* 2000; Martin 2001; Siddall & Burreson 1998). However, with regard to the position of the Clitellata within the Annelida, molecular analysis has indicated that clitellates form a clade within the Polychaeta and that polychaetes are a paraphyletic or polyphyletic group (Kojima 1998; McHugh 2000; Martin 2001). Oligochaeta, defined by having few setae on their outer body surfaces and refers to many aquatic and terrestrial worms, including the earthworms, was suspected and confirmed to be paraphyletic with leeches and/or branchiobdellids lying within the oligochaete clade based on both morphological and molecular data (Jamieson *et al.* 1987; Jamieson 1988; Siddall & Burreson 1998; Siddall *et al.* 2001). The inclusion of leeches and branchiobdellids

within the Oligochaeta would thus render the name Oligochaeta synonymous with Clitellata (Siddall *et al.* 2001; Jamieson *et al.* 2002). Earthworms, having multilayered clitella, were found to form a single clade and thus Jamieson (1988) assigned them to Crassicitellata. The acquisition of a multilayered clitellum was deduced to be a monophyletic event (Jamieson *et al.* 2002; James & Davidson 2012). The monophyly of Clitellata was further supported in recent studies with Tubificidae/Echiura/Capitellidae as sister taxa in Struck *et al.* (2011), with Terebelliformia/Arenicolidae as sister taxa in Struck *et al.* (2015) and Weigert *et al.* (2014), and with Terebelliformia/Arenicolidae/Maldanidae as sister taxa in Weigert *et al.* (2015).

Sims (1980) proposed superfamilies for earthworms based on the ovarian characters first recognized by Gates (1976). These include Criodriloidea, Lumbricoidea, Biwadriloidea, Glossoscolecoidea, and Megascolecoidea. James & Davidson's (2012) molecular analyses based on 28S, 18S, and 16S gene sequences, found that Criodriloidea is nested within Lumbricoidea, and that Biwadriloidea and Glossoscolecoidea are not supported and have unresolved positions. Megascolecoidea, defined by having a large, fan to rosette-shaped ovary and prostate glands associated with the male pores, on the other hand is a monophyletic group. James and Davidson (2012) proposed a limited Megascolecoidea, which only include the families of Megascolecidae, Ocnerodrilidae, and Acanthodrilidae as opposed to Sims (1980) and Omodeo (2000), who included Eudrilidae and Octochaetidae in the superfamily.

Among the families of Megascolecoidea, Megascolecidae is the most speciose, with 55 genera especially concentrated around the Asia-Pacific but also distributed in some parts of North and South America and in Madagascar (Blakemore 2000; BOLD

Systems). Megascolecidae includes members with diverse prostate gland types (generally racemose structure), whose ducts generally are joined by the sperm ducts in combined male and prostatic pore(s) on segment 18 or nearby and the spermathecal pores open into some or all of the intersegmental furrows from 4/5 to 9/10 (rarely intra-segmentally) (Sims & Easton 1979; Blakemore 2000; Jamieson & Ferraguti 2006; James & Davidson 2012). *Pheretima* s. lat. used to be the largest genus of Megascolecidae comprising more than 1,400 names, which include numerous synonyms, invalid names, and *lapsae* updated from Sims & Easton (1972). Sims and Easton (1972) reallocated species in the group into 'convenient' species groups comprising 10 genera using computer-based phenetic analyses. Blakemore (2007) reported an approximate of 930 valid species from the 1,400 names and updated Sims and Easton's list of the pheretimoid species to a total of 13 genera belonging to the group. The members are defined by having a perichaetine setal arrangement around the segmental equators except the first and the last segment of the body. They have a short clitellum xiv–xvi. Prostate glands have racemose structure and there is a pair of caeca on the intestine in a single segment in most species. The testes is contained within testes sacs. Also, the gizzard originates in viii and the excretory system is meronephridial (Sims & Easton 1972).

1.5 Status of the diversity of Philippine earthworms

Research on earthworm taxonomy in the Philippines began way back in the late 1800's but surveys on earthworm biodiversity in the Philippines has been given more attention than ever since the issue on the destruction by the earthworms of the

Banaue rice terraces, a National Cultural Treasure of the Philippines, was reported. *Pheretima* species were identified to be responsible for the soil erosion (Joshi *et al.* 1999). Over the last decade, the number of Philippine species dramatically increased from less than 10 known species to an estimate of 200 native pheretimoid species representing eight genera (Flores 2008; Aspe & James 2014). This was made possible by the efforts of the Philippine Terrestrial Annelids and Gastropods Survey project headed by Dr. Samuel James and funded by the National Science Foundation in the USA. The eight genera that compose the Philippine native species include *Pheretima*, *Isarogoscolex*, *Pithemera*, *Amynthas*, *Pleionogaster*, *Polypheretima*, *Dendropheretima*, and *Archipheretima*. Studies show that the Philippine archipelago may be the center of species radiation for *Pheretima* while Indochina may be the center of species radiation for *Amynthas* (Aspe & James 2016).

Most of the surveys on earthworms in the Philippines focused on Luzon Island until the last decade when collection on Mindanao and associated islands started. I was designated as the lead Field Biologist for the Mindanao Phase during this earthworm biodiversity project. This dissertation reports a total of 39 new pheretimoid species of Mindanao: Chapter 2 describes the morphology of 18 new species of *Pheretima* from Mt. Malindang, one of the priority sites for conservation in the Philippines, located at the base of Zamboanga peninsula in western Mindanao (Mallari *et al.* 2001; CI *et al.* 2006); Chapter 3 describes one species of *Polypheretima* and three species *Pithemera* from Mt. Malindang; and Chapter 4 describes 11 new species of *Pheretima* (two are of subgenus *Parapheretima*), three new species of *Amynthas*, two new species of *Polypheretima*, and one new species of *Pithemera* from other areas around Mindanao

and associated islands. The species described in Chapters 2, 3 and 4 were published in *Zootaxa*, *Journal of Natural History*, and *Zoological Studies*, respectively.

It is not reliable to infer evolutionary relationships among the pheretimoid species by simply using morphological data. Thus, molecular data is essential to reconstruct relationships among earthworms and to test the reliability of morphological characters that are used in taxonomy. James (2005a) conducted a preliminary molecular phylogeny in this group, which include the morphospecies from Luzon Island, using the data of mitochondrial 16S rDNA and nuclear 28S rDNA. Although his analyses of the combined data produced a tree topology that is more or less consistent with morphological data, all of the basal nodes were weakly supported. Chapter 5 of this thesis tackles a molecular phylogenetic study of the pheretimoid species of Mindanao and associated islands, using the mitochondrial 16S rDNA and COI, and the nuclear 28S rDNA and histone H3 genes, in attempt to produce a better resolved phylogeny among the pheretimoid species (Aspe *et al.*, in review).

CHAPTER 2

New species of *Pheretima* (Clitellata: Megascolecidae) from the Mt. Malindang Range, Mindanao Island, Philippines

2.1 Introduction

Until recently, knowledge of the native earthworm fauna of the Philippines was very limited. Non-specialist biologists in the Philippines erroneously identified all earthworms there as *Lumbricus terrestris* Linnaeus, 1758, a species common to North America and Europe but not detected in recent studies in the Philippines. Organized research on earthworm diversity in the Philippines began after Lawrence Heaney and collaborators discovered that the Isarog shrew-rat (*Rhynchomys isarogensis* Musser & Freeman 1981) and *Chrotomys gonzalesi* Rickart & Heaney, 1991 feed exclusively on earthworms. The desire of the mammalogists to identify the worms the rat feeds on led to the discovery of 10 new species collected in 1993, all belonging to perichaetine genera in the *Pheretima* complex (Sims & Easton 1972) in the family Megascolecidae (James 2004).

Pheretima Kinberg, 1867, a Southeast Asian group with a range extending from northern Australia to Myanmar and northward to Korea, became the largest genus of earthworms in the Megascolecidae *sensu* Gates (1959). Using computer-based phenetic analyses, Sims and Easton (1972) and Easton (1979) reallocated species in *Pheretima* s. lat. (pheretimoid species) into 'convenient' species groups comprising 10 genera (*Amyntas*, *Archipheretima*, *Pheretima*, *Planapheretima*, *Metapheretima*, *Pithemera*, *Ephemitra*, *Metaphire*, *Polypheretima* and *Pleionogaster*).

Blakemore (2007) estimated that among more than 1400 nominal taxa of pheretimoid earthworms (which include numerous synonyms, invalid names, and *lapsus*) there are roughly 930 valid species and subspecies in *Pheretima* auct. He acknowledged around 40 valid species of *Pheretima* s. str., with the distributional range restricted to the Indo-Australian archipelago, Sumatra, and the Philippines.

As the result of taxonomic studies in the last decade, around 200 species of native earthworms representing eight genera are now known from the Philippines (Blakemore 2007; Flores 2008; James 2004, 2005b, 2006, 2009; James *et al.* 2004; Hong & James 2004, 2008a–c, 2009, 2010, 2011a, b). Among these are 46 new species of *Pheretima sensu* Sims & Easton (1972), reported in studies conducted mostly in mountainous forested areas on Luzon Island (James *et al.* 2004; Hong & James 2008a–c, 2009, 2010, 2011a, b), but also in one study in the Mt. Kitanglad Range, Mindanao Island (James 2004). The eighteen new species from Mt. Malindang reported in this chapter were described in Aspe & James (2014).

2.2 Material and methods

Study area. The Malindang Range is a large volcanic complex at the base of the Zamboanga Peninsula, western Mindanao Island, Philippines (Fig. 2.1). The forests in this mountain range are the only remaining natural forests representing the tropical Zamboanga biogeographic zone (Mallari *et al.* 2001), one of 15 such zones in the Philippines. Reaching 2425 m asl., Mt. Malindang (~ 8°18'N, 123°39'E) is the highest mountain on the Zamboanga Peninsula, covering 53,262 ha and consisting of

46% lower montane and upper montane (mossy) forest, 25% bushland, 6% denuded land, and 23% cultivated land (Mallari *et al.* 2001).

Earthworms were collected in primary forest, disturbed forest, grassland, and agricultural habitats at various elevations in four geographically separate barangays (= precincts): Barangay (Brgy) Lake Duminagat in the municipality of Don Victoriano; Brgy Sibucal and Brgy Toliyok in Oroquieta City; and Brgy Small Potongan in the municipality of Concepcion. The terrain in the forested areas was very rugged, with steep grades and many cliff faces, making access extremely difficult. Surrounded by humid primary forest in Brgy Lake Duminagat is a crater lake about 9 ha in area called Lake Duminagat. The primary forest in this barangay had never been logged and remained largely undisturbed by human activities. The vegetation was dense and lush; trees were thickly covered with moss, ferns, and lichens, and ground was thickly covered with moss, roots, and leaf litter. The dominant trees included *Viburnum* sp., *Lithocarpus*, *Caldeluvia*, *Pometia*, *Macaranga dipterocarpifolia*, and *Polyosma philippinensis*.

Trees in the disturbed forest, regrown after deforestation by humans, were dominated by dipterocarps (those identified included *Lithocarpus mindanaensis*, *L. philippinensis*, and *Polyosma philippinensis*) and tended to be more closely spaced than in the primary forest, and to have more undergrowth (saplings, shrubs, and tree ferns). The ground was covered with thick leaf litter, roots, bryophytes, and lichens.

Sampling. The earthworms described in this paper were collected in an ecological and distributional study at Mt. Malindang during the periods 9–15 Oct.

2003 and 18–25 Feb. 2004. Sampling methods and the locations of sampling sites were previously reported in Aspe (2006) and Aspe *et al.* (2009). A summary is as follows. In each barangay, six scattered plots 20 m x 20 m in extent were established, with an average distance of 75 m between plots. On each plot, 0.075 m³ (0.5 m x 0.5 m square x 0.3 m deep) of soil was examined from each of 10 quadrats in randomly selected spots. This gave a total of 4.5 m³ of soil sampled at each of the five collecting sites (see Table 2.1), which equals a surface area of 15 m². In the Lake Duminagat Disturbed category, two sites were lumped (Disturbed Forest and Logged Over) in Aspe *et al.* (2009: Table 2), and thus represents 12 plots and 9 m³ of soil sampled, equaling 30 m². The earthworms collected in each quadrat were preliminarily sorted to species and counted. Additional haphazard sampling was done outside the plots to test for patch effects in the quadrats. Tree bark, ferns, mosses, vines, and the insides of rotten logs were also checked for earthworms. Earthworms collected were rinsed in tap water, killed in 10% ethanol, and placed in Saranex sealable plastic bags filled with a volume of 10% formalin that was at least three times the total volume of the earthworms. After two days, the formalin was replaced with 80% ethanol. Elevations were read by GPS (Magellan Map 410; Luzon map datum) if a satellite signal was detectable, or with an altimeter if not. Elevation is expressed in this paper as meters above sea level (m asl).

Examination and descriptions. Worms were sorted in the field to putative species using body size, coloration and number of spermathecal pores as identifying characters. Some of the worms were released alive after collection and counting, due

to limitations on the total number of specimens we were allowed to take in the collecting agreements with the Protected Area and Wildlife Bureau and the indigenous community. Among the preserved specimens, external and internal characters were examined for a representative subset. Without exception, these examinations confirmed the original assignment of specimens to putative species. It is therefore assumed that all specimens listed in Table 2.1 are correctly identified. Some specimens were unfortunately lost from our field collection due to unforeseeable circumstances, and so the number of specimens listed in Table 2.1 is higher than indicated in the descriptions.

All descriptions are based on external examination and on dorsal dissection under a stereomicroscope, following the terminology and conventions of Easton (1979). Descriptions of body color are based on living specimens. Body dimensions refer to fixed material. The degree of separation between pores is expressed as a proportion of the circumference of the worm; for example, 'spermathecal pores 0.13 circumference apart ventrally' means the distance between the pores is 0.13 the circumference of the worm at that point, with the circumference calculated as π times segment diameter. The generic diagnosis and assignment to species groups follows Sims & Easton (1972). While the species described share many character states diagnostic for the genus, shared characters were included to facilitate information retrieval from the separate descriptions. Line drawings were prepared with Adobe Illustrator ver. CS5.

While there appeared to be 22 previously undescribed *Pheretima* species at Mt. Malindang, species with single specimen for morphological examination were not

formally named and included here. In this paper, the usual practice of illustrating earthworms with drawings of the external anterior-ventral aspect is departed. Instead, schematic drawings in the dorsal view of the internal morphology showing the structure and location of organs are presented. The reasons for this break from tradition are 1) the most useful first pass at identification involves overall color pattern and the size of mature individuals, neither of which is evident in drawings of external aspect presented at the same size rather than the same scale; 2) the external aspect is quite stereotyped within *Pheretima* species groups (e.g. *Pheretima* species herein do not possess genital markings that vary in pattern); 3) artifacts of preservation and degree of sexual maturity further limit the utility of a single drawing of external morphology in species identification; and 4) most of the characters used in species discrimination are internal, and the large proportion of descriptions typically devoted to internal anatomy reflects this.

Holotypes and some of the paratypes are deposited in the National Museum of the Philippines Annelid Collection (NMA), P. Burgos St., Manila, Philippines. Other paratypes are deposited in the Annelid Collection of the Zoological Reference Collection (ZRC.ANN) of the Lee Kong Chian Natural History Museum (formerly Raffles Museum of Biodiversity Research), Faculty of Science, National University of Singapore, Singapore.

2.3 Results

Eighteen new species from Mt. Malindang, Mindanao Island were described. All of them belong to the subgenus *Pheretima* (*Pheretima*) Kinberg, 1867,

distinguished from the other subgenus, *Pheretima* (*Parapheretima*) Cognetti, 1912, by the absence of secretory diverticula on the coelomic surface of the copulatory bursae. Eleven species belong to the *P. sangirensis* (Michaelsen, 1891) species group of Sims & Easton (1972), characterized by having a pair of spermathecal pores in the intersegmental furrow of 7/8 and by lacking penial sheaths in the copulatory bursae: *P. maculodorsalis* Aspe & James 2014, *P. tigris* Aspe & James 2014, *P. immanis* Aspe & James 2014, *P. lago* Aspe & James 2014, *P. nunezae* Aspe & James 2014, *P. boniaoi* Aspe & James 2014, *P. malindangensis* Aspe & James 2014, *P. misamisensis* Aspe & James 2014, *P. wati* Aspe & James 2014, *P. longiprostata* Aspe & James 2014, and *P. nolani* Aspe & James 2014. One species, *P. longigula* Aspe & James 2014, belongs to the *P. montana* (Kinberg, 1867) species group, characterized by having a pair of spermathecal pores in the intersegmental furrow of 7/8 and penial sheaths in the copulatory bursae. Two species, *P. vergrandis* Aspe & James 2014 and *P. conceptionensis* Aspe & James 2014 are monothecate. Three species (*P. adevai* Aspe & James 2014, *P. lluchi* Aspe & James 2014, and *P. potonganensis* Aspe & James 2014) belong to the *P. darnleiensis* (Fletcher, 1887) species group, characterized by having either four or five pairs of spermathecae from segments vi to ix, with a fifth pair variably present in segment v. An athecate species, *P. subanensis* Aspe & James 2014, was also described. In addition to *Pheretima*, three *Pithemera* and one *Polypheretima* species were also detected, which were described in Aspe & James (2015) and are reported in Chapter 3.

Table 2.1 shows the frequency and relative abundance of *Pheretima* species at the five collecting sites where *Pheretima* was detected. *Pheretima wati*, *P.*

misamisensis, and *P. potonganensis* were the most widely distributed among sites (frequency 1 for *P. wati* and 0.8 for *P. misamisensis* and *P. potonganensis*). *Pheretima adevai*, *P. wati* and *P. potonganensis* showed the highest relative abundance across all sites and plots (19.4%, 11.8% and 11.2%, respectively, of all individuals collected). The sites with highest species diversity were in disturbed forest in Barangays Lake Duminagat and Sibucal (20 and 17 species, respectively). The four sites where we found *Pheretima* to be species-rich and common were all above 900 m in elevation.

TAXONOMY

Megascolecidae Rosa, 1891

***Pheretima* (*Pheretima*) Kinberg, 1867**

Type species. *Pheretima montana* Kinberg, 1867

Generic diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired within copulatory pouches opening on segment xviii; one or more pairs of spermathecal pores in intersegmental furrows between 4/5 and 8/9. Clitellum annular, covering three segments (xiv to xvi). Single female pore midventrally on xiv. Genital markings usually absent. Internally, esophageal gizzard usually originating in viii; a pair of caeca originating in xxvii, extending forward; septa in 4/5–7/8, 10/11–12/13, thickened or slightly thickened, lacking in 8/9 or 9/10

in some species. Ovaries and funnels free in xiii. Male sexual system holandric, with paired testes and funnels enclosed in sacs in x and xi, and seminal vesicles in xi and xii. Spermathecae a single pair, multiple pairs, or sometimes single and located midventrally. Nephridia on spermathecal duct present. One pair of prostate glands, racemose. Copulatory bursae present; secretory diverticula on coelomic surface of copulatory pouches lacking.

***Pheretima maculodorsalis* Aspe & James 2014**

(Figs 2.2A, 2.3A, 2.3B)

Material examined. Holotype: adult (NMA 4505), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, J. Adeva, Oct. 9–15, 2003. Paratypes: two juveniles (NMA 4531), same collection data as for holotype.

Etymology. The species name is derived from the Latin ‘macula’ (spot) and ‘dorsalis’ (pertaining to the back) and refers to the oval spots along the dorsal midline.

Diagnosis. Large worm, adult length 226–235 mm; dark red stripes in dorsal intersegmental furrows in head region, replaced by oval dots in post-clitellar segments; one pair of spermathecal pores closely spaced at intersegment 7/8; spermatheca with irregularly rounded ampulla, stout muscular duct, stalked diverticulum with 2–3 lobed receptacle; very long caeca extending from xxvii to xxi.

Description. In living animals, head segments striped dark red in intersegments, non-pigmented equators; in post-clitellar segments, stripes replaced by dorsal oval dots, which are also of dark red coloration. Length 226–235 mm (n=3 adults, including non-type material); diameter 11–13 mm at x, 9 mm at xx; body cylindrical in cross-section, tail narrowing abruptly in last 8 segments; 115–122 segments. First dorsal pore at 12/13; spermathecal pores one pair at 7/8, 0.09 circumference apart ventrally, with small thickened lips, ventral surface of ½ vii–viii thickened. Female pore single in xiv, openings of copulatory bursae paired in xviii, 0.13 circumference apart ventrally, 2–4 setae between openings. Clitellum annular, from xiv to xvi. Setae evenly distributed around segmental equators; 73–74 setae on vii, 63–75 setae on xx, dorsal setal gaps present, no ventral gaps.

Septa 5/6–7/8 and 10/11–13/14 muscular, 8/9 membranous, 9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard extending from viii to x, esophagus with low vertical lamellae x–xiii, intestinal origin xvii, caeca originating in xxvii, extending forward to xxi, ventral margins slightly incised; typhlosole originates in xxvii, simple fold slightly less than dorsal vessel diameter; intestinal wall with 50–54 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xiii; extra-esophageal vessel joins ventral esophageal wall in xi, receives efferent parieto-esophageal vessel in xiii.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in viii, with nephridia on ducts; each spermatheca with irregularly rounded ampulla, stout muscular duct, stalked diverticulum attached to duct near ampulla, terminating in 2–3 lobed receptacles, stalks short. Spermathecae contain small, ovate spermatophores with very slender tails about half length of spermatophore body. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates in xvii to xx, each a single, dense, racemose mass; short straight muscular duct entering posterior margin of copulatory bursa; paired large copulatory bursae extend from xviii to xxi; coelomic surfaces of paired hemispheric copulatory bursae muscular, secretory diverticula lacking; roof of copulatory bursae with two pads, posterior pad bifurcate, both pads with small lumen within glandular tissue; small penis between pads; penial sheaths in copulatory bursae absent. Bursal floor has thick wrinkles, no other projections.

Remarks. *Pheretima maculodorsalis* Aspe & James 2014 belongs to the *P. sangirensis* species group in Sims & Easton (1972), characterized by spermathecal pore(s) opening only in 7/8 and absence of penial sheaths in the copulatory bursae. Members of this group may have no septa in either intersegments 8/9 or 9/10 or both; the caeca are either simple or have short pockets on the ventral margins; the male system is holandric, with paired testis sacs; and the copulatory pouches are simple, with short conical penes. In Sims & Easton (1972), the *P. sangirensis* group was composed of *P. sangirensis*, *P. ceramensis* Cognetti, 1922, and *P. crassicystis*

Michaelsen, 1896. Michaelsen (1900) reassigned *P. crassicystis* as a subspecies of *P. sangirensis*. Blakemore (2007) acknowledged Michaelsen's (1900) reassignment of *P. sangirensis* subspecies: *P. s. sangirensis*, *P. s. crassicystis*, and *P. s. chica* Michaelsen, 1896. The subspecies vary in size (140 mm x 3.5–4.5 mm in *P. s. sangirensis*; 240 mm x 8 mm in *P. s. crassicystis*; and 54–120 mm in *P. s. chica*) and color (dark purple brown in *P. s. sangirensis*; purplish gray in *P. s. crassicystis*; and purple in *P. s. chica*). Also, the first dorsal pore in *P. s. sangirensis* is located in 11/12 while it is in 12/13 in *P. s. crassicystis* and *P. s. chica*. Another species, *P. unicystis* Lee, 1981 from Vanua Tu, was added to the species group, but *P. unicystis* differs from the other members in the group by having the clitellum located in ½ xiv–½ xvi and in consistently having only one spermatheca located on the right side of 7/8. Blakemore (2007) considered *P. unicystis* to be a possible junior synonym of *P. montana* Kinberg, 1867. *Pheretima maculodorsalis* differs markedly from *P. sangirensis* (and subspecies; see Table 2.2 for comparison) and *P. ceramensis* in pigmentation pattern (pigmented over the entire dorsum in *P. ceramensis*), the distance between male pores and spermathecal pores (about 0.2 circumference apart and slightly closer set, respectively in *P. ceramensis*) (James, 2004), the origin of the intestine (xv in *P. ceramensis*), and the number of intestinal vessels (36 in *P. ceramensis*), among other characters. *Pheretima maculodorsalis* is similar to *P. s. crassicystis* in size (240 mm) and the location of the dorsal pore, but the latter is entirely pigmented, has no septum in 8/9, has caeca extending from xxvii–xxii, and has the prostate extending from xvii–xix. James (2004) reviewed the *P. sangirensis* group and added to this group 10 new species (*P. quincunxia*, *P. diesmosi*, *P. monoporata*, *P. viciniporta*, *P. baungonensis*, *P.*

paucisetosa, *P. alba*, *P. virgata*, *P. rubida* and *P. asurgo* Blakemore, 2006 (a replacement name for *P. rugosa* James, 2004 to avoid homonymy with *P. houletti rugosa* Gates, 1926) from the Mt. Kitanglad range in Mindanao. Hong & James (2008b) added another two species (*P. lagunaensis* and *P. mariae*) to this group from Mt. Makiling on Luzon Island. *Pheretima maculodorsalis* is a large worm, and among the species at Mt. Kitanglad is most similar in size to *P. virgata*, which reaches 290 mm. The two species differ in the intestinal origin (xvi in *P. virgata*), the pigmentation pattern (stripes in *P. virgata*), the number of intestinal vessels (42 in *P. virgata*), and the number and shape of pads in the copulatory bursae. Other large worms on Mt. Malindang are *P. tigris* Aspe & James 2014, *P. immanis* Aspe & James 2014, and *P. lago* Aspe & James 2014. *Pheretima maculodorsalis* differs from them (Table 2.2) in pigmentation pattern; the length of the caeca; the shape, size and position of prostate glands and copulatory pouches; and the spermathecal pores, which are closer together. *Pheretima maculodorsalis* is the only species of the *sangirensis* group at Mt. Malindang that has the intestinal origin in xvii.

Occurrence. *Pheretima maculodorsalis* was found in primary and disturbed forest at two of five forest sites in Brgy Lake Duminagat, at elevations of 1479–2027 m asl. It occurred in soil and rotting logs (Table 2.1).

***Pheretima tigris* Aspe & James 2014**

(Figs 2.2B, 2.3C)

Material examined. Holotype: adult (NMA 4506) Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe and J. Adeva, Oct. 9–15, 2003. Paratypes: three juveniles (NMA 4532), same collection data as for holotype. Other material: two adults (ZRC.ANN.0016), Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range, 8°24'04" N, 123°36'47" E, 848 m asl, coll. Nonillon Aspe, M. Lluch and J. Adeva, Feb. 18–25, 2004.

Etymology. The species name is the Latin 'tigris' (tiger), referring to the striped body.

Diagnosis. Large worm with adult length of 230–283 mm; dark red to purple dorsal pigment stripes in intersegmental furrows, equators non-pigmented; one pair of spermathecal pores at 7/8; spermathecae with ovate to pyriform ampullae; relatively small prostates extending from xvi to xviii; 56–58 intestinal vessels; very large, elongate caeca extending from xxvii to xix; penes absent.

Description. Living animals have iridescent, dark red to purple dorsal stripes at intersegmental furrows; pigment almost black in formalin; equators non-pigmented. Length 230–283 mm (n=3 adults); diameter 8–10 mm at x, 11–14 mm at xx; body cylindrical in cross-section, tail narrowing abruptly in last 6 segments; 113–123 segments. First dorsal pore at 12/13; spermathecal pores one pair at 7/8, 0.13 circumference apart ventrally; large indistinct pads paired in viii behind spermathecal pores; female pore single in xiv, openings of copulatory bursae paired in xviii, 0.14 circumference apart ventrally, 0–4 setae between openings. Clitellum annular,

extending from xiv to xvi. Setae evenly distributed around segmental equators; 53–66 setae on vii, 48–61 setae on xx, dorsal and ventral gaps absent.

Septa 5/6 and 7/8 slightly muscular, 6/7 and 10/11–15/16 muscular, 8/9 membranous, 9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located on body wall anterior and posterior to septa. Large gizzard extending from viii to x; esophagus with low vertical lamellae x–xiii; intestinal origin xvi; caeca originate in xxvii, extend forward to xix, broad base diminishes to narrow tip, several small ventral pockets; typhlosole originates in xxvii, three-pronged origin composed of main central ridge with two short branches posterior to beginning of ridge, then simple fold 1/6 lumen diameter; intestinal wall with 56–58 longitudinal blood vessels. Intestine narrow with thick villous lining in xvi–xxvi, intestine much wider after xxvii.

Hearts in x to xiii, esophageal, but x and xi very small; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; spermathecae paired, postseptal in viii, with nephridia on ducts; each spermatheca with large ovate to pyriform ampulla, stout muscular duct, stalked diverticulum attached to duct ental near ampulla, terminating in oblong receptacle wider at distal end; stalk short, thick. One or two spermatophores in each ampulla, nearly spherical, with long curved tail and ragged, 'dirty' end that may have been a plug in spermathecal pore. Male sexual system holandric; testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii, that

in xii with long flattened dorsal lobe; vesicles of xi in testes sacs; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; each prostate densely racemose, extending from xvi to xviii, muscular duct attached to surface of hemispheric to elliptical copulatory bursa in xvii to xix, entering posterior dorsal face of copulatory bursa; paired copulatory bursae extend from xvii to xix coelomic surfaces of copulatory bursae muscular, secretory diverticula lacking; floor of bursae with 5 small pads forming U-shaped array around posterior side of opening; pyramidal penial mound directed to opening from posterior bursal roof; penes absent.

Remarks. *Pheretima tigris* Aspe & James 2014 belongs to the *P. sangirensis* group in Sims & Easton (1972) but differs from *P. sangirensis* pigmentation pattern, intestinal origin, and number of intestinal vessels, and in lacking penes (Table 2.2). Anterior septa are present except at 9/10, unlike most other species in the *P. sangirensis* group, where septa 8/9/10 are absent. *Pheretima tigris* is a large worm, similar in size to *P. ceramensis* and *P. s. crassicystis* (140–440 mm and 240 mm, respectively) (James, 2004), but the latter two species are entirely pigmented and have shorter caeca (xxvii–xx and xxvii–xxiv, respectively). In addition, *P. ceramensis* has the intestinal origin in xv and has fewer longitudinal blood vessels (36), and *P. s. crassicystis* has no dorsal setal gap and lacks a septum in 8/9. Among the species at Mt. Kitanglad (James 2004), *P. tigris* is most similar to *P. virgata* James, 2004 in size and pigmentation pattern, the origin of the intestine and typhlosole, and the absence of penes, but differs from the latter in the number of setae (76 in vii and 80 in xx in *P. virgata*), the number of longitudinal blood vessels in the intestine (42 in *P. virgata*), the extent of the copulatory bursae (xviii in *P. virgata*), and the number and shape of

pads in the copulatory bursae. *Pheretima tigris* differs from *P. maculodorsalis* in pigmentation pattern, the origin of the intestine, the extent of the prostate glands, the absence of penes, and in the number and the shape of pads in the copulatory pouch.

Occurrence. *Pheretima tigris* was found at elevations of 915–2027 m asl, commonly in primary forest in Brgy Lake Duminagat and less commonly in disturbed forest in Brgy Small Potongan. It occurred in soil and rotting logs (Table 2.1).

***Pheretima immanis* Aspe & James 2014**

(Figs 2.2C, 2.3D)

Material examined. Holotype: adult (NMA 4507), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe and J. Adeva, Oct. 9–15, 2003. Paratype: one adult, tail end missing (ZRC.ANN.0017), same data as for holotype.

Etymology. The species name is from the Latin 'immanis' (huge, enormous), referring to the large size.

Diagnosis. Adults large, reaching 365 mm in length; thick dark purple to black dorsal stripes at intersegmental furrows, equators unpigmented; one pair of spermathecal pores at 7/8; spermathecae, prostate glands and copulatory bursae small relative to body size; penes lacking; 28–32 intestinal vessels.

Description. Living animals with iridescent, broad, dark blue-purple to black dorsal pigment stripes at intersegmental furrows; stripes narrow ventrally to fine points, non-pigmented equators widest ventrally. Length 365 mm (n=1 adult), diameter 17 mm at x, 18 mm at xx; body cylindrical in cross-section; 119 segments. First dorsal pore at 12/13; spermathecal pores one pair at 7/8, 0.12 circumference apart ventrally; female pore single in xiv, openings of copulatory bursae paired in xviii, 0.14 mm circumference apart ventrally, 5 setae between openings. Clitellum annular, extending from xiv to xvi. Setae unevenly distributed; 61–69 setae on vii; 63–68 setae on xx; no dorsal or ventral gaps.

Septa 5/6 and 7/8 slightly muscular, 10/11–15/16 muscular, 8/9 thin, 9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located at septum/body wall junction mainly on body wall at anterior and posterior faces of septa. Large gizzard extending from viii to x, esophagus with low vertical lamellae from x to xiii; intestine originates in xvi; caeca originate in xxvii, extend forward to xx; typhlosole originates in xxvii, simple fold $\frac{1}{4}$ lumen diameter. Intestinal wall with 28–32 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels vi, vii and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; spermathecae paired, postseptal in viii, with nephridia on ducts; each spermatheca with large, rounded rectangular ampulla, stout muscular duct, stalked diverticulum attached to duct near ampulla, terminating in oblong

receptacle wider at distal end, attached by its side near narrow ental end; stalks short. Four spermatophores present in each ampulla. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles xi and xii each with dorsal lobe; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; each prostate densely racemose, in xvii and xviii, muscular duct attached to surface of hemispheric copulatory bursa, entering posterior dorsal face of copulatory bursa; paired small copulatory bursae extend from xvi to xvii; coelomic surfaces of copulatory bursae muscular, secretory diverticula lacking; floor of bursae with two small pads lateral to opening; penes absent.

Remarks. A member of the *P. sangirensis* group, *P. immanis* Aspe & James 2014 is by far the largest of any earthworm species previously known from the Philippines. Other large-sized worms in the Philippines include *P. virgata* James, 2004 (length 290 mm) from Mt. Kitanglad; *P. barligensis* Hong & James, 2011b (length 225–255 mm) from Mt. Province on Luzon Island; *P. callosa* Gates, 1937 (length 330 mm) from Benguet on Luzon Island; and *P. maculodorsalis* (length 226–235 mm), *P. tigris* (length 230–283 mm), and *P. lago* (length 223–315 mm) described herein. *Pheretima immanis* differs from these species in pigmentation pattern; the origins of the intestine; the shape and size of spermathecae, diverticula, prostates and copulatory pouches; and the lengths of the caeca. *Pheretima immanis*, with one pair of spermathecae in viii, differs from the large worms *P. barligensis* (4 pairs in 5/6–8/9) and *P. callosa* (3 pairs in 6/7–8/9). *Pheretima immanis* is most similar to *P. tigris* in having dorsal stripes, in the arrangement of septa and the origins of the intestine and typhlosole, and in lacking penes; both also lack setal gaps on the dorsum and ventrum.

However, mature individuals of *P. immanis* reach a larger size, and the dorsal stripes are much thicker than those of *P. tigris*. Internally, the two species differ in the extent of the caeca, the size and position of prostate glands and copulatory bursae (Table 2.2), the number of intestinal vessels, and the shape and size of spermathecae. Other large worms in the *P. sangirensis* group are *P. ceramensis* (140–440 mm) and *P. s. crassicystis* (240 mm), but these two species markedly differ from *P. immanis* in having pigmentation all over the body. Moreover, *P. ceramensis* has the intestinal origin in xv and has shorter caeca (xxvii–xxiv); *P. crassicystis* has no septa in 8/9, the prostate is a bit longer (xvii–xix), and the caeca are shorter (xxvii–xxii). The largest *Pheretima* species in the world, which Blakemore *et al.* (2007) identified as *P. darnleiensis*, reaches 700 mm in length; that species differs markedly from *P. immanis* in having 4 or 5 pairs of spermathecal pores located in 5/6–8/9.

Occurrence. *Pheretima immanis* was found at elevations of 915–2027 m, but was somewhat more common at elevations above around 1480 m than at lower elevations (Table 2.1).

***Pheretima lago* Aspe & James 2014**

(Fig. 2.4A)

Material examined. Holotype: adult (NMA 4508), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Oriental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., Mindanao Island, Philippines, coll. Nonillon

Aspe, Nolan Aspe, and J. Adeva, Oct. 9–15, 2003. Paratypes: one adult (NMA 4533); two adults (ZRC.ANN.0018), same collection data as for holotype.

Etymology. The species name ‘lago’ means ‘large worm’ in the Cebuano dialect of the Philippines.

Diagnosis. Worms large, up to 315 mm long; dorsum dark, gradually fading towards ventral side; one pair of spermathecal pores at 7/8; relatively small spermathecae, diverticula with 2–4 chambered receptacles; intestine originating in xiv; hearts in xi to xiii, lacking in x, prostate glands located mostly anterior to copulatory bursae; 36–38 intestinal vessels.

Description. Living individuals with dark-brown to black dorsum anteriorly, lighter posteriorly, with gradually widening, non-pigmented equators; head setal rings with very thin non-pigmented area. Length 223–315 mm (n=4 adults); diameter 10–11 mm at x, 10–11 mm at xx; body cylindrical in cross-section, tail narrowing abruptly in last 6 segments; 116–134 segments. First dorsal pore at 12/13; inconspicuous spermathecal pores one pair in 7/8, 0.18–0.24 circumference apart ventrally; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.15 circumference apart ventrally, 0–2 setae between openings. Clitellum annular, extending from xiv to xvi. Setae unevenly distributed; 49 setae on vii, 53 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6–7/8, 10/11–13/14 muscular, 8/9 thin, 9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments sparser on segmental equators. Large gizzard extending from ix to x; esophagus with low vertical lamellae within x to xiii; intestinal origin in xiv; slender caeca originating in xxvii,

extending forward to xxi, ventral margins slightly incised; typhlosole originates in xxvii, simple fold of 1/5 lumen diameter; intestinal wall with 36–38 longitudinal blood vessels.

Hearts in xi to xiii, esophageal; hearts in x much reduced, hidden under membrane that is perhaps remnant of septum 9/10; commissural vessels vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from xi to xiii; extra-esophageal vessel joins ventral esophageal wall in x, receives efferent parieto-esophageal vessel in xiii, with upper and lower branches.

Ovaries and funnels free in xiii; spermathecae paired, preseptal in vii, with nephridia on ducts; each spermatheca with large irregular rounded ampulla, stout muscular duct, stalked diverticulum attached to duct near ampulla, terminating in 2–4 chambered receptacle; stalks long, muscular. Spermathecal ducts fluted internally and off center from duct axis, ducts bearing rosette-shaped structure engorged with blood. Numerous ovate to pyriform spermatophores in each ampulla, tails longer than spermatophore body. Male sexual system holandric; testes and funnels enclosed in ventrally paired sacs in x and xi; seminal vesicles in xi and xii, each with long digitate dorsal lobe; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; each prostate racemose, with 6–7 main lobes in xiv to xviii; short, curved muscular duct enters anterior surface of copulatory bursa; paired elongate copulatory bursae extend from xvii to xx, coelomic surface of copulatory bursae muscular, secretory diverticula lacking; posterior portion of bursae filled with solid glandular tissue; long penis in anterior chamber of bursa; half-circle collar around

anterior base of penis; copulatory bursae lack penial sheaths. Three long ridges in bursae, lateral to opening, one each on roof, floor, and lateral face of chamber.

Remarks. *Pheretima lago* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972) but differs from all subspecies of *P. sangirensis* in having septa in 8/9, preseptal spermathecae in vii, and the intestine originating in xiv. It is a large worm, similar in size to *P. ceramensis* and *P. s. crassicystis*, but *P. ceramensis* has the intestine originating in xv and has shorter caeca (xxvii–xxiv), and *P. s. crassicystis* has no dorsal setal gap, the septum in 8/9 is lacking, and the prostates extend from xvii–xix. *Pheretima lago* is the second largest *Pheretima* species from Mt. Malindang next to *P. immanis*. *Pheretima lago* differs from *P. immanis* and another large worm, *P. tigris*, in pigmentation pattern, in having a dorsal setal gap, in the origin of the gizzard, in having spermathecal diverticula with chambered receptacles, in the number of hearts, in the extent of the caeca, in the number of intestinal vessels, and in having long penes (Table 2.2). *Pheretima lago* is similar to *P. callosa* James, 2004 in size, but the latter has 3 pairs of spermathecal pores in 6/7–8/9, the intestinal origin in xv, and prostates confined to xviii.

Occurrence. *Pheretima lago* was found at elevations of 900–2030 m asl. It was common at higher elevations in primary forest in Brgy Lake Duminagat, but uncommon at lower elevations (Table 2.1).

***Pheretima nunezae* Aspe & James 2014**

(Fig. 2.4B)

Material examined. Holotype: adult, amputee (NMA 4509), Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratype: one juvenile (NMA 4534), same data as for holotype.

Etymology. The species is named in honor of Dr. Olga Nuneza, one of our collaborators in the Malindang Biodiversity Research Program and a professor at Mindanao State University-Iligan Institute of Technology, Iligan, Philippines.

Diagnosis. Large worm, dark gray-brown dorsally, non-pigmented ventrally, clitellum gray; one pair of spermathecal pores at 7/8; septa from 4/5 to 13/14, except for 9/10; relatively small prostates extending from xvii to xix, copulatory bursae confined to xviii; 20–23 intestinal vessels.

Description. In living animals, dorsum dark gray-brown anteriorly, fading posteriorly; ventrum non-pigmented; equators non-pigmented; clitellum gray, darker than adjacent segments. Length of posteriorly incomplete worm 116 mm; diameter 8.5 mm at x; 9 mm at xx; body cylindrical in cross-section. First dorsal pore at 12/13; paired spermathecal pores at 7/8, 0.28 circumference apart ventrally; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.22 circumference apart ventrally, 9 setae between openings. Clitellum annular, extending from xiv to xvi. Setae evenly distributed, 46 setae on vii, 51 setae on xx, dorsal gap present, ventral gap absent.

Septa 4/5/6/7/8 slightly muscular, 8/9 membranous, 9/10 absent, 10/11–15/16 slightly muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard extending from ix to x; esophagus with wide-angled, chevron-shaped lamellae, extending from x to xiii; intestine originates in xv; caeca originate in xxvii, extend forward to xxiv, with pocketed ventral margin; typhlosole simple fold, about 1/8 lumen diameter, originating at 26/27. Intestinal wall with 20–23 longitudinal blood vessels.

Hearts in xi to xiii, esophageal commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; spermathecae paired in vii, one preseptal and the other postseptal; spermathecae irregularly shaped, with nephridia on ducts; each spermatheca with angular, apically attached oval ampulla, short thick muscular duct with slight bulge for diverticulum attachment; single-stalked diverticulum attached to face of duct, terminating in thick, sausage-shaped receptacle, stalk shorter than receptacle. Each spermatheca contains 2 spermatophores. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with short, spherical dorsal lobe; vasa deferentia slender, free from body wall, passing around lateral face of copulatory bursae en route to ental end of prostatic ducts; each prostate racemose, with three lobes, extending from xvii to xix, wrapped around lateral margins of copulatory bursa; muscular duct attached to surface of copulatory bursa along entire length of latter and enters apex. Ductlets of lobes meet

vasa deferentia at common junction with muscular prostatic duct. Copulatory bursae hemispherical in xviii; coelomic surface of bursa muscular, secretory diverticula lacking; bursal floor with 2 pads lateral to center; elongate fold with longitudinal groove extending across medial edges of each pad; opening composed of sphincter valve surrounded by narrow ring; bursal roof with pair of folds or pads medial to small, rounded quadrangular penial projection directed posteriorly; copulatory bursae lacking penial sheaths.

Remarks. *Pheretima nunezae* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972). It differs from all subspecies of *P. sangirensis* in having a septum in 8/9, the gizzard originating in ix, hearts absent in x, and fewer intestinal vessels and setae in xx. It differs from *P. ceramensis* Cognetti, 1922 in the origin of the gizzard (viii in *P. ceramensis*); the number of setae (60 setae per segment in *P. ceramensis*; James 2004); the number of hearts and intestinal vessels (x–xiii and 36, respectively in *P. ceramensis*); and the number of pads on the floor of the copulatory bursae. *Pheretima nunezae* differs from most of the Malindang species in the *sangirensis* group in size, particularly in width (except that of *P. maculodorsalis* and *P. tigris*); in having wide spacing between the spermathecal pores and between male pores, especially in large worms; in the shape of the spermathecae and spermathecal diverticula; in the size and position of the prostate glands and copulatory bursae; in the number of intestinal vessels; and in the length of the caeca. No other species known in the Philippines resembles *P. nunezae*.

Occurrence. A few *Pheretima nunezae* were found in disturbed forest in Brgys Sibucal and Small Potongan at elevations of 902–1067 m asl; overall, it was

one of the least common species, comprising 2.1% of all specimens collected (Table 2.1).

***Pheretima boniao* Aspe & James 2014**

(Fig. 2.4C)

Material examined. Adult (NMA 4518), Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range, 8°24'04"N, 123°36'47"E, 848 m asl, Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch and J. Adeva, Feb. 18–25, 2004. Paratype: one adult (NMA 4531), same data as for holotype.

Etymology. The species is named in honor of Dr. Renato Boniao, a collaborator at Malindang Biodiversity Research Program and a professor at Mindanao State University-Naawan, Naawan, Misamis Oriental, Philippines.

Diagnosis. Body with purplish brown stripes; closely spaced spermathecal pores in 7/8; closely spaced male pores appearing as one in xviii; spermathecae round; elongate prostate glands from xvi to xxi.

Description. In living animals, purplish brown dorsal stripes at intersegmental furrows; equators non-pigmented; ventral side non-pigmented; clitellum gray. Length 101–133 mm; diameter 5–5.5 mm at x; 5.5–6 mm at xx; body cylindrical in cross-section, tail tapered; 98–108 segments. Paired spermathecal pores at 7/8, 0.14 circumference apart ventrally; female pore single in xiv; openings of copulatory

bursae very closely spaced in floor of pit 0.6 mm wide in xviii, 0.03 circumference apart ventrally, no setae between openings. Clitellum annular, extending from xiv to xvi. Setae more numerous on ventral side, 59–78 setae on vii, 62–68 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6/7/8 thin, 10/11–13/14 slightly muscular, 8/9 very thin, 9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7, nephridia of intestinal segments mainly on body wall on anterior and posterior faces of septa, located at septum/body wall junction. Large gizzard in viii–xi; esophagus with circumferential lamellae from xi to xiii; intestine originates in xiv; caeca originate in xxvii, extend forward to xxiv, with serrate ventral margin; typhlosole a simple fold of about 1/6 lumen diameter, originating in xxvii; intestinal wall with 33–45 longitudinal blood vessels.

Hearts in xi–xiii, esophageal; commissural vessels vi, vii, and ix lateral, absent in viii; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; paired spermathecae post-septal in viii, with nephridia on ducts; each spermatheca consists of small rounded rectangular ampulla, very large bulbous muscular duct, expanding ectally, and single stalked diverticulum attached to posterior face of muscular bulb of duct, terminating in round receptacle attached by its end; stalks become abruptly stout midway towards ectal end.

Spermathecal duct walls thick, complexly corrugated. Spermatophores lacking. Male sexual system holandric; testes and funnels enclosed in ventrally joined sacs in x and xi; seminal vesicles in xi and xii, each with long dorsal lobe; vasa deferentia slender,

free from body wall, passing around anterior lateral face of copulatory bursae en route to ental end of prostatic ducts; prostates in xvi to xxi, each racemose, 4 lobed, wrapped around lateral margin of copulatory bursa; muscular duct attaches to dorsal face of copulatory bursa at center, runs along surface, and enters anterior face. Ductlets from anterior prostatic lobes join vas deferens and posterior ductlets at common junction with muscular prostatic duct. Copulatory bursae ovate, extending from xvii to xx; coelomic surface of copulatory bursa muscular; the outlet of the two bursae exit towards a common male pore; secretory diverticula lacking. Floor of bursae lacks pads; roof with small anterior pad; penis absent.

Remarks. *Pheretima boniaoi* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972). It differs from all subspecies of *P. sangirensis* in having striped pigmentation, in having a closer space between the spermathecal pores, especially the male pores, which appear as one, and in having more elongate prostates. The new species is similar to *P. tigris*, *P. immanis*, and *P. virgata* from Kitanglad (James, 2004) in having dorsal striped pigmentation, but the latter three are much larger worms. *Pheretima boniao* is similar to *P. vicinipora* from Mt. Kitanglad (James, 2004) and to *P. wati* Aspe & James 2014 in having closely spaced spermathecal pores (although these are much closer together in *P. vicinipora*; 0.04 circumference apart) and male pores, but the latter two are smaller and are pigmented all over. Another distinctive character of *P. boniaoi* is the stalks of the spermathecal diverticula, which become abruptly stout midway towards the ectal end.

Occurrence. Only two individuals of *Pheretima boniaoi* were found in disturbed forest in Brgys Small Potongan and Brgy Lake Duminagat, at elevations of 915–1024 and 1479–1662 m, respectively (Table 2.1).

***Pheretima malindangensis* Aspe & James 2014**

(Figs 2.2E, 2.5A)

Material examined. Holotype: adult (NMA 4513) Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: 2 adults (NMA 4536); one adult (ZRC.ANN.0020), same collection data as for holotype.

Etymology. The species is named after Mt. Malindang National Park.

Diagnosis. Small worm with adult length reaching 60–81 mm, purplish brown; distance between spermathecal pores and male pores relatively short; spermathecal ampulla small, rounded, rectangular, with large, bulbous, muscular duct; gizzard large, extending from viii to xi; intestinal origin in xvi; hearts in xi to xiii, absent in x; elongate prostate glands extending from xvi to xxi; copulatory bursae large and ovate extending from xvii to xx; caeca extending from xxvii to xxiii. Penial setae present on penial body of copulatory bursae.

Description. Living animals with dorsum dark purplish-brown anteriorly, fading to medium brown posteriorly; equators non-pigmented; ventral side non-

pigmented; clitellum gray. Length 60–81 mm (n=3 adults); diameter 4.0–4.5 mm at x, 4.0–5.0 mm at xx; body cylindrical in cross-section, tail blunt; 69–96 segments. First dorsal pore 12/13; paired spermathecal pores at 7/8, 0.16 circumference apart ventrally, with prominent internal ridges leading into each pore. Female pore single in xiv; openings of copulatory bursae paired in xviii, 0.11 circumference apart ventrally, no setae between openings. Clitellum annular, extending from xiv to xvi. Setae evenly distributed, 43–47 setae on vii, 50–57 on xx, no dorsal gap, ventral gap present.

Septa 5/6/7/8 and 10/11–13/14 slightly muscular, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments mainly on body wall at anterior and posterior faces of septa, located at septum/body wall junction. Large gizzard extending from viii to xi; esophagus with circumferential lamellae within xi and xii, with digitiform internal texture from xiii to xiv; intestine originates in xvi; caeca originate in xxvii, extending forward to xxiii, with serrate ventral margins; typhlosole a simple fold about 1/6 lumen diameter, originating in xxvii; intestinal wall with 30 longitudinal blood vessels.

Hearts in xi to xiii, esophageal, absent in x; commissural vessels vi, vii, and ix lateral; viii extending to gizzard; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; paired spermathecae post-septal in viii, with nephridia on ducts; ampulla small, rounded, rectangular; spermathecal duct large, bulbous, muscular, its wall thick and corrugated; single stalked diverticulum attached to posterior face of muscular bulb of duct, terminating in bean-shaped receptacle

attached by its end, stalks longer than receptacle, increasing in diameter ectally. Two spermatophores present in each ampulla. Male sexual system holandric; testes and funnels enclosed in unpaired ventral sacs in x and xi; seminal vesicles in xi and xii, each with short dorsal lobe; vasa deferentia slender, free from body wall, passing around anterior lateral face of copulatory bursae en route to ental end of prostatic ducts; each prostate racemose, 4 or 5 lobed, from xvi to xxi, wrapped around lateral margin of copulatory bursa; muscular duct enters dorsal face of copulatory bursa just posterior of center. Ductlet from anterior prostatic lobes joins vas deferens, posterior ductlet at common junction with muscular prostatic duct. Large, ovate copulatory bursae extending from xvii to xx, broader in anterior portion; coelomic surface of copulatory bursa muscular, secretory diverticula lacking. Floors of bursae with anterior and posterior pads flanking opening, gutter leading from opening up to base of penis; roofs with single angular posterior pad and large anterior glandular mass. Large blocky centrally placed penis bearing 5 penial setae; copulatory bursae lack penial sheaths.

Remarks. *Pheretima malindangensis* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972). It is unique in having setae on the penial body. It differs from all subspecies of *P. sangirensis* in having closer spacing between the spermathecal pores and between the male pores, in lacking a dorsal setal gap (also lacking in *P.s. crassicystis*), and in having a ventral setal gap; in the origin of intestine; and in lacking hearts in x (Table 2.2). It is similar in size and coloration to *P. wati*, *P. longiprostata* Aspe & James 2014, and *P. nolani* Aspe & James 2014 (see descriptions below), but differs (apart from the setae on the penis) in the male pore spacing, in

lacking a dorsal setal gap, and having a ventral setal gap; in the number of hearts, the origin of intestine, and shape of spermathecae; and in the size, shape, and position of the prostates and copulatory bursae (Table 2.2). *Pheretima malindangensis* does not closely resemble any species in the *sangirensis* group at Mt. Kitanglad (James 2004).

Occurrence. *Pheretima malindangensis* was found in Brgys Sibucal and Lake Duminagat, at elevations of 902–2027 m asl, with more individuals found in Brgy Sibucal. It occurred in the soil and above ground in substrates such as rotting logs (Table 2.1).

***Pheretima misamisensis* Aspe & James 2014**

(Fig. 2.5B)

Material examined. Holotype: adult (NMA 4516) Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: two adults (NMA 4537); two adults (ZRC.ANN.0021), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., coll. Nonillon Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species is named after Misamis Occidental Province, where this species was collected.

Diagnosis. Small, brown worm, reaching 55–65 mm in adult length; one pair of spermathecal pores widely spaced at 7/8; intestinal origin in xv; elongate prostate glands extending from xvi to xxii; low, circular copulatory bursae in xvii to xix; short caeca extending from xxvii to xxv.

Description. In living animals, dorsum very dark red-brown to black anteriorly, medium red-brown posteriorly; equators non-pigmented; ventral side non-pigmented; clitellum dark. Length 55–65 mm, diameter 3.5 mm at x (n=5 adults), 3–4 mm at xx; 90–103 segments; body cylindrical in cross-section, tail blunt. First dorsal pore 12/13; paired spermathecal pores sublateral on 7/8; 0.3 circumference apart ventrally; female pore single in xiv, openings of copulatory bursae paired in xviii, 0.23 circumference apart ventrally, 6 or 7 setae between openings. Clitellum annular, extending from xiv to xvi. Setae on ventrum more closely spaced compared with that of the dorsum, 42–51 setae on vii, 43–48 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6/7/8 and 10/11–13/14 slightly muscular, 8/9 very thin, 9/10 partial. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in viii to x; esophagus with circumferential lamellae from xi to xii, digitiform internal texture from xiii to xiv; intestinal origin in xv; caeca originating in xxvii, extending forward to xxv; typhlosole rudimentary; intestinal wall with 32 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends to between x and xiii;

extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; paired spermathecae preseptal in vii, with nephridia on ducts. Each spermatheca consists of blunt reniform ampulla; equally long, stout duct; and single stalked diverticulum attached to duct near body wall, terminating in blunt ovate receptacle; stalks longer than spermathecal duct. Male sexual system holandric; testes and funnels enclosed in paired ventral sacs in x and xi, but funnels of x extend into xi; seminal vesicles in xi and xii, each with short, acinous dorsal lobe; vasa deferentia slender, free from body wall, passing over anterior lateral face of copulatory bursae en route to ental end of prostatic ducts; prostates in xvi to xxii, each racemose, 5- or 6-lobed, wrapped around lateral margin of copulatory bursa; muscular duct enters posterior dorsal face of copulatory bursa. Ductlets from anterior prostatic lobes join vas deferens; posterior ductlets join anterior ductlets at junction with muscular prostatic duct. Copulatory bursae low circular domes extending from xvii to xix. Coelomic surface of copulatory bursa muscular, secretory diverticula lacking; walls consist of outer muscular layer, inner glandular layer; floors of bursae with one large anterior pad, inner posterior dorsal face with very short, conical penis; penial sheaths in copulatory bursae absent.

Remarks. *Pheretima misamisensis* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972). It is similar to *P.s. chica* (Michaelsen, 1896) in size (54–120 mm) but differs in color (purple in *P.s. chica*); the spacing of the spermathecal pores and male pores (0.25 and 0.2 circumference apart, respectively, in *P.s. chica*); and in the number of setae in the post-clitellar region (>60 in *P.s. chica*).

Pheretima misamisensis is most similar to *P. wati* Aspe & James 2014 in size, the origins of the gizzard and intestine, and the number of hearts (Table 2.2). However, in *P. misamisensis* the spermathecal and male pores are widely spaced, whereas they are more closely spaced in *P. wati*; in *P. misamisensis*, septa 9/10 is absent, whereas in *P. wati* septa 8/9 is absent. These two species also differ in the number of setae on vii; shape of the spermathecae; size and position of the prostates and copulatory bursae; and length of the caeca. *Pheretima misamisensis* is similar in length to *P. quincunxia*, *P. rugosa*, and *P. rubida* at Mt. Kitanglad (James, 2004), but differs from the latter three in the spacing between spermathecal pores (0.13 circumference apart in *P. quincunxia*, 0.15 circumference apart in *P. rugosa*, and 0.12 circumference apart in *P. rubida*), the origin of the intestine (xvi in *P. quincunxia* and *P. rubida*; xvii in *P. rugosa*), the absence of septa in 9/10, and the length of the prostate (xvii–xix in *P. quincunxia*; xvi–xx in *P. rugosa* and *P. rubida*). *Pheretima misamisensis* also differs from *P. rubida* and *P. rugosa* in the location of the first dorsal pore, which is in 11/12, and from *P. quincunxia* in pigmentation (the latter is unpigmented).

Occurrence. *Pheretima misamisensis* was not found on any of the sampling plots, but was detected in haphazard samples at four of the five sites listed in Table 2.1; it occurred at elevations above ~900 m in Brgys Sibucal, Small Potongan, and Lake Duminag, but was not found at 238–271 m in Brgy Toliyok. It was found in soil and rotting logs (Table 2.1).

***Pheretima wati* Aspe & James 2014**

(Fig. 2.5C)

Material examined. Holotype: adult (NMA 4517) Brgy Sibucal, Oroquieta City, Misamis Oriental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: five adults (NMA 4538), same collection data as for holotype. Other material: three adults (ZRC.ANN.0022), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., coll. Nonillon Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species name 'wati' is the word for small earthworm in Cebuano dialect.

Diagnosis. Small, purplish-brown worm reaching 67–75 mm in adult length; one pair of spermathecal pores at 7/8; male pores very closely spaced; relatively large, elongate spermathecae; intestinal origin in xv; caeca extending from xxvii to xxii; long prostate extending from xv to xxii, copulatory bursae elongate extending from xvii to xxi.

Description. Living animals purplish-brown dorsally, fading to yellow brown ventrally; equators pigmented. Length 67–75 mm (n=9 adults); diameter 3.5–4.0 mm at x; 4.0 mm at xx; body cylindrical in cross-section, tail narrowing gradually to sharp point; 90–104 segments. First dorsal pore at 12/13; spermathecal pores paired at 7/8, 0.17 circumference apart ventrally; female pore single in xiv; depressed region where the copulatory bursae open in xviii; copulatory bursae paired, 0.08 circumference apart ventrally, no setae between openings. Clitellum annular, extending from xiv to

xvi. Setae more closely spaced on ventrum than on dorsum, 59–71 setae on vii, 52–60 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6–7/8 and 9/10–13/14 thin, 8/9 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Gizzard large in viii to x; esophagus with low, vertical lamellae from x to xiii; intestinal origin in xv; caeca originate in xxvii, extend forward to xxii; typhlosole a simple fold about 1/3 lumen diameter, originating at 27/28; intestinal wall with 34–38 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels lateral in vi, vii, and ix, lacking in viii; supra-esophageal vessel from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii, spermathecae post-septal in viii, with nephridia on ducts; large glandular mass on interior ventral surface of viii, partially obscuring spermathecal ducts and diverticula; spermatheca with ovate to pyriform ampulla, slender muscular duct, stalked diverticulum attached ectally to duct, terminating in ovate receptacle; stalk shorter than spermathecal duct. One spermatophore present in each ampulla. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with short, round, knobby dorsal lobe; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates in xv to xxii, each racemose with 5 or 6 separate lobes, distributed around dorsal-lateral margin of copulatory bursa; muscular duct enters posterior dorsal surface of copulatory bursa. Ductlets from anterior

prostatic lobes join vas deferens and posterior ductlets at common junction with muscular prostatic duct. Copulatory bursae elongately bean-shaped extending from xvii to xxi. Coelomic surface of copulatory bursa muscular, secretory diverticula lacking; floors of bursae with medial longitudinal ridge, roofs with posterior glandular pad and penial platform with small, central peak; anterior half of bursa occupied by glandular mass.

Remarks. A member of the *P. sangirensis* group of Sims & Easton (1972), *P. wati* Aspe & James 2014 is similar to all subspecies of *P. sangirensis*; to *P. nunezae*, *P. misamisensis*, and *P. longiprostata* from Mt. Malindang; and to *P. baungonensis* from Mt. Kitanglad (James 2004) in having the intestinal origin in xv. However, it has more setae on vii than the other species, and its male pores are much more closely spaced. Moreover, it differs from the others in the shape and length of the spermathecae, the septal arrangement (absent in 9/10 in *P. baungonensis*), and the extent and shape of prostates and copulatory bursae (xvii–xix and xviii, respectively in *P. baungonensis*), and the caeca are much shorter in *P. baungonensis*. The new species is similar to *P. vicinipora* from Mt. Kitanglad (James, 2004) in having very closely spaced male pores, but the new species has its first dorsal pore in 12/13 (13/14 in *P. vicinipora*), its spermathecal pores are more distant than in *P. vicinipora* (0.08), and its caeca and prostates are more extensive (xxvii–xxv and xvi–xix, respectively, in *P. vicinipora*). Among the Malindang species, *P. wati* has the second most extensive prostate glands relative to body length after *P. longiprostata*; the prostates extend across 8 segments from xv to xxii. Like *P. tigris* and *P. immanis*, *P. wati* lacks penes,

but unlike them, the male pores are very close together, though not joined as one. The glandular mass in viii is also unique.

Occurrence. *Pheretima wati* was found at all sites, at 238–2027 m asl, and was most common at 915–1024 m in Brgy Small Potongan. It occurred both in soil and above ground in substrates such as rotting logs. It was one of the more common species, comprising 11.8% of all worms collected (Table 2.1).

***Pheretima longiprostata* Aspe & James 2014**

(Fig. 2.6A)

Material examined. Holotype: adult (NMA 4519) Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range (8°24'04"N, 123°36'47"E), 900 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratype: one adult (NMA 4539), same collection data as for holotype.

Etymology. The species name derives from the Latin 'longus' (long) and 'prostata' (prostate gland), referring to the long prostate glands.

Diagnosis. Very small, yellowish-brown worm reaching 37–41 mm in adult length; first dorsal pore at 11/12; one pair of spermathecal pores at 7/8; spermathecae small with oval ampulla and stout duct; intestinal origin in xv; elongate prostate glands extending from xv to xxiii; short caeca extending from xxvii to xxv; 20 intestinal vessels.

Description. In living animals, dorsum light yellowish-brown anteriorly, fading posteriorly; equators non-pigmented; ventral side non-pigmented; clitellum gray. Length 37–41 mm (n=2 individuals); diameter 3.5 mm at x, 3 mm at xx; body cylindrical in cross-section, tail tapering; 82-90 segments. First dorsal pore at 11/12; paired spermathecal pores at 7/8, 0.16 circumference apart ventrally; female pore single on xiv; openings of copulatory bursae paired on xviii, 0.16 circumference apart ventrally, 0–5 setae between openings. Clitellum annular, extending from xiv to xvi. Setae evenly distributed, 35–40 setae on vii, 37–47 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6/7/8 thin, 8/9/10 absent, 10/11–13/14 thin. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Gizzard large, extending from viii to x; esophagus with lamellae from xi to xiii; intestinal origin in xv; caeca originate in xxvii, extending forward to xxv; typhlosole a simple fold of about 1/4 lumen diameter, originating at 26/27. Intestinal wall with 20 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; paired spermathecae pre-septal in vii, with nephridia on ducts; each spermatheca with transversely placed oval ampulla, short thick muscular duct with slight bulge for diverticulum attachment, single stalked diverticulum attached to posterior face of duct, terminating in ovate receptacle; stalk

longer than receptacle. Spermathecal duct walls thick, complexly corrugated. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with short dorsal lobe; vasa deferentia slender, free from body wall, passing around lateral face of copulatory bursae en route to ental end of prostatic ducts; relatively large and extensive prostates in xv to xxiii, each racemose, bilobed, wrapped around lateral margins of copulatory bursa; muscular duct attached over half of its length to medial face of copulatory bursa, running along surface and entering at apex. Single ductlets from anterior prostatic lobes join vas deferens; these join one or two posterior ductlets at common junction with muscular prostatic duct. Copulatory bursae ovate, extending from xvii to xx, attached to body wall; coelomic surface of copulatory bursa muscular, secretory diverticula lacking. Interiors of bursae lacking pads; large penis from dorsal apex fills most of bursal interior; copulatory bursae lack penial sheaths.

Remarks. *Pheretima longiprostata* Aspe & James 2014 belongs to the *P. sangirensis* group of Sims & Easton (1972). Individuals of this species were by far the smallest among the *P. sangirensis* group at Malindang, and are among the smallest *Pheretima* reported from the Philippines. In size and color, *P. longiprostata* is most similar among the Malindang species to *P. vergrandis* Aspe & James 2014 (see description below). However, the two species differ in the location of the first dorsal pore, the number and position of spermathecae and male pores, the length of the caeca, and the size and shape of the prostate glands. *Pheretima longiprostata* has the most elongate prostate glands relative to body size among the known Philippine *Pheretima* species, extending 9 segments from xv to xxiii (see Table 2.2). *Pheretima wati* has

prostate glands similar in size to those of *P. longiprostata*, extending 8 segments from xv to xxii, but the two species differ in size, coloration, location of the first dorsal pore, septal arrangement, length of the caeca, the shape of the spermathecae, and the number of intestinal vessels. Prostate glands function to produce fluids in which sperm cells can be transferred between worms during copulation (Edwards & Bohlen 1996), and these glands may also help to provide the cocoon with nutrients. However, the physiological significance of long or large prostate glands is not clear. Some species in *Pheretima* and in *Amyntas* (Kinberg, 1867) lack prostate glands; in these cases, the clitellum may be larger so as to supply the necessary nutrients to the cocoon. This remains to be investigated.

Occurrence. *Pheretima longiprostata* was uncommon, with only a few individuals found in disturbed forest in Brgys Small Potongan and Sibucal. It was not observed above 1067 m asl. Individuals occurred in the soil and on rotten logs (Table 2.1).

***Pheretima nolani* Aspe & James 2014**

(Fig. 2.6B)

Material examined. Holotype: adult (NMA 4520) Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 900 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: two adults (ZRC.ANN.0028), Brgy Lake

Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., coll. Nonillon Aspe, Nolan Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species is named after Nolan Aspe, who assisted in the fieldwork.

Diagnosis. Worm reaching 89–97 mm in adult length; body purplish brown; one pair of spermathecal pores at 7/8; spermathecae small, irregular, knobby, with short, thick duct; single-stalked diverticula terminating in sausage-shaped receptacle; intestinal origin in xv; prostate glands from xv to xx; caeca extending from xxvii to xxii; 42–44 intestinal vessels.

Description. In living animals, dorsum purplish black anteriorly, fading to medium brown posteriorly; narrow equators non-pigmented; ventral side non-pigmented. Length 89–97 mm (n=2 adults); diameter 5 mm at x; 4.5 mm at xx; body cylindrical in cross-section, tail narrowing gradually to sharp point; 111 segments. First dorsal pore at 12/13; paired spermathecal pores at 7/8, 0.14 circumference apart ventrally; female pore single on xiv; openings of copulatory bursae paired on xviii, 0.12 circumference apart ventrally, 2 setae between openings. Clitellum annular, extending from xiv to xvi. Setae on ventrum more closely spaced compared with that of the dorsum, 33–48 setae on vii, 42 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6/7/8 thinly muscled, 8/9 membranous, 9/10 absent, 10/11–13/14 slightly muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of

septa, at septum/body wall junction. Large gizzard extending from viii to x; esophagus with lamellae extending from xi to xiii; intestine originates in xv; caeca originates in xxvii, extends forward to xxii, with serrate ventral margins; typhlosole a simple fold of about 1/6 lumen diameter, originating at 26/27; intestinal wall with 42–44 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; paired spermathecae pre-septal in vii, with nephridia on ducts. Spermatophores spherical, with small appendage. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with short dorsal lobe; vasa deferentia slender, free from body wall, passing around anterior face of copulatory bursae en route to ental end of prostatic ducts; prostates in xv to xx, each racemose, 4 lobes, wrapped around lateral margin of copulatory bursa, muscular duct attached over most of its length to lateral face of copulatory bursa, running along surface and entering dorsal face. Two or 3 ductlets from anterior prostatic lobe join vas deferens; 2 or 3 posterior ductlets join at common junction with muscular prostatic duct. Copulatory bursae hemispherical extending from xvii to xix; coelomic surface of each bursa muscular, secretory diverticula lacking. Floors of bursae with some folds adjacent to opening but lacking pads; roofs with small posterior pad, large penis attached to anterior internal wall of bursa, only the tip free; copulatory bursae lack penial sheaths.

Remarks. This species belongs to the *P. sangirensis* group of Sims & Easton (1972). It differs from all subspecies of *P. sangirensis* in having the spermathecal pores and male pores more closely spaced, in having fewer setae in the post-clitellar region, in having a septum in 8/9, and in having more extensive prostate glands. Among the Malindang *sangirensis* species, *Pheretima nolani* Aspe & James 2014 is similar to *P. wati* in having setal gaps and in the relative spacing between the spermathecal pores and between the male pores, in the origins of the gizzard and intestine, and in the number of hearts. However, *P. wati* is smaller, has more setae around the equatorial segments, has fewer intestinal vessels, lacks penes, and lacks a septum at 8/9, whereas *P. nolani* lacks a septum at 9/10. The prostate of *P. nolani* extends for 6 segments from xv to xx, whereas that in *P. wati* extends 8 segments from xv to xxii. The shape of the spermathecae also differs, and the copulatory bursae in *P. nolani* are shorter than in *P. wati*. Compared with *Pheretima* species from Mt. Kitanglad and Luzon Island, *P. nolani* is most similar to *P. baungonensis* James, 2004 in terms of the size, number, and location of spermathecae, but the latter is dark brown in color; lacks dorsal setal gaps; has the spermathecal pores and male pores spacings 0.32 circumference apart and 0.19 circumference apart, respectively; has the first dorsal pore is in 13/14; has smaller prostate glands and copulatory bursae; has fewer longitudinal intestinal vessels; and has penes.

Occurrence. *Pheretima nolani* was common than other species, especially in primary forest in Brgy Lake Duminagat; it was most abundant in the highest elevation range (1845-2027 m), but was not observed in Brgys Small Potongan and Toliyok, at lower elevations (Table 2.1).

***Pheretima longigula* Aspe & James 2014**

(Figs 2.2D, 2.6C)

Material examined. Holotype: adult (NMA 4511) Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe and J. Adeva, Oct. 9–15, 2003. Paratypes: three adults (NMA 4535); two adults (ZRC.ANN.0019), same collection data as for holotype.

Etymology. The species name is derived from the Latin 'longus' (long) and 'gula' (throat), referring to the long esophagus.

Diagnosis. Slender worm reaching 139–186 mm in adult length; red-brown dorsally; one pair of spermathecal pores at 7/8; two pairs of seminal vesicles extending from xi to xiv; slender esophagus; intestine begins in xxi; penes with sheath; bilobed copulatory bursae in xvii to xviii; prostates posterior to copulatory bursae in xix, xx.

Description. Animals in life with red-brown dorsum; equators pigmented. Length 139–186 mm (n=6 adults); diameter 3.5–4.0 mm at x, 3.8–4.5 mm at xx; body cylindrical in cross-section; 99–110 segments. First dorsal pore at 12/13; pair of crescent shaped spermathecal pores, concave edge of pores anterior to 7/8, 0.24–0.27 circumference apart ventrally; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.17–0.21 circumference apart ventrally, 0–4 setae between

openings. Clitellum annular, extending from xiv to xvi. Setae unevenly distributed, 28–47 setae on vii, 27–51 setae on xx, no dorsal or ventral gaps.

Septa 5/6–7/8 and 10/11–13/14 muscular, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on septa, rather few and very small. Anterior internal organs all elongate; large gizzard extending from viii to x, esophagus has chevron-patterned lamellae from x to xi, low vertical lamellae from xii to xiii; esophagus long, slender, extending from 15/16 to xix; intestine originates in xxi; caeca originate in xxvii, extend forward to xxii, simple with smooth ventral margin; typhlosole originates gradually from xxxiii, simple fold, 1/3 lumen diameter; intestinal wall with 32–36 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii and xiv.

Ovaries and funnels free in xiii; spermathecae paired, preseptal in vii, with nephridia on ducts; ampulla ovate, spermathecal ducts bulbous, muscular, expanding ectally, with three large ridges on internal posterior side, parallel to one another and duct axis; stalked diverticulum attached to duct near ampulla, terminating in short ovate to lanceolate receptacle; stalk short, curved. Spermatophores lenticular to spherical, with short, thin tail curved back onto body of spermatophore. One individual has a misplaced small spermatheca at intersegment 8/9 on the right side of the body wall. Male sexual system holandric; testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi–xii and xii–xiv; vasa deferentia slender,

free from body wall en route to ental end of prostatic ducts, traveling along medial surface of copulatory bursae; each prostate racemose in xix to xx, muscular duct entering medial posterior face of copulatory bursa; copulatory bursae bilobed, elongate in xvii and xviii, anterior to the prostates. Coelomic surface of copulatory bursa muscular, secretory diverticula lacking; bursae have glandular mass with long, arched pad directed towards opening; posterior portion containing long, nearly cylindrical penis with circular sheath entirely within both lobes of the bursa.

Remarks. *Pheretima longigula* Aspe & James 2014 belongs to the *P. montana* group of Sims & Easton (1972), characterized by having penis sheaths, which the *P. sangirensis* group lacks. The *Pheretima montana* group once comprised seven species, but Blakemore's (2007) review of the group concluded that all but two of these species, *P. hahli* Ude, 1905 and *P. vitiensis* Beddard, 1892, are synonymous to *P. montana*. The only Philippine species detected so far in the *montana* group, *P. longigula* is longer than *P. montana* (length= 70–135 mm) although *P. montana* is thicker (5 mm diameter). The spermathecal pores are more widely spaced in *P. montana* (0.5 circumference apart) and the spermathecal duct is longer and more slender compared to that of *P. longigula*. *Pheretima longigula* differs from *P. hahli* and *P. vitiensis* in the origin of the intestine (xv in *P. hahli* and *P. vitiensis*), the size of the gizzard (viii–ix in *P. hahli*), the length of the prostate (xvii–xix in *P. hahli* and *P. vitiensis*), the origin of the caeca (xxvi in *P. hahli* and *P. vitiensis*), and color (yellow brown in *P. hahli* and *P. vitiensis*). *Pheretima vitiensis* is metandric according to Beddard (1892), but Michaelsen (1900) apparently found differently in placing this species in synonymy with *P. montana*. Most *Pheretima* species in the Philippines have

the intestine originating in xv or xvi, and *P. longigula* is unique in having the intestinal origin in xxi, with a correspondingly long esophagus. This species is also unique in the shape of the copulatory bursae and their position relative to the prostate glands, and in having the typhlosole originating in xxxiii, whereas in most other species, it originates in xxvii.

Occurrence. *Pheretima longigula* was uncommon (2.1% of total specimens), occurring at high elevations (1479–2027 m) in primary and disturbed forest in Brgy Lake Duminagat (Table 2.1).

***Pheretima adevai* Aspe & James 2014**

(Fig. 2.7A, B)

Material examined. Holotype: (NMA 4524) Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range, 8°24'04" N, 123°36'47" E, 900 m asl, Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch and J. Adeva, Feb. 18–25, 2004. Paratypes: three adults (NMA 4543), same collection data as for holotype. Other material: two adults (ZRC.ANN.0025), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., coll. Nonillon Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species is named after Julius Adeva, who assisted in the fieldwork.

Diagnosis. Slender brown worm reaching 110–131 mm in adult length; four pairs of spermathecal pores in 5/6–8/9; space between spermathecal pores wider than space between male pores; first dorsal pore at 12/13; intestinal origin in mid-xv; prostates in xviii to xix; caeca extending from xxvii to xxiii.

Description. In living animals, dorsum brown, darker anteriorly; equators pigmented. Length 110–131 mm (n=6 adults); diameter 5 mm at x, 4 mm at xx; body cylindrical in cross-section; 83–99 segments. First dorsal pore at 12/13; spermathecal pores at 5/6/7/8/9, 0.25 circumference apart ventrally; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.16 circumference apart ventrally, 3–7 setae between openings. Clitellum annular, extending from xiv to xvi. Setae unevenly distributed, 32–37 setae on vii, 36–39 setae on xx, no dorsal or ventral gaps. Septa 5/6/7/8 and 10/11–13/14 muscular, 8/9 present ventrally, 9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in ix to x; esophagus with low vertical lamellae extending from x to xiii; intestine originates in mid-xv; caeca originate in xxvii, extend forward to xxiii, with smooth, simple ventral margin; typhlosole rudimentary; intestinal wall with 34–38 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; spermathecae paired in vi to ix, with many nephridia on ducts; each spermatheca with ovate, spherical, or pyriform ampulla, short thick non-muscular duct, stalked diverticulum attached to anterior face of duct near ampulla, terminating in short, oblong receptacle containing 2 or 3 rounded masses of sperm; stalk short. No spermatophores were observed in spermathecal ampullae. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with long, narrow dorsal lobe; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates in xviii to xix, each racemose and bilobed in shape of butterfly wings; short muscular duct enters apex of copulatory bursa in xviii. Coelomic surface of copulatory bursa muscular, secretory diverticula lacking, walls thin; each bursa ovate-hemispheric, with elongate, conical penis on bursa roof; one spherical pad anterior and one posterior to opening.

Remarks. *Pheretima adevai* Aspe & James 2014 belongs to the *P. darnleiensis* species group of Sims & Easton (1972). Sims & Easton (1972) synonymized 15 species under the name *P. darnleiensis* Fletcher, 1887, all characterized by having either 4 or 5 pairs of spermathecal pores, located in 5/6–8/9, with an optional fifth pair in 4/5. Incongruently, Darmawan *et al.* (2012), described nominal *P. darnleiensis* from Darmaga, Indonesia, to have 4 pairs of spermathecal pores, from 4/5–7/8. Blakemore *et al.* (2007) further expanded the synonymy to include very large worms (about 700 mm long) from Mt. Kinabalu and Borneo, and possibly some of the *Pheretima dubia* group (three pairs of spermathecae vii-ix). After examining several of the species included in the synonymy of *P. darnleiensis*, Hong &

James (2011a) suggested that species-level differences had been ignored, or had been discounted against the large number of spermathecae. They argued that the synonymy was excessive and buried significant morphological and geographical diversity in an increasingly meaningless concept of *P. darnleiensis*, and concluded that it is not probably useful to place into synonymy morphologically distinguishable taxa of greatly differing sizes. In any case, synonymy decisions in the *P. darnleiensis* group need to be reviewed, and the issue needs to be addressed with both morphological and molecular data.

Pheretima adevai, *P. lluchi* Aspe & James 2014, and *P. potonganensis* Aspe & James 2014 (see below) at Mt. Malindang are the only species in the *P. darnleiensis* group presently known from Mindanao Island. *Pheretima adevai* differs from *P. darnleiensis* in the location of the first dorsal pore, the spacing between the spermathecal pores and between the male pores, and the number of setae between the male pores (Table 2.3). *Pheretima adevai* is similar to *P. lluchi* in size and in the origin of the gizzard, but the two differ in the location of the first dorsal pore, the spermathecal and male pore spacings, the origin of the intestine, the number of intestinal vessels, and the extent of the prostates and copulatory bursae (Table 2.3).

Seven species in the *darnleiensis* group were recently described from Luzon (*P. cabigati* Hong & James, 2008a from Banaue; *P. pugnatoris* and *P. tabukensis* Hong & James, 2010 from Kalinga; *P. margaritata*, *P. kalbaryonensis*, and *P. thaii* Hong & James, 2011a from Kalbaryo; and *P. barligensis* Hong & James, 2011b from Mountain. Province), aside from three other species placed synonymy (Sims & Easton 1972) with *P. darnleiensis*: *Perichaeta belli* Rosa, 1898 from Mindoro Island;

Perichaeta vaillanti Beddard, 1912; and *Pheretima benguetensis* Beddard, 1912.

Perichaeta belli is 75 mm long, has zebra-like brown bands dorsally, has 48 setae in vii, has 8 setae between the male pores, and has very short caeca in xxvi–xxv.

Perichaeta benguetensis is 190 mm in length, has purplish blue pigmentation, has the first dorsal pore in 7/8, and lacks seta in 8/9/10. These features differ markedly from those in *P. adevai*. Length and other pertinent characters in *Pe. vaillanti* are unavailable for comparison with the other species. Among recently described species, *P. margaritata* and *P. pugnatoris* are most similar to *P. adevai* in size and in the origin of the intestine (xv), but differ from *P. adevai* in the location of the first dorsal pore (9/10 in *P. margaritata*; 11/12 in *P. pugnatoris*), in the number of setae on vii (24 in *P. margaritata*; 16–19 in *P. pugnatoris*), in lacking a septum in 8/9, in the origin of the gizzard in viii, and in the length of the caeca (xvii–xxv and xxvii–xxiv in *P. margaritata* and *P. pugnatoris*, respectively). In addition, *P. adevai* has the male pores closer together than in *P. margaritata*, and a shorter prostate than in *P. pugnatoris*.

Occurrence. *Pheretima adevai* was the most abundant species at Malindang, comprising 19.4% of all individuals collected (Table 2.1). Although it was detected in disturbed habitat in Brgy Small Potongan at 915–1024 m elevation, it was most common in Brgy Lake Duminagat at elevations of 1479–2027 m. It mostly inhabited soil, but some individuals were collected on rotten logs and other substrates above ground (Table 2.1).

***Pheretima lluchi* Aspe & James 2014**

(Fig. 2.7C)

Material examined. Holotype: (NMA 4525) Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: three adults (NMA 4544), same collection data as for holotype. Other paratypes: two adults (ZRC.ANN.0026), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1357 m asl., coll. Nonillon Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species is named after Myko Lluch, who assisted in the fieldwork.

Diagnosis. Brown, slender worm reaching 104–135 mm in adult length; four pairs of spermathecal pores in 5/6 to 8/9; distance between spermathecal pores same as that between male pores; first dorsal pore in 13/14; intestinal origin in xvi; prostates in xviii to xx; caeca from xxvii to xxiv.

Description. In living animals, dorsum brown, darker anteriorly; equators pigmented. Length 104–135 mm (n=6 adults); diameter 4 mm at x, 4.5 mm at xx; body cylindrical in cross-section, tail blunt; 71–104 segments. First dorsal pore at 13/14; spermathecal pores at 5/6/7/8/9, 0.2 circumference apart ventrally; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.2 circumference apart ventrally, 5-6 setae between openings. Clitellum annular, extending from xiv to xvi.

Setae evenly distributed, 29-30 setae on vii, 39-45 setae on xx, no dorsal or ventral gaps.

Septa 5/6/7/8 and 10/11–13/14 muscular, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in ix to x; esophagus with circumferential lamellae from xi to xiii, pebbly texture in xiv; intestine originates in xvi; caeca originate in xxvii, extend forward to xxiv, simple, with smooth ventral margin; typhlosole rudimentary; intestinal wall with 28–30 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral, viii extending to gizzard; supra-esophageal vessel extends from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; ovisacs lacking; spermathecae 4 pairs in vi to ix, with many nephridia on ducts; each spermatheca with ovate to spherical ampulla and short, thick, non-muscular duct; stalked diverticulum attached to lateral face of duct at middle, terminating in short, banana-shaped receptacle; stalk about as long as receptacle. Spermatophores lacking. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, lacking dorsal lobes; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; each bilobed prostate racemose in xviii to xix; short, muscular duct enters apex of copulatory bursa in xviii, ectal half of duct attached to copulatory bursa surface.

Coelomic surface of copulatory bursa muscular, secretory diverticula lacking; bursa a low, ovate dome; conical penis with thick base projects from bursa roof.

Remarks. *Pheretima lluchi* Aspe & James 2014 belongs to the *P. darnleiensis* group of Sims & Easton (1972). It differs from *P. darnleiensis* in the location of the first dorsal pore, in the number of setae between male pores, in the origin of intestine, and in the length of the prostates (Table 2.3). *Pheretima lluchi* furthermore differs from *Perichaeta belli* (Rosa, 1898) and *P. benguetensis* (Beddard, 1912), which Sims & Easton (1972) synonymized with *P. darnleiensis*, in color and pigmentation pattern (brown bands in *Pe. belli*; purplish blue pigmentation in *P. benguetensis*), in the number of setae (more in *Pe. belli*; 40), in the location of the first dorsal pore (7/8 in *P. benguetensis*), and in the extent of the caeca (xxvi–xxv in *Pe. belli*; xxv–xx in *P. benguetensis*), among other characters. *Pheretima lluchi* is similar to *P. adevai* in the size and origin of the gizzard, but the two species differ in the location of the first dorsal pore, the origin of the intestine, the lengths of the caeca and prostates, and markedly in the spacings of the spermathecal and male pores (Table 2.3). *Pheretima lluchi* is similar to *P. margaritata* and *P. pugnatoris* in size, septal arrangement, and the origin of the gizzard, but differs from them in the number of setae on vii (24 in *P. margaritata*; 16–19 in *P. pugnatoris*), the location of the first dorsal pore (9/10 in *P. margaritata*; 11/12 in *P. pugnatoris*), the origin of the intestine (xv in the two latter species), the length of the caeca and prostates (xxvii–xxv and xvii–xviii, respectively in *P. margaritata*, and xxvii–xxiv and xvii–xix, respectively in *P. pugnatoris*), and markedly in the spacings of the spermathecal and male pores (0.25–0.28 and 0.26 circumference apart, respectively, in *P. margaritata*; 0.26–0.29 and 0.17–0.18 in *P.*

pugnatoris). Other Philippine *Pheretima* species with more than one pair of spermathecae are *P. castilloi* James *et al.*, 2004; *P. callosa* James, 2004; and *P. philippina* Rosa, 1891. These species differ from *P. lluchi* in size and in having 3 pairs of spermathecae (5/6–7/8 in *P. castilloi*; 6/7–8/9 in *P. callosa* and *P. philippina*).

Occurrence. *Pheretima lluchi* was uncommon, comprising 1.3% of the total individuals found on plots; we detected it in Brgys Sibucal and Lake Duminagat, at elevations of 902–2027 m. It inhabited both the soil and substrates above ground, such as rotten logs. The species was not observed at lower elevations (Table 2.1).

***Pheretima potonganensis* Aspe & James 2014**

(Fig. 2.7D)

Material examined. Holotype: (NMA 4526) Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range (8°24'04"N, 123°36'47"E), 900 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: three adults (NMA 4545), same collection data as for holotype. Other material: 12 adults (ZRC.ANN.0027), Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004.

Etymology. The species is named after Brgy Small Potongan, the type locality.

Diagnosis. Brown worms reaching 63–89 mm in adult length; 4 pairs of spermathecal pores from 5/6 to 8/9; first dorsal pore at 12/13; no dorsal setal gap, ventral setal gap present; septa all present from 5/6 to 13/14; intestinal origin in xiv; prostates from xvii to xix; caeca from xxvii to xx.

Description. In living animals, dorsum purple-brown, darker anteriorly; ventral side slightly pigmented anteriorly; equators pigmented; clitellum gray. Length 63–89 mm (n= 16 adults); diameter 4 mm at x, 4 mm at xx; body cylindrical in cross-section; 69–96 segments. First dorsal pore at 12/13; inconspicuous spermathecal pores paired at 5/6/7/8/9; female pore single in xiv; openings of copulatory bursae paired in xviii, white transverse slits, 0.17 circumference apart ventrally, 4 setae between openings. Five annuli per segment in ix–xiii. Clitellum brown, annular, extending from xiv to xvi. Setae on ventrum more closely spaced than those on dorsum, 32–34 setae on vii, 28–44 setae on xx, dorsal gap absent, ventral gap present. Septa 5/6–13/14 thin, but 13/14 slightly muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in ix to x; esophagus with chevron-patterned lamellae extends from xi to xiii; intestine originates in xiv; caeca originate in xxvii, extend forward to xx, simple, with smooth ventral margin; typhlosole rudimentary; intestinal wall with 26 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral, those in viii extend to gizzard; supra-esophageal vessels in x to xiii; extra-esophageal

vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii, ovisacs lacking; spermathecae paired from vi to ix, with many nephridia on ducts; each spermatheca with ovate to quadrangular ampulla, short non-muscular duct, stalked diverticulum attached to duct near body wall, terminating in short ovate receptacle; stalk almost as long as ampulla. Male sexual system holandric; testes and funnels enclosed in midventral sac in x, paired sacs in xi; seminal vesicles in xi and xii, each with short dorsal lobe; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates from xvii to xix; each racemose, bilobed, wrapped around copulatory bursa in xviii-xix; short, muscular duct enters apex of copulatory bursa in xviii. Copulatory bursae in xviii to xix. Coelomic surface of copulatory bursae muscular, secretory diverticula lacking; bursae low, circular domes; penis an irregular lump with terminal crease, extending from bursa roof; one horizontally directed pad anterior and the other posterior to opening in each bursa; an angular lateral projection between them may be a third pad.

Remarks. *Pheretima potonganensis* Aspe & James 2014 belongs to the *P. darnleiensis* group of Sims & Easton (1972). It differs from *P. darnleiensis* in the location of the first dorsal pore, male pore spacing, the number of setae between the male pores, the presence of ventral gaps, the presence of a septum in 8/9, and the origin of the intestine (Table 2.3). It also differs markedly from *Perichaeta belli* and *P. benguetensis*, which Sims & Easton (1972) synonymized with *P. darnleiensis*, in coloration and pigmentation pattern and in the extent of caeca, among other characters.

Individuals of *P. potonganensis* are smaller than those of *P. adevai* and *P. lluchi*, and unlike the latter two species, septa are present from 5/6 to 13/14 (Table 2.3).

Pheretima potonganensis also differs in having ventral setal gaps and in the size of the prostate glands, and the caeca are markedly longer than in *P. adevai* and *P. lluchi*, extending 8 segment lengths from xxvii to xx. *Pheretima potonganensis* differs from *P. lluchi* also in the location of the first dorsal pore. *Pheretima potonganensis* is similar to *P. tabukensis* from Kalinga, Luzon in the distance between male pores relative to body size, and both have septa from 5/6 to 13/14, but these species differ in the number of setae in vii (19–20 in *P. tabukensis*), the location of the first dorsal pore (11/12 in *P. tabukensis*), the origin of the intestine (xv in *P. tabukensis*), and the lengths of the prostates and caeca (xvii–xviii and xxvii–xxv, respectively, in *P. tabukensis*) (Hong & James 2010).

Numerous small, round outgrowths attached on the body wall inside some specimens of *P. potonganensis* were observed. These occurred in different body regions, but were concentrated mostly in the spermathecal region. It is speculated that these are some type of parasitic cysts inside the earthworms, but this remains to be investigated.

Occurrence. *Pheretima potonganensis* was the second-most abundant species after *P. adevai*, comprising 11.2% of all individuals found on plots. It was also relatively widespread across Mt. Malindang, occurring in disturbed forest at elevations of 238–1662 m, although it was not found in primary forest at higher elevations. It occurred both in the soil and above ground on substrates such as rotten logs (Table 2.1).

***Pheretima vergrandis* Aspe & James 2014**

(Fig. 2.8A, B)

Material examined. Holotype: adult (NMA 4521) Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 900 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, Nolan Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratypes: four adults (NMA 4541), same collection data as for holotype. Other material: four adults (ZRC.ANN.0023), Brgy Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8°17'55"N, 123°37'01"E), 1500 m asl., coll. Nonillon Aspe and J. Adeva, Oct. 9–15, 2003.

Etymology. The species name is from the Latin 'vergrandis' (small, tiny), referring to the small size of individuals of this species.

Diagnosis. Very small, light-brown worm reaching 35–75 mm in adult length; single mid-ventral spermathecal pore on 7/8; single male pore mid-ventral on xviii; first dorsal pore at 13/14; spermatheca with irregularly rounded ampulla, bulbous muscular duct expanding ectally with single stalked diverticulum attached to middle of duct; hearts from x to xiii; elongate racemose prostates in xvi to xxi; columnar copulatory bursae in xviii to xx; caeca extending from xxvii to xxiv.

Description. Living animals with very light-brown dorsum; no stripes; clitellum pale; body cylindrical in cross-section, tail narrowing gradually to sharp

point. Length 35–75 mm (n=10 adults); diameter 2.3–3.0 mm at x; 2.3–2.7 mm. at xx; 85–99 segments. First dorsal pore 13/14; single mid-ventral spermathecal pore with wide white lips at 7/8; female pore single in xiv; openings of copulatory bursae united mid-ventrally in xviii. Clitellum annular, extending from xiv to xvi. Setae unevenly distributed, 37–42 setae on vii, 50–54 setae on xx, dorsal and ventral gaps present.

Anterior septa all delicate, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located on body wall at septum/body wall junction and on anterior and posterior faces of septa. Large gizzard extends from viii to x; esophagus with villous-pebbly lining extends from x to xiii; intestinal origin in xv; caeca originate in xxvii, extend forward to xxiv; typhlosole a simple fold of about 1/5 lumen diameter, originating at 27/28; intestinal wall with 24–28 longitudinal blood vessels.

Hearts in x to xiii, esophageal, absent in x; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; single spermatheca preseptal in vii, with nephridia on ducts; spermatheca with irregularly rounded ampulla, bulbous muscular duct expanding ectally; duct internally corrugated, with two large internal ridges on anterior side; single stalked diverticulum attached to middle of duct, terminating in spherical receptacle; stalk short. Spermatophores spherical, with short, narrow tail. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with long dorsal lobe; vasa deferentia slender, free

from body wall en route to ental end of prostatic ducts; prostates in xvi to xxi; each racemose, wrapped around dorsolateral margin of copulatory bursa; muscular duct partially attached to and entering posterior face of copulatory bursa. Copulatory bursae in xviii to xx; columnar with rounded apex, attached to body wall at end; oriented towards common opening through body wall. Coelomic surface of copulatory bursae muscular; secretory diverticula lacking; roof with slender, tapering penis.

Remarks. Among the few monothecate species placed in *Pheretima* is *P. ambonensis* Cognetti, 1913 (Sims & Easton 1972). Originally described from Ambon, Indonesia, *P. ambonensis* has a single spermathecal pore located midventrally in 7/8, with 2 spermathecal diverticula. It is 125 mm long with 98 segments, and has 32–40 setae in the pre-clitellar and 50–60 setae in the post-clitellar areas. Its male pores are 0.2 circumference apart ventrally (James 2004), the intestine originates in xv, and penes are present. *Pheretima vergrandis* differs from *P. ambonensis* in size, and in having a single diverticulum and a single male pore. Other monothecate species in the Philippines are *P. monothecca* James, 2004 and *P. monoporata* James, 2004, both from Mt. Kitanglad; *P. arayatensis* James *et al.*, 2004 from Mt. Arayat in Luzon; and *P. concepcionensis* Aspe & James 2014, also from Mt. Malindang. In contrast to *P. vergrandis*, these species all have 2 spermathecal diverticula and 2 male pores, like *P. ambonensis*. In addition, the spermathecal pore is in 5/6 in *P. monothecca*, and in 8/9 in *P. arayatensis* (Table 2.4). Individuals of *P. vergrandis* are among the smallest in *Pheretima* species recorded in the Philippines. The condition of having a single diverticulum seems to be derived from two spermathecae present ancestrally. A single diverticulum could have resulted from the loss of one spermatheca (in this case, the

left, as the one present is consistently offset toward the right side), or it could have resulted from fusion. Likewise, two male openings is the ancestral condition, but the two pores have fused externally in species like *P. vergrandis*.

Occurrence. *Pheretima vergrandis* was moderately common (6.9% of all individuals collected on plots) in Brgys Sibucal and Lake Duminagat, at elevations of 902–1662 m. It was not observed in Brgys Small Potongan or Toliyok. The species inhabited soil and rotten logs (Table 2.1).

***Pheretima conceptionensis* Aspe & James 2014**

(Fig. 2.8C)

Material examined. Holotype: adult, amputee (NMA 4522), Brgy Small Potongan, municipality of Concepcion, Misamis Occidental Province, Mt. Malindang Range (8°24'04"N, 123°36'47"E), 900 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Paratype: one adult, posterior end missing (NMA 4542), same collection data as for holotype.

Etymology. The species name refers to the municipality of Concepcion, the type locality.

Diagnosis. Brown worm; single spermathecal pore located midventrally at 7/8; first dorsal pore 12/13; single spermatheca post-septal in viii, with nephridia on ducts; spermatheca with blocky, ovate ampulla, bulbous muscular duct expanding ectally

with two stalked diverticula attached to middle of duct; prostate glands in xvii to xix; copulatory bursae confined to xviii; penis present; caeca extending from xxvii to xxv.

Description. In living animals, dorsum very dark brown anteriorly, fading to medium brown posteriorly; equators pigmented; clitellum lighter, ventral side non-pigmented. Length of holotype >89 mm; diameter 5.2 mm at x; 4.2 mm at xx; body cylindrical in cross-section, tail narrowing gradually to sharp point; >104 segments. First dorsal pore at 12/13; single midventral spermathecal pore with elevated white lips at 7/8; female pore single in xiv; openings of copulatory bursae paired on xviii, 0.14 circumference apart ventrally, 2 setae between openings. Clitellum annular, extending from xiv to xvi. Setae evenly distributed, 40-50 setae on vii, 56-68 setae on xx, dorsal and ventral gaps present.

Septa 5/6/7/8 and 10/11–13/14 slightly muscular, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard extends from viii to x; esophagus with low vertical lamellae extends from x to xiii; intestine originates in xvi; caeca originate in xxvii, extend forward to xxv; typhlosole a simple fold of about 1/4 lumen diameter, originating at 26/27; intestinal wall with 30–35 longitudinal blood vessels.

Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xv; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiii.

Ovaries and funnels free in xiii; single spermatheca post-septal in viii, with nephridia on ducts; spermatheca with blocky, ovate ampulla; three spermatophores present; bulbous muscular duct expanding ectally, two stalked diverticula attached to middle of duct, terminating in ovate receptacles; stalks about same length as spermathecal duct and receptacles. Male sexual system holandric; testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii, each with long, slender dorsal lobe; vasa deferentia slender, free from body wall, passing over anterior lateral face of copulatory bursae en route to ental end of prostatic ducts; prostates in xvii to xix, each racemose, 3- to 4-lobed, wrapped around dorsal-lateral margin of copulatory bursa; muscular duct enters posterior face of copulatory bursa. Copulatory bursae in xviii hemispherical. Coelomic surface of bursae muscular, secretory diverticula lacking; floor of bursae thick, cleft by 5 furrows converging on opening; roof with blunt ovate penis.

Remarks. *Pheretima conceptionensis* Aspe & James 2014 is similar to *P. ambonensis* Cognetti, 1913 and *P. monoporata* James, 2004 in having the spermathecal pore located in intersegment 7/8 and the spermatheca with two identical diverticula. It differs from *P. ambonensis* in the location of the first dorsal pore (11/12 in *P. ambonensis*), in the presence of dorsal and ventral setal gaps (both lacking in *P. ambonensis*), in the male pore spacing (0.14 circumference apart ventrally, compared to 0.2 circumference in *P. ambonensis*) (James, 2004), in having the intestinal origin in xvi rather than xv; in having the spermatheca post-septal in viii (in vii in *P. ambonensis*), and in the two diverticula terminating in ovate receptacles with long stalks about the same length as the spermathecal duct and receptacle (the two

diverticula have much shorter chambers and stalks in *P. ambonensis*). *Pheretima conceptionensis* differs from *P. monoporata* in size (*P. monoporata* is smaller), in the number of setae (fewer in *P. monoporata*), in the ventral setal gap (lacking in *P. monoporata*), in septal arrangement (setae in 8/9/10 present in *P. monoporata*), in the origin of the intestine (vii in *P. monoporata*), in the size of the prostate (slightly larger in *P. monoporata*), and in the length of caeca (longer in *P. monoporata*) (Table 2.4). *Pheretima conceptionensis* is similar to *P. monotheca* in having two diverticula on the spermathecal duct, but the latter species is smaller, has fewer setae, lacks ventral gaps, has the spermathecal pore in 5/6, has closer male pore spacing, has the origin of the intestine in xviii, and has longer caeca (Table 2.4). *Pheretima conceptionensis* is similar to *P. vergrandis* in the arrangement of septa, origin of the gizzard in viii, origin of the intestine, and the presence of penes, but the former species is larger, has the first dorsal pore at 12/13, has two male pores on xviii, and has shorter caeca and prostate glands (Table 2.4).

Occurrence. *Pheretima conceptionensis* was uncommon, comprising 3.4% of individuals on plots; it was found in disturbed forest in Brgys Sibucal, Small Potongan, and Lake Duminagat, at elevations of 915–1662 m. It occurred both in the soil and above ground, on substrates such as on rotten logs (Table 2.1).

***Pheretima subanensis* Aspe & James 2014**

(Fig. 2.8D)

Material examined. NMA 4523 Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl., Mindanao Island, Philippines, coll. Nonillon Aspe, M. Lluch, and J. Adeva, Feb. 18–25, 2004. Other material: one adult, tail end missing (ZRC.ANN.0024), same collection data as above.

Etymology. The species is named after the indigenous tribe of Mt. Malindang, the “Subanen”.

Diagnosis. Small, purplish brown worm reaching around 67 mm in adult length; ventral gap absent; first dorsal pore at 12/13; hearts paired in xi to xiii, absent in x; spermathecae absent; racemose prostate glands in xvii to xx, with columnar copulatory bursae in xviii to xix; penes lacking; caeca in xxvii to xxiii.

Description. In living animals, dorsum dark purplish brown anteriorly, fading to medium brown posteriorly; equators pigmented; clitellum off-white; ventral side non-pigmented. Length 67 mm (holotype); diameter 3.5 mm at x; 3.5 mm at xx; body cylindrical in cross-section, tail blunt; 119 segments. First dorsal pore at 12/13; spermathecal pores absent; female pore single in xiv; openings of copulatory bursae paired on xviii, 0.21 circumference apart ventrally, 5 setae between openings. Clitellum brown, annular, extending from xiv to xvi. Setae unevenly distributed, 36 setae on vii, 45 setae on xx, dorsal gap present, ventral gap absent.

Septa 5/6/7/8, 10/11–13/14 thinly muscular, 8/9/10 absent. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall at anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in ix to x; esophagus with circumferential lamellae extending

from xi to xii, with digitiform internal texture from xiii to xiv; intestine originates in xv; caeca originate in xxvii, extend forward to xxiii; typhlosole a simple fold of about 1/4 lumen diameter, originating at 26/27.

Hearts in xi to xiii, esophageal; commissural vessels in vi, vii, ix lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xiii; extra-esophageal vessels join ventral esophageal wall in x, receive efferent parieto-esophageal vessels in xiv.

Ovaries and funnels free in xiii; spermathecae absent. Male sexual system holandric; testes and funnels appear reduced compared to other species, enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii, each with short dorsal lobe; vasa deferentia slender, free from body wall, passing around base of copulatory bursae en route to ental end of prostatic ducts; prostates in xvii to xx, each racemose, 3-lobed. Ductlet from anterior prostatic lobes joins vas deferens and posterior ductlet at common junction with muscular prostatic duct. Copulatory bursae in xviii to xix columnar, with rounded apex, attached to body wall at end; oriented towards common opening through body wall. Coelomic surface of bursae muscular, secretory diverticula lacking; penes absent.

Remarks. Sims & Easton (1972) stated that it was not possible for them to distinguish between athecate forms of *Pheretima* and *Metaphire* Sims & Easton, 1972 provided that the only character that distinguishes the two genera is the presence of nephridia on the spermathecal ducts in *Pheretima* and the absence of nephridia in that position in *Metaphire*. The two genera are identical in other characters such as the origin of the intestinal caeca in xxvii and the presence of copulatory bursae. Sims &

Easton (1972) noted that several species assigned to *Metaphire* might belong in *Pheretima sensu stricto*, and *vice versa*, since the presence or absence of nephridia on the spermathecal ducts was often not mentioned in descriptions. By the same token, generic assignment to *Pheretima* or *Metaphire* is problematic for athecal worms, because spermathecal ducts are lacking, making the presence or absence of nephridia on the ducts a moot point.

Here, it is proposed that the relative size of the copulatory bursae may be a distinguishing character between *Pheretima* and *Metaphire*, with species in *Pheretima* (e.g., this study) tending to have a more prominent dome-shaped, intra-coelomic copulatory bursae than those in *Metaphire* (e.g., Ohfuchi, 1938; 1957; Tsai *et al.* 2004b; Bantaowong *et al.* 2011a), and if this is the case, it would be useful in assigning athecal worms to one or the other of these genera (Chang *et al.* 2009). Homology among copulatory bursae has not been clear; some authors consider only invaginations of the body wall into the coelom to comprise copulatory bursae, whereas others also consider intramural chambers and even shallow indentations to be copulatory bursae. In the case of *Pheretima* vs. *Metaphire* as defined by Sims & Easton (1972), the type of invagination is the same in the two genera: a large or small bursa visible from within the body, and this is the source of the difficulty in assigning athecal worms in the *Pheretima* complex with caeca and copulatory bursae to one or the other of these genera. The criterion of having a large or small, invaginated copulatory bursa would seem to exclude from either genus any species whose male pore invaginations are entirely within the body wall (intramural), or whose male pores lie in shallow indentations.

Examples of species originally identified as *Pheretima* but reassigned to *Metaphire* by Sims & Easton (1972) because nephridia on the spermathecal ducts were not mentioned in descriptions are members of the octothecal, holandric *M. ignobilis* Gates, 1935 species group, which are most similar to *P. darnleiensis*. Members of this group apparently have only intramural male pores but not intra-coelomic copulatory bursae as in true *Pheretima*. One member of this group, *M. riukiensis* Ohfuchi, 1957, has only thick epidermal lips pressed together covering the male pores, rather than an invagination. None of these species would be considered synonyms of *P. darnleiensis*, and it is clear that a major taxonomic revision of *Metaphire* is necessary.

Joshi *et al.* (1999) reported an athecal earthworm 200–300 mm long from Ifugao, Luzon, but it is likewise not clear whether this species belongs in *Pheretima* or in *Metaphire*. James (personal correspondence) however, thought that this species is most likely a *Pheretima* basing on the size and shape of the copulatory bursae. *Pheretima subanensis* Aspe & James 2014 is similar to *P. malindangensis*, *P. vergrandis*, and *P. potonganensis* in relative size and coloration, but it is athecal, whereas the latter three are bithecal, monothecate and octothecal, respectively. The athecal species also differs from the others in male pore spacing, setal gaps, the size and position of the prostate glands and copulatory bursae, and the intestinal origin in xv rather than in xvi.

Gates (1972) listed the following as indicators that lumbricid or megascolecid earthworms are parthenogenetic: (1) testes and/or seminal vesicles retained in the juvenile state in adult specimens; (2) absence of spermatozoal iridescence in male

funnels and/or spermathecae; (3) spermatophores lacking or, if present, lack spermatozoa inside. In *Pheretima subanensis*, the testes appear to be reduced, and it was observed to have no spermatozooidal iridescence in the sperm funnels. These observations may indicate that this species is parthenogenetic.

Occurrence. *Pheretima subanensis* was found in disturbed forest in Brgy Small Potongan, at 915–1024 m elevation (Table 2.1).

2.4 Discussion

Edwards & Bohlen (1996) noted that earthworm communities typically contain from one to 15 species. Most earthworm communities contain around three to six species, with a remarkable degree of consistency among different habitats and different geographic regions, both temperate and tropical. Earthworm diversity is higher than the norm at Mt. Malindang, with at least 22 species (the 18 *Pheretima* species described in Aspe & James, 2014, and three *Pithemera* and one *Polypheretima* species described in Aspe & James, 2015) and also at Mt. Kitanglad (18 species; James 2004) (Table 2.5). Aspe *et al.* (2009) reported that species diversity in high elevations is high compared in the lower elevations and that the disturbed areas in the lower elevations, especially in the grasslands and agricultural areas, the exotic *Pontoscolex corethrurus* Müller, 1857 was abundant, with an average of 45 individuals/m². The results agree with the study conducted by Tsai *et al.* (2004a) in Taiwan wherein the number of native species decreased while the number of exotic species increased with the decrease in elevation. Species richness was high in the forested sites situated in the higher elevations, but abundance is observed to be

extremely low (mostly < 1 individual/m²). This is due to the network of thick moss and roots that mats the soil, which also makes digging more difficult. Based on experience in the other sites in the Philippines, this is the usual case in pristine habitats.

Blakemore (2007) listed a total of 40 valid *Pheretima* species in the Pacific region, described from 1886 to 2004. Prior to 2004, only three valid *Pheretima* species were known from the Philippines: a highly questionable report of *P. darnleiensis* from Benguet (near Banaue), Luzon Island and from Mindoro Island; *P. philippina* from Cebu Island; and a questionable record of *P. urceolata* Horst, 1893 from Mt. Apo, Mindanao Island (Blakemore, 2007). From 2004 to present (including this study), 64 new *Pheretima* species have been added, bringing the total number of *Pheretima* earthworms for the Philippines to 67 species. Table 2.5 shows the diversity of the *Pheretima* species reported by site and by island in the Philippines. Among these species, only two, *P. darnleiensis* and *P. urceolata*, are known to occur outside the country (nominal *P. darnleiensis* has been reported from Darnley Island (Torres Strait), Papua New Guinea, Fiji, Malaysia, Indonesia, and Singapore; *P. urceolata*, from Indonesia). The known ranges of the rest of the species are all restricted to areas around the type localities. This pattern indicates a remarkable degree of endemism, both among local areas, among islands in the Philippines, and in the Philippines as a whole, and suggests that many species remain to be detected in the Philippines. Furthermore, there is no knowledge at all of the breadth of ranges of earthworm species in the Philippines, although the lack of species in common between Mt. Malindang and Mt. Kitanglad, about 125 km apart, indicates the ranges are quite limited in size.

James (2004) suggested that *Pheretima* species on islands such as Indonesia and Borneo that neighbor the Philippines are unlikely to have colonized the Philippines in the past two million years, nor has there likely been genetic exchange among islands, because water depths between islands now range from 200 to 1000 m, too deep to have permitted land connections during the Pleistocene (Heaney, 1985). In addition, the Zamboanga Peninsula, where Mt. Malindang is located, was separate from the rest of Mindanao until the end of the Miocene, as it is part of the Greater Sunda Shelf in the west, whereas the rest of Mindanao originated from the east (Hall 1996). This may explain why the *Pheretima* species assemblage at Mt. Malindang is so different from that at Mt. Kitanglad (James 2004). On the other hand, there is increasing evidence that dispersal across water by rafting, including dispersal by soil-dwelling invertebrates and vertebrates (de Queiroz 2005; Vidal *et al.* 2008), may be more common than previously thought, and so lack of close evolutionary connections among earthworms among the Philippine Islands, and between the Philippines and neighboring archipelagos, cannot be assumed a priori. Future molecular studies can elucidate the evolutionary relationships among species and populations at several levels: between the Zamboanga Peninsula and the rest of Mindanao; among the Philippine Islands; and between the Philippine archipelago and other archipelagos and continental areas in southeastern Asia.

This paper adds new members to the *Pheretima* (*Pheretima*) *sangirensis*, *montana*, and *darnleiensis* groups of Sims & Easton (1972), new monothecate species related to *P. ambonensis*, and an athebate *Pheretima* species. It is expected that additional *Pheretima* species will be detected in the Philippines.

To facilitate the further study of Philippine earthworms, a key to the *Pheretima* species described herein from the Mt. Malindang Range, is provided. Until more is known about the taxonomy and distributions of earthworms on Mindanao and in the rest of the Philippines, however, this key should be used with caution in identifying earthworms from outside the Mt. Malindang Range.

Key to species of *Pheretima* reported from the Mt. Malindang Range

1 One pair of spermathecae	2
- Four pairs of spermathecae	9
- Single spermatheca	10
- No spermatheca, length around 67 mm.....	<i>Pheretima subanensis</i>
2 Large worms, adult width \geq 8 mm.....	3
- Small or slender worms, adult width $<$ 8 mm.....	6
3 Dorsum with oval pigmented dots on intersegmental furrows, adult length 226–235 mm	<i>Pheretima maculodorsalis</i>
- Dorsum with stripes on intersegmental furrows.....	4
- Dorsum pigmented all over.....	5
4 Dorsum with dark red to purple stripes on intersegmental furrows, adult length 230–283 mm	<i>Pheretima tigris</i>
- Dorsum with thick, dark purple to black stripes on intersegmental furrows, adult length up to 365 mm	<i>Pheretima immanis</i>
5 Dorsum dark, gradually fading towards ventral side, intestine originating in xiv, adult length 223–315 mm	<i>Pheretima lago</i>

- Dorsum dark gray-brown anteriorly, fading posteriorly, intestine originating in xv
..... *Pheretima nunezae*
- Dorsum with stripes on intersegmental furrows, male pores appearing as one,
adult length 101–133 mm..... *Pheretima boniaoi*
- Dorsum pigmented all over..... 7
- 6 Intestine originating in xv, dorsal setal gap present, ventral setal gap absent..... 8
- Intestine originating in xvi, dorsal setal gap absent, ventral setal gap present,
dorsum purplish brown, adult length 69–96 mm..... *Pheretima malindangensis*
- Intestine originating in xxii, no dorsal or ventral setal gaps, dorsum red-brown,
adult length 139–186 mm..... *Pheretima longigula*
- 7 Prostates in xv–xx, caeca in xvii–xxii, adult length 89–97 mm
..... *Pheretima nolani*
- Prostates in xv–xxii, caeca in xxvii–xxii, adult length 67–75 mm
..... *Pheretima wati*
- Prostates in xvi–xxii, caeca in xxvii–xxv, adult length 55–65 mm
.....*Pheretima misamisensis*
- Prostates in xv–xxiii, caeca in xxvii–xxv, adult length 37–41 mm
.....*Pheretima longiprostata*
- 9 Distance between male pores less than distance between spermathecal pores,
first dorsal pore in 12/13, adult length 110–131 mm..... *Pheretima adevai*
- Spermathecal pores and male pores have the same distance between pores, first
dorsal pore in 13/14, adult length 104–135 mm..... *Pheretima lluchi*

- Spermathecal pores inconspicuous, first dorsal pore at 12/13, adult length 63–89 mm..... *Pheretima potonganensis*
- 10 One spermathecal diverticulum, single male pore, first dorsal pore at 13/14, adult length 35–50 mm..... *Pheretima vergrandis*
- Two spermathecal diverticula, male pores paired, first dorsal pore at 12/13, average adult length ca. 90 mm..... *Pheretima conceptionensis*

CHAPTER 3

**New *Polypheretima* and *Pithemera* (Clitellata: Megascolecidae) species from the
Mt. Malindang Range, Mindanao Island, Philippines**

3.1 Introduction

Mt. Malindang Range Natural Park (MMRNP) comprises a complex of inactive volcanoes located at the foot of the Zamboanga Peninsula, which extends westward from central Mindanao Island, Philippines. Comprising an area of 533,000 km², the Park ranges in altitude from 600 m asl. in the lowlands to 2404 m asl. at the top of the highest peak, Mt. Malindang (8.2166°N, 123.6333°E). About 330,000 km² remain covered with relatively undisturbed primary forest, while the rest includes bushland, denuded land, and cultivated land. The complex topography includes a 60 km² crater lake (Lake Duminagat), two other sunken crater areas of more than 200 km² each, and numerous canyons and ravines.

Due to its rugged topography, altitudinal extent, and tropical location, MMRNP has a diverse flora and fauna including species endemic to the Mt. Malindang Range, especially among vertebrates (SEARCA 2006). To date, however, the faunal diversity of the Mt. Malindang Range has been only patchily inventoried, with most attention to vertebrates (amphibians, reptiles, birds, and mammals) and much less to invertebrate groups that nonetheless are functionally important members of tropical forest ecosystems. One such latter group is the earthworms, which play important functional roles that affect soil properties and have community-wide effects (e.g., Anderson 1988; Fragoso & Lavelle 1992; Jouquet *et al.* 2006; Lavelle *et al.* 2006, Boniao *et al.* 2012). Studies of earthworm taxonomy in the Philippines over the

past decade (James 2004a, 2005b, 2006, 2009; James *et al.* 2004; Hong & James 2004, 2008a, 2008b, 2008c, 2009, 2010, 2011a, 2011b, Aspe & James 2014) have indicated high diversity and possibly high local endemism, and suggest that many species remain to be discovered. This series of reports describing new earthworm species focused on various sites on Luzon Island, with studies on Mindanao Island conducted only at Mt. Kitanglad (James 2004a) and Mt. Malindang (Aspe & James 2014).

A study of the diversity and distribution of earthworms on Mt. Malindang detected 23 species, including 18 species in *Pheretima*, three in *Pithemera*, one in *Polypheretima*, and the introduced species *Pontoscolex corethrurus* Müller, 1857 (Aspe 2006, Aspe *et al.* 2009). The 18 *Pheretima* species, which were reported in Chapter 1 were described in Aspe & James (2014) while the species reported in this chapter were described in Aspe & James (2015).

3.2 Material and methods

Site description. Samples were collected from 9 to 15 October 2003 in primary and disturbed forests at different elevations in Barangay [=Precinct] Lake Duminagat in the municipality of Don Victoriano, Barangay Small Potongan in the municipality of Concepcion, and Barangays Sibucal and Toliyok in Oroquieta City (Fig. 3.1B). All are in Misamis Occidental Province. The primary forest had remained largely undisturbed by human activities. The vegetation was dense and lush; trees were stout and thickly covered with moss, ferns, and lichens. The ground was also thickly covered with moss, roots, and leaf litter. Trees in the disturbed forest, regrown after deforestation by humans, were dominated by dipterocarps tended to be more

closely spaced than in the primary forest, and tended to have more undergrowth (saplings, shrubs, and tree ferns). The ground was also covered with thick leaf litter, roots, bryophytes, and lichens. The terrain in the forested areas was very rugged, with steep grades and many cliff faces, making access very difficult. Surrounded by humid primary forest in Brgy Lake Duminagat is a crater lake about 60 km² in area called Lake Duminagat. The geographical coordinates of collecting sites were determined by GPS (Magellan Map410); elevations were determined by GPS if a satellite signal was detectable, or with an altimeter if not. The map datum used in the GPS readings was Luzon.

Sampling. Sampling methods and the locations of sampling sites were previously reported in Aspe (2006) and Aspe *et al.* (2009). A summary is as follows: In each barangay, six scattered plots 20 m x 20 m in extent were established, with an average distance of 75 m between plots. On each plot, 10 quadrats (0.5 m x 0.5 m square x 0.3 m deep) in randomly selected spots were dug and searched for earthworms. The earthworms collected were preliminarily sorted to species and counted to assess relative abundance. Additional haphazard sampling was done outside the plots to further assess species' distributions across all sites. Tree bark, ferns, mosses, vines, and the insides of rotten logs were also checked for earthworms. Earthworms collected were cleaned in tap water, killed in 10% ethanol, and placed in Saranex sealable plastic bags filled with a volume of 10% formalin that was at least three times the total volume of the earthworms. After two days, the formalin was replaced with 80% ethanol for long-term preservation.

Descriptions. The descriptions given below are based on the terminological

conventions of Easton (1979), and the classification is that of Sims & Easton (1972). Descriptions of body colour are based on living specimens. Body dimensions refer to fixed material. The degree of separation between pores is expressed as a proportion of the circumference of the worm; for example, 'male pores 0.23 circumference apart ventrally' means the distance between the pores is 0.23 the circumference of the worm at that point, with the circumference calculated as π times segment diameter. While many character states are shared among species within genera, generic characters of the three species in *Pithemera* are repeated to aid in identification. All descriptions are based on external examination and dorsal dissection under a stereomicroscope. Illustrations were prepared with Adobe Illustrator ver. CS5. Holotypes and some paratypes are deposited in the Annelid Collection of the National Museum of the Philippines (NMA), Manila. Other paratypes are deposited in the Annelid Collection of the Zoological Reference Collection (ZRC.ANN) of the Lee Kong Chian Natural History Museum, Faculty of Science, National University of Singapore, Singapore.

3.3 Results

Four new species from Mt. Malindang, Mindanao Island are described:

Polypheretima mindanaoensis Aspe & James 2015, *Pithemera malindangensis* Aspe & James 2015, *Pi. duminagati* Aspe & James 2015, and *Pithemera donvictoriano* Aspe & James 2015 Figure 3.1B shows the location in Mt. Malindang where *Polypheretima mindanaoensis* and species of *Pithemera* were collected. Table 3.1 shows the frequency, site density, and relative abundance of the four species at the five collecting sites on Mt. Malindang. The numbers of *Pheretima* individuals (Aspe

& James, 2014) are also included to indicate the overall relative abundance and frequency of *Polypheretima* and *Pithemera* species in the earthworm community. Among species in the latter two genera, *Pithemera malindangensis* was the most abundant, with a relative abundance of 11.7%, while *Pithemera duminagati* showed the highest frequency of occurrence (0.6), though it was only collected through haphazard sampling outside the plots. The sites with the highest species diversity were in disturbed forest in Barangays Lake Duminagat and Sibucal (20 and 17 species, respectively). The sites with highest species diversity and abundance were all above 900 m in elevation. *Polypheretima mindanaoensis* and the three *Pithemera* species were not detected in Barangay Small Potongan, and only one individual of *Pithemera donvictoriano* was collected in Barangay Toliyok at a lower elevation.

TAXONOMY

Megascolecidae Rosa, 1891

***Polypheretima* Michaelsen, 1934**

Type species. *Perichaeta stelleri* Michaelsen, 1892

Generic diagnosis. Body cylindrical; setal arrangement perichaetine; annular clitellum covering segments xiv–xvi; pair of male pores in xviii on circular porophores which may be within copulatory bursae; ventral genital markings present or absent; oesophageal gizzard in viii; intestine begins in xv or xvi; nephridia on spermathecal

ducts lacking; caeca lacking; male sexual system usually holandric, with testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii; spermathecal pores small, spermathecal diverticula simple and usually ectal in origin; prostates racemose; copulatory bursae may or may not be present; ovaries free in xiii; oviducts lead to single or closely paired opening (Easton, 1979). Hong and James (2008a) observed that most *Polypheretima* species on Luzon have white pigmentation and are generally smaller than *Pheretima* species.

***Polypheretima mindanaoensis* Aspe & James 2015**

(Fig. 3.2A, 3.3A)

Material examined. Holotype: adult (NMA 4527), Barangay Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mt. Malindang Range (8.2986°N, 123.6169°E), 1357 m asl, Mindanao Island, Philippines; coll. Nonillon Aspe, Nolan Aspe and J. Adeva, 9–15 October 2003. Paratypes: one adult, one juvenile (NMA 4545); two adults (ZRC.ANN.0013); same collection data as for holotype.

Etymology. The species is named for Mindanao Island.

Diagnosis. Body white; stout, adult length 90–118 mm; paired, sublateral genital markings on each of xix to xxvi; intestinal origin in xv; small spermathecae present in batteries or lacking.

Description. White, clitellum pinkish-grey. Body stout, adult length 90–118

mm; diameter 5.1 mm at x, 7 mm at xx; 140–141 segments (n= four adults); body cylindrical in cross-section, tail narrowing gradually to sharp point. First dorsal pore at 12/13; spermathecal pores lacking or inconspicuous; female pore single in xiv, male pores on paired low papillae on xviii, 0.23 circumference apart, 10 setae between openings. Clitellum annular, from xiv to xvi. Setae pointed posteriorly, unevenly distributed around segmental equators; 41–53 setae on vii, 44–46 setae on xx; dorsal and ventral setal gaps lacking. Genital markings widely paired on xix to xxv and/or xxvi, between the sixth and seventh setal lines.

Septa 4/5/6/7/8 muscular, 8/9 absent, 9/10 present around dorsal vessel and hearts; 10/11 to 13/14 thickly muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located at septum-body wall junction, mainly on body wall near anterior and posterior faces of septa. Oesophageal gizzard large, extending from viii–x; oesophagus with lamellar inner surface extend from xi–xiii; intestinal origin in xv, caeca lacking; typhlosole a simple fold of about one-sixth lumen diameter originating in xvi; intestinal wall without longitudinal blood vessels but with 2 pairs of vertical vessels per segment.

Hearts in x to xii, oesophageal. Commissural vessels in vi, vii, and ix, lateral; lacking in viii. Supra-oesophageal vessel extending from x to xii; extra-oesophageal vessels joining ventral oesophageal wall in x, receiving efferent parieto-oesophageal vessels in xiv.

Ovaries and funnels free in xiii, spermathecae lacking in two adults, one adult individual with five spermathecae closely lining up on the left side of vi, another adult individual with three spermathecae closely lining up on the left side and five

spermathecae closely lining up on the right in vi and two spermathecae on left and two on right in vii; spermathecae small, ampulla pyriform, spermathecal duct short, slender; diverticulum stalk long and slender, attached ectally to duct, with one kink, terminating in short, sausage-shaped receptacle; no nephridia on spermathecal ducts. Male sexual system holandric, testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii; pseudovesicles in xiii; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates in xvi to xxi, each prostate racemose, trilobed but compact; copulatory bursae lacking.

Remarks. *Polypheretima mindanaoensis* Aspe & James 2015, the first member of *Polypheretima* reported from Mindanao Island, belongs to the *Po. elongata* Perrier, 1872 species group of Easton (1979), characterised by having a pair of genital markings in xix, successive segments in line with the male pores, paired batteries of up to 28 spermathecae in vi and/or vii, and a shallow copulatory bursae with no stalked glands. Easton (1979) included five species in the group: *Po. elongata*; *Po. everetti* Beddard & Fedarb, 1895; *Po. kinabaluensis* Beddard & Fedarb, 1985; *Po. phacellotheca* Michaelsen, 1899; and *Po. stelleri* Michaelsen, 1892. *Polypheretima mindanaoensis* markedly differs from *Po. elongata* and *Po. everetti* in size (355 x 4 in *Po. elongata*; 300 x 12 in *Po. everetti*), in the number of segments covered by the genital markings (extending from xix to xxii in *Po. elongata*; xix to xxi in *Po. everetti*), in the number of setae on vii (usually 80–130 in *Po. elongata*; 130 in *Po. everetti*) (Table 3.2). *Polypheretima mindanaoensis* (white) differs in colour from *Po. everetti* and *Po. kinabaluensis* (reddish purple and red, respectively). Those individuals of *Polypheretima mindanaoensis* that have spermathecae have fewer spermathecae in

each battery than *Po. kinabaluensis* (6–12 in 5/6/7), *Po. phacellotheca* (9–12 in 5/6), or *Po. stelleri* (up to 28 in 5/6/7), and individuals have fewer setae (41–53) on vii than *Po. phacellotheca* (80) or *Po. stelleri* (130). Finally, *Po. mindanaoensis* differs from all congeners in the *Po. elongata* species group in lacking copulatory bursae. Congeners reported from the Philippines include *Po. fruticosa* Hong & James, 2008a, *Po. perlucidula* Hong & James, 2008a, and *Po. bannaworensis* Hong & James, 2008a, from Banaue, Luzon Island; *Po. pagudpudensis* Hong & James, 2011a from Kalbaryo, Luzon Island; *Po. monticola* Beddard, 1912 from Mt. Pulong (Mt. Pulag), Benguet Province, and from Luzon Island; and *Po. everetti* and *Po. elongata*, both collected on Balabac Island, Palawan. *Polypheretima perlucidula*, *Po. bannaworensis*, *Po. pagudpudensis*, and *Po. monticola* belong to the *Po. bifaria* species group of Easton (1979), characterised by having pairs of spermathecae in 5/6–8/9 or only in 6/7 or 7/8, while *Po. fruticosa* is closely related to *Po. voeltzkowi* Michaelsen, 1907, characterised by having a pair of spermathecae in only in 5/6. The new species also differs from the other species in the number of setae between male pores, in the genital marking pattern, and in the size and segmental position of the prostate glands (Table 3.2).

Spermathecae function to receive and store sperm released by the male pores of the partner during copulation; loss of spermathecae usually means a loss of male function. Loss of spermathecae, reduction of testes, and lack of spermatozoal iridescence in the sperm funnels in earthworms are indicative of reproduction by parthenogenesis (Gates 1972), in which the egg develops into a new individual without being fertilised by sperm. In the case of *Po. elongata*, which have been

introduced into many parts of the world (see Table 3.4), Easton (1979) observed that athecate individuals are especially numerous in introduced populations, presumably reproducing parthenogenetically.

In one specimen of *Po. mindanaoensis*, the ventral nerve cord has a solid dark core, perhaps related to parasitism. Several nematodes near an empty nerve cord swelling in xvii were observed.

Occurrence. *Polypheretima mindanaoensis* was found at two of five collecting sites. In all, eight individuals were collected in disturbed forests in Barangays Lake Duminagat and Sibucal at elevation of 902–1662 m asl. The soil it inhabited was covered with thick leaf litter and roots, bryophytes and lichens. It was not observed on rotten logs.

***Pithemera* Sims & Easton, 1972**

Type species. *Perichaeta bicincta* Perrier, 1875

Generic diagnosis. Body cylindrical; setae numerous, regularly arranged around each segment; clitellum annular, covering two or two and a half segments from xiv to xv, and/or half of xvi; spermathecal pores small, three to five pairs from 4/5 to 8/9; female pore single or paired in xiv; genital markings present or absent; oesophageal bursae absent; intestinal caeca originating in xxii (in *Pheretima*, caeca originating in xxvii); spermathecal ducts without nephridia (as in *Polypheretima*); male system holandric or metandric; prostate glands racemose; copulatory bursae

lacking (Sims & Easton, 1972). Hong and James (2008a) observed that *Pithemera* species are generally lighter in pigmentation and smaller in size than *Pheretima* species.

***Pithemera malindangensis* Aspe & James 2015**

(Fig. 3.2B, 3.3B)

Material examined. Holotype: adult (NMA 4528), Mt. Malindang Range (8.2986°N, 123.6169°E), Barangay Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mindanao Island, Philippines; 1357 m asl; coll. Nonillon Aspe, Nolan Aspe and J. Adeva, 9–15 October 2003. Paratypes: three adults (NMA 4547); two adults (ZRC.ANN.0014); all same collection data as for holotype.

Etymology. The species is named for Mt. Malindang.

Diagnosis. White worm; adult length 91–144 mm; clitellum from xiv through half of xvi; five pairs of spermathecal pores, 4/5 to 8/9; distance between male pores 0.2 circumference apart; no genital markings; intestinal origin in xiv; prostate glands from xvi–xix.

Description. White, clitellum grey-brown. Adult length 91–144 mm (n= six adults); diameter 4 mm at x, 3.5 mm at xx; body cylindrical in cross-section, tail blunt; 118–126 segments. First dorsal pore at 13/14, inconspicuous in most specimens; paired spermathecal pores 4/5/6/7/8/9, inconspicuous; female pores paired in xiv, male pores on paired circular porophores forming mounds in xviii, 0.2 circumference apart,

two to four setae between openings. Clitellum annular, extending from xiv through half of xvi. Setae evenly distributed around segmental equators, 58 setae on vii, 30–40 setae on xx; dorsal setal gap absent, ventral gap present. Genital markings lacking.

Septa 5/6/7/8 muscular, 8/9 absent, 9/10–13/14 muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located at septum-body wall junction, mainly on body wall near anterior and posterior faces of septa. Gizzard large, extending from viii to ix; oesophagus with internal lamellar sections extending from xi to xiii; intestinal origin in xiv; caeca from xxii to xxi; typhlosole a simple fold of about one-fifth lumen diameter, originating in xxii; intestinal wall lacks longitudinal blood vessels.

Hearts in x–xii, oesophageal. Commissural vessels in vi, vii, and ix, lateral; lacking in viii. Supra-oesophageal vessel extending from x to xii; extra oesophageal vessels joining ventral oesophageal wall in x, receiving efferent parieto-oesophageal vessels in xiv.

Ovaries and funnels free in xiii. Spermathecae five pairs in v to ix; duct short, slender; small, spherical to ovate ampulla greater than duct length; diverticulum stalk short, attached ectally to duct, terminating in short, sausage-shaped receptacle. Male sexual system holandric; testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii; vasa deferentia slender, free from body wall, those on each side separate until xiv, joining ental end of prostatic ducts; each prostate racemose, broad, multilobed, in xvi to xix; ectal half of duct expands to form muscular spindle; ducts in hairpin loop. Transverse muscle bands present above body wall at 16/17 and 18/19, the latter much larger. Copulatory bursae lacking.

Remarks. *Pithemera malindangensis* Aspe & James 2015 belongs to the *Pi. bicincta* (Perrier, 1875) species group of Sims & Easton (1972), which initially comprised two species, *Pi. bicincta* and *Pi. violacea* Beddard, 1895. Michaelsen (1910), Ohfuchi (1957), and Shen & Tsai (2002), however, considered *Pi. violacea* to be a junior synonym of *Pi. bicincta*. *Pithemera bicincta*, first collected from Mindoro Island, has the first spermathecal pores in 4/5, and the intestinal caeca are paired and positioned laterally. *Pithemera malindangensis* is similar to *Pi. bicincta* in the arrangement and number of spermathecae and septa, the number and locations of hearts, and the length of the caeca, but the former is larger, lacks genital markings, and has the intestinal origin in xvi rather than xv. Other members of the *bicincta* species group reported from the Philippines (all from Luzon Island except for *Pi. duminagati* Aspe & James 2015 described below) include *Pi. rotunda* and *Pi. philippinensis* (James *et al.* 2004); *Pi. duhuani*, *Pi. fragumae*, *Pi. ifugaoensis*, and *Pi. triangulata* (Hong & James 2008a); and *Pi. glandis*, *Pi. fusiformis*, and *Pi. levii* (Hong & James 2011a). *Pithemera malindangensis* and *Pi. duminagati* are similar to most or all other members of the *bicincta* group in the origin of the gizzard, number of hearts, and length and location of the caeca, but differ from the others in having the intestinal origin in xvi rather than xv. The Malindang species are also white and lack genital markings. *Pithemera malindangensis* is larger than *Pi. duminagati*, the male pores are more distant, and it has only two pairs of dense tufts of nephridia (on the anterior faces of 5/6/7), whereas the latter has three pairs (on 5/6/7/8) (Table 3.3).

Occurrence. *Pithemera malindangensis* was the most abundant among the four species, comprising 11.7% of all earthworms collected. It was common in

primary and disturbed forests in Barangay Lake Duminagat at elevations of 1479–2027 m asl. The soil it inhabited was covered with thick leaf litter and roots, bryophytes, and lichens. It was not observed on rotten logs.

***Pithemera duminagati* Aspe & James 2015**

(Fig. 3.2C, 3.3C)

Material examined. Holotype: adult (NMA 4529), Mt. Malindang Range (8.2986°N, 123.6169°E), Barangay Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mindanao Island, Philippines; 1357 m asl; coll. Aspe, *et al.*, 9–15 October 2003. Paratypes: two adults (NMA 4548), same collection data as for holotype.

Etymology. The species is named for Barangay Lake Duminagat, the type locality.

Diagnosis. Very small, white worm; adult length 36–37 mm; clitellum from xiv through half of xvi; 5 pairs of spermathecal pores, 4/5 to 8/9; distance between male pores 0.13 circumference apart; no genital markings; intestinal origin in xv; prostate glands from xvi–xxi.

Description. White; clitellum grey-brown. Adult length 36–37 mm (n= three adults); diameter 2.5 mm at x, 2 mm at xx; body cylindrical in cross-section, tail blunt; 88–95 segments. First dorsal pore at 12/13; paired spermathecal pores in 4/5/6/7/8/9, inconspicuous; female pores paired in xiv; male pores on paired porophores in xviii,

0.13 circumference apart, zero to three setae between openings. Clitellum annular, extending from xiv to half of xvi. Setae unevenly distributed, 43–48 setae on vii, 31–43 setae on xx; no dorsal or ventral gaps. Genital markings lacking.

Septa 5/6/7/8 slightly muscular, 8/9 absent, 9/10–13/14 thinly muscular. Dense tufts of nephridia on anterior faces of 5/6/7/8; nephridia of intestinal segments located at septum/body wall junction, mainly on body wall near anterior and posterior faces of septa. Gizzard large, extending from viii to ix; oesophagus with lamellar section from xi to xiii; intestinal origin in xv; caeca from xxii to xxi; typhlosome a simple fold of about one-quarter lumen diameter, originating in xix, full size in xxii; intestinal wall lacks longitudinal blood vessels but has two pairs of vertical vessels per segment.

Hearts in x–xii, oesophageal; commissural vessels in vi, vii, and ix, lateral; lacking in viii; supra-oesophageal vessel extends from x to xii; extra oesophageal vessels joining ventral oesophageal wall in x, receiving efferent parieto-oesophageal vessels in xiv.

Ovaries and funnels free in xiii; ovisacs in xiv; spermathecae five pairs from v to ix; duct short, slender; small, ovate ampulla; diverticulum stalk short, attached ectally to duct, terminating in short, pyriform receptacle; receptacle a small sphere lacking spermatozoal iridescence. Male sexual system holandric; testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii; vasa deferentia slender, free from body wall, joining ental end of prostatic ducts; each prostate racemose, broad, 3 or 4 lobed, present from xvi to xxi; ectal half of duct expands to form muscular spindle; ducts in hairpin loop. Ental portion of duct with two lumens. Copulatory bursae lacking.

Remarks. *Pithemera duminagati* Aspe & James 2015 belongs to the *Pi. bicincta* group of Sims & Easton (1972). Although *Pi. duminagati* is similar to *Pi. bicincta* in some characters, it differs from the latter in size, in lacking genital markings, in the distance between male pores, in the relative size of the prostates, and in the origin of the intestine (Table 3.3). *Pithemera duminagati* is similar to *Pi. rotunda* James *et al.* 2004, another member of the *bicincta* group, in size and in being white, in septal arrangement, in intestinal origin, and in the length of caeca, but *Pi. duminagati* has fewer setae between the male pores, lacks genital markings, and has proportionally larger prostate glands. Among the three *Pithemera* species from Mt. Malindang, *Pi. duminagati* is more similar to *Pi. malindangensis* in the extent of the clitellum, number of spermathecae, and septal arrangement. Like *Pi. donvictoriano*i Aspe & James 2015 (see below), *Pi. duminagati* is smaller in body size than *Pi. malindangensis* and has three pairs of dense tufts of nephridia on 5/6/7/8, whereas the latter has only two pairs on 5/6/7.

Occurrence. *Pithemera duminagati* was found outside the sampling plots at three of five sites: in primary and disturbed forest in Barangay Lake Duminagat and in disturbed forest in Barangay Sibucal, at elevation of 902–2027 m asl. The soil it inhabited was covered with thick leaf litter and roots, bryophytes and lichens. It was not observed on rotten logs.

***Pithemera donvictoriano*i Aspe & James 2015**

(Fig. 3.2D, 3.3D)

Material examined. Holotype: adult (NMA 4530), Mt. Malindang Range (8.2986°N, 123.6169°E), Barangay Lake Duminagat, municipality of Don Victoriano, Misamis Occidental Province, Mindanao Island, Philippines; 1357 m asl; coll. Aspe, *et al.*, 9–15 October 2003. Paratypes: two adults (NMA 4549 and ZRC.ANN.0015); both same collection data as for holotype.

Etymology. The species is named for the municipality of Don Victoriano, the type locality.

Diagnosis. White worm; adult length 41–58 mm; clitellum annular from xiv to xv; 4 pairs of spermathecal pores, 5/6 to 8/9; male pores closely spaced; intestinal origin in xiv; prostate glands from xvi–xix.

Description. White; clitellum dark brown. Length 41–58 mm (n=3 adults); diameter 2.5–3.0 mm. at x; 2.5–3.0 mm at xx; body cylindrical in cross-section, tail blunt; 85–100 segments. First dorsal pore at 12/13; paired spermathecal pores in 5/6/7/8/9, inconspicuous; female pores paired in xiv; male pores on lateral faces of slightly elevated cones in xviii, 0.11 circumference apart, 8 setae between openings. Clitellum annular, extending from xiv to xv. Setae evenly distributed around segmental equators, 42–48 setae on vii, 41–57 setae on xx; no dorsal or ventral gaps. Genital markings lacking.

Septa 5/6/7/8 slightly muscular, 8/9 absent, 9/10–12/13 thinly muscular. Dense tufts of nephridia on anterior faces of 5/6/7/8; nephridia of intestinal segments located at septum/body wall junction, mainly on body wall near anterior and posterior faces of septa. Gizzard large, from viii to ix; oesophagus with lamellar sections from xi to xiii;

intestinal origin in xiv; caeca from xxii to xxi; typhlosole a simple fold of about one-sixth lumen diameter, originating in xxii, maximum diameter in xxiv; intestinal wall without longitudinal blood vessels.

Hearts in x–xii, oesophageal; commissural vessel in vi, vii, and ix, lateral; viii extending to gizzard; supra-oesophageal vessel extending from x to xii; extra-oesophageal vessels joining ventral oesophageal wall in x, receiving efferent parieto-oesophageal vessels in xiv.

Ovaries and funnels free in xiii; ovisacs lacking; spermathecae four pairs in vi to ix; duct short, slender; ampulla small, narrow ovate; diverticulum stalk short, attached ectally to duct, terminating in short, ovate receptacle. Male sexual system holandric; testes and funnels enclosed in annular sacs in x and xi; with sacs enclosing hearts; seminal vesicles in xi–xii; vasa deferentia slender, free from body wall, joining ental end of prostatic ducts; prostates in xvi to xix; each prostate racemose, broad, 4-lobed, lobes deeply incised; ectal half of duct expands to form muscular spindle; ducts in hairpin loop. Ental portion of duct with three lumens. Copulatory bursae lacking.

Remarks. *Pithemera donvictoriano* Aspe & James 2015 belongs to the *Pi. pacifica* group of Sims & Easton (1972) and is the only member of this species group so far reported from the Philippines. This group is characterised by having four spermathecae, with the first spermathecal pore in 5/6, in contrast to *Pi. bicincta* group, members of which have the first pair of spermathecal pores in 4/5. Like the other *Pithemera* species on Malindang, it is white and lacks genital markings, and the caeca extend from xvii to xxi. Like *Pi. duminagati*, it has smaller average body size than *Pi. malindangensis*, and it has three pairs of dense tufts of nephridia, on 5/6/7/8, whereas

Pi. malindangensis has only two pairs, on 5/6/7. *Pithemera donvictoriano*i differs from all other Philippine *Pithemera* species in that the clitellum extends from xiv through xv, rather than from xiv through half of xiv (Table 3.3).

Occurrence. *Pithemera donvictoriano*i was rare, with only one individual collected inside a plot in disturbed forest in Barangay Toliyok, although it was also detected in disturbed forest in Barangay Lake Duminagat. The soil it inhabited was covered with thick leaf litter and roots, bryophytes, and lichens. It was not observed on rotten logs.

3.4 Discussion

Blakemore (2007) listed 14 valid *Pithemera* species distributed in East Asia and the Pacific region, described from 1938 to 2004. He also listed 38 valid *Polypheretima* species distributed from East Asia and the Pacific region to South America and Africa, described from 1872 to 1984. In addition, Nguyen *et al.* (2014, 2015) listed a total of 13 *Polypheretima* species from Vietnam, which include four recently described species. Prior to 2004, only one *Pithemera* and three *Polypheretima* species were known from the Philippines: *Pithemera bicincta* from Mindoro Island; *Polypheretima monticola* from Benguet Province, Luzon Island; and *Po. elongata* and *Po. everetti*, both from Balabac Island, Palawan Province. From 2004 to present (including this study), 12 *Pithemera* and five *Polypheretima* species were added to the Philippine fauna, bringing the totals to 13 and eight, respectively.

Table 3.4 shows the diversity of *Polypheretima* and *Pithemera* species reported from the various countries or regions where they occur. The Philippines has the

highest diversity for *Pithemera*, with 13 species, followed by Papua New Guinea and the Pacific Islands, each area with six species. For *Polypheretima*, Indonesia has the highest diversity, with 18 species, followed by Vietnam with 13 species, and then Papua New Guinea and the Philippines, each area with eight species. Among the species of *Polypheretima*, *Po. elongata* is the most widespread, having been reported from 10 regions around East Asia, the Pacific, and Africa. This is followed by *Po. taprobanae* and *Po. everetti*, reported from six and four regions, respectively, around Asia, the Pacific, South America, and Madagascar. The most widespread *Pithemera* species is *Pi. bicincta*, which occurs in six regions around East Asia and the Pacific. Among the species in the Philippines, *Po. elongata*, *Po. everetti*, and *Pi. bicincta* are widely distributed, both locally and globally. Joshi *et al.* (1999) reported *Po. elongata* and *Pi. bicincta* at Ifugao, Luzon Island. The known ranges of the rest of the *Pithemera* and *Polypheretima* species are all restricted to the type localities, indicating a high degree of endemism, both among local areas and islands in the Philippines, and in the Philippines as a whole. This pattern suggests that many species remain to be detected in the Philippines.

To facilitate the further study of earthworms at Mt. Malindang and on Mindanao Island, a key to the species that are from the Mt. Malindang Range is provided. The key also includes Genus *Pheretima* and the exotic species *Pontoscolex corethrurus*. The most easily located external features are used at the beginning of the key. Until more is known about the taxonomy and distributions of earthworms on Mindanao and in the rest of the Philippines, however, this key should be used with caution in identifying earthworms from outside the Mt. Malindang Range.

Key to earthworm species from the Mt. Malindang Range

- 1 Clitellum nine segments, from xiv–xxii, interrupted ventrally, setae lumbricine in arrangement*Pontoscolex corethrurus*
- Clitellum three segments, female pore single2
- Clitellum two or 2½ segments, female pores paired*Pithemera* (3)
2. Body pigmented, genital markings lacking; spermathecae, nephridia on ducts, and caeca present *Pheretima*
- Body white, genital markings paired in xix–xxvi, spermathecae lacking or in batteries, caeca absent*Polypheretima mindanaoensis*
3. Clitellum two segments in xiv–xv, four pairs of spermathecae, 5/6–8/9
..... *Pithemera donvictoriano*
- Clitellum 2½ segments, five pairs of spermathecae, 4/5–8/9 4
4. Intestinal origin in xiv, prostate glands in xvi–xix, male pores more distant, adult length 91–144 mm *Pithemera malindangensis*
- Intestinal origin in xv, prostate glands in xvi–xxi, male pores closer, adult length 36–37 mm *Pithemera duminagati*

CHAPTER 4

New species of *Pheretima*, *Amyntas*, *Polypheretima*, and *Pithemera* (Clitellata: Megascolecidae) from Mindanao and associated islands, Philippines

4.1 Introduction

Mindanao is the southernmost major island group in the Philippines, consisting of Mindanao Island and small surrounding islands. It is surrounded by four seas: the Sulu Sea to the west, the Philippine Sea to the east, the Celebes Sea to the south, and the Mindanao Sea to the north. With a land area of 104,630 km², Mindanao is the second largest island in the Philippines next to Luzon. The island is mountainous, and is home to Mount Apo, the highest mountain in the country. Mindanao initially consisted of separate islands, but these merged in the Miocene due to closing of the Eurasian and Australian plates, which affected minor plates in between (Hall 1996). Partly due its complex geological history, Mindanao has very high species diversity (Heaney & Rabor 1982; Heaney *et al.* 2006; Heaney & Tabaranza 2006; Welton *et al.* 2010; James 2004; Aspe & James 2014). However, as in other parts of the Philippines, the biodiversity on Mindanao is under severe threat due to habitat alteration, overexploitation of natural resource, urban development, and increasing human population. Specific threats include mining and logging operations, and land conversion for industry, agriculture, and urban development. Overall, less than six percent of the original forest of the Philippines now remains, and 491 species of animals are listed as globally threatened on the 2004 IUCN Red List of Threatened Species (CI *et al.* 2006). Adequate knowledge of the remaining natural resources is crucial to enacting effective laws and designing conservation management strategies.

Prior to 2004, knowledge on Philippine earthworms was very limited. Taxonomic studies over the past decade, however, have resulted in the identification of around 200 species of native earthworms representing eight genera in the Philippines (Flores 2008; James 2004, 2005a, 2006, 2009; James *et al.* 2004; Hong & James, 2004, 2008a–c, 2009a, 2010, 2011a, b; Aspe & James 2014, 2015). These indicate high diversity and high local endemism of earthworms in the Philippines. Most of the Philippine earthworm species detected in the last decade were described from Luzon and associated islands, with only around 20% from Mindanao, where James (2004) described 18 species from Mt. Kitanglad, and Aspe & James (2014, 2015) described 22 species from Mt. Malindang. Chapter 4 reports 17 new earthworm species in the genera *Pheretima* (*Pheretima*), *Pheretima* (*Parapheretima*), *Amyntas*, *Polypheretima*, and *Pithemera*, from various localities on Mindanao, Camiguin, and Dinagat Islands (Aspe & James 2016).

4.2 Material and methods

Collecting localities. Collection sites were chosen based primarily on the Key Conservation Sites identified by the Haribon Foundation (Mallari *et al.* 2001). These sites roughly correspond to those identified as Priority Sites for Conservation in the Philippines, which are actually or potentially managed for conservation (CI *et al.* 2006). Collection was conducted intermittently from December 2003 to December 2012. The collection sites (Fig. 4.1) include Mt. Timpoong on Camiguin Island, approximately 10 km north of the Misamis Peninsula (11–15 October 2004); Cagdianao Municipality on Dinagat Island northeast of Mindanao (23–26 April 2004);

the Mt. Kitanglad Range and Mt. Musuan in Bukidnon Province in central Mindanao (26–31 October 2012); the Mt. Apo National Park; the Mt. Hamiguitan Range Wildlife Sanctuary in Davao Occidental Province in southeastern Mindanao (7–10 May 2006); the Mt. Malindang Range in Misamis Occidental Province at the base of the Zamboanga Peninsula (18–23 February 2004); and the Mt. Timolan Protected Landscape in Zamboanga del Sur Province (18–20 December 2012). Figure 4.2 shows the mountainous landscapes of two of the collection sites, Mt. Timpoong and Mt. Apo. Since most of the collection sites are in areas protected by the Protected Areas and Wildlife Bureau (PAWB) of the Department of Environment and Natural Resources (DENR), Prior Informed Consent certificates were secured from the Protected Area Management Board for these sites before collecting was conducted.

Sampling. Collecting was done from soil in primary and secondary forests at the high elevations away from human settlements or trails to increase the likelihood that the earthworms collected are native species. Other habitats including tree bark, ferns, mosses, and the insides of rotten logs were also checked for earthworms. Upon collection, earthworms were rinsed in tap water, killed in 10% ethanol, and placed in Saranex sealable plastic bags filled with a volume of 10% formalin that was at least three times the total volume of the earthworms. After two days, the formalin was replaced with 80% ethanol. Elevations were read by GPS (Magellan Map 410; Luzon map datum) if a satellite signal was detectable, or with an altimeter if not. Elevation is expressed in this paper as meters above sea level (m asl).

Examination and descriptions. Worms were sorted in the field to putative species, using body size, coloration, and number and location of spermathecal pores as

identifying characters. External and internal characters were examined later in the laboratory for a representative subset of specimens of each species. All descriptions are based on external examination and on dorsal dissection under a stereomicroscope, following the terminology and conventions of Easton (1979). Descriptions of body color are based on living specimens. Body dimensions refer to fixed material. The degree of separation between pores is expressed as a proportion of the circumference of the worm; for example, 'spermathecal pores 0.21 circumference apart ventrally' means the distance between the pores is 0.21 the circumference of the worm at that point, with the circumference calculated as π times segment diameter. The generic diagnoses and assignment to species groups follow Sims & Easton (1972). While the species described share many character states diagnostic for the genus, shared characters were included to facilitate information retrieval from the separate descriptions. For convenience in species comparison, the new species were initially compared with the other members of the same species group by their sizes and by the spermathecal pores spacing and the male pores spacing. However, it is assured that the combinations of all the morphological characters were used to examine and compare all the members of the species group. Line drawings were prepared with Adobe Illustrator ver. CS5. Holotypes and paratypes are deposited in the National Museum of the Philippines Annelid Collection (NMA), P. Burgos St., Manila, Philippines. Other paratypes are deposited in the Annelid Collection of the Zoological Reference Collection (ZRC.ANN) of the Lee Kong Chian Natural History Museum, Faculty of Science, National University of Singapore, Singapore.

4.3 Results

Seventeen new earthworm species were detected on Mindanao and associated islands. Table 4.1 lists the species found, and the total number of species currently known, at each site. Nine of the 17 new species belong to the subgenus *Pheretima* (*Pheretima*) Kinberg 1867, which differs from the other subgenus, *Pheretima* (*Parapheretima*) Cognetti 1912, in lacking secretory diverticula on the coelomic surface of the copulatory bursae. Among the new *Pheretima* species, five (*P. acia* n. sp., *P. dinagatensis* n. sp., *P. enormis* n. sp., *P. hamiguitanensis* n. sp., and *P. lantapanensis* n. sp.) belong to the *P. urceolata* group of Sims and Easton (1972), characterized by having a pair of spermathecal pores on intersegments 5/6; three (*P. timpoongensis* n. sp., *P. camiguinensis* n. sp., *P. sibucalensis* n. sp. and *P. apoensis* n. sp.) belong to the *P. sangirensis* group of Sims and Easton (1972), characterized by having a pair of spermathecal pores on intersegments 7/8. Two new species, *P. (Parapheretima) pandanensis* n. sp. and *P. (Paraph.) boaensis* n. sp., are members of the subgenus *Pheretima* (*Parapheretima*), characterized by having secretory diverticula on the coelomic surface of the copulatory bursae. Three new species are in *Amyntas*, characterized by lacking nephridia on the spermathecal ducts and lacking copulatory bursae. These include *A. dinagatensis* n. sp., which is athecate; *A. cagdianaensis* n. sp., which belongs to the *A. supuensis* group of Sims and Easton (1972), characterized by having a pair of spermathecal pores at 8/9; and *A. talaandigensis* n. sp., which belongs to the *A. rimosus* group of Sims and Easton (1972), characterized by having intrasegmental spermathecae in 6, 7, 8, and 9. Two species are in *Polypheretima*: *Po. bukidnonensis* n. sp., which belongs to the *Po.*

elongata group, has a pair of genital markings in xix, successive segments in line with the male pores, and paired batteries of up to 28 spermathecae in vi and/or vii; *Po. zamboangensis* n. sp. either has spermathecal pores at intersegments 5/6/7 or lacks spermathecal pores, has 220+ segments, and has a proandric male sexual system. Finally, one new species is in *Pithemera*. *Pithemera nolani* n. sp. is unique among congeners in having only a pair of spermathecal pores in 5/6 and in possessing copulatory bursae, and it is by far the largest species of *Pithemera* in the Philippines.

TAXONOMY

Megascolecidae Rosa, 1891

Genus *Pheretima* Kinberg, 1867

Subgenus *Pheretima* Kinberg, 1867

Type species. *Pheretima montana* Kinberg, 1867

Generic diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired within copulatory bursae opening on segment xviii; one or more pairs of spermathecal pores in intersegmental furrows between 4/5 and 8/9. Clitellum annular, covering three segments (xiv to xvi). Single midventral female pore on xiv. Genital markings usually absent. Internally, esophageal gizzard usually originating in viii; a pair of caeca originating in xxvii, extending forward. Ovaries and

funnels free in xiii. Male sexual system holandric, with paired testes and funnels enclosed in sacs in x and xi, and seminal vesicles in xi and xii. Spermathecae one pair, multiple pairs, sometimes single and located midventrally, or sometimes lacking. Nephridia present on spermathecal duct(s). One pair of prostate glands, racemose. Copulatory bursae present; secretory diverticula lacking on coelomic surface of copulatory bursae.

***Pheretima acia* n. sp.**

(Fig. 4.2A)

Material examined. Holotype: preclitellate (NMA 4585), Brgy (Barangay) Boa, municipality of Cagdianao, Dinagat Province, (10°05'53"N, 125°39'42"E), 250 m asl, Dinagat Island, Philippines, coll. N. Aspe, J. Adeva, 23–26 Apr. 2004.

Paratypes: two adults (NMA 4601), same collection data as for holotype.

Etymology. The species name is from the Latin *acia* (thread, yarn), referring to the small, thread-like body.

Diagnosis. Very small, brown worm with thread-like body; adult length 38–47 mm, diameter 1–1.2 mm; pigmented segmental equator; pair of spermathecal pores at intersegment 5/6; prostates small in xvii to xviii; penes present.

Description. Brown, equators pigmented. Length 33–47 mm (n= 3 adults); diameter 1.2 mm at x, 1 mm at xx; body circular in cross-section, tail tapering; 87–94 segments. First dorsal pore at 12/13, paired spermathecal pores at 5/6, spermathecal

pores 0.8 mm (0.21 circumference apart ventrally). Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 0.8 mm (0.24 circumference apart ventrally), 3 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equators in some segments; 26–35 setae on vii, 29–38 setae on xx, dorsal and ventral setal gaps lacking. Genital markings lacking.

Septa 4/5–7/8 and 10/11–13/14 thin, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv; caeca simple, originating in xxvii, extending forward to xxvi; typhlosole originating in xxvi, simple fold, 1/3 lumen diameter; intestinal wall with 38 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, with nephridia on ducts. Each spermatheca with ovate ampulla; slender, bulbous, muscular duct; single stalked diverticulum attached to the ental portion of the right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalk short, terminating in ovate to sausage-shaped receptacle. Male sexual system holandric; testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates small, in xvii to xviii; each prostate a single, dense, racemose mass; short muscular duct entering anterior margin of copulatory bursa. Copulatory bursae round, in xvii–xix; coelomic surfaces

muscular, secretory diverticula lacking; roof with thick pad, floor with thin pad; short conical penis present.

Remarks. *Pheretima acia* n. sp. belongs to the *P. urceolata* species group of Sims & Easton (1972), characterized by having spermathecal pores opening only on intersegment 5/6. Sims & Easton (1972) assigned *P. urceolata* Horst, 1893 and *P. baweanensis* Michaelsen, 1928 to this species group, but Gates (1961) synonymized these two species. Currently, there are 20 species members of the *urceolata* group, including the new species described here. *Pheretima acia* differs markedly from any member of the *P. urceolata* group and from the rest of the congeners in having a very small, thread-like body. Other relatively short members of *P. urceolata* group have body diameters two to three times that of *P. acia*: *P. dinagatensis* n. sp. (65 mm x 2.5–3 mm), *P. lantapanensis* n. sp. (>58 mm x 2.5–3 mm), *P. abiadai* Hong & James, 2008c (33–60 mm x 2.2–3 mm), *P. nagaensis* Hong & James, 2008c (36–53 mm x 2.5–3 mm), *P. viracensis* Hong & James, 2009a (38–61 mm x 2–2.6 mm), and *P. doriae* Hong & James, 2009a (34–45 mm x 2–2.4 mm). Like *Pheretima acia*, all these species except *P. lantapanensis* have penes, but the spermathecal pores in *P. acia* are more closely spaced (0.21 circumference) than those of *P. dinagatensis* (0.31), *P. abiadai* (0.25), *P. nagaensis* (0.29), *P. viracensis* 0.32–0.36) and *P. doriae* (0.32). The male pores in *P. acia* are closer (0.24) than those in *P. doriae* (0.32) and more widely spaced compared to that of *P. abiadai* (0.18–0.2). *Pheretima acia* has small prostates, which only extends in two segments (xvii–xviii) while the other species have relatively bigger prostates, covering three to five segments (xv–xix). Although *P. lantapanensis* also has prostates that extends in two segments, they are in xvi–xvii. In

addition, the caeca in *P. acia* are very short, covering only two segments extending only from xxvii–xxvi, whereas the other species have caeca that are longer, extend from xxvii–xxv or –xxiv or –xxiii (Table 4.2). No other species in the *P. urceolata* group closely resemble *P. acia*.

***Pheretima dinagatensis* n. sp.**

(Fig. 4.2B)

Material examined. Holotype: adult (NMA 4586), Brgy Boa, municipality of Cagdianao, Dinagat Province, (10°05'53"N, 125°39'42"E), 250 m asl, Dinagat Island, Philippines, coll. N. Aspe, J. Adeva, 23–26 Apr. 2004. Paratypes: two adults, amputee (NMA 4602); three adults, amputee (ZRC.ANN 0055), same collection data as for holotype.

Etymology. The species is named after Dinagat Island.

Diagnosis. Worm with adult length of 65 mm; reddish brown dorsal stripes at intersegmental furrows, ventrum pale; pair of spermathecal pores at intersegment 5/6; first dorsal pore 11/12; spermathecal pore distance 0.31 circumference apart ventrally; setae between male pores numerous, 12–14; penes present.

Description. Reddish brown dorsal stripes at intersegmental furrows, pale ventrum, equators non-pigmented. Length 65 mm (n=1 adult); diameter 2.5 mm at x, 3 mm at xx; body circular in cross-section, tail tapering; 127 segments. First dorsal pore 11/12, paired spermathecal pores at 5/6, positioned on the lateral margins, distance

between spermathecal pores 2.4 mm (0.31 circumference apart ventrally). Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 2.3 mm (0.24 circumference apart ventrally), 12–14 setae between openings. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equator of some segments; 39 setae on vii, 43–48 setae on xx, dorsal and ventral setal gaps lacking. Genital markings lacking.

Septa 4/5–7/8 and 10/11–13/14 membranous, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xiv or xv; caeca simple, originating in xxvii, extending forward to xxiii, may be folded up or tucked under intestine in xxv or xxiv; typhlosole originating in xxvi, simple fold, 1/3 lumen diameter, intestinal wall with 31–33 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, with nephridia on ducts; each spermatheca with round ampulla and slender, bulbous, muscular duct; single stalked diverticulum attached to the ental portion of right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalks short, terminating in small, round to oval receptacle. Male sexual system holandric; testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xv or xvi to xx; each

prostate a single, dense, racemose mass with four lobes; muscular duct entering center of copulatory bursa, forming a coil. Copulatory bursae round in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with two thick pads, floor pads lacking; short conical penis present.

Remarks. *Pheretima dinagatensis* belongs to *P. urceolata* group of Sims & Easton (1972). Members of *P. urceolata* group with body dimensions similar to *P. dinagatensis* n. sp. include *P. lantapanensis* n. sp., *P. abiadai*, *P. nagaensis*, *P. viracensis*, and *P. doriae* but the former differs from the latter four in having dorsal stripes. *Pheretima dinagatensis* is also similar to *P. lantapanensis* n. sp. in the location of the first dorsal pore but differs from the latter in having more setae between the male pores and more setae in the post-clitellar area (8 and 31–36, respectively in *P. lantapanensis*), and in having penes. *Pheretima dinagatensis* has more body segments (127) and has more setae between the male pores (12–14) than *P. abiadai* (74–78 segments and 6–9 setae, respectively), *P. nagaensis* (57–76 and 7–10, respectively) *P. viracensis* (83–96 and 7–10, respectively), and *P. doriae* (77–86 and 5–8, respectively). The first dorsal pore in *P. dinagatensis* is on 11/12 in *P. dinagatensis* but on 12/13 in *P. abiadai* and *P. nagaensis*. Like *P. abiadai*, *P. nagaensis*, *P. viracensis* and *P. doriae*, the spermathecal ampulla is round, however, the spermathecal duct is slender whereas in others it is short. In addition, *P. dinagatensis* has longer caeca (xxvii–xxiii) compared to that of *P. abiadai*, *P. nagaensis*, *P. viracensis* and *P. doriae*, which extend from xxvii–xxiv or -xxv. No other species in the *P. urceolata* group closely resemble *P. dinagatensis*.

***Pheretima enormis* n. sp.**

(Fig. 4.2C)

Material examined. Holotype: adult (NMA 4587), municipality of San Isidro, Davao Occidental Province, Mt. Hamiguitan (06°44'03"N, 126°12'15"E), 1430 m asl, Mindanao Island, Philippines, coll. Nolan Aspe, J. Cantil, 7–10 May 2006. Paratypes: one adult (NMA 4603); two adults, amputee (ZRC.ANN 0056), same collection data as for holotype.

Etymology. The species name is from the Latin 'enormis' (huge, enormous), referring to the large body size.

Diagnosis. Very large worm with adult dimension 305–347 mm x 13.5–16.5 mm; purplish brown dorsum, ventrum pale; equators non-pigmented, making the body appear striped; pair of spermathecal pores at intersegment 5/6; first dorsal pore in 13/14; setae in vii 45–46, setae in xx 75–80; 7 setae between male pores; penis lacking.

Description. Purplish brown dorsum, lighter ventrum; equators non-pigmented, making the body appear striped. Length 305–347 mm (n=2 adults); diameter 13.5–15.5 mm at x, 13–16.5 mm at xx; body cylindrical in cross-section, tail tapering; 110–147 segments. First dorsal pore at 13/14; paired spermathecal pores at 5/6, distance between spermathecal pores 8.5 mm (0.17 circumference ventrally apart). Female pore single in xiv; openings of copulatory bursae paired in xviii, distance between openings 7.5 mm (0.14 circumference apart ventrally), 7 setae between openings. Clitellum annular, from xiv to xvi. Setae irregularly distributed

around equators in some segments; 45–46 setae on vii, 75–80 setae on xx, dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 4/5–7/8 and 10/11–13/14 muscular, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv; caeca simple, originating in xxvi, extending forward to xxiii; typhlosole originating in xxvii, simple fold, 1/3 lumen diameter; intestinal wall with 38 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, with nephridia on ducts; each spermatheca with round to ovate ampulla and bulbous, muscular duct; stalked diverticulum attached to the right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalks long, convoluted, with two kinks, terminating in long, sausage-shaped receptacle, receptacle longer than the ampulla. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvi to xix; each prostate a single, dense, racemose mass; short muscular duct from lateral margin of prostate entering center of copulatory bursa. Copulatory bursae ovate in xvii–xix, with bulge in center forming a dome; coelomic surfaces muscular, secretory diverticula lacking; roof with two pads, floor pad lacking; blocky penis present.

Remarks. *Pheretima enormis* n. sp. belongs to *P. urceolata* group of Sims & Easton (1972). While most members of *P. urceolata* group have body dimensions within 33–116 mm x 1.2–5.4 mm, *P. enormis* has an adult body size of 305–347 mm x 13–16.5 mm. Other *Pheretima* species with large body size include *P. maculodorsalis* Aspe & James, 2014 (226–235 mm); *P. tigris* Aspe & James, 2014 (230–283); *P. immanis* Aspe & James, 2014 (365 mm); *P. lago* Aspe & James, 2014 (223–315 mm); *P. virgata* James, 2004 (290 mm); *P. ceramensis* Cognetti, 1922 (140–440 mm); *P. barligensis* Hong & James, 2011b (225–255 mm); *P. callosa* Gates, 1937 (330); and a large earthworm that Blakemore *et. al.* (2007) identified as *P. darnleiensis* Fletcher, 1887 (700 mm). *Pheretima enormis* differs from these species in having one pair of spermathecal pores on 5/6, whereas the others have one pair on 7/8 (*P. maculodorsalis*, *P. tigris*, *P. immanis*, *P. lago*, *P. virgata*, and *P. ceramensis*), four pairs on 5/6–8/9 (*P. barligensis* and *P. darnleiensis*), or three pairs on 6/7–8/9 (*P. callosa*). In addition, *P. enormis* has the first dorsal pore at 13/14 (12/13 in the other species), has more setae (7) between male pores (2–4 in *P. maculodorsalis*, 0–4 in *P. tigris*, 4 in *P. virgata*, 5 in *P. immanis*, and 0–2 in *P. lago*), and has a different pigmentation pattern than the other species (the dorsal intersegmental furrows have red oval dots in *P. maculodorsalis*, stripes in *P. tigris* and *P. virgata*, and very thick stripes in *P. immanis*).

***Pheretima hamiguitanensis* n. sp.**

(Fig. 4.2D)

Material examined. Holotype: adult (NMA 4588), municipality of San Isidro, Davao Occidental Province, Mt. Hamiguitan (06°44'03"N, 126°12'15"E), 1430 m asl, Mindanao Island, Philippines, coll. Nolan Aspe, J. Cantil, 7–10 May 2006. Paratypes: three adults (NMA 4604), two adults, same collection data as for holotype, one adult, Brgy Baracatan, Davao City, Mt. Apo National Park (7°00'04" N, 125°21'55" E), 1524 m asl, Mindanao Island, Philippines, coll. N. Aspe, A. Solis, Dec. 11–14, 2003.

Etymology. The species is named for Mt. Hamiguitan, the type locality.

Diagnosis. Worm with adult length 81–90 mm; dorsum brown, ventrum pale, equators non-pigmented; pair of spermathecal pores at intersegments 5/6; setae in vii 38–42; spermathecae with ovate ampulla, spermathecal diverticulum terminating in elongate receptacle that is longer than the ampulla; penis lacking.

Description. Brown dorsum, lighter ventrum, equators non-pigmented, making the body appear striped. Length 81–90 mm (n=2 adults); diameter 3–3.5 mm at x, 3.5–4 mm at xx; body round in cross-section, tail tapering; 108–111 segments. First dorsal pore at 12/13, paired spermathecal pores at 5/6, distance between spermathecal pores 0.7 mm (0.07 circumference apart ventrally). Female pore single in xiv; openings of copulatory bursae paired in xviii, distance between openings 1.3 mm (0.12 circumference apart ventrally), 2 setae between openings. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equators in some segments, 38–42 setae on vii, 44–47 setae on xx; dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 4/5–7/8 membranous, 10/11–13/14 muscular, 8/9/10 lacking. Dense tufts

of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv or xvii; caeca simple originating in xxvii, extending forward to xxiv; typhlosole and longitudinal blood vessels not observed due to damage. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, with nephridia on ducts; each spermatheca with ovate ampulla, bulbous, muscular duct, and stalked diverticulum; diverticulum attached to ectal portion of right face of duct near ampulla of right spermatheca, and on left face of duct of left spermatheca; stalks short, terminating in elongate receptacle, receptacle longer than ampulla. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates variably in xvii to xx, or xv to xix; each prostate a dense, racemose mass, with three lobes; muscular duct from anterior margin of prostate entering to lateral margin of copulatory bursa. Copulatory bursae ovate in xviii–xix; coelomic surface muscular, secretory diverticula lacking; roof with thick pad, floor pad lacking; penis lacking.

Remarks. *Pheretima hamiguitanensis* n. sp. belongs to the *P. urceolata* group of Sims & Easton (1972). Members of *P. urceolata* group with body dimensions relatively similar to *P. hamiguitanensis* n. sp. include *P. bicoloris* Hong & James, 2009a (71–98 mm x 3.8–4.6 mm); *P. kitangladensis* James, 2004 (108 mm x 3.6–3.8 mm); *P. baletei* James, 2004 (>78 mm x 4.3–4.5 mm); *P. bukidnonensis* James, 2004

(76 mm x 3.5 mm); and *P. makilingensis* Hong & James, 2008b (110 mm x 4–4.2 mm). However, *P. hamiguitanensis* has more closely spaced spermathecal pores (0.07 circumference) than *P. kitangladensis* (0.28), *P. balettei* (0.25), *P. bukidnonensis* (0.38), and *P. makilingensis* (0.15). Its male pores are more closely spaced (0.12 circumference) than those of *P. bicolensis* (0.14), *P. kitangladensis* (0.2), *P. balettei* (0.25), *P. bukidnonensis* (0.17) but are more distantly spaced than those of *P. makilingensis* (0.08). It has fewer setae (2) between male pores than *P. kitangladensis*, *P. bukidnonensis*, *P. makilingensis* (all with 8 setae), and *P. balettei* (6 setae). There are more setae in the pre-clitellar area (38–47 per segment) than in the other species (29–34 in *P. bicolensis*, 24 in *P. balettei* and *P. bukidnonensis*, and 35 in *P. makilingensis*). *Pheretima hamiguitanensis* also differs from *P. bicolensis*, *P. balettei* and *P. makilingensis* in lacking penes. In addition, the shape of the spermathecae differs from the other species (diverticulum with small oval receptacle in *P. bicolensis*, club-shaped receptacle in *P. kitangladensis*, short club-shaped receptacle in *P. balettei*, ovate receptacle in *P. bukidnonensis*, and globular to ovate receptacle in *P. makilingensis*). No other species in the *P. urceolata* group closely resemble *P. hamiguitanensis*.

***Pheretima lantapanensis* n. sp.**

(Fig. 4.2E)

Material examined. Holotype: adult, amputee (NMA 4589), Brgy Songco,

municipality of Lantapan, Bukidnon Province, Mt. Kitanglad Range (8°05'47"N, 124°55'21"E), 2200 m asl, Mindanao Island, Philippines, coll. N. Aspe, 27–31 Oct. 2012. Paratype: one adult, amputee (NMA 4605), same collection data as for holotype.

Etymology. The species is named for the municipality of Lantapan, Bukidnon Province, where this species was collected.

Diagnosis. Worm with adult diameter 2.5–3 mm; dorsum brown, ventrum pale, equators pigmented; pair of spermathecal pores at intersegment 5/6; setae in vii 30–39; gizzard origin in ix; prostates small, xvi–xvii; penis lacking.

Description. Brown dorsum, pale ventrum, equators pigmented. Length >58 mm; diameter 2.5 mm at x, 3 mm at xx (n=2 adults); body circular in cross-section. First dorsal pore at 11/12, paired spermathecal pores at 5/6, distance between spermathecal pores 3 mm (0.38 circumference apart ventrally). Female pore single in xiv; openings of copulatory bursae paired in xviii, distance between openings 2.2 mm (0.23 circumference apart ventrally), 8 setae between openings. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equator in some segments; 30–39 setae on vii, 31–36 setae on xx, dorsal setal gaps present, ventral setal gaps lacking. Genital markings lacking.

Septa 4/5–7/8 and 10/11–13/14 thin, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv; caeca simple, originating in xxvii, extending forward to xxiii, may be folded up or tucked under the intestine in xxiv; typhlosole originating in xxvi, simple fold slightly less than lumen diameter; intestinal

wall with 33 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, with nephridia on ducts. Each spermatheca with pear-shaped ampulla; slender, bulbous, muscular duct; single stalked diverticulum attached to ectal portion of right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalks short, terminating in large, ovate receptacle. Male sexual system holandric; testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates small in xvi to xvii; each prostate a dense, racemose mass with two lobes; thin duct entering from anterior margin of prostate to center of copulatory bursa. Copulatory bursae round in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with two thick pads, floor pads lacking; penis lacking.

Remarks. *Pheretima lantapanensis* n. sp. belongs to the *P. urceolata* group of Sims & Easton (1972). Members of the *P. urceolata* group with body width similar to *P. lantapanensis* include *P. dinagatensis*, *P. abiadai*, *P. nagaensis*, and *P. viracensis*. *Pheretima lantapanensis* differs from these species in having pigmented segmental equators and in lacking penes. The distance between spermathecal pores in *P. lantapanensis* is very wide (0.38) compared to that of *P. dinagatensis* (0.31), *P. abiadai* (0.25), *P. nagaensis* (0.29) and *P. viracensis* (0.32–0.36). The new species has relatively fewer setae (30–39) on the post-clitellar segments and the extent of the prostates is only xvi–xvii compared with that of *P. dinagatensis* n. sp. (43–48 and

xv/xvi–xix, respectively), *P. abiadai* (42–47 and xvii–xix, respectively), *P. nagaensis* (41–48 and xvii–xx, respectively), and *P. viracensis* (40–45 and xvii–xix, respectively). *Pheretima lantapanensis* also has large pear-shaped spermathecal ampulla with slender duct and prominent ovate diverticula, whereas the others have relatively small, round ampulla with short ducts. Although the spermathecal duct in *P. dinagatensis* is also slender, its diverticular receptacle is round and small. No other member of the *P. urceolata* group closely resembles *P. lantapanensis*.

***Pheretima timpoongensis* n. sp.**

(Fig. 4.3A)

Material examined. Holotype: adult (NMA 4590), Brgy Pandan, Mambajao City, Camiguin Province, Mt. Timpoong (9°10'45"N, 124°43'15"E), 1350 m asl, Camiguin Island, Philippines, coll. N. Aspe, J. Adeva, 11–15 Oct. 2004. Paratypes: seven adults (NMA 4606); six adults (ZRC.ANN 0059), same collection data as for holotype.

Etymology. The species is named for Mt. Timpoong, where this species was collected.

Diagnosis. Large, purplish brown worm with adult dimensions 147–225 mm x 7–9.5 mm; ventrum pale; equators non-pigmented; pair of spermathecal pores at intersegment 7/8; large gizzard in ix–x or x–xi; intestinal origin in xv or xvi; prostates extend from xvii–xx; penes present.

Description. In living animals, purplish brown dorsum, lighter ventrum, equators non-pigmented. Length 147–225 mm (n=14 adults); diameter 7.3–9.5 mm at x, 7–8.5 mm at xx; body circular in cross-section, tail tapering; 98–119 segments. First dorsal pore at 12/13; paired spermathecal pores at 7/8, spermathecal pores 6 mm (0.20 circumference apart ventrally). Female pore single in xiv; openings of copulatory bursae paired in xviii, distance between openings 5.4 mm (0.23 circumference apart ventrally), 2–5 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equator in some segments; more closely spaced on ventrum; 29–43 setae on vii, 49–73 setae on xx; dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 4/5–6/7 membranous, 7/8 and 10/11–13/14 muscular, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low, vertical lamellae x–xiii, intestinal origin in xv or xvi, caeca simple originating in xxvii, extending forward to xxiv or xxiii; typhlosole originating in xxvii, simple fold, 1/3 lumen diameter; intestinal wall with 31–44 longitudinal blood vessels. Hearts in x to xiii; hearts esophageal.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in viii, with nephridia on ducts; each spermatheca with ovate ampulla, stout muscular duct, and stalked diverticulum. Diverticulum attached to the ectal portion of left face of duct of right spermatheca, and on right face of duct of left spermatheca; stalks short, with one coil, terminating in ovate receptacle. Spermathecae contain two small, ovate spermatophores. Male sexual system holandric; testes and funnels enclosed in paired

sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles in xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvii to xx, each a dense, racemose mass with two or three lobes; thick muscular duct from lateral margin of prostate entering posterior margin of copulatory bursa. Copulatory bursae ovate-hemispherical in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with two pads, floor with folding encircling the opening; penes present; penis sheath lacking.

Remarks. *Pheretima timpoongensis* n. sp. belongs to *P. sangirensis* species group of Sims & Easton (1972), which differs from the *P. montana* Kinberg, 1867 group in having spermathecal pore(s) opening only at 7/8, and no penial sheaths in the copulatory bursae. Members of the *sangirensis* group have a holandric male system, with paired testis sacs; the copulatory bursae are simple, and some species have short, conical penes. Blakemore (2007) acknowledged three valid subspecies in *P. sangirensis*: *P. s. sangirensis*; *P. s. crassicystis* Michaelsen, 1896; and *P. s. chica* Michaelsen, 1896. Currently there are 26 species members of *P. sangirensis* group, including the new species described here. *Pheretima timpoongensis* is larger (147–225 mm x 7–9.5 mm) than *P. s. sangirensis* (140 mm x 3.5–4.5 mm) and *P. s. chica* (54–120 mm) but shorter than *P. s. crassicystis* (240 mm x 8 mm). The spermathecal pores in *Pheretima timpoongensis* (0.2 circumference) are closer than in *P. sangirensis* (0.25–0.28) and there are fewer setae between male pores in the new species (2–5 vs 6–10 in *P. sangirensis*; particularly 10 in *P. s. crassicystis*). Also, there is no dorsal setal gap in *P. s. crassicystis*, the caeca are not simple and they have stubby pockets that are deeply divided from one another, the stalk of the spermathecal diverticulum

originates entally, and the copulatory bursae are fairly flat, in contrast to that of the new species. Among other species in the *P. sangirensis* group, *P. timpoongensis* is similar to *P. lago* Aspe & James, 2014 and *P. nunezae* Aspe & James, 2014 from Mt. Malindang (the smaller *P. lago* specimens (223–315 mm x 10–11 mm) overlap in size with the larger *P. timpoongensis* specimens; the *P. nunezae* specimens were amputees but width (8.5–9 mm) are similar) in the number of setae around segments, and in having penes. However, *P. lago* is dark brown, has fewer setae between male pores (0–2), has closer male pores (0.15 circumference apart ventrally), has stalked diverticulum originating entally and terminating in a receptacle with 2–4 chambers, the prostates are located anterior of the copulatory bursae, and the duct from the prostate enters the anterior margin of the copulatory bursa. On the other hand, *P. nunezae* is dark gray-brown, has more setae (9) between the male pores, has greater distance (0.28 circumference) between the spermathecal pores, has fewer blood vessels on the intestinal wall (20–23), has smaller copulatory bursae (xviii), has stalked diverticulum originating entally and terminating in sausage-shaped receptacle, has relatively smaller prostates (xvii–xix), and the duct from the prostate enters the lateral margin of the copulatory bursa. *Pheretima timpoongensis* is also similar to *P. apoensis* n. sp. (see below) from Mt. Apo in relative size and in the number of setae around equators. However, *P. timpoongensis* have greater distance between male pores, the stalked diverticulum originating ectally is less stout, the duct from the prostate enters the posterior margin of the copulatory bursa, and penes are present. *Pheretima timpoongensis* also have some relatively overlapping features with some individuals of *P. camiguinensis* n. sp. (described below): the color, the number of

setae on the segments, the number of setae between male pores, the position of the gizzard and the intestinal origin, the length of caeca and relative size of the prostates. However, *P. timpoongensis* is larger, have wider space between spermathecal pores and between male pores, and penes are present.

***Pheretima camiguinensis* n. sp.**

(Fig. 4.3B)

Material examined. Holotype: adult (NMA 4591), Brgy Pandan, Mambajao City, Camiguin Province, Mt. Timpoong (9°10'45"N, 124°43'15"E), 1350 m asl, Camiguin Island, Philippines, coll. N. Aspe, J. Adeva, 11–15 Oct. 2004. Paratypes: nine adults (NMA 4607); nine adults (ZRC.ANN 0060), same collection data as for holotype.

Etymology. The species is named for Camiguin Island.

Diagnosis. Reddish brown worm with adult length 63–122 mm; pair of spermathecal pores at intersegment 7/8; spermathecal pores 0.17 circumference apart, male pores 0.15–0.18 circumference apart; 32–34 setae on vii, 39–44 setae on xx; penis lacking.

Description. In living animals, reddish brown dorsum, pale ventrum, equators non-pigmented. Length 63–122 mm (n=19 adults); diameter 3.5–4.8 mm at x, 3.5–5.2 mm at xx; body circular in cross-section, tail tapering; 87–103 segments. First dorsal pore at 12/13, paired spermathecal pores at 7/8, distance between spermathecal pores

1.9–2.8 mm (0.17 circumference apart ventrally). Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 2–2.5 mm (0.15–0.18 circumference ventrally apart), 1–4 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equator in some segments; 32–34 setae on vii, 39–44 setae on xx, dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 5/6–7/8 and 10/11/12 thick; 4/5, 8/9, and 13/14 thin; 9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x; esophagus with low, vertical lamellae x–xiii; intestinal origin in xv; caeca originating in xxvii, extending forward to xxiv, xxiii, or xxii; caeca may be folded over, or tucked under the intestine in xxiii; typhlosole originating in xxvii, simple fold slightly less than lumen diameter; intestinal wall with 26–30 longitudinal blood vessels. Hearts in x to xiii; heart esophageal.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in viii, with nephridia on ducts; each spermatheca with ovate ampulla, stout muscular duct, and stalked diverticulum attached to ectal portion of left face of duct of right spermatheca, and on right face of duct of left spermatheca; stalks short, with one coil, terminating in ovate receptacle. Spermathecae contain two small, ovate spermatophores. Male sexual system holandric; testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvi to xx (or xxi in some individuals); some individuals have

asymmetrical prostates (e.g. xvi to xx on left and xvi to xxi on right); each prostate a dense, racemose mass, with three to five lobes; thick muscular duct from lateral margin of prostate entering posterior margin of copulatory bursa. Copulatory bursae ovate-hemispherical in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with thick pad, floor pads lacking; short, blocky penis present.

Remarks. *Pheretima camiguinensis* n. sp. belongs to the *P. sangirensis* group of Sims & Easton (1972). The new species is similar in size to *P. s. chica* Michaelsen, 1896; *P. baungonensis* James, 2004; *P. quincunxia* James, 2004; *P. malindangensis* Aspe & James, 2014; and *P. wati* Aspe & James, 2014. However, the spermathecal pores (0.17 circumference in *P. camiguinensis*) are farther apart in *P. s. chica* (0.25–0.28) and *P. baungonensis* (0.32) and closer in *P. quincunxia* (0.13). The male pores are closer in *P. camiguinensis* (0.15–0.18 circumference) than in *P. baungonensis* (0.19) but farther than in *P. wati* (0.08). *Pheretima camiguinensis* has more setae per segment (32–34 in vii, 39–44 in xx) than *P. quincunxia* (16–20; 20–30) but fewer than *P. malindangensis* (43–47; 50–57) and *P. wati* (59–71; 52–60). The prostates in *P. camiguinensis* (xvii–xx/ xxi) are larger than in *P. baungonensis* and *P. quincunxia* (xvii–xix for both), but both the prostates and copulatory bursae are smaller than in *P. malindangensis* (xvi–xxi and xvii–xx, respectively) and *P. wati* (xv–xxii and xv–xxi, respectively). *Pheretima camiguinensis* differs from *P. baungonensis* in that the latter has the first dorsal pore at 13/14, more setae (8) between male pores, and shorter caeca (xxvii–xxv). *Pheretima camiguinensis* (reddish brown) differs in color from *Pheretima s. chica* (purple) and *P. quincunxia* (non-pigmented). No other member of the *P. sangirensis* group closely resembles *P. camiguinensis*.

Although there are variations in the size of the gizzard and prostates, and in the length of the caeca, among individuals of *P. camiguinensis*, molecular data for the mitochondrial 16S and CO1 genes reveal that these are intraspecific variations (0–0.013 [n = 4] and 0.002 [n = 2], respectively, in terms of K2P; Aspe & James, unpublished data). Numerous parasitic nematodes were commonly observed near the caeca in individuals of *P. camiguinensis*.

***Pheretima sibucalensis* n. sp.**

(Fig. 4.3C)

Material examined. Adult, amputee (NMA 4510), Brgy Sibucal, Oroquieta City, Misamis Occidental Province, Mt. Malindang Range (8°19'31"N, 123°38'02"E), 991 m asl, Mindanao Island, Philippines, coll. N. Aspe, M. Lluch, and J. Adeva, 18–23 Feb. 2004.

Etymology. The species is named after Brgy Sibucal of Oroquieta City, where this species was collected.

Diagnosis. Dark gray-brown worm, diameter 6.5–7 mm, ventrum and equators non-pigmented; pair of spermathecal pores at 7/8; spermathecal pores and male pores, 0.21 and 0.17 circumference apart, respectively; 70 setae on vii, 89 setae on xx; 9 setae between male pores; prostate extends from xvii to xxi; penes lacking.

Description. Living animals with dorsum dark gray-brown anteriorly, fading posteriorly; ventrum non-pigmented; equators non-pigmented; clitellum gray, darker than adjacent segments. Length > 165 mm (n=1 adult, amputee); diameter 6.5 mm at x; 7 mm at xx; body circular in cross-section. First dorsal pore at 12/13, paired spermathecal pores at 7/8, 0.21 circumference apart; female pore single in xiv; openings of copulatory bursae paired in xviii, 0.17 circumference apart, depressed area with 9 setae between openings. Clitellum annular, extending from xiv to xvi. Setae evenly distributed, 70 setae on vii, 89 setae on xx, dorsal gap present, ventral gap absent.

Septa 4/5/6/7/8 slightly muscular, 8/9 membranous, 9/10 absent, 10/11–13/14 slightly muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body wall on anterior and posterior faces of septa, at septum/body wall junction. Large gizzard in viii–x; esophagus with lamellae from x to xiii; intestinal origin in xiv; caeca originate in xxvii, extend forward to xxii, with pocketed ventral margin; typhlosole in xxvii/xxvi, simple fold of about 1/8 lumen diameter, intestinal wall with 38 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; viii extends to gizzard.

Ovaries and funnels free in xiii; paired spermathecae preseptal in vii, with nephridia on ducts; each spermatheca with round ampulla; short, bulbous, muscular duct; stalked diverticulum attached to the ental portion of left face of duct near ampulla of the right spermatheca, and on left face of duct of left spermatheca; stalks short, terminating in bean-shaped receptacle. Each spermatheca contains two spermatophores. Male sexual system holandric; testes and funnels enclosed in paired

sacs in x and xi; seminal vesicles in xi and xii, each with short, spherical dorsal lobe; vasa deferentia slender, free from body wall, passing around lateral face of copulatory bursae en route to ental end of prostatic ducts; each prostate racemose from xvii to xxi; muscular duct from lateral margin of prostate entering posterior margin of copulatory bursa. Ductlets of lobes meet vasa deferentia at common junction with muscular prostatic duct. Copulatory bursae ovate in xvii–xx; coelomic surfaces muscular, secretory diverticula lacking; roof with thick pad, floor pad lacking; penes lacking.

Remarks. *Pheretima sibucalensis* n. sp. belongs to the *P. sangirensis* group of Sims & Easton (1972). It differs from *P. sangirensis* in having more setae on segmental equators (70–89 vs. 40–60+), closer spermathecal pores, the intestinal origin in xiv rather than xv, larger prostates, and no penes. *Pheretima sibucalensis* is similar to *P. nunezae* Aspe & James, 2014 from Mt. Malindang, in size, coloration, presence of dorsal gaps and absence of ventral gaps, arrangement of the septa, origin of the gizzard, and the length of the caeca. However, *P. sibucalensis* has shorter distance between male pores and between spermathecal pores (0.21 and 0.17, respectively) compared to that of *P. nunezae* (0.28 and 0.22, respectively). The setae on the segmental equators in *P. sibucalensis* is more numerous (70 in vii and 89 in xx, respectively) than that of *P. nunezae* (46 in vii, 51 in xx, respectively). The new species also differs from *P. nunezae* in the intestinal origin (xiv vs. xv), in the number of blood vessels on the intestinal wall (38 vs. 28–33), in the extent of caeca (xxvii–xxii vs. xxvii–xxiv), in the extent of the prostate and copulatory bursae (xvii–xxi vs. xvii–xix and xvii–xx vs. xviii), and in lacking penes. *Pheretima timpoongensis* n. sp., which also closely resembles *P. nunezae*, differs from *P. sibucalensis* in having larger

body diameter, fewer setae on each segment, in having a wider space between male pores, in having fewer setae between the male pores, and in lacking penes. *Pheretima sibucalensis* does not closely resemble any other members of the *P. sangirensis* group.

***Pheretima apoensis* n. sp.**

(Fig. 4.3D)

Material examined. Holotype: adult (NMA 4592), Brgy Baracatan, Davao City, Mt. Apo National Park (7°00'04" N, 125°21'55" E), 1524 m asl, Mindanao Island, Philippines, coll. N. Aspe, A. Solis, Dec. 11–14, 2003. Paratypes: seven adult (NMA 4608), five adults same collection data as for holotype, two adults, municipality of Maramag, Bukidnon Province, Mt. Musuan Range (7°52'53.8"N, 125°03'57.6"E), 400 m asl, Mindanao Island, Philippines, coll. N. Aspe, 26 Oct. 2012; three adults (ZRC.ANN 0061), same collection data as for holotype; two adults, same collection data as for the ones collected in Mt. Musuan.

Etymology. The species is named after Mt. Apo, where this species was first collected.

Diagnosis. Large worm, adult body dimension 163–350 mm x 7.2–12 mm; 102–120 segments; dorsum purplish brown, ventrum pale; equators non-pigmented; spermathecal pores lacking in athecate individuals, at 7/8 in thecate individuals; 37–48 setae on vii, 50–58 setae on xx; 4–5 setae between male pores; male pores 0.15–0.16 circumference apart; penis lacking.

Description. In living animals, purplish brown dorsum, lighter ventrum, equators non-pigmented making the worm appear striped. Length 163–350 mm (n= 13 adults); diameter 7.2–11 mm at x, 8–12 mm at xx; body cylindrical in cross-section, tail tapering; 102–120 segments. First dorsal pore 12/13, spermathecal pores lacking in some individuals, thecate individuals with paired spermathecal pores at 7/8, distance between spermathecal pores 0.16 circumference apart ventrally. Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 0.15–0.16 circumference apart ventrally, 4–5 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equators in some segments; 37–48 setae on vii, 50–58 setae on xx, dorsal setal gaps present, ventral setal gaps lacking. Genital markings lacking.

Septa 4/5–8/9 membranous, 11/12–13/14 thick, 9/10/11 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in ix–xi, esophagus with low vertical lamellae x–xiii, intestinal origin in xv, caeca simple originating in xxvii, extending forward to xxvi or xxiv; typhlosome originating in xxvii, simple fold, 1/3 lumen diameter, intestinal wall with 32–38 longitudinal blood vessels. Hearts in xi to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae lacking in some individuals, thecate individuals with a pair of spermathecae postseptal in viii, with nephridia on ducts; each spermatheca with round ampulla, short, stout muscular duct, stalked diverticulum attached to the ental portion of the left face of duct of the right

spermatheca, and on right face of duct of left spermatheca, stalks short, terminating in small, round receptacle. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall en route to ental end of prostatic ducts; prostates in xv or xvii to xix, each a single, dense, racemose mass with 3 lobes; short muscular duct entering middle area of the surface of copulatory bursa. Copulatory bursae ovate-hemispheric in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with thick pad, floor pads lacking; penis lacking.

Remarks. *Pheretima apoensis* n. sp., belonging to the *P. sangirensis* group of Sims & Easton (1972), is a large worm similar to *P. ceramensis* Cognetti, 1922 from Indonesia, *P. lago* Aspe & James, 2014 from Mt. Malindang, and *P. timpoongensis* n. sp. from Camiguin Island, in relative size and in having pigmentation over the entire dorsal area that gradually fade toward the ventrum. However, *P. apoensis* differs from these species in lacking penes. It has closer male pores than in *P. ceramensis* (0.2 circumference apart) and in *P. timpoongensis* (0.23 circumference apart), has closer spermathecal pores (for athecate individuals) than in *P. lago* and in *P. timpoongensis* (0.18–0.24 and 0.2 circumference apart, respectively), and has fewer setae than in *P. ceramensis* (60 per segment). Its prostates also are in different position compared to that of *P. lago* (positioned in xiv–xviii, anterior of the copulatory bursae) and its stalked diverticulum has different structure (stalked diverticulum in *P. lago* terminating in a receptacle with 2–4 chambers). Although *P. virgata* James, 2004 from Mt. Kitanglad, and *P. tigris* Aspe & James, 2014, *P. maculodorsalis* Aspe & James,

2014, and *P. immanis* Aspe & James, 2014 from Mt. Malindang are also similar to *P. apoensis* in size, these species significantly differ from the new species in pigmentation pattern from *P. virgata* and *P. tigris* (with dorsal intersegmental stripes in *P. virgata* and *P. tigris*; with mid-dorsal intersegmental oval dots in *P. maculodorsalis*; and with very thick dorsal intersegmental stripes in *P. immanis*).

The *P. apoensis* that were collected from Mt. Apo (nine adults; 163–188 x 7.2–9 mm) are smaller compared to the ones in Bukidnon Province (four adults; 324–350 x 10–12 mm) and all individuals collected from Mt. Musuan are athecate. Also, the caeca from *P. apoensis* collected from Mt. Apo are longer by two segments and the prostates are more extensive (xv–xix vs. xvii–xix). The *P. apoensis* from Mt. Musuan and the *P. apoensis* from Mt. Apo have a genetic divergence of 0.011 (or 1.1%) based on 16S using K2P model, which indicates that the two are conspecific. On another note, a study on cryptic species of hormogastrid earthworms showed an upper limit of 4.27% intraspecific divergence in *Hormogaster elisae* using K2P based on 16S (Novo et al. 2010). It is most likely that geographic isolation (e.g. the distance between Mt. Apo and Mt. Musuan is approximately 110 km) may have caused divergence in some morphological features such as the body size and the loss of spermathecae, in this case, the *P. apoensis* from Mt. Musuan. A study on the reproductive traits of *Aporrectodea trapezoides* showed that isolation of an earthworm in a controlled environment can trigger the earthworm to reproduce parthenogenetically (Férrandez et al. 2010). *Pheretima apoensis* was also observed in Mt. Kitanglad Range, which is close to Mt. Musuan.

Subgenus *Parapheretima* Cognetti, 1912

Type species. *Pheretima aberrans* Cognetti, 1911

Diagnosis. *Pheretima* species with a secretory diverticulum discharging into each copulatory bursa.

***Pheretima (Parapheretima) pandanensis* n. sp.**

(Fig. 4.3E)

Material examined. Holotype: adult (NMA 4593), Brgy Pandan, Mambajao City, Camiguin Province, Mt. Timpoong (9°10'45"N, 124°43'15"E), 1350 m asl, Camiguin Island, Philippines, coll. N. Aspe, J. Adeva, 11–15 Oct. 2004. Paratypes: five adults (NMA 4609); four adults (ZRC.ANN 0062), same collection data as for holotype.

Etymology. The species is named for Brgy Pandan of Mambajao City, Camiguin Province, where this species was collected.

Diagnosis. Dark reddish brown worm, adult length 85–150 mm; ventrum pale; pair of spermathecal pores at intersegment 7/8; male pores and spermathecal pores 0.23 and 0.23–0.25 circumference apart; 50–55 setae on xx; 4–9 setae between male pores; single secretory diverticulum projecting from lateral surface of each copulatory bursa; long, tapering penis.

Description. In living animals, dark reddish brown dorsum, lighter ventrum,

equators non-pigmented. Length 86–150 mm (n=10 adults); diameter 4–5 mm at x, 4–5 mm at xx; body circular in cross-section, tail tapering; 85–104 segments. First dorsal pore at 12/13, paired spermathecal pores at 7/8, distance between spermathecal pores 3 mm (0.23 circumference apart ventrally), spermathecal pores inconspicuous in some individuals. Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 3–4 mm (0.23–0.25 circumference apart ventrally), 4–9 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equators; 36–44 setae on vii, 50–55 setae on xx, dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 6/7/8 and 10/11–12/13 muscular, 4/5/6 and 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xiv; caeca simple, originating in xxvii, extending forward to xxv or to xxiv, margins serrated; typhlosole originating in xxvii, simple fold slightly less than lumen diameter, intestinal wall with 31–53 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in viii, with nephridia on ducts. Each spermatheca with round ampulla, very large, short, bulbous, muscular duct; single stalked diverticulum attached to ectal portion of left face of duct close to the ampulla of the right spermatheca, and on right face of duct of left spermatheca; stalks terminating in elongate receptacle, becoming bulbous abruptly midway toward duct. Spermathecae contained two small, ovate spermatophores,

lacking in some individuals. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostate glands in two parts, in xvii–xviii, and in xix–xx, each part dense, racemose mass with two or three lobes; long duct from each prostate joins others to form thick, coiling, muscular duct connecting to copulatory bursa. Each copulatory bursa hemispheric in xvii–xix; coelomic surface muscular, with single secretory diverticulum projecting from lateral surface; roof with two pads, floor with thin pads; long, tapering penis arises from padded roof.

Remarks. *Pheretima (Parapheretima) pandanensis* n. sp. and *P. (Paraph.) boaensis* n. sp. (see next description) are the first *P. (Paraph.)* species reported from the Philippines. *Pheretima (Paraph.) pandanensis* n. sp. differs from other *P. (Paraph.)* species in the location of the spermathecae, which are paired in intersegment 7/8. Other *P. (Paraph.)* species are either monothecate (members of the *P. (Paraph.) barbara* Cognetti, 1913 species group), or if bithecate, have the spermathecae located at intersegment 6/7 [*P. (Paraph.) bernhardi* Gates, 1948 or members of the *P. (Paraph.) beauforti* Cognetti, 1913 species group] or at intersegments 5/6/7 [*P. (Paraph.) saba* Sims & Easton, 1972]. *Pheretima (Paraph.) pandanensis* is similar to *P. camiguinensis* n. sp. in size and coloration, but differs from the latter in having a wider distance between spermathecal pores and between male pores, in having more setae between the male pores, and in having more post-clitellar setae on the segmental equators (Table 4.3).

***Pheretima (Parapheretima) boaensis* n. sp.**

(Fig. 4.3F)

Material examined. Holotype: adult (NMA 4594), Brgy Boa, municipality of Cagdianao, Dinagat Province, (10°05'53"N, 125°39'42"E), 250 m asl, Dinagat Island, Philippines, coll. N. Aspe, J. Adeva, 23–26 Apr. 2004. Paratypes: two adults (NMA 4610); three adults (ZRC.ANN 0063), same collection data as for holotype.

Etymology. The species is named after Brgy Boa in the municipality of Cagdianao, Dinagat Province, where this species was collected.

Diagnosis. Reddish brown worm, adult length 94–133 mm; ventrum pale; single spermathecal pore midventrally at intersegment 5/6; single muscular secretory diverticulum projecting from anterior margin of each copulatory bursa; 6–9 setae between male pores; penis lacking.

Description. Reddish brown dorsum, lighter ventrum, equators pigmented. Length 94–133 mm (n=6 adults); diameter 3.5 mm at x, 4–4.5 mm at xx; body circular in cross-section, tail tapering; 94–146 segments. First dorsal pore at 12/13, single midventral spermathecal pore at 5/6. Female pore single in xiv, openings of copulatory bursae paired in xviii, distance between openings 2.3 mm (0.16 circumference apart ventrally), 6–9 setae between openings. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equators in some segments; 41–46 setae on vii, 57–59 setae on xx, dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 4/5/6 thin, 7/8 and 10/11–13/14 muscular, 8/9/10 lacking. Dense tufts of

nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xiv or xv, caeca simple originating in xxvii, extending forward to xxiii. One individual with caeca folded upward in xxiii, extending about length of one segment. Typhlosole originating in xxvi, simple fold, 1/3 lumen diameter; intestinal wall with 35–39 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Single midventral spermatheca positioned on right side of the body, postseptal in vi, with nephridia on duct. Spermatheca with irregularly round ampulla; bulbous, muscular duct; two stalked diverticula attached ectally to duct, terminating in bean-shaped receptacle, stalk short. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testis sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvii to xix (one individual has the prostates in xvi to xviii); each prostate a dense, racemose mass with three lobes; muscular duct entering anterior margin of copulatory bursa. Each copulatory bursa round in xviii; coelomic surface muscular, with single, muscular, banana-shaped secretory diverticulum projecting from the anterior margin; roof with thick pad, floor pads lacking; short, blocky penis present.

Remarks: Having a single midventral spermatheca indicates that *Pheretima* (*Parapheretima*) *boaensis* n. sp. belongs to the *P. barbara* group of Sims & Easton (1972). However, this species group is characterized by having a midventral

spermatheca in 6/7 and 7/8. Sims & Easton (1972) assigned to this species group *P. (Paraph.) barbara barbigua* [replacement name for *P. (Paraph.) barbara ambigua* Cognetti, 1913 by Blakemore (2004)]; *P. (Paraph.) barbara barbara* Cognetti, 1913; and *P. (Paraph.) hellwigiana* Cognetti, 1913. The new species differs from these species in having a single midventral spermatheca in 5/6. The shape of the secretory diverticula also differs; in the other species, the diverticula are more elongate, form kinks, and project from the posterior end of the copulatory bursae. *Pheretima (Parapheretima) boaensis* differs from *P. (Paraph.) pandanensis* n. sp. in the number and location of the spermathecae, distance between male pores, and projection of the secretory diverticula from the anterior end of the copulatory bursae.

Genus *Amyntas* Kinberg, 1867

Type species. *Amyntas aeruginosus* Kinberg, 1867

Generic diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired and superficial, opening on xviii. Spermathecal pores small or large, usually paired (bithecate) but occasionally numerous (polythecate) or single (monothecate). Spermathecal pores positioned either intersegmental or intrasegmental between 4/5 and 8/9. Clitellum annular, covering three segments (xiv to xvi). Single female pore midventrally on xiv. Genital markings present or absent. If genital markings present, variable in number and forming complex pattern on

segments near male pores. Esophageal gizzard usually originating in viii; esophageal bursae lacking; pair of caeca originating in xxvii, extending forward. Ovaries and funnels free in xiii. Male sexual system holandric, metandric, or proandric. Spermathecae a single pair, multiple pairs, sometimes single and located midventrally, or sometimes lacking. Nephridia on spermathecal duct lacking. One pair of prostate glands, racemose. Copulatory bursae lacking.

***Amyntas dinagatensis* n. sp.**

(Fig. 4.4A)

Material examined. Holotype: preclitellate (NMA 4595), Brgy Boa, municipality of Cagdianao, Dinagat Province, (10°05'53"N, 125°39'42"E), 250 m asl, Dinagat Island, Philippines, coll. N. Aspe, J. Adeva, 23–26 Apr. 2004. Paratypes: two preclitellate individuals (NMA 4611); three preclitellate individuals (ZRC.ANN 0064), same collection data as for holotype.

Etymology. The species is named for Dinagat Island.

Diagnosis. Purplish brown worm, body dimension 240 mm x 7–9 mm (longest preclitellate specimen among samples); ventrum pale; equators non-pigmented, making the body appear striped; spermathecal pores and spermathecae lacking; 20 setae between male pores; intestinal origin in xiii; caeca long, from xxvii to xxi; prostate glands small, rudimentary, confined to xviii.

Description. Purplish brown dorsum, lighter ventrum, equators non-

pigmented, making the body appear striped. Large preclitellates with length 223–240 mm (n = 6 individuals); diameter 6.5–9 mm at x, 6–7 mm at xx; body circular in cross-section, tail tapering; 163–167 segments. First dorsal pore at 12/13, spermathecal pore absent. Female pore single in xiv, openings of male pores paired in xviii, distance between pores 6 mm (0.27 circumference apart ventrally), 20 setae between pores. Setae irregularly distributed around equators in some segments; 35–48 setae on vii, 70–73 setae on xx, dorsal setal gaps present, no ventral gaps. Genital markings lacking.

Septa 5/6/7 membranous, 7/8 and 10/11–12/13 muscular, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xii, intestinal origin in xiii, caeca simple originating in xxvii, extending forward to xxi; typhlosole originating in xxvi, simple fold, about 1/5 lumen diameter, intestinal wall with 43 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae lacking. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with short spherical dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; racemose prostates small, rudimentary, confined to xviii.

Remarks. The main distinguishing characters that separate *Amyntas* from *Pheretima* are the absence of nephridia on the spermathecal ducts and the absence of

copulatory bursae in *Amynthas* (Sims & Easton 1972). Despite having no spermathecae to determine if nephridia is present on the spermathecal ducts or not, we assign *A. dinagatensis* n. sp. in *Amynthas* on the basis of the absence of copulatory bursae. Even at preclitellate stage, the spermathecae and the copulatory bursae in thecate *Pheretima* species should already be evident.

Prior to the last decade, seven *Amynthas* species had been reported from the Philippines, all from Luzon Island: *A. albobrunneus* Beddard, 1912; *A. americanorum* Beddard, 1912; *A. orientalis* Beddard, 1912; *A. pauaiensis* Beddard, 1912; *A. sodalis* Beddard, 1912; *A. robustus* Vaillant, 1889; and *A. corticis* Kinberg, 1867. However, due to the lack of information on the location of the caeca on Beddard's (1912) five *Amynthas* species, and because the types of these species could not be located (Reynolds & Cook 1976), Hong & James (2004) proposed that placement of these species in *Amynthas* be considered provisional. Furthermore, Beddard's (1912) *Amynthas* species have share some features with Philippine *Pithemera* (James *et al.* 2004), leading Hong & James (2004) to suspect that the four species might actually be *Pithemera*.

Since only preclitellate *A. dinagatensis* specimen were collected, the new species is provisionally assigned to the *A. illotus* group of Sims & Easton (1972), which includes *Amynthas* species without spermathecae. Tsai *et al.* (2002) acknowledged five species in this group: *A. hohuanmontis* Tsai *et al.* 2002; *A. illotus* Gates, 1932; *A. assacceus* Chen, 1935; *A. sheni* Chen, 1935; and *A. oyuensis* Ohfuchi, 1937. Blakemore (2007) eliminated this species group, with species assigned to it either considered have doubtful identity (given the status *species inquirenda*) and in

need of further investigation, or synonymized with other species. *Amyntas dinagatensis*, despite having no clitellate specimen, differs markedly from other Philippine *Amyntas* in its relatively large size (240 mm x 7–9 mm; adult body dimensions of other species range from 32–48 mm x 2.7–3.5 mm (*A. mindoroensis* Hong & James, 2004) to 84–111 mm x 4.6–5.8 mm (*A. heaneyi* James, 2004); in having numerous setae (20) between male pores (6–9 in *A. isarogensis* Hong and James 2004; 4–7 in *A. malinaoensis* Hong & James, 2004; 2–6 in *A. heaneyi* James, 2004; and 0 in *A. philippinensis* Hong & James, 2004, *A. mindoroensis* and *A. halconensis* Hong & James, 2004); in having the intestinal origin in xiii (in xv, xvi, or xvii in the others); and in having longer caeca (xxvii–xxi) than the others (xxvii–xxv, xxiv, or xxiii). Although *A. dinagatensis* is similar in size to *A. americanorum* (200 mm x 8 mm) and *A. robustus* (113–244 mm x 5.6–7.5 mm), in contrast, the new species appears to have stripes because the segmental equators don't have pigmentation, whereas the equators are pigmented in *A. robustus* while *A. americanorum* has pale brown coloration. The new species also lacks genital markings around the spermathecal pores, while the latter two have, and the genital markings are especially numerous in *A. americanorum*. The intestinal origin of *A. dinagatensis* is in xiii while it is in xiv or xv in *A. robustus* and *A. americanorum*. Also, the caeca is long, covering seven segments, extending from xvii–xxi, while it only covers three segments in the latter two. In addition, the prostate glands are small, rudimentary, and confined to xviii in *A. dinagatensis*, but these are larger in *A. americanorum* (xvi–xxii) and *A. robustus* (xvi–xx or xxi).

***Amyntas cagdianoensis* n. sp.**

(Fig. 4.4B)

Material examined. Holotype: adult, amputee (NMA 4596), Brgy Boa, municipality of Cagdiano, Dinagat Province, (10°05'53"N, 125°39'42"E), 250 m asl, Dinagat Island, Philippines, coll. N. Aspe, J. Adeva, 23–26 Apr. 2004.

Etymology. The species is named for the municipality of Cagdiano, Dinagat Province, where this species was collected.

Diagnosis. Brown worm, body width 2.6–3 mm; closely paired spermathecal pores at intersegment 8/9; first dorsal pore 11/12; spermathecal pores and male pores 0.12 and 0.11, respectively; 3 setae between male pores; genital markings lacking; proandric male sexual system.

Description. Brown dorsum, pale ventrum, equators pigmented. Length >52 mm (n=1, adult amputee); diameter 2.6 mm at x, 3 mm at xx; body circular in cross-section. First dorsal pore at 11/12, paired spermathecal pores closely paired at 8/9, distance between spermathecal pores 1 mm (0.12 circumference apart ventrally). Female pore single in xiv, openings of male pores paired in xviii, distance between pores 1 mm (0.11 circumference apart ventrally), 3 setae between pores. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equators in some segments; 40 setae on vii, 39 setae on xx, dorsal setal gaps present, ventral gaps lacking. Genital markings lacking.

Septa 4/5–7/8 and 10/11–13/14 thin, 8/9/10 lacking. Dense tufts of nephridia

on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in ix–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv; caeca simple, originating in xxvii, folding upward in xxv, extending for two segments; typhlosole originating in xxvi, simple fold, about 1/4 lumen diameter, intestinal wall with 24 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in ix, no nephridia on ducts. Each spermatheca with pyriform ampulla; short, slender, muscular duct; single stalked diverticulum attached to ectal portion of right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalks long and convoluted, terminating in sausage-shaped receptacle. Male sexual system proandric, testes and funnels enclosed in paired sacs in x; seminal vesicles xi, each with short spherical dorsal lobe; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xv to xix; each prostate a dense, racemose mass with four lobes; stout U-shaped muscular duct from lateral margin of prostate widens toward body wall, then narrows slightly just before body wall.

Remarks. *Amyntas cagdianaensis* n. sp. belongs to the *A. supuensis* group of Sims & Easton (1972), characterized by having a pair of spermathecal pores at 8/9. Four species are assigned to this group: *A. antefixus* Gates, 1935 and *A. supuensis* Michaelsen, 1896 (both described from Halmahera, Indonesia), and *A. dinghumontis* Zhang *et al.* 2006 and *A. liaoi* Zhang *et al.* 2006 (both described from Guandong, China). The new species is smaller than *A. antefixus* (85–120 mm x 3.5–5 mm) and *A.*

supuensis (115 mm x 6 mm) and lacks genital markings, whereas *A. antefixus* has the genital markings at the midventral line on iii, iv and v, and *A. supuensis* has them around the spermathecal and male pores. The first dorsal pore is at 11/12 in *A. cagdianaensis*, but at 12/13 in *A. antefixus* and *A. supuensis*. Also, the male pores in *A. antefixus* are distantly spaced and located towards the lateral margins, while in *A. cagdianaensis*, the male pores are much closer, (1 mm, 0.11 circumference apart ventrally). Moreover, the spermathecal diverticulum in *A. antefixus* has short stalk while in the new species it is long and convoluted. *Amyntas cagdianaensis* is larger than *A. dinghumontis* (13–60 mm x 0.6–2 mm) and *A. liaoi* (55 mm x 1.1–2 mm), and although *A. dinghumontis* lacks genital markings like *A. cagdianaensis*, the spermathecal and male pores of the former are more widely spaced (0.25 and 0.17–0.25, respectively). *Amyntas liaoi* differs from *A. cagdianaensis* in having genital markings around the spermathecal and male pores, and more setae (8) between male pores. No Philippine congener closely resembles *A. cagdianaensis* n. sp.

***Amyntas talaandigensis* n. sp.**

(Fig. 4.4C, 4.4D)

Material examined. Holotype: adult (NMA 4597), Brgy Songco, municipality of Lantapan, Bukidnon Province, Mt. Kitanglad Range (8°05'47"N, 124°55'21"E), 2200 m asl, Mindanao Island, Philippines, coll. N. Aspe, 27–31 Oct. 2012. Paratypes: one adult (NMA 4613); two adults (ZRC.ANN 0066), same collection data as for

holotype.

Etymology. The species is named for the Talaandig indigenous tribe of Mt. Kitanglad.

Diagnosis. Brown worm, adult length 85–110 mm; pair of spermathecal pores on small protrusions at intrasegments anterior of setal equators of 6, 7, 8, 9; first dorsal pore at 13/14; genital markings lacking; intestinal origin in xvi, prostate glands small, confined to xviii.

Description. Brown dorsum, pale ventrum, equators non-pigmented. Length 85–110 mm (n=4 adults); diameter 3.2–4.5 mm at x, 3.7–4.5 mm at xx; body circular in cross-section, tail tapering; 85–105 segments. First dorsal pore at 13/14, paired spermathecal pores on small protrusions at intrasegments anterior of setal equators of 6, 7, 8, 9; spermathecal pores inconspicuous on 6; distance between spermathecal pores 1.8 mm (0.13 circumference apart ventrally). Female pore single in xiv, openings of male pores paired in xviii, distance between pores 2.8 mm (0.20 circumference apart ventrally), 12–13 setae between pores. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equators in some segments; 32–37 setae on vii, 40–45 setae on xx, dorsal and ventral setal gaps present. Genital markings lacking.

Septa 4/5–6/7 and 12/13/14 thin, 7/8 and 10/11/12 muscular, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia in intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xvi; caeca simple, originating in xxvii, extending forward to xxiv; typhlosole originating in xxvi,

simple fold slightly less than lumen diameter, intestinal wall with 27–40 longitudinal blood vessels. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, vii, viii and ix, nephridia on ducts lacking. Each spermatheca with pyriform ampulla; long, slender, muscular duct; single stalked diverticulum attached to the ectal portion of right face of duct of right spermatheca, and on left face of duct of left spermatheca; stalk long, terminating in ovate receptacle. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with short, spherical dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates very small, attached to wall in xviii; stout U-shaped muscular duct widens towards body wall, then narrows slightly just before body wall.

Remarks. *Amyntas talaandigensis* n. sp. belongs to the *A. rimosus* group of Sims & Easton (1972), which is characterized by having intrasegmental spermathecae in 6, 7, 8, and 9. Sims and Easton assigned to this group *A. rimosus rimosus* Gates, 1931 and *A. rimosus effeminatus* Gates, 1932, both described from Myanmar. However, Blakemore (2007) suggested that the latter subspecies is probably a synonym of the nominal subspecies. The new species differs from *A. r. rimosus* in the location of the first dorsal pore (at 13/14, compared to 12/13 in *A. r. rimosus*), the absence of genital markings or distinctly demarcated porophores around the male pores (Gates in 1931 described *A. r. rimosus* to have a pair of thickly crescent-shaped genital markings in xviii, but in 1972, he described the species to have distinctly

demarcated porophores around the male pores instead of genital markings), the origin of the intestine (in xvi, compared to xv in *A. r. rimosus*), and the size of the prostate glands (in xviii, compared to covering five segments from xv–xix in *A. r. rimosus*). Another species with ventral intrasegmental spermathecal pores is *A. monstriferus* (Kobayashi 1936) from Korea. This species is much larger (235 mm) than *A. talaandigensis*, has genital markings on viii, and has manicate intestinal caeca. Hong & James (2009b) included *A. ani*, also from Korea, in this species group; *Amyntas ani* is larger (164–171 mm x 7.8 mm) than *A. talaandigensis*, has more segments and more setae around segments vii and xx (125–130, 65 and 68, respectively), and has longer prostate glands (xvii–xx). *Amyntas talaandigensis* is the second *Amyntas* species described from Mt. Kitanglad Range, following *Amyntas heaneyi* (James 2004).

Genus *Polypheretima* Michaelsen, 1934

Type species. *Perichaeta stelleri* Michaelsen, 1892

Generic diagnosis. Body cylindrical; setal arrangement perichaetine; annular clitellum covering segments xiv–xvi; pair of male pores in xviii on circular porophores that may be within copulatory bursae; ventral genital markings present or absent; esophageal gizzard in viii; intestine begins in xv or xvi; nephridia on spermathecal ducts lacking; caeca lacking; male sexual system usually holandric, with testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii; spermathecal

pores small, spermathecal diverticula simple and usually ectal in origin; prostates racemose; copulatory bursae may or may not be present; ovaries free in xiii; oviducts lead to single or closely paired opening (Easton 1979).

***Polypheretima bukidnonensis* n. sp.**

(Fig. 4.5A, 4.5B)

Material examined. Holotype: adult (NMA 4598), municipality of Maramag, Bukidnon Province, Mt. Musuan Range (7°52'53.8"N, 125°03'57.6"E), 400 m asl, Mindanao Island, Philippines, coll. N. Aspe, 26 Oct. 2012. Paratype: one adult, amputee (NMA 4614), same collection data as for holotype.

Etymology. The species is named for the province of Bukidnon.

Diagnosis. Brown worm, adult length 131 mm; paired spermathecal battery pores at intersegments 5/6/7; 7–11 pyriform-shaped spermathecae in each battery; male pores 0.22 circumference apart; 39–45 setae on vii, 46–58 setae on xx; genital markings widely paired on xix to xxi, in line with the male pores; gizzard origin in viii; copulatory bursae lacking.

Description. Brown dorsum, pale ventrum, equators pigmented. Length 131 mm (n=1 adult); diameter 5–6.5 mm at x, 5.5–6.5 mm at xx; body circular in cross-section, tail tapering; 147 segments. First dorsal pore at 12/13, paired spermathecal battery pores at intersegments 5/6/7. Female pore single in xiv, openings of male pores paired in xviii, distance between pores 3.8–4.5 mm (0.22 circumference apart

ventrally), 6–7 setae between pores. Clitellum annular, from xiv to xvi. Setae unevenly distributed around equators in some segments; 39–45 setae on vii, 46–58 setae on xx, dorsal setal gaps present, ventral setal gaps lacking. Paired genital markings widely spaced on xix to xxi, in line with male pores.

Septa 4/5 membranous, 5/6–7/8 and 10/11–13/14 thin, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 4/5, 5/6, and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xv, caeca lacking; typhlosole originating in xxvi, simple fold, 1/3 lumen diameter. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecal batteries paired, postseptal in vi and vii. One adult individual with eight spermathecae closely aligned on left side and nine spermathecae closely aligned on right side in vi, and eight spermathecae closely aligned on left side and 10 spermathecae closely aligned on right side in vii. Another adult individual with eight spermathecae closely aligned on left side and 11 spermathecae closely aligned on right in vi, and seven spermathecae on left and 11 on right in vii. Spermathecae small, ampulla pyriform, spermathecal duct short, slender; diverticulum stalk long and slender, attached ectally to duct, terminating in short, sausage-shaped receptacle; no nephridia on spermathecal ducts. Male sexual system holandric, testes and funnels enclosed in paired ventral sacs in x and xi; seminal vesicles in xi and xii; pseudovesicles in xiii; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvii to xix, each prostate

racemose, compact; muscular duct from lateral margin of prostate widens towards body wall, then narrows slightly just before body wall; copulatory bursae lacking.

Remarks. *Polypheretima bukidnonensis* n. sp. belongs to the *Po. elongata* Perrier, 1872 group of Easton (1979), characterized by having paired genital markings in xix and successive segments in line with the male pores, paired batteries of up to 28 spermathecae in vi and/or vii, and a shallow copulatory bursae with no stalked glands. Easton (1979) included five species in the group: *Po. elongata*; *Po. everetti* Beddard & Fedarb, 1895; *Po. kinabaluensis* Beddard & Fedarb, 1985; *Po. phacellotheca* Michaelsen, 1899; and *Po. stelleri* Michaelsen, 1892. *Polypheretima mindanaoensis* Aspe & James, 2015 from Mt. Malindang was recently added to this species group. Among members of *Po. elongata* group, *Po. bukidnonensis* is similar to *Po. elongata* and *Po. everetti* in having paired genital markings widely spaced on xix to xxi or xxi, in line with the male pores. However, *Po. elongata* and *Po. everetti* are large worms (355 mm and 300 mm, respectively); the male pores are more widely spaced (0.25 circumference apart ventrally); there are many more setae per segment (up to 130), whereas *Po. bukidnonensis* has only 39–58 setae; and these species have copulatory bursae, whereas *Po. bukidnonensis* lacks them. *Polypheretima elongata* is also non-pigmented and rarely has more than three spermathecae per battery in 5/6/7, while *Po. everetti* has 6–17 spermathecae per battery in 5/6/7, and there are 16 setae between male pores. *Polypheretima bukidnonensis* is similar to *Po. mindanaoensis* in size (90–118 mm x 5.1–7 mm) and in lacking copulatory bursae, but the latter is non-pigmented and the genital markings extend from xix to xxvi. In addition, *Po. mindanaoensis* has fewer spermathecae (0–5) per battery in 5/6/7, and its gizzard originates in viii.

***Polypheretima zamboangensis* n. sp.**

(Fig. 4.5C, 4.5D)

Material examined. Holotype: adult (NMA 4599), Brgy Timolan, municipality of Tigbao, Zamboanga del Sur Province, Mt. Timolan (7°48'00.0"N 123°15'00.0"E), 1000 m asl, Mindanao Island, Philippines, coll. N. Aspe, 18–20 Dec. 2012. Paratypes: five adults (NMA 4615); four adults (ZRC.ANN 0068), same collection data as for holotype.

Etymology. The species is named for the province of Zamboanga del Sur.

Diagnosis. Slender worm, adult body dimension 223–306 mm x 3.5–5; body non-pigmented; 227–248 segments; spermathecal pores paired and inconspicuous at intersegments 5/6/7, or lacking; 60–84 setae on vii, 69–70 setae on xx; male pores 0.31 circumference apart; gizzard in x; metandric male sexual system.

Description. Body non-pigmented, equators non-pigmented. Length 223–306 mm (n=10 adults); diameter 3.5–4 mm at x, 4–5 mm at xx; body circular in cross-section, tail tapering; 227–248 segments. First dorsal pore at 12/13; paired spermathecal pores at intersegments 5/6/7, inconspicuous, lacking in some specimens. Female pore single in xiv, openings of male pores paired in xviii, distance between openings 3.9 mm (0.31 circumference apart ventrally), 8–9 setae between openings. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equators in some segments; 60–84 setae on vii, 69–70 setae on xx; two first ventral setal lines

prominent; dorsal and ventral setal gaps present. Paired genital markings widely spaced on xix to xxii, in line with male pores.

Septa 4/5–9/10 muscular, 11/12–13/14 thin, 10/11 lacking. Dense tufts of nephridia on anterior faces of 5/6–8/9; nephridia of intestinal segments located mainly on body near septum/body wall junction. Gizzard in x–xi, esophagus with low vertical lamellae x–xiii, intestinal origin in xvii, caeca lacking; typhlosole originating in xx, simple fold, about 1/5 lumen diameter. Hearts in xi to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. In one specimen, spermathecae two pairs, postseptal in vi and vii, spermathecal ampulla pyriform, spermathecal duct short, slender; stalked diverticulum attached to ental portion of left face of duct near ampulla of right spermatheca, and on left face of duct of left spermatheca; stalks short, terminating in small ovate receptacle; no nephridia on spermathecal ducts. Spermathecae lacking in other specimens. Male sexual system metandric, testes and funnels enclosed in paired ventral sacs in xi; seminal vesicles in xii and xiii; pseudovesicles in xiii; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvi to xix, each prostate racemose, compact, with five minor lobes; stout U-shaped muscular duct from lateral margin of prostate widens towards body wall, then narrows slightly just before body wall; copulatory bursae lacking.

Remarks. A metandric male sexual system and paired spermathecal pores in 5/6/7 indicate that *Po. zamboangensis* n. sp. belongs to the *Po. huoensis* Easton, 1979 species group. However, members of this species group are confined to Papua New

Guinea (Easton 1979) and have a different genital marking pattern (genital markings present on vii–ix, xvii, and xix). Ten species in *Polypheretima* have been reported from the Philippines: *Polypheretima monticola* Beddard, 1912 from Benguet Province, Luzon Island; *Po. elongata* Perrier, 1872 and *Po. everetti* Beddard & Fedarb, 1895, both from Balabac Island, Palawan Province; *Po. fruticosa* Hong & James, 2008a, *Po. perlucidula* Hong & James, 2008a, and *Po. bannaworensis* Hong & James, 2008a, from Banaue, Luzon Island; *Po. pagudpudensis* Hong & James, 2011a from Kalbaryo, Luzon Island; and *Po. mindanaoensis* Aspe & James, 2015, *Po. bukidnonensis* n. sp. (reported here), and *Po. zamboangensis* n. sp., from Mindanao Island. Among these species, *Po. zamboangensis* is similar to *Po. perlucidula* and *Po. pagudpudensis* in having a pair of spermathecae in vi and vii and in lacking body pigmentation. However, the latter two species are smaller (*Po. perlucidula*, 38–56 mm x 1.3–1.5 mm; *Po. pagudpudensis*, 56–63 mm x 2.1–2.2 mm); *Po. perlucidula* lacks genital markings, and there are only epidermal thickenings on segment xviii in *Po. pagudpudensis*. In addition, in the latter two species the pre-clitellar setae are more numerous than the post-clitellar setae (50 and 23, respectively, in *Po. perlucidula*; 63 and 27 in *Po. pagudpudensis*), the origin of gizzard is in viii, and the male sexual system is holandric. *Polypheretima zamboangensis* has more segments (227–248) than all other Philippine *Polypheretima* species (*Po. fruticosa*, 105–107; *Po. perlucidula*, 117–131; *Po. bannaworensis*, 67–95; *Po. pagudpudensis*, 126–136; *Po. mindanaoensis* M, 140–141; *Po. bukidnonensis* n. sp., 147; *Po. everetti*, 126) except for *Po. elongata* (136–297). But the male pores in *Po. zamboangensis* are more distant (0.31 circumference apart ventrally) compared to that of *Po. elongata* (0.25

circumference apart ventrally), the gizzard is bigger (x–xi vs. ix in *Po. elongata*), the intestinal origin is in xvii (vs. xv in *Po. elongata*), and the prostates are smaller (xvi–xix vs. xvi–xxi in *Po. elongata*). The other species have holandric male sexual system and either have spermathecal batteries in 5/6/7 (*Po. bukidnonensis* n. sp., *Po. mindanaoensis*, *Po. elongata*, and *Po. everetti*), have four pairs of spermathecae in 5/6–8/9 (*Po. monticola* and *Po. bannaworensis*), or have only one pair in 5/6 (*Po. fruticosa*).

Genus *Pithemera* Sims and Easton, 1972

Type species. *Perichaeta bicincta* Perrier, 1875

Generic diagnosis. Body cylindrical; setae numerous, regularly arranged around each segment; clitellum annular, covering two, two and a half, or three segments from xiv to xv, and/or half of xvi; spermathecal pores small, three to five pairs from 4/5 to 8/9; female pore single or paired in xiv; genital markings present or absent; intestinal caeca originate in or near xxii (in *Pheretima*, caeca originate in xxvii); spermathecal ducts lack nephridia (as in *Amyntas* and *Polypheretima*); male system holandric or metandric; prostate gland racemose; copulatory bursae may be present or absent.

***Pithemera nolani* n. sp.**

(Fig. 4.5E)

Material examined. Holotype: adult (NMA 4600), municipality of San Isidro, Davao Occidental Province, Mt. Hamiguitan (06°44'03"N, 126°12'15"E), 1430 m asl., Mindanao Island, Philippines, coll. Nolan Aspe, J. Cantil, 7–10 May 2006. Paratypes: five adults (NMA 4616); four adults (ZRC.ANN 0069), same collection data as for holotype.

Etymology. The species is named after Nolan Aspe, who assisted in the fieldwork.

Diagnosis. Reddish brown worm, adult length 143–159 mm; equators non-pigmented making the body appear striped; pair of spermathecal pores at intersegment 5/6; first dorsal pore at 11/12; 9–11 setae between male pores; spermathecae large, ovate; intestinal origin in xiv; copulatory bursae present.

Description. Reddish brown dorsum, pale ventrum, equators non-pigmented, making the body appear striped. Length 143–159 mm (n=5 adults); diameter 7.5 mm at x, 7 mm at xx; body circular in cross-section, tail tapering; 100–114 segments. First dorsal pore at 11/12, paired spermathecal pores at 5/6, distance between spermathecal pores 4.5 mm (0.19 circumference apart ventrally). Female pore single in xiv, openings of male pores paired in xviii, distance between pores 4.5 mm (0.20 circumference apart ventrally), 9–11 setae between pores. Clitellum annular, from xiv to xvi. Setae irregularly distributed around equators in some segments; 34–36 setae on vii, 56–59 setae on xx, dorsal setal gaps present, ventral setal gaps lacking. Genital markings lacking.

Septa 4/5–7/8 and 13/14 thin, 10/11–12/13 muscular, 8/9/10 lacking. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard in viii–x, esophagus with low vertical lamellae x–xiii, intestinal origin in xiv; caeca simple, originating in xxiii, extending forward to xxi; typhlosole originating in xxiii, simple fold slightly less than lumen diameter. Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix lateral; those in viii extend to gizzard.

Ovaries and funnels free in xiii. Spermathecae paired, postseptal in vi, nephridia on ducts lacking. Each spermatheca large, with ovate ampulla; bulbous, muscular duct; single stalked diverticulum attached to right face of duct of right spermatheca, and to left face of duct of left spermatheca; stalks long, terminating in sausage-shaped receptacle. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles in xi, xii, each with digitate dorsal lobe; vasa deferentia slender, free from body wall on way to ental end of prostatic ducts; prostates in xvi to xx or xv to xix; each prostate a dense, racemose mass with four lobes; muscular duct entering from the lateral margin of prostate to posterior margin of copulatory bursa. Copulatory bursae round in xvii–xix; coelomic surfaces muscular, secretory diverticula lacking; roof with two thick pads, floor pads lacking; penis lacking.

Remarks. Sims & Easton (1972) include the absence of copulatory bursae as one of the distinguishing characters of *Pithemera*. But despite the presence of copulatory bursae in *Pi. nolani* n. sp., the new species is assigned in *Pithemera* rather than in *Pheretima* on the basis of having no nephridia on the spermathecal ducts and in

having the caecal origin near xxii. Therefore, we amend the generic diagnosis of *Pithemera* Sims & Easton 1972, to include species with copulatory bursae.

Pithemera species reported from the Philippines include: *Pi. bicincta* Perrier, 1875; *Pi. rotunda* James *et al.* 2004; *Pi. philippinensis* James *et al.* 2004; *Pi. duhuani* Hong & James, 2008a; *Pi. fragumae* Hong & James, 2008a; *Pi. ifugaoensis* Hong & James, 2008a; *Pi. triangulata* Hong & James, 2008a; *Pi. glandis* Hong & James, 2011a; *Pi. fusiformis* Hong & James, 2011a; *Pi. levii* Hong & James, 2011a; *Pi. malindangensis* Aspe & James, 2015; *Pi. duminagati* Aspe & James, 2015; *Pi. donvictoriano*i Aspe & James, 2015; and *Pi. nolani* n. sp. *Pithemera nolani* n. sp. differs from the other *Pithemera* species in having only a pair of spermathecal pores in 5/6, whereas the others have either five pairs in 4/5–8/9 (*Pi. bicincta*, *Pi. rotunda*, *Pi. philippinensis*, *Pi. duhuani*, *Pi. fragumae*, *Pi. ifugaoensis*, *Pi. triangulata*, *Pi. glandis*, *Pi. fusiformis*, *Pi. levii*, *Pi. malindangensis* and *Pi. duminagati*), four pairs in 5/6–8/9 (*Pi. donvictoriano*i), four pairs in 4/5–7/8 (*Pi. levii*), or three pairs in 4/5–6/7 (*Pi. triangulata*). *Pithemera nolani* also markedly differs among congeners in possessing copulatory bursae. In addition, *Pi. nolani* is also the largest (143–159 mm x 3.5 mm) species of *Pithemera* in the Philippines; other species range from 20–22 mm x 1.7–1.8 mm (*Pi. fusiformis*) to 91–144 mm x 3.5–4 mm (*Pi. malindangensis*), with most having maximum lengths less than 100 mm.

4.4 Discussion

Blakemore (2007) listed a total of 40 valid *Pheretima* species in SE Asia and the Pacific region, described from 1886 to 2004. The list includes 18 Philippine

species that were described in James (2004) and James *et al.* (2004). From 2004 to the present, including the new species described here, there are now 80 known *Pheretima* species in the Philippines, comprising 76% of the world's *Pheretima*. Aspe & James (2015) likewise stated that the Philippines has the highest diversity for *Pithemera*, with now 14 species, comprising 47% of the world's *Pithemera*. These figures suggest that the archipelago may be the center of species radiation for these groups. For *Polypheretima*, of the 68 valid species recorded, Indonesia has the highest diversity with 26% of the total number, followed by Vietnam with 19%, and the Philippines with 15%. For *Amyntas*, with a record of around 470 valid species, China showed to have the highest diversity (17%) followed by Korea (12%), Myanmar and Indonesia (each with 11%), and Taiwan (10%) (Blakemore 2007, Hong 2007, Tsai *et al.* 2010, Bantaowong *et al.* 2011b, Shen 2012, Blakemore *et al.* 2013, Hong & James 2013, Shen *et al.* 2013, 2014, Sun *et al.* 2013, Zhao *et al.* 2013). These figures also suggest that Indochina may be the center of species radiation for these groups.

De Bruyn *et al.* (2014) conducted meta-analyses of geological, climatic, and biological data sets from SE Asia to determine which areas have been the sources of long-term biological diversity, particularly in the pre-Miocene, Miocene, and Plio-Pleistocene intervals. Their study included published molecular phylogenies of taxa representing plants, insects and spiders, freshwater crustaceans, freshwater molluscs, freshwater fishes, herpetofauna, birds, and mammals. Despite having no data for earthworms, their results, which suggest that Borneo and Indochina were the major “evolutionary hotspots” and were the source of species dispersal of a diverse range of fauna and flora in the region, including the Philippines, in the early Miocene, seem to

agree with the suggestion regarding the species radiation in earthworms. Some species may have likely dispersed from Borneo to the Philippines (except Palawan) through the Sulu Archipelago “land bridges” during sea level minima, with subsequent interspecific competition between invaders and members of the original populations (Sims & Easton 1972; Oliveros & Moyle 2010). For other species, dispersal across water may have been achieved by rafting (de Queiroz 2005; Vidal *et al.* 2008). The geographical origins and affinities of the Borneo and Philippine earthworms may extend to Australasia, where some native earthworm species are regarded as being congeneric with the Asian species (Sims & Easton 1972).

Mindanao itself is a collision complex of three arc systems: the East Mindanao arc, which includes Dinagat Island and the Mt. Hamiguitan Range; the Central Mindanao volcanic arc, which includes Camiguin Island, Mt. Kitanglad, Mt. Musuan, and Mt. Apo; and the West Mindanao arc (Corpus 1992) (Figure 1). The East Mindanao arc, which originated near the Australian plate, collided and coalesced with the Central Mindanao volcanic arc around the Early Miocene (~20 Ma) as the Philippine Sea Plate was moving northward towards a subduction zone in the Philippine trench (Hall 1996). The East/Central Mindanao complex collided and coalesced with the West Mindanao arc by strike-slip fault motion by the end of Miocene (~5 Ma) by progressive merging of the West Sangihe arc with Talaud-Halmahera arc system (Corpus 1992; Hall 1996). By the late Pliocene (~1 Ma), the Zamboanga Peninsula, which is a part of Sunda Shelf from the north, coalesced with the rest of Mindanao (Hall 1996). This geological history may provide hint on why the Mindanao earthworm fauna is so diverse.

It is unknown whether the distributions of the species reported here were broader prior to human invasion. What is seen as their present distributions could be artifacts of human disturbance and/or limited sampling, which was conducted in undisturbed forests to the exclusion of other sites. Future molecular studies can elucidate the phylogeny and geographical distribution of species and populations at several levels: among the West Mindanao, Central Mindanao, and East Mindanao arcs; among the Philippine Island groups; and among the Philippine archipelago and other archipelagos and continental areas in SE Asia. In a preliminary molecular phylogenetic analysis of genera in the *Pheretima* group (James 2005b) based on combined data from the 16S and 28S rRNA genes, the *sangirensis* group formed a clade with 100% nodal support. However, the analysis included only Luzon specimens, and a broader analysis including specimens from Mindanao and Visayas Islands is needed to confirm monophyly of this group. In the same analysis, *Pithemera* and *Polypheretima* from Luzon formed a strongly supported clade, whereas the Philippine *urceolata* species group emerged as polyphyletic. A phylogenetic study with broader taxonomic and geographical coverage will be necessary for a clear understanding of the evolutionary relationships among pheretimoid earthworms in the Philippines.

The number of species collected from the sites here (except Mt. Malindang and Mt. Kitanglad) is fewer, with only one to five species per site (Table 4.1) compared to the number of species collected at Mt. Malindang (22 species in Aspe & James 2014, 2015) and at Mt. Kitanglad (18 species in James 2004). This is due to that the sampling duration in the sites reported here was shorter (three to six days) compared to the sampling duration and effort reported in Aspe & James (2014, 2015) and James

(2004), which took several weeks. However, it is highly anticipate that there are still more species waiting to be discovered in these, sites and in the rest of the Philippines, especially when the earthworm composition in the Visayas group of islands would already be included in the examination, with each island being ecologically unique . The morphological distinctions among the known species are relatively clear at present, but as the described diversity continues to increase, it is expected that the distinctions become more subtle, and verification from molecular data will be needed. For now, a key to the species reported herein from Mindanao, Camiguin, and Dinagat islands, is provided, which is useful for regional studies. This key can be used together with the keys provided in James (2004) and Aspe & James (2014, 2015) to identify species, but this key should be used with caution in identifying earthworms from outside Mindanao and associated islands.

Key to earthworms reported from Mindanao, Camiguin, and Dinagat islands

- 1 Nephridia on spermathecal ducts lacking..... 2
- Nephredia on ducts present; copulatory bursae present..... (*Pheretima*) 6
- 2 Copulatory bursae lacking..... 3
- Copulatory bursae present, intestinal caeca originating in xxiii; reddish brown dorsum; equators non-pigmented, making the body appear striped; pair of spermathecal pores at 5/6; adult length 143–159 mm..... *Pithemera nolani* n. sp.
- 3 Intestinal caeca originating in xxvii..... (*Amyntas*) 4
- Intestinal caeca lacking..... (*Polypheretima*) 5
- 4 Spermathecae lacking, purplish brown dorsal, equators non-pigmented making the

- body appear striped, first dorsal pore 12/13, 20 setae between male pores, 240 mm
x 7–9 mm..... *Amyntas dinagatensis* n. sp.
- Spermathecae paired at intersegments 8/9; brown dorsum; first dorsal pore at
11/12; 3 setae between male pores; body width 2.6–3 mm
..... *Amyntas cagdianaensis* n. sp.
- Spermathecae paired at intrasegments 6, 7, 8, 9; brown dorsum; first dorsal pore at
13/14; 12–13 setae between male pores; adult length 85–110 mm
..... *Amyntas talaandigensis* n. sp.
- 5 Spermathecae in pairs at 5/6/7; body non-pigmented; proandric male system; adult
length 223–306 mm..... *Polypheretima zamboangensis* n. sp.
- Spermathecae in batteries at 5/6/7; dorsum brown; holandric male system; adult
length 131 mm..... *Polypheretima bukidnonensis* n. sp.
- 6 Secretory diverticula projecting from copulatory bursae present
..... *P. (Parapheretima)* 7
- Secretory diverticula projecting from copulatory bursae lacking
..... *P. (Pheretima)* 8
- 7 Spermathecae paired at 7/8; secretory diverticula projecting from lateral surface of
copulatory bursae; long, tapering penes *P. (Paraph.) pandanensis* n. sp.
- Single midventral spermatheca at 5/6; secretory diverticula projecting from
anterior margin of copulatory bursae; penes lacking... *P. (Paraph.) boaensis* n. sp.
- 8 Spermathecae at 5/6.....(*P. urceolata* species group) 9
- Spermathecae at 7/8.....(*P. sangirensis* species group) 12
- 9 Body length \leq 90 mm..... 10

- Body 305–347 mm in length; purplish black dorsum; equators non-pigmented, making the body appear striped; first dorsal pore at 13/14; penes lacking
..... *Pheretima enormis* n. sp.
- 10 Brown pigmentation over entire dorsum..... 11
- Segmental equators non-pigmented making the body appear striped; body 81–90 mm x 3–4 mm; first dorsal pore at 12/13; 2 setae between male pores; penis lacking..... *Pheretima hamiguitanensis* n. sp.
- Reddish-brown dorsal stripes; first dorsal pore at 11/12; 12–14 setae between male pores; penes present..... *Pheretima dinagatensis* n. sp.
- 11 Body 33–47 mm x 1–1.2 mm, thread-like; first dorsal pore at 12/13; 3 setae between male pores; penes present..... *Pheretima acia* n. sp.
- Body >58 mm x 2.5–3 mm; first dorsal pore at 11/12; 8 setae between male pores; penis lacking..... *Pheretima lantapanensis* n. sp.
- 12 Large worm, body 163–350 mm x 7.2–12 mm; purplish black dorsum; may have no spermathecae; spermathecal pores 0.16 circumference apart for individuals with spermathecae; male pores 0.15–0.16 circumference apart; penis lacking
.....*Pheretima apoensis* n. sp.
- Large worm, body 147–225 mm x 7–9.5 mm; purplish brown dorsum; spermathecal pores 0.2 circumference apart; male pores 0.23 circumference apart; penes present.....*Pheretima timpoongensis* n. sp.
- Body 63–122 mm x 3.5–5.2 mm; reddish brown dorsum; spermathecal pores 0.17 circumference apart; male pores 0.15–0.18 circumference apart; penis lacking
.....*Pheretima camiguinensis* n. sp.

- Body >165 mm x 6.5–7 mm; spermathecal pores 0.21 circumference apart; male pores 0.17 circumference apart; penis lacking..... *Pheretima sibucalensis* n. sp.

CHAPTER 5

A molecular phylogenetic study of pheretimoid species (Clitellata: Megascolecidae) in Mindanao and associated islands, Philippines

5.1 Introduction

Over the past decade the knowledge on earthworm taxonomic studies in the Philippines dramatically increased and the results indicate an astonishingly high diversity and high local endemism of earthworms in the country. The Philippines now has an estimate of 200 native species that are all pheretimoids representing eight genera in Megascolecidae (Aspe & James 2014), a large earthworm family widely distributed in Australia and the Pacific region, in North America, in Madagascar, and especially in eastern Asia (BOLD Systems). Previous taxonomic works on Philippine earthworms done were concentrated on Luzon (e.g. Hong & James 2004a, 2009, 2011), the largest island located on the northern part of the country and there was only one study on Mindanao (James 2004a), the second largest island located on the southern part of the country. The discovery of new species does not appear to stop anytime soon as more species continue to be described. Recently, more species from Mindanao have been added to the growing list of Philippine species (e.g. Aspe & James 2014, 2015, 2016), which shows that *Pheretima* Kinberg, 1867 is the most speciose genus among the native earthworms in the country.

Pheretima was the largest genus of earthworms in the family Megascolecidae until Sims & Easton (1972), based on phenetic treatment of morphological data, reallocated members of this group into 'convenient' species groups comprising 10 genera (*Pheretima* s. lat. or *Pheretima* group of genera.), which present earthworm taxonomists follow and collectively refer to as pheretimoid species. The general characteristics of pheretimoid species include having perichaetine setal arrangement, meronephridial excretory system, single gizzard in viii, a pair of racemose prostates opening through male pores in xviii, and testes contained within testis sacs. Sims & Easton (1972) assigned pheretimoid species with nephridia on the spermathecal ducts, with copulatory bursae, and with caeca originating in xxvii to *Pheretima* s. str. On the other hand, they assigned pheretimoid species with no nephridia on the spermathecal ducts, with no copulatory bursae, and with caeca originating in xxvii to *Amyntas* Kinberg, 1867. They also assigned pheretimoid species with no nephridia on the spermathecal ducts but with copulatory bursae, and with caeca originating in xxvii to *Metaphire* Sims & Easton, 1972. For members of *Pheretima* s. str. with no secretory diverticula projecting from the copulatory bursae, they assigned them to the subgenus *Pheretima* while those that have, they assigned them to the subgenus *Parapheretima* Cognetti, 1912. As pheretimoid species are morphologically widely varied, Sims & Easton (1972) also assigned species groupings for different genera primarily basing on the number and position of spermathecal pores: e.g. members of the subgenus *Pheretima* with a pair of spermathecal pores at intersegments 5/6 belong to the *P. urceolata* Horst 1893 group; those with a pair of spermathecal pores at intersegments 7/8 belong to the *P. sangirensis* Michaelsen 1891 group; those with four pairs of

spermathecal pores at intersegments 5/6/7/8/9 belong to the *P. darnleiensis* Fletcher 1887 group.

James (2005a) conducted a preliminary molecular phylogenetic study to verify the intrageneric relationships among pheretimoids and as well the species groups in *Pheretima* s. str. as assigned by Sims & Easton (1972). Previous and recent molecular phylogenetic studies on pheretimoids are on *Amyntas* and *Metaphire* in China and Taiwan (eg. Chang *et al.* 2008, Sun *et al.* 2013, Zhao *et al.* 2015) and James' (2005a) work was the first molecular phylogenetic study on Philippine earthworms that includes species in the *Pheretima* s. str. The specimens he worked on were collected from Luzon group of islands using the data of mitochondrial 16S rDNA and nuclear 28S rDNA. The trees generated from 16S and 28S respectively poorly resolved the phylogeny and although the tree generated from the combined gene markers have better resolution, most of the internal nodes were weakly supported (< 0.8 posterior probability) and many of the branches formed polytomy. To produce a well-resolved phylogeny at different taxonomic levels, it is recommended that a combination of three or more genes with different mutation rates and having a total length of more than 2000 bp should be used (Novo *et al.* 2010, 2011, Pérez-Losada *et al.* 2009, Chang *et al.* 2008, Chang & James 2011).

This paper is a molecular phylogenetic study of the pheretimoid specimens collected from Mindanao and associated islands with the goal to verify Sims & Easton's (1972) generic and species-group assignments that were based on morphological data. This work also attempts to resolve the intergeneric and

interspecific phylogeny of the pheretimoid taxa using four gene markers with different mutation rates having a total length of more than 2000 bp.

5.2 Material and methods

Collection sites and sampling. Collection of specimens was conducted intermittently from May 2003 to April 2004. The collection sites were chosen based primarily on the Key Conservation Sites of the Philippines identified by the Haribon Foundation (Mallari *et al.* 2001). These include Mt. Timpoong in Camiguin Island, approximately 10 km north of the Misamis Peninsula; Cagdianao Municipality in Dinagat Island northeast of Mindanao; Mt. Tago and Mt. Musuan in Bukidnon Province, both in central Mindanao; Mt. Apo in Davao del Sur Province; Mt. Busa in Sarangani Province; and Mt. Malindang Range in Misamis Occidental Province at the base of the Zamboanga Peninsula (Fig. 5.1). As most of the sites are protected areas under the Protected Areas and Wildlife Bureau (PAWB) of the Department of Environment and Natural Resources (DENR), Prior Informed Consent certificates were obtained from the Protected Area Management Board for the respective sites prior to collection. Sampling was done from soil, ferns, mosses, and the insides of rotten logs in primary and secondary forests at high elevations away from human settlements or trails. Worms were sorted in the field to putative species, using body size, coloration, and number and location of spermathecal pores as identifying characters. The earthworms were rinsed in tap water, killed in 10% ethanol and then preserved in 95% ethanol.

Morphological examination. External and internal characters were examined in the laboratory using a stereomicroscope. The generic diagnoses and assignment to genus and species groups follow Sims & Easton (1972). Eighteen morphospecies from Mindanao and associated islands were examined and identified (Table 2). Among these, 12 are identified as *Pheretima* (*Pheretima*): six are of the *P. sangirensis* species group of Sims & Easton (1972), namely *P. malindangensis* Aspe and James, 2014, *P. boniaoi* Aspe and James, 2014, *P. apoensis*, Aspe and James, 2016, *P. timpoongensis* Aspe and James, 2016, *P. camiguinensis* Aspe and James, 2016, and a *Pheretima* morphospecies from Mt. Busa (hereafter, *Pheretima* Busa1); and five morphospecies are of the *P. urceolata* species group of Sims and Easton [9], which include *Pheretima* Apo1, *Pheretima* Apo3, and *Pheretima* Apo4 from Mt. Apo, *Pheretima* Busa2 from Mt. Busa, and *Pheretima* Tago1 from Mt. Tago. One morphospecies is identified as the monothecate *P. vergrandis* Aspe and James, 2014. One *Pheretima* morphospecies collected from Mt. Musuan is assigned to *P. apoensis* due to morphological similarity despite being athecate and despite having relatively larger body size. There are also two species of the *P. (Parapheretima)*, namely *P. (Parapheretima) pandanensis* Aspe and James, 2016 from Camiguin Island and *P. (Parapheretima) boaensis* Aspe and James, 2016 from Dinagat Island. Members of the subgenus *Parapheretima* are distributed in Borneo and western New Guinea and these are the first records of *Parapheretima* in the Philippines. There are also three species of *Amyntas*, which include *A. dinagatensis* Aspe and James, 2016 from Dinagat Island, one *Amyntas* morphospecies from Mt. Apo and a morphospecies from Mt. Tago, hereafter, *Amyntas* Apo1 and *Amyntas* Tago1, respectively. Table 5.3 shows the summary of

the comparison of morphological features among the pheretimoid taxa included in the analyses.

DNA extraction, amplification, and sequencing. Total genomic DNA was extracted from muscle tissues of the specimens using the DNeasy Blood & Tissue Kit (Qiagen, USA). Regions of the mitochondrial 16S rRNA (hereafter, 16S), cytochrome *c* oxidase subunit I (COI), nuclear 28S rRNA (28S) and histone H3 (H3) genes were amplified using the polymerase chain reaction (PCR). The mixture (total volume 10 μ l) contained 1 μ l DNA and 9 μ l PCR-mix (3.76 μ l sterile dH₂O, 2.68 μ l of 2 μ g/ μ l bovine serum albumin (BSA), 0.45 μ l of each primer [forward and reverse primers, 10 pmol/ μ l], 0.9 μ l of 10 \times buffer, 0.71 μ l of dNTP, 0.05 μ l Ex *Taq*-polymerase). The cycling profile was as follows: denaturation for 30 sec at 95°C, annealing for 30 sec at 50°C, and extension for 1 min at 72°C for 35 cycles with an initial denaturation step for 1 min at 95°C and a final extension step for 7 min at 72°C. An alternative cycling profile was followed to amplify genes that failed to be amplified using the above-mentioned cycling profile: denaturation for 1 min at 94°C, annealing for 1 min at 48°C, and extension for 1 min at 72°C for 35 cycles with an initial denaturation step for 4 min at 95°C. PCR amplifications were confirmed by electrophoresis in 2% agarose gel, visualized by SYBR Green. Sequencing reactions were performed with BigDye Terminator Cycle Sequencing Kit ver 3.1 (Applied Biosystems, USA) using 0.8 pmol/ μ l of the same primers as for amplification. Sequencing was done by an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, USA). Table 5.1 shows the summary of the primers used and the lengths of the genes acquired.

Data analysis. The sequences were aligned using MUSCLE (Edgar 2004). Phylogenetic reconstructions for individual genes and for combined (concatenated) data set were performed with the maximum-likelihood (ML) method (raxmlGUI ver 1.2) (Stamatakis 2006) and with the Bayesian inference (MrBayes ver 3.2.1) (Huelsenbeck & Ronquist 2001). Gene concatenation was done using FASconCAT (Kuck & Meusemann 2010). PAUP* ver 4.0 beta 10 (Swofford 2002) was used to test for incongruence among data set. For the ML analysis, GTR+G+I was selected as the best-fit model in jModelTest ver 2.1.4 (Darriba *et al.* 2012) (see Appendix for the parameters used). For the Bayesian analysis, a best-fit model for each gene was selected in jModeltest ver 2.1.4: GTR+G+I for 16S and COI; F81 for 28S; and HKY+G for H3. Nodal supports were assessed by bootstrapping with 1000 pseudoreplicates for ML. Posterior probability values and tree topology for BI were calculated with 1×10^7 generations with trees sampled every 100 generations, and 25% of the tree samples were discarded as burn-in. The tree was rooted using *Dichogaster* sp. (Acanthodrilidae) as outgroup.

5.3 Results

There was difficulty in obtaining DNA from the specimens and in PCR amplification as the specimens were collected from the field around 11 years ago. Purification of the DNA and the addition of BSA to the PCR-mix somehow improved the results of amplification although amplification remained unsuccessful for many of the samples. Regardless of modifications made to the reaction mix and PCR profile, amplification was unsuccessful for many specimens, suggesting DNA degradation

and/or primer mismatch: out of 50 specimens extracted for DNA, 16S was successfully amplified for only 18 specimens*; COI and H3 fragments were successfully amplified for 10 and 11 specimens, respectively; and despite addition of DMSO to the PCR mix as being used in James (2005), amplification of 28S was unsuccessful for all but one specimen (Table 5.2).

The intraspecific sequence divergence for the pheretimoid species of Mindanao calculated using K2P model based on 16S sequences ranged from 0.000–0.011 (0%–1.1%) (Table 5.4). On the other hand, the interspecific sequence divergence rate for the same group of species ranged from 0.022–0.142 (2.2–14.2%).

The resulting tree for the combined data of 16S, COI, 28S, and H3 (Fig. 5.2) showed the clade for the Mindanao pheretimoid species is strongly supported with posterior probability of 1.0 and is moderately supported with bootstrap value of 69%. There are three major clades that have formed: a clade composed of *Amynthas* Tago1 and *Amynthas* Apo1, strongly supported with posterior probability of 1.0 and bootstrap value of 100%; a weakly supported clade that is composed of members of the *P. urceolata* group, *Amynthas dinagatensis* and *P. (Parapheretima) boaensis*; and a clade that is supported with 0.99 posterior probability and 61% bootstrap value, composed of *P. (Parapheretima) pandanensis*, members of the *P. sangirensis* group, the monothecate *P. vergrandis*, and the morphospecies that is assigned to *P. apoensis*. The clade comprised of *Amynthas* Apo1 and *Amynthas* Tago1 separated from *Amynthas dinagatensis*, making the genus *Amynthas* non-monophyletic. The two *Parapheretima* species are separated: *P. (Parapheretima) boaensis* is a sister taxon to *Amynthas dinagatensis* (with posterior probability of 0.99 and bootstrap value of 57%)

within the weakly supported clade that also contains the members of the *P. urceolata* group, while *P. (Parapheretima) pandanensis* formed a clade with members of the *P. sangirensis* group supported with 0.99 posterior probability and 61% bootstrap value. This makes the subgenus *Parapheretima* non-monophyletic. A sub-clade composed of members of the *P. urceolata* group (*Pheretima* Busa2, *Pheretima* Apo4 and *Pheretima* Tago1) within the weakly supported clade is well-supported with 0.98 posterior probability and 81% bootstrap value. The position of the monothecate *P. vergrandis* among members of the *P. sangirensis* group is supported with 0.90 posterior probability and 59% bootstrap value. Also, the position of the athecate morphospecies from Mt. Musuan that is assigned to *P. apoensis* together with the thecate *P. apoensis* from Mt. Apo is strongly supported with 1.0 posterior probability and 100% bootstrap value.

5.4 Discussion

The analyses of the combined data produced a tree more or less consistent with morphology-based expectation (Fig. 5.2). Despite having limited taxa and limited genes included in the analysis, some interesting points are raised that need to be pondered by the community of earthworm systematists. These can serve as hypotheses to be tested with expanded data. *Amyntas* differs from *Pheretima* s. str. in having no nephridia on the spermathecal ducts and in having no copulatory bursae whereas the latter possess both organs. Sims & Easton (1972) acknowledged that there are *Amyntas* and *Pheretima* that are athecate and in such case, the athecate species may be assigned to either of the genera based on having copulatory bursae or not. Even at

preclitellate stage, the spermathecae and the copulatory bursae in thecate *Pheretima* species should already be evident (e.g. James 2004a). An additional problem is that athecate individuals with copulatory bursae could also belong to *Metaphire*, a genus differing from *Pheretima* by lacking nephridia on the spermathecal ducts. Nonetheless, the issue between athecate *Pheretima* and *Metaphire* has been addressed in Aspe & James (2014). The non-monophyly of *Amyntas* as shown in the tree here shows that the character used by Sims & Easton (1972), particularly the absence of copulatory bursae, to define *Amyntas* appear homoplasious. It is suggested in James (2005a) and Chang *et al.* (2008) that *Amyntas* may either be paraphyletic or polyphyletic implying that the development or loss of either the nephridia on spermathecal ducts and/or the secondary male pores happened more than once in the pheretimoid taxa. In another study on molecular phylogeny of pheretimoids in Hainan Island, China, it is proposed that *Amyntas* and *Metaphire* be reconsidered as one genus (Zhao *et al.* 2015). Our results reinforce these older concerns about the monophyly of pheretimoid genera.

The phylogenetic relationship between *Amyntas dinagatensis* and *P. (Parapheretima) boaensis* is supported with 0.99 posterior probability and 57% bootstrap value, despite having very little morphological similarity: *Amyntas dinagatensis* is athecate (vs. with single spermatheca at intersegment 5/6 in *P. (Parapheretima) boaensis*), has larger body size (240 mm × 7–9 mm vs. 94–133 mm × 3.5–4.5 mm in *P. (Parapheretima) boaensis*), the segmental equators are not pigmented (vs. pigmented equators in *P. (Parapheretima) boaensis*), the distance between male pores is wider (0.27 circumference apart ventrally vs. 0.16 circumference apart ventrally in *P. (Parapheretima) boaensis*), more setae between

male pores (20 vs. 6–9), and the copulatory bursae are absent (vs. present in *P. (Parapheretima) boaensis*) (Aspe & James 2016). What is common though between these two species is that both were collected from Dinagat Island while the rest of the species within the clade are from mainland Mindanao. Thus, the clade for these two species reflects geographic affinity.

The separate positions of the two members of the subgenus *Parapheretima* as shown in the tree of the combined data (Fig. 2) suggests that the presence of secretory diverticula that Sims & Easton (1972) used to define *Parapheretima* is homoplasious. In other words, the respective secretory diverticula on the copulatory bursae of *P. (Parapheretima) boaensis* and *P. (Parapheretima) pandanensis* evolved independently. The position of *P. (Parapheretima) pandanensis*, which has a pair of spermathecae at intersegment 7/8, among the members of the *P. sangirensis* group, which is also defined by the same character, is rather well-supported with 0.99 posterior probability and 61% bootstrap value. On the other hand, *P. (Parapheretima) boaensis*, which has a single spermatheca midventrally in intersegment 5/6, is nested among the weakly supported clade that contains members of the *P. urceolata* group, defined by having a pair of spermathecal pores at intersegment 5/6. Therefore, it appears in this case (we do not intend this as a general rule) that the location of spermathecae is a better indicator of phylogeny than the presence of secretory diverticula on the copulatory bursae. If this is proven true, the subgenus *Parapheretima* should be discarded unless further morphological and molecular investigation supports the existence of a clade containing the type species of the subgenus and other members with secretory diverticula. However, at this point, we

cannot draw any conclusion because the position of *P. (Parapheretima) boaensis* among the members of *P. urceolata* group is weakly supported. Further phylogenetic studies should be done involving other species of the (*P.*) *Parapheretima* in Borneo and western New Guinea to confirm the results and determine the placement of (*P.*) *Parapheretima* among the pheretimoids.

As mentioned above, the members of the *P. urceolata* group formed a weakly supported clade that includes *Amyntas dinagatensis* and *P. (Parapheretima) boaensis*. Within this clade is a well-supported clade (0.98 posterior probability and 81% bootstrap value) that contains *Pheretima* Busa2, *Pheretima* Apo4 and *Pheretima* Tago1, partially reflecting Sims & Easton's (1972) species-group assignment based on the location of spermathecae. In James (2005a), members of the *P. urceolata* group did not form a clade and it is suggested that some members of the *P. urceolata* group may be derived from an octothecal ancestor (with spermathecae in intersegments 5/6/7/8/9), which also gave rise to the hexathecal species (with spermathecae in intersegments 5/6/7/8 and in 6/7/8/9, respectively). Here, we do not support any conclusions about the monophyly of the *P. urceolata* group.

The *P. sangirensis* group formed a well-supported clade with 1.0 posterior probability in James (2005a). Here, a clade that is composed of members of the *P. sangirensis* group is supported with posterior probability of 0.82, with the inclusion of the monothecate *P. vergrandis* and the athecate morphospecies assigned to *P. apoensis*. It is hypothesized in James (2004a) and Aspe & James (2014) that monothecate species must have evolved from bithecate species with the fusion of two spermathecae into one because the single spermatheca in these species have two identical diverticula.

Such is the case in *P. ambonensis* Cognetti, 1913, *P. monothecca* James, 2004a and *P. concepcionensis* Aspe & James, 2014. In *P. vergrandis* (with midventral spermatheca at intersegment 7/8), the single spermatheca has only one diverticula, which could have resulted from either a loss of one spermatheca in bithecate ancestor or fusion of two spermathecae into one, followed by a loss of a diverticulum. The more parsimonious option would be to reject the fusion hypothesis, but we suggest that much more detailed investigation is necessary to be certain of the evolutionary history. The clade that is formed for *P. vergrandis*, *P. boniaoi* and *P. malindangensis* also reflects geographic affinity having these species being collected from the same locality, in the Mt. Malindang Range.

The relationship between the athecate morphospecies from Mt. Musuan provisionally assigned to *P. apoensis* and the thecate *P. apoensis* from Mt. Apo is strongly supported (with posterior probability of 1.0 and bootstrap value of 100%). The genetic divergence of 0.011 (or 1.1%) using K2P model based on 16S (Table 5.4) indicates that the two taxa are conspecifics and the population recently diverged. On another note, a study on cryptic species of hormogastrid earthworms showed an upper limit of 4.27% intraspecific divergence in *Hormogaster elisae* using K2P based on 16S (Novo *et al.* 2010). It is most likely that geographic isolation (e.g. the distance between Mt. Apo and Mt. Musuan is approximately 110 km) may have allowed divergence in some morphological features such as the body size (188 mm × 9 mm in *P. apoensis* from Mt. Apo vs. 324 mm × 10 mm in *P. apoensis* from Mt. Musuan) and the loss of spermathecae, in this case, the *P. apoensis* from Mt. Musuan. A study on the reproductive traits of *Aporrectodea trapezoides* showed that isolation of an

earthworm in a controlled environment can trigger the earthworm to reproduce parthenogenetically (Férrnandez *et al.* 2010).

In general, several of the nodes have support values that are very weak and have formed polytomies, which is most likely due to the lack of data, preventing obtaining a more credible estimate of the relationships among the pheretimoid taxa. The results of this study could have improved if more 28S data had been available. The addition of genes with divergence rates slower than that of COI and 16S but faster than that of 28S and H3, such as 12S and ITS2, may also improve the resolution. Moreover, working on newly collected specimens would have reduced the difficulty in obtaining DNA from the specimens and in PCR amplification. Further molecular and morphological investigation should be done to trace the pheretimoid phylogeny and be able to establish a more robust system of classification.

SUMMARY, CONCLUSION, AND RECOMMENDATION

In only a decade, the number of the Philippine native earthworms rose from less than 10 species to around 200 species, of which all are pheretimoid representing eight genera in the Megascolecidae. This dissertation reports a total of 39 pheretimoid species from Mindanao. With the new species described, there are now 80 known *Pheretima* s. str. species in the Philippines, comprising 76% of the world's *Pheretima*, and there are now 14 species of *Pithemera*, comprising 47% of the world's *Pithemera*. These figures suggest that the Philippine archipelago may be the center of species radiation for these groups. There are now 16 species of *Amynthas* in the Philippines representing less than 1% of the world's *Amynthas* and also there are now 10 species of *Polypheretima* in the Philippines representing 15% of the world's *Polypheretima*. The high diversity of the two latter species in mainland Asia and Indonesia, respectively, suggests that Indochina may be the center of species radiation for these two genera. The known ranges of the Philippine species are restricted to areas around the type localities. This pattern indicates a remarkable degree of endemism, both among local areas, among islands in the Philippines, and in the Philippines as a whole, and suggesting that many species remain to be detected in the Philippines.

Among the *Pheretima* (*Pheretima*), which is the most speciose among the earthworm genera in the Philippines, there are now currently 27 species in the *sangirensis* group, 20 species in the *urceolata* group, 11 species in the *darnleiensis*

group, one species in the *montana* group, four monothecate species, and one athecate species, in the Philippines. In addition, the two *Pheretima* (*Parapheretima*) reported here are the first records of *Parapheretima* in the country. It is proposed in Aspe & James (2014; also in Chapter 2) that the relative size of the copulatory bursae be a distinguishing character between *Pheretima* and *Metaphire*, with species in *Pheretima* tending to have more prominent dome-shaped, intra-coelomic copulatory bursae than those in *Metaphire*. This character would be useful in assigning athecal worms to one or the other of these genera.

With regards to the phylogenetic analyses, despite having limited taxa and limited genes included in the analyses, the combined data set produced well-resolved results generating phylogeny more or less consistent with morphology-based expectation. Sims & Easton's (1972) taxonomic assignment on genus *Amyntas* and subgenus *Parapheretima* do not reflect phylogeny and the characters they used to define these taxa (absence of copulatory bursae and presence of secretory diverticula on the copulatory bursae, respectively) are homoplasious. The species grouping in *Pheretima* based on the location of spermathecae is partially reflected in the pheretimoid phylogeny. Particularly, members of the *P. sangirensis* group form a strongly supported clade with the inclusion of *P. (Parapheretima) pandanensis* (also with spermathecal pores at 7/8), the monothecate with spermathecal pores at 7/8, and athecate conspecific of *P. apoensis*. Also, members of the *P. urceolata* group form a weakly supported clade with the inclusion of the monothecate *P. (Parapheretima) boaensis* (with spermathecal pores at 5/6). Finally, spermathecal fusion or loss can

occur in pheretimoid evolution, such as in the case of the monothecate *P. vergrandis* and the athecate conspecific of *P. apoensis*.

In general, several of the nodes of the tree based on combined data set have support values that are very weak and have formed polytomies, which is most likely due to insufficient data, preventing obtaining a more credible estimate of the relationships among the pheretimoid taxa. The results could have improved if more data were available. The addition of genes with divergence rates slower than that of COI and 16S but faster than that of 28S and H3, such as 12S and ITS2, may also improve the resolution. Further molecular work including more taxa is needed to be able to establish a more robust system of classification of the pheretimoid species and come up with a better-resolved phylogeny. The Visayas islands in central Philippines is expected to harbor an even a richer diversity of species and inclusion of the earthworms from the islands in this area and the specimens from Luzon will provide a more or less complete phylogenetic information of the Philippine earthworms. Moreover, a consolidated phylogenetic study in the East Asian archipelagos will provide insights into the evolutionary, biogeographic, and ecological processes involved in island radiations of soil-dwelling animals.

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TABLES

TABLE 2.1. Population density for *Pheretima* species at sampling sites on Mt. Malindang, and frequency of occurrence of species across sites (Modified from Aspe *et al.*, 2009). Data are given for only five of nine sites, as no *Pheretima* individuals were found at four of the sites (agricultural areas and grasslands in Brgys Small Potongan and Toliyok). + indicates individuals collected outside sampling plots. Density values for each site are individuals collected per 4.5 m³ of soil examined on plots (from Aspe & James, 2014).

Barangay Habitat Elevation	Lake Duminagat Primary Forest (1845–2027 m asl)	Lake Duminagat Disturbed Forest (1479–1662 m asl)	Sibucal Disturbed Forest (902–1067 m asl)	Small Potongan Disturbed Forest (915–1,024 m asl)	Toliyok Disturbed Forest (238–271 m asl)	Total (%) individuals on all plots	Frequency of occurrence among sites
Geographical Coordinates	8°17'55" N 123°37'01" E	8°18'22.1"N 123°37'12.1"E	8°19'31" N 123°38'02" E	8°24'04" N 123°36'47" E	8°25'51" N 123°42'21" E		
Species							
<i>P. maculodorsalis</i>	11	4	-	-	-	13 (5.6)	0.4
<i>P. tigris</i>	12	+	-	2	-	14 (5.9)	0.6
<i>P. immanis</i>	+	11	-	4	-	15 (6.3)	0.6
<i>P. lago</i>	11	2	2	-	-	15 (6.3)	0.6
<i>P. nunezae</i>	-	-	2	3	-	5 (2.1)	0.4
<i>P. boniaoi</i>	-	+	-	+	-	+	0.4
<i>P. malindangensis</i>	4	1	8	-	-	13 (5.5)	0.6
<i>P. misamisensis</i>	+	+	+	+	-	+	0.8
<i>P. wati</i>	1	8	6	13	+	28 (11.8)	1
<i>P. longiprostata</i>	-	-	+	4	-	4 (1.7)	0.4
<i>P. nolani</i>	14	7	+	-	-	21 (8.9)	0.6
<i>P. longigula</i>	5	+	-	-	-	5 (2.1)	0.4
<i>P. adevai</i>	23	23	-	+	-	46 (19.4)	0.6
<i>P. lluchi</i>	+	+	3	-	-	3 (1.3)	0.6
<i>P. potonganensis</i>	-	+	8	6	12	26 (11.2)	0.8
<i>P. vergrandis</i>	-	+	15	1	-	16 (6.9)	0.6
<i>P. concepcionensis</i>	-	+	1	7	-	8 (3.4)	0.6
<i>P. subanensis</i>	-	-	-	+	-	+	0.2
Total species	12	16	15	11	2	22	

TABLE 2.2. Comparison of species in the *Pheretima sangirensis* and *P. montana* groups at Mt. Malindang (from Aspe & James, 2014).

Character	<i>Pheretima sangirensis</i> * Michaelsen, 1891	<i>Pheretima maculodorsalis</i> Aspe & James 2014	<i>Pheretima tigris</i> Aspe & James 2014	<i>Pheretima immanis</i> Aspe & James 2014	<i>Pheretima lago</i> Aspe & James 2014	<i>Pheretima nunezae</i> Aspe & James 2014
Body pigmentation	Dorsally pigmented all over	Dorsal intersegments with oval dots	Dorsal intersegments with stripes	Dorsal intersegments with stripes	Dorsally pigmented all over	Dorsally pigmented all over
Length	54–240	226–235	230–283	365	223–315	> 116
Width	4–8	9–13	8–14	18–17	10–11	8.5–9
Setae vii; xx	40; 60+	73–74; 63–75	53–66; 48–61	61–69; 63–68	49; 53	46; 51
Setae bet. male pores	6	2–4	0–4	5	0–2	9
Sper. pores spacing	0.25–0.28	0.09	0.13	0.12	0.18–0.24	0.28
Male pores spacing	0.17–0.25	0.13	0.14	0.14	0.15	0.22
Setal gaps D; V**	+/-; -	+; -	-; -	-; -	+; -	+; -
Septa in 5/6-13/14	- in 8/9/10	- in 9/10	- in 9/10	- in 9/10	- in 9/10	+ in 4/5; - in 9/10
Origin of gizzard	viii	viii	viii	viii	ix	ix
Origin of intestine	xv	xvii	xvi	xvi	xiv	xv
Caeca	from xxvii	xxvii–xxi	xxvii–xix	xxvii–xx	xxvii–xxi	xxvii–xxiv
Origin of typhlosole	absent in xxx–xl	xxvii	xxvii	xxvii	xxvii	xxvii/xxvi
Intestinal vessels	36	50–54	56–58	28–32	36–38	20–23
Location of hearts	x–xiii	x–xiii	x–xiii	x–xiii	xi–xiii	xi–xiii
Prostate glands	xvii–xix	xvii–xx	xvi–xviii	xvii–xviii	xiv–xviii	xvii–xix
Copulatory bursae	present	xviii–xxi	xvii–xix	xvi–xvii	xvii–xx	xviii
Penes	+	+	-	-	+	+

* including all subspecies; data based on Michaelsen (1891, 1899, 1900)

**D, dorsal gap; V, ventral gap; +, present; –, absent.

Table 2.2 continued

Character	<i>Pheretima boniaoi</i> Aspe & James 2014	<i>Pheretima malindangensis</i> Aspe & James 2014	<i>Pheretima misamisensis</i> Aspe & James 2014	<i>Pheretima wati</i> Aspe & James 2014	<i>Pheretima longiprostata</i> Aspe & James 2014	<i>Pheretima nolani</i> Aspe & James 2014	<i>Pheretima longigula</i> Aspe & James 2014
Body pigmentation	Dorsal intersegment with stripes	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over
Length	101–133	60–81	55–65	67–75	37–41	89–97	139–186
Width	5–6	4–5	3–4	3.5–4	3–3.5	4.5–5	3.5–4.5
Setae vii; xx	59–78; 62–68	43–47; 50–57	42–51; 43–48	59–71; 52–60	35–40; 37–47	33–48; 42	28–47; 27–51
Setae bet. male pores	0	0	6–7	0	0–5	2	0–4
Sper. pores spacing	0.14	0.16	0.3	0.17	0.16	0.14	0.24
Male pores spacing	0.03	0.11	0.23	0.08	0.16	0.12	0.17
Setal gaps D; V*	+; -	-; +	+; -	+; -	+; -	+; -	-; -
Septa in 5/6-13/14	- in 9/10	- in 8/9/10	- in 9/10	- in 8/9	- in 8/9/10	- in 9/10	- in 8/9/10
Origin of gizzard	viii	viii	viii	viii	viii	viii	viii
Origin of intestine	xvii	xvi	xv	xv	xv	xv	xxi
Caeca	xxvii–xxiv	xxvii–xxiii	xxvi–xxv	xxvii–xxii	xxvii–xxv	xxvii–xxii	xxvii–xxii
Origin of typhlosole	xxvii	xxvii	xxvii	xxvii/xviii	xxvii/xxvi	xxvii/xxvi	xxxiii
Intestinal vessels	33–45	30	32	34–38	20	42–44	32–36
Location of hearts	xi–xiii	xi–xiii	x–xiii	x–xiii	x–xiii	x–xiii	x–xiii
Prostate glands	xvi–xxi	xvi–xxi	xvi–xxii	xv–xxii	xv–xxiii	xv–xx	xviii–xx
Copulatory bursae	xvii–xx	xvii–xx	xvii–xix	xvii–xxi	xvii–xx	xvii–xix	xvii–xviii
Penes	-	+	+	-	+	+	+

TABLE 2.3. Comparison of species in the *Pheretima darnleiensis* group at Mt. Malindang (from Aspe & James, 2014).

Character	<i>Pheretima darleiensis</i> [*] Fletcher 1887	<i>Pheretima adevai</i> Aspe & James 2014	<i>Pheretima lluchi</i> Aspe & James 2014	<i>Pheretima potonganensis</i> Aspe & James 2014
Body pigmentation	?	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over
Length	75–170	110–131	104–135	63–89
Location of spermathecal pores	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9	5/6/7/8/9
First dorsal pore	11/12	12/13	13/14	12/13
Setae vii; xx	12–35; 38–45	32–37; 36–39	29–30; 39–45	32–34; 28–44
Setae bet. male pores	12	3–7	5–6	4
Sper. pores spacing	0.2	0.25	0.2	?
Male pores spacing	0.2	0.16	0.2	0.17
Setal gaps D; V	?; -	-; -	-; -	-; +
Septa in 5/6-13/14	+ or - in 8/9; - in 9/10	- in 9/10	- in 8/9/10	+
Origin of gizzard	ix/viii	ix	ix	ix
Origin of intestine	xv	xv	xvi	xiv
Caeca	xxvii–xxiv	xxvii–xxiii	xxvii–xxiv	xxvii–xx
Origin of typhlosole	?	xxvii	xxvii/xviii	xxvii
Intestinal vessels	?	34–38	28–30	26
Location of hearts	x–xiii	x–xiii	x–xiii	x–xiii
Prostate glands	xviii–xix	xviii–xix	xviii–xx	xvii–xix
Copulatory bursae	?	xviii	xviii–xix	xviii–xix
Penes	+	+	+	+

^{*}Description from Fletcher (1887) and Hong & James (2011b); excluding the description by Blakemore *et al.* (2007).

TABLE 2.4. Comparison of monothecate Philippine *Pheretima* species, including the athecate *Pheretima subanensis* Aspe & James 2014 (from Aspe & James, 2014).

Character	<i>Pheretima monoporata</i> James 2004a	<i>Pheretima monothecca</i> James 2004a	<i>Pheretima arayatisensis</i> James <i>et al.</i> 2004a	<i>Pheretima vergrandis</i> Aspe & James 2014	<i>Pheretima concepcionensis</i> Aspe & James 2014	<i>Pheretima subanensis</i> Aspe & James 2014
Body pigmentation	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over	Dorsally pigmented all over
Length	60–70	62	88–141	35–75	>89	67
Spermathecal pore	7/8	5/6	8/9	7/8	7/8	0
First dorsal pore	12/13 (4); 13/14 (1)	12/13	12/13	13/14	12/13	12/13
Setae vii; xx	26–34; 32–36	36; 32	54; 87	37–42; 50–54	40–50; 56–68	36; 45
Setae bet. male pores	0	3	5–6	0	2	5
Male pores spacing	0.05	0.11	?	0	0.14	0.21
Setal gaps D; V	+; -	+; -	?	+; +	+; +	+; -
Spermathecal diverticulum	two	two	two	one	two	0
Septa in 5/6-13/14	+ in 4/5, - in 13/14	+ in 4/5; - in 8/9/10 and 13/14	- in 8/9	- in 8/9/10	- in 8/9/10	- in 8/9/10
Origin of gizzard	viii	viii	viii	viii	viii	ix
Origin of intestine	xvii	xviii	xv	xv	xvi	xv
Caeca	xxvii–xxiii	xxvii–xxiii	xxvii–xxii	xxvii–xxiv	xxvii–xxv	xxvii–xxiii
Origin of typhlosole	xxvii	xxvii	xxvii	xxvii/xviii	xxvii	xxvii
Intestinal vessels	18–20	26	?	24–28	30–35	?
Location of hearts	x–xiii	x–xiii	x–xiii	x–xiii	x–xiii	xi–xiii
Prostate glands	xvi, xvi–xx	xviii (?)	xviii	xvi–xxi	xvii–xix	xvii–xx
Copulatory bursae	xviii	xviii	xviii (?)	xviii–xx	xviii	xviii–xix
Penes	-	+	+	+	+	-

TABLE 2.5. Diversity of *Pheretima* species reported from various localities and islands in the Philippines. None of the species has been reported at more than one locality in the Philippines, and only two species (see the footnotes) also occur outside the Philippines (from Aspe & James, 2014).

Island	Number of <i>Pheretima</i> species	Total species per island
Luzon		30
Kalbaryo	4	
Kalinga	6	
Mt. Province & Banaue ¹	9	
Mt. Arayat	2	
Mt. Makiling	2	
Mt. Isarog	7	
Catanduanes	2	2
Mindanao		32
Mt. Kitanglad	15	
Mt. Malindang	18	
Mt. Apo ²	1	
Cebu	1	1
Total species	67	

¹Includes nominal *P. darnleiensis*, also reported from Papua New Guinea, Fiji, Malaysia, Indonesia, and Singapore.

²Includes *P. urceolata*, also reported from Indonesia.

TABLE 3.1. Density and frequency of earthworm species found at collecting sites on Mt. Malindang (Modified from Aspe et al. 2009, table 2). Data are given for only five of nine sites, as no individuals were found at two of the sites (agricultural areas and grasslands in Barangays Small Potongan and Toliyok). + indicates individuals were also collected outside sampling plots. The density value for each site is individuals collected per 4.5 m³ of soil examined on plots. *Aspe and James (2014) (from Aspe & James, 2015).

Barangay Type of forest Elevation (meters asl) Species	Primary Forest Lake Duminagat (1845–2027 m asl)	Disturbed Forest Lake Duminagat (1479–1662 m asl)	Disturbed Forest Sibucal (902–1067 m asl)	Disturbed Forest Small Potongan (915–1,024 m asl)	Disturbed Forest Toliyok (238–271 m asl)	Total (%) individuals on all plots	Frequency of occurrence among sites
*18 <i>Pheretima</i> species	79+	56+	46+	38+	12	232 (85)	-
<i>Polypheretima mindanaoensis</i>	-	7	1	-	-	8 (2.9)	0.4
<i>Pithemera malindangensis</i>	23	9	-	-	-	32 (11.7)	0.4
<i>Pithemera duminagati</i>	+	+	+	-	-	+	0.6
<i>Pithemera donvictorianoii</i>	-	+	-	-	1	1 (0.37)	0.4
Total individuals	102+	72+	47+	38+	13	273 (100)	
Total species	14	20	17	11	3	26	

TABLE 3.2. Comparison of characters among *Polypheretima* species in the Philippines. +, present; -, absent (from Aspe & James, 2015).

Character	<i>Polypheretima mindanaoensis</i> Aspe & James 2015	<i>Polypheretima elongata</i> Perrier 1872	<i>Polypheretima everetti</i> Beddard & Fedard 1895	<i>Polypheretima monticola</i> Beddard 1912	<i>Polypheretima fruticosa</i> Hong & James 2008a	<i>Polypheretima perlucidula</i> Hong & James 2008a	<i>Polypheretima bannaworensis</i> Hong & James 2008a	<i>Polypheretima pagudpudensis</i> Hong & James 2011a
Body pigmentation	white	white	Reddish purple	?	brown dorsal	white	brownish dorsal	white
Length	90–118	355	300	130	39–61	38–56	51–74	56–63
Setae vii; xx	41–53; 44–46	Usually 80 to 130; ?	Numerous, up to 130; ?	?	44; 41	50; 23	39; 46	63; 27
Setae bet. male pores	10	?	6–12	?	9	12	6–7	8
Sper. pores spacing	-	?	?	?	0.25	0.28	0.22–0.25	?
Male pores spacing	0.23	0.25	0.25	?	0.27	0.32	0.24	0.23
Location of spermathecae	0 or up to 5 spermathecae in each battery in 5/6, or 5/6/7	Rarely more than 3 spermathecae in each battery in 5/6/7	6–12 spermathecae in each battery in 5/6/7	Paired in 5/6–8/9	Paired in 5/6	Paired in 5/6/7	Paired in 5/6–8/9	Paired in 5/6/7
Genital markings	Paired in xix–xxvi	Paired in ix–xxii	Paired in xix–xxi	Paired in ix, xvii, xix–xx	Paired in xviii or xix	-	-	Paired in xvii, xix, xx
Septa in 5/6–13/14	+ in 4/5; - in 8/9	- in 9/10	?	- in 5/6–8/9 and 13/14	- in 8/9	+	+	+
Origin of gizzard	viii	?	viii	viii	viii	viii	viii	viii
Origin of typhlosole	xvi	?	?	?	xx	xxii	xxii	xxii
Prostate glands	xvi–xxi	?	?	?	xviii–xxii	xvii–xx	xvi–xx	xvii–xxii

TABLE 3.3. Comparison of characters among *Pithemera* species in the Philippines. +, present; -, absent (from Aspe & James, 2015).

Character	<i>Pithemera malindangensis</i> Aspe & James 2015	<i>Pithemera duminagati</i> Aspe & James 2015	<i>Pithemera donvictorianoii</i> Aspe & James 2015	<i>Pithemera bicincta</i> Perrier 1875	<i>Pithemera rotunda</i> James <i>et. al.</i> 2004	<i>Pithemera philippinensis</i> James <i>et. al.</i> 2004
Body pigmentation	white	white	white	?	white	pigmented
Length	91–144	36–37	41–58	40–80	31–44	51–54
Cover of clitellum	xiv–1/2 xvi	xiv–1/2 xvi	xiv–xv	xiv–1/2xvi	xiv–1/2 xvi	xiv– 1/2xvi
Spermathecal pores	4/5–8/9	4/5–8/9	5/6–8/9	4/5–8/9	4/5–8/9	4/5–8/9
Setae vii; xx	58; 30–40	43–48; 31–43	42–48; 41–57	42; 46	49–54; 48–61	56–68; 49–61
Male pore setae	2–4	0–3	8	?	9	9
Male pore spacing	0.2	0.13	0.11	0.2	?	?
Female pores	paired	paired	paired	paired	paired	paired
Genital markings	-	-	-	paired in xviii–xix	Paired in xviii–xxii	-
Septa in 5/6–13/14	- in 8/9	- in 8/9	- in 8/9 and 13/14	- in 8/9	- in 8/9	- in 8/9
Dense tufts of nephridia	5/6/7	5/6/7/8	5/6/7/8	?	?	?
Origin of intestine	xiv	xv	xiv	xv	xv	xv
Caeca	xxii–xxi	xxii–xxi	xxii–xxi	xxii–xxi	xxii–xxi	xxii–xxi
Origin of typhlosole	xxii	xix	xxii	?	xxii	xxii
Location of hearts	x–xii	x–xii	x–xii	x–xii	x–xii	x–xii
Prostate glands	xvi–xix	xvi–xxi	xvi–xix	xvi–xix, xx	xvi–xx	xvii–xix

Table 3.3 continued

Character	<i>Pithemera duhuani</i> Hong & James 2008a	<i>Pithemera fragumae</i> Hong & James 2008a	<i>Pithemera ifugaensis</i> Hong & James 2008a	<i>Pithemera triangulata</i> Hong & James 2008a	<i>Pithemera glandis</i> Hong & James 2011a	<i>Pithemera fusiformis</i> Hong & James 2011a	<i>Pithemera levii</i> Hong & James 2011a
Body pigmentation	brown dorsal	white	light brown	light brown	brown dorsal	brown dorsal	Brown dorsal
Length	53–59	53–101	38–65	47–76	62–108	20–22	50–65
Cover of clitellum	xiv–1/2xvi	xiv–1/2xvi	xiv–xvi	xiv–1/2xvi	xiv–1/2xvi	xiv–1/2xvi	xiv–1/2xvi
Spermathecal pores	4/5–8/9	4/5–8/9	4/5–8/9	4/5–6/7	4/5–8/9	4/5–8/9	4/5–7/8
Setae vii; xx	40; 39	66; 69	41; 44	41; 44	53; 61	36; 47	54; 63
Male pores setae	6–7	10–15	8–10	1–3	8–11	10	6–7
Male pores spacing	0.32–0.37	0.24	0.15–0.24	0.1	0.18	0.31	0.13
Female pores	paired	paired	paired	paired	paired	single	paired
Genital markings	paired in xvii, xix or xviii only	paired in xvii– xviii	paired in xx	-	-	Paired in xix– xx	Paired in xviii– xix
Septa in 5/6–13/14	- in 8/9	- in 8–10	- in 8/9	- in 8/9	- in 8/9 and 13/14	- in 8/9	- in 8/9
Dense tufts of nephridia	?	?	?	?	?	?	?
Origin of intestine	xv	xv	xv	xv	xv	xv	xv
Caeca	xxii–xxi	xxii	xxii–xxi	xxii	xxii–xx	xxii–xxi	xxii–xxi
Origin of typhlosole	xxii	xxii	xxii	xxii	xxii	-	xxii
Location of hearts	x–xii	xi–xii	x–xii	x–xii	x–xii	xi–xiii	x–xii
Prostate glands	xvi–xix	xvi–xix	xvi–xix	xviii	xvii–xix	xvii–xix	xvii–xix

TABLE 3.4. Diversity of *Polypheretima* and *Pithemera* species around the world. Symbols indicate species with broad ranges: **Polypheretima elongata*; +*Polypheretima everetti*; @*Polypheretima taprobanae*; #*Pithemera bicincta*. These data are from Blakemore (2003, 2007), James *et al.* (2004); James (2004b), Hong & James (2008a, 2011a), Tsai *et al.* (2000), and Nguyen *et al.* (2014, 2015) (from Aspe & James, 2015).

Country/Region	<i>Polypheretima</i>	<i>Pithemera</i>
Korea	–	1 [#]
Japan	1 [*]	1 [#]
Taiwan	1 [*]	2 [#]
Philippines	8 ^{*+}	13 [#]
Vietnam	13 ^{*@}	–
Thailand	1 [*]	–
Malaysia	5 [*]	–
Indonesia	18 ^{*+}	1 [#]
Papua New Guinea	8 [*]	6 [#]
Australia	1 [@]	–
Pacific Islands (Fiji, Samoa, New Caledonia, Hawaii)	4 ^{*+@}	6 [#]
Brazil	1 [@]	–
Africa	1 [*]	–
Madagascar	3 [@]	–
India	1 [@]	–

TABLE 4.1. The list of new species collected in each site in Mindanao and associated islands. * indicates that the species is found in more than one locality (Aspe & James 2016).

Locality	Current total no. of earthworm species	New species
Mt. Timpoong, Camiguin Island	3	<i>Pheretima (Pheretima) timpoongensis</i> n. sp. <i>Pheretima (Pheretima) camiguinensis</i> n. sp. <i>Pheretima (Parapheretima) pandanensis</i> n. sp.
Cagdianao, Dinagat Island	5	<i>Pheretima (Pheretima) acia</i> n. sp. <i>Pheretima (Pheretima) dinagatensis</i> n. sp. <i>Pheretima (Parapheretima) boaensis</i> n. sp. <i>Amyntas dinagatensis</i> n. sp. <i>Amyntas cagdianaoensis</i> n. sp.
Mt. Kitanglad Range, Bukidnon	21	<i>Pheretima (Pheretima) lantapanensis</i> n. sp. <i>Amyntas talaandigensis</i> n. sp. <i>Pheretima (Pheretima) apoensis</i> n. sp.*
Mt. Apo	2	<i>Pheretima (Pheretima) apoensis</i> n. sp.* <i>Pheretima (Pheretima) hamigitanensis</i> n. sp.*
Mt. Musuan, Bukidnon	2	<i>Pheretima (Pheretima) apoensis</i> n. sp.* <i>Polypheretima bukidnonensis</i> n. sp.
Mt. Hamiguitan Range, Davao Occidental	3	<i>Pheretima (Pheretima) enormis</i> n. sp. <i>Pheretima (Pheretima) hamigitanensis</i> n. sp.* <i>Pithemera nolani</i> n. sp.
Mt. Malindang Range, Misamis Occidental	23	<i>Pheretima (Pheretima) sibucalensis</i> n. sp.
Mt. Timolan, Zamboanga del Sur	1	<i>Polypheretima zamboangensis</i> n. sp.

TABLE 4.2. Comparison of species in the *Pheretima urceolata* group reported in Chapter 4 (Aspe & James 2016).

Characters	<i>Pheretima urceolata</i> Horst, 1893*	<i>Pheretima acia</i> n. sp.	<i>Pheretima dinagatensis</i> n. sp.	<i>Pheretima enormis</i> n. sp.	<i>Pheretima hamiguitanensis</i> n. sp.	<i>Pheretima lantapanensis</i> n. sp.
Length	70–80	33–47	65	305–347	81–90	>58
Width x, xx	4, 4	1.2, 1	2.5, 3	13.5–15.5, 13–16.5	3–3.5, 3.5–4	2.5, 3
Dorsal pigmentation	?	brown	Reddish brown stripes	Purplish brown	brown	brown
Segment equator	?	pigmented	unpigmented	unpigmented	unpigmented	pigmented
Segments	92–104	87–94	127	110–147	108–111	?
1 st dorsal pore	12/13	12/13	11/12	13/14	12/13	11/12
Setal gaps D, V	+, -	-, -	-, -	+, -	+, -	+, -
Sperm. pore distance	0.45	0.21	0.31	0.17	0.07	0.38
Male pore distance	0.1	0.24	0.24	0.14	0.12	0.23
Setae between openings	?	3	12–14	7	2	8
Setae vii, xx	20, 42	26–35, 29–38	39, 43–48	45–46, 75–80	38–42, 44–47	30–39, 31–36
Gizzard	viii-?	viii-x	viii-x	viii-x	viii-x	viii-x
Intestinal origin	xvi	xv	xiv/xv	xv	xv/ xvii	xv
Caeca	xxvii–xxiii/xxi	xxvii–xxvi	xxvii–xxiii	xxvi–xxiii	xxvii–xxiv	xxvii–xxiii
Prostate	xvii–xviii or xviii	xvii–xviii	xv/xvi–xix	xvi–xix	xvii–xx/ xv–xix	xvi–xvii
Copulatory bursae	?	xvii–xix	xvii–xix	xvii–xix	xvii–xix	xvii–xix
Penes	+	+	+	+	-	-

* based from type examined by Gates (1961)

TABLE 4.3. Comparison of species in the *Pheretima sangirensis* group and *Pheretima* (*Parapheretima*) species reported in Chapter 4.

Characters	<i>Pheretima sangirensis</i> * (Michaelsen, 1891)	<i>Pheretima timpoongensis</i> n. sp.	<i>Pheretima camiguinensis</i> n. sp.	<i>Pheretima sibucalensis</i> n. sp.	<i>Pheretima apoensis</i> n. sp.	<i>Pheretima</i> (<i>Parapheretima</i>) <i>pandanensis</i> n. sp.	<i>Pheretima</i> (<i>Parapheretima</i>) <i>boaensis</i> n. sp.
Length	54–240	147–225	63–122	>165	163–350	86–150	94–133
Width x, xx	4–8	7.3–9.5, 7–8.5	3.5–4.8, 3.5–5.2	6.5, 7	7.2–11, 8–12	4–5, 4–5	3.5, 4–4.5
Dorsal pigmentation	Variation of purple	Purplish brown	Reddish brown	Dark gray-brown	Purplish brown	Dark reddish brown	Reddish brown
Segment equators	?	unpigmented	unpigmented	unpigmented	unpigmented	unpigmented	pigmented
Segments	?	98–119	87–103	?	102–120	85–104	94–146
1 st dorsal pore	?	12/13	12/13	12/13	12/13	12/13	12/13
Spermathecal pores	Paired at 7/8	Paired at 7/8	Paired at 7/8	Paired at 7/8	-, paired at 7/8	Paired at 7/8	Single midventral at 5/6
Sperm. pore distance	0.25–0.28	0.2	0.17	0.21	-, 0.16	0.23	-
Male pore distance	0.17–0.25	0.23	0.15–0.18	0.17	0.15–0.16	0.23–25	0.16
Setae between openings	6–10	2–5	1–4	9	4–5	4–9	6–9
Setae vii, xx	40; 60+	29–43, 49–73	32–34, 39–44	70, 89	37–48, 50–58	36–44, 50–55	41–46, 57–59
Gizzard	viii–?	ix–x/ ix–xi	viii–x/ ix–xi/ ix–x	ix–xi	ix–xi	ix–x	ix–x
Intestinal origin	xv	xv/ xvi	xv	xiv	xv	xiv	xiv/xv
Caeca	xxvii–?	xxvii–xxiv/ xxiii	xxvii–xxiv/ xxiii/ xxii	xxvii–xxii	xxvii– xxvi/xxiv	xxvii–xxv/ xxiv	xxvii–xxiv/ xxiii
Prostate	xvii–xix	xvii–xx	xvii–xx/ xxi	xvii–xxi	xv/xvii–xix	two parts, in xvii– xviii, and xix–xx	xvii–xix/ xvi– xviii
Copulatory bursae	present	xvii–xix	xvii–xix	xvii–xx	xvii–xix	xvii–xix	xviii
Secretory diverticula	-	-	-	-	-	+	+
Penes	+	+	-	-	-	+	-

TABLE 4.4. Comparison of *Amynthas*, *Polypheretima*, and *Pithemera* species reported in Chapter 4 (Aspe & James 2016).

Characters	<i>Amynthas dinagatensis</i> n. sp.	<i>Amynthas cagdianoensis</i> n. sp.	<i>Amynthas talaandigensis</i> n. sp.	<i>Polypheretima bukidnonensis</i> n. sp.	<i>Polypheretima zamboangensis</i> n. sp.	<i>Pithemera nolani</i> n. sp.
Length	240	>52	85–110	131	223–306	143–159
Width x, xx	9, 7	2.6, 3	3.2–4.5, 3.7–4.5	5–6.5, 5.5–6.5	3.5–4, 4–5	3.5, 3.5
Dorsal pigmentation	Dark brown	brown	brown	brown	unpigmented	Reddish brown
Segment equator	unpigmented	pigmented	unpigmented	pigmented	unpigmented	unpigmented
Segments	163–167	?	85–105	147	227–248	100–114
1 st dorsal pore	12/13	11/12	13/14	12/13	12/13	11/12
Setal gaps D, V	+, -	+, -	-, -	+, -	+, +	+, +
Spermathecal pores	-	Paired at 8/9	Paired at 6,7,8,9	Batteries at 5/6/7	-, paired at 5/6/7	Paired at 5/6
Sperm. pore distance	-	0.12	0.13	-	inconspicuous	0.19
Male pore distance	0.27	0.11	0.2	0.22	0.31	0.20
Setae between openings	20	3	12–13	6–7	8–9	9–11
Setae vii, xx	35–48, 70–73	40, 39	32–37, 40–45	39–45, 46–58	60–84, 69–70	34–36, 56–59
Genital markings	-	-	-	Paired in xix– xxi	Paired in xix– xxii	-
Gizzard	viii–x	viii–x	viii–x	viii–x	x–xi	viii–x
Intestinal origin	xiii	xv	xvi	xv	xvii	xiv
Caeca	xxvii–xxi	xxvii–xxiii	xxvii–xxiv	-	-	xxiii–xxi
Prostate	xviii	xv–xix	xviii	xvi/xvii–xix	xvi–xix	xvi–xx/ xv– xix
Copulatory bursae	-	-	-	-	-	xvii–xix

TABLE 5.1. Summary of the primers used for the 16S rDNA, COI, 28S rDNA and Histone3 sequences

Amplified Region (size in bp; genetic position)	Primer name	Primer sequence (5'-3')	Reference
16S (455; 1-455)	16sAr	CCGGTCTGAACTCAGATCACGT	Palumbi [19]
	16sBr	CGCCTGTTTATCAAAAACAT	Palumbi [19]
16S (373; 1-455)	16SF-ME	GCAAAGGTAGCATAATCACTTGC	This study
	16SR-ME	AATTTTGCCTATATAGATACCCTAAGC	This study
COI (641; 456-1097)	LCO1490	ACTTCAGGGTGACCAAAAAATCA	Folmer et al. [20]
	HCO2198	GGTCAACAAATCATAAAGATATTGG	Folmer et al. [20]
COI (591; 456-1097)	COI-F_N	TTTGAGCCGGAATAATTGG	This study
	COI-R_N	TCGAAGAATGATGTATTTAGGTTTCG	This study
28S (756; 1098-1854)	C1'	ACCCGCTGAATTTAAGCAT	Jamieson <i>et al.</i> [21]
	D2	TCCGTGTTTCAAGACGG	Jamieson <i>et al.</i> [21]
H3 (333; 1855-2185)	H3aF	ATGGCTCGTACCAAGCAGACVGC	Colgan <i>et al.</i> [22]
	H3aR	ATATCCTTRGGCATRATRGTGAC	Colgan <i>et al.</i> [22]

TABLE 5.2. Taxon list and sequences obtained (O). Outgroup sequences were obtained from GenBank.

Taxon	Remark	16S	28S	COI	H3
<i>Pheretima</i> Apo1	<i>P. urceolata</i> group	O		O	O
<i>Pheretima</i> Apo3	<i>P. urceolata</i> group	O		O	O
<i>Pheretima</i> Apo4	<i>P. urceolata</i> group	O		O	
<i>Pheretima</i> Busa2	<i>P. urceolata</i> group	O			
<i>Pheretima</i> Tago1	<i>P. urceolata</i> group	O			
<i>Pheretima apoensis</i> n. sp. (Mt. Apo)	<i>P. sangirensis</i> group	O			
<i>Pheretima apoensis</i> n. sp. (Mt. Musuan)	athecate morphospecies	O		O	O
<i>Pheretima timpoongensis</i> n. sp.	<i>P. sangirensis</i> group	O		O	O
<i>Pheretima camiguinensis</i> n. sp.	<i>P. sangirensis</i> group	O		O	O
<i>Pheretima</i> Busa1	<i>P. sangirensis</i> group	O			
<i>Pheretima malindangensis</i>	<i>P. sangirensis</i> group	O		O	O
<i>Pheretima boniaoi</i>	<i>P. sangirensis</i> group	O		O	O
<i>Pheretima vergrandis</i>	monothecate morphospecies	O		O	O
<i>P. (Parapheretima) pandanensis</i> n. sp.	Subgenus <i>Parapheretima</i>	O			O
<i>P. (Parapheretima) boaensis</i> n. sp.	Subgenus <i>Parapheretima</i>	O		O	O
<i>Amyntas dinagatensis</i> n. sp.	<i>Amyntas</i>	O			O
<i>Amyntas</i> Tago1	<i>Amyntas</i>	O	O		
<i>Amyntas</i> Apo1	<i>Amyntas</i>	O			
<i>Pontodrilus litoralis</i>	outgroup	AF406568	AY101576	LC018740.1	
<i>Dichogaster</i> sp.	outgroup	AF406571	AY101555		
Out of 50 specimens		18	1	10	11

*16S sequences of other representatives of some species here are not included in the ML and MrBayes phylogenetic reconstructions.

TABLE 5.3. Comparison of morphological features among pheretimoid taxa included in the analyses. “O” means present.

Diagnostic character	Genus <i>Amyntas</i>		Genus <i>Pheretima</i>	
		Subgenus <i>Parapheretima</i>	Subgenus <i>Pheretima</i>	
			<i>P. urceolata</i> group	<i>P. sangirensis</i> group
Nephridia on spermathecal duct		O	O	O
Spermathecae in 5/6	O	O	O	
Spermathecae in 7/8	O	O		O
Copulatory bursae		O	O	O
Secretory diverticula		O		

TABLE 5.4. Intraspecific and interspecific sequence divergences of some Mindanao pheretimoid species calculated using K2P model based on 16S sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Pheretima (Parapheretima) boaensis1</i>														
2 <i>Pheretima (Parapheretima) boaensis2</i>	0.011													
3 <i>Pheretima (Parapheretima) pandanensis1</i>	0.112	0.105												
4 <i>Pheretima (Parapheretima) pandanensis2</i>	0.116	0.101	0.008											
5 <i>Pheretima apoensis1</i>	0.142	0.126	0.084	0.087										
6 <i>Pheretima apoensis2</i>	0.142	0.126	0.084	0.087	0.000									
7 <i>Pheretima apoensis (Musuan)</i>	0.142	0.126	0.090	0.094	0.011	0.011								
8 <i>Pheretima camiguinensis1</i>	0.102	0.087	0.061	0.064	0.067	0.067	0.080							
9 <i>Pheretima camiguinensis2</i>	0.102	0.087	0.061	0.064	0.067	0.067	0.080	0.000						
10 <i>Pheretima camiguinensis3</i>	0.102	0.087	0.061	0.064	0.067	0.067	0.080	0.000	0.000					
11 <i>Pheretima camiguinensis4</i>	0.105	0.091	0.058	0.061	0.064	0.064	0.077	0.005	0.005	0.005				
12 <i>Pheretima timpoongensis1</i>	0.105	0.090	0.055	0.058	0.074	0.074	0.084	0.022	0.022	0.022	0.022			
13 <i>Pheretima timpoongensis2</i>	0.105	0.090	0.058	0.055	0.077	0.077	0.087	0.028	0.028	0.028	0.028	0.005		
14 <i>Pheretima timpoongensis3</i>	0.105	0.090	0.055	0.058	0.074	0.074	0.084	0.022	0.022	0.022	0.022	0.000	0.005	
15 <i>Pheretima timpoongensis4</i>	0.105	0.090	0.058	0.055	0.077	0.077	0.087	0.028	0.028	0.028	0.028	0.005	0.000	0.005

FIGURES

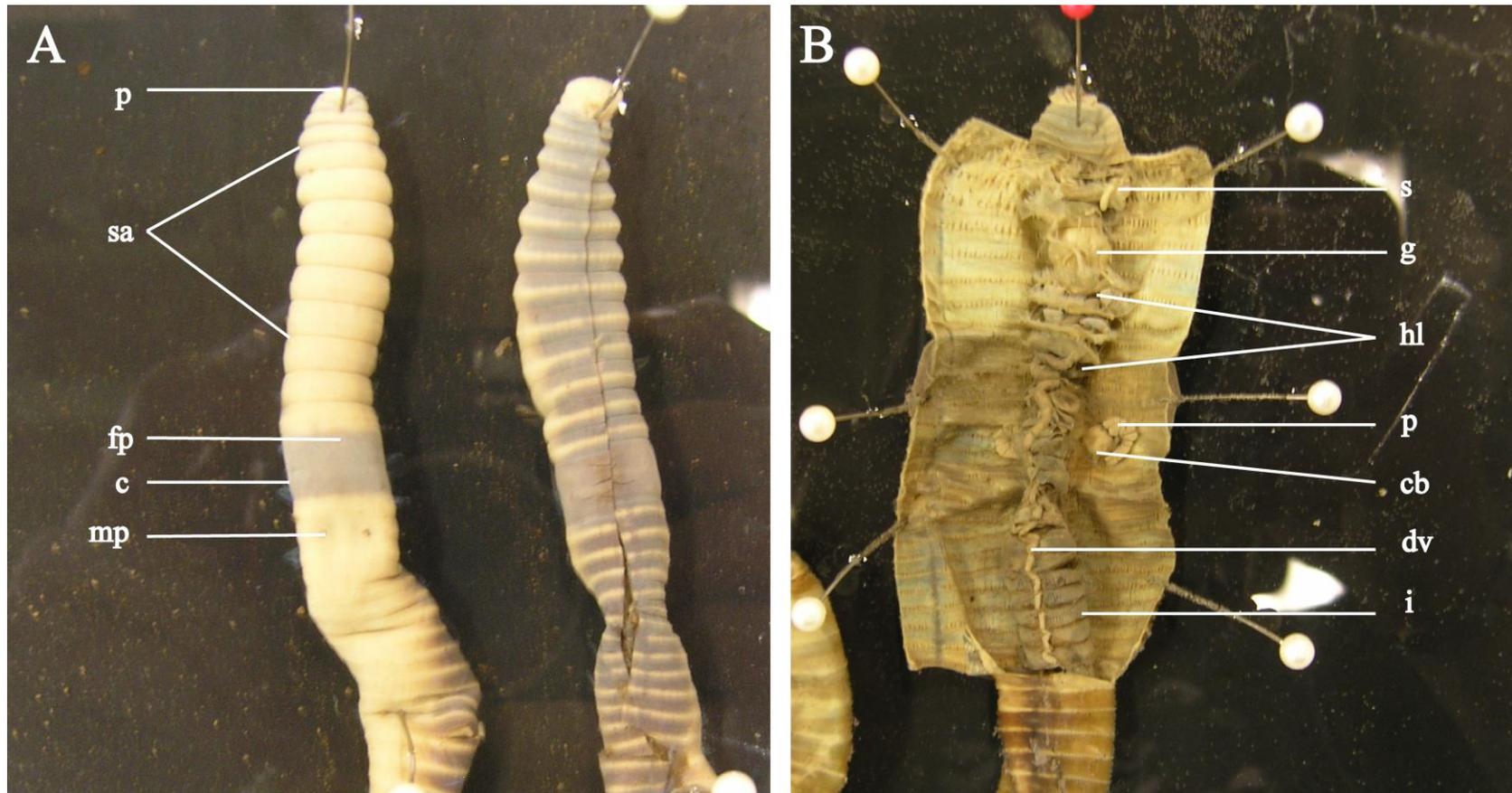


FIGURE 1.1. (A) The external morphology of a *Pheretima* species on ventral side (left) and dorsal side (right) showing the prostomium (p), the spermathecal area (sa), the female pore (fp), the clitellum (c), and the male pores (mp). (B) The internal morphology of a *Pheretima* species on dorsal side showing the spermathecae (s), the gizzard (g), the location of the hearts (hl), the prostate gland (p), the copulatory bursae (cb), the dorsal vessel (dv), and the intestine (i).

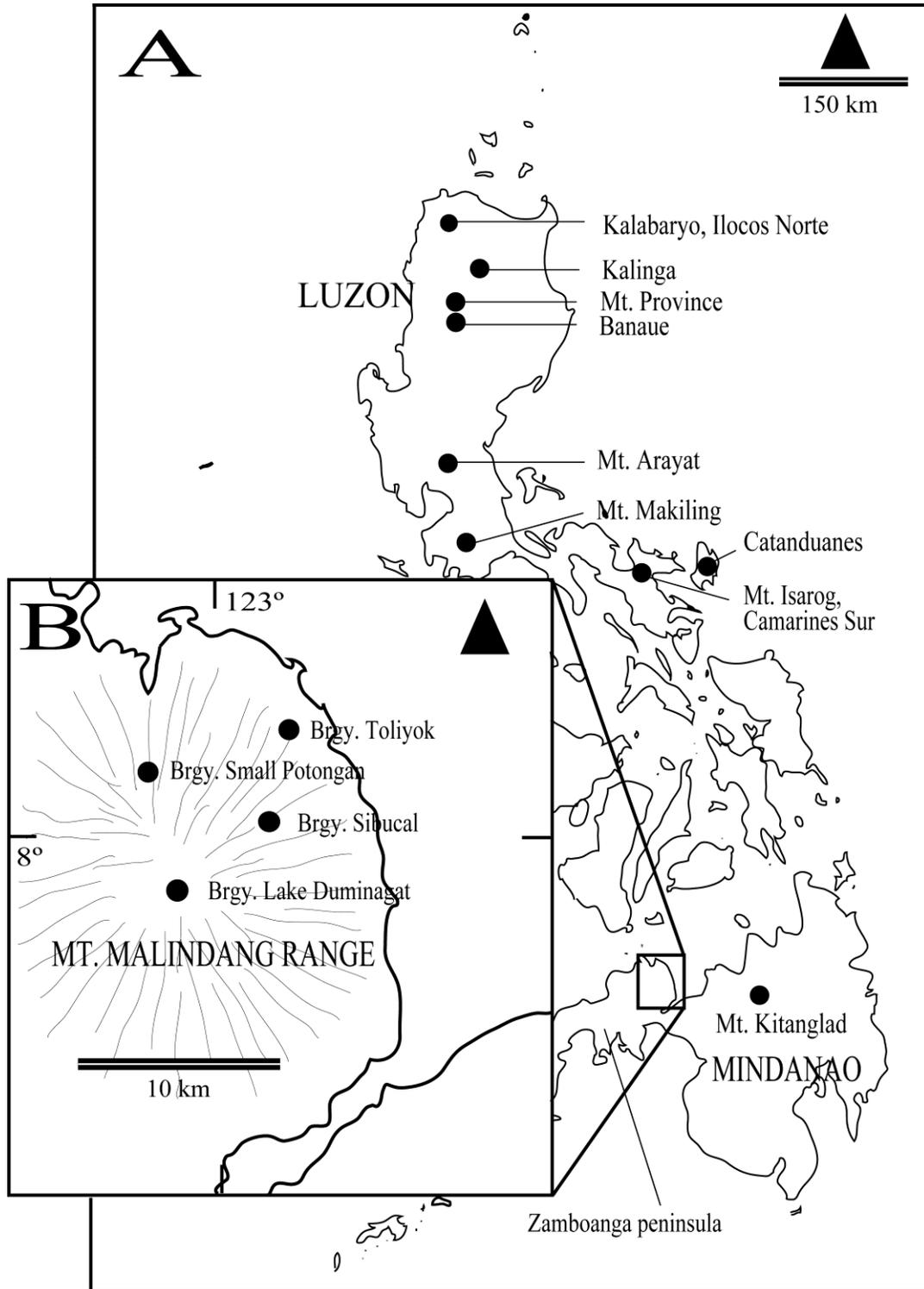


FIGURE 2.1. (A) Map of the Philippines showing localities where new earthworm species described in the last decade were collected. (B) Map of Mt. Malindang showing locations of the barangays where the new *Pheretima* species reported in this study were collected (from Aspe & James, 2014).



FIGURE 2.2. Photographs of living *Pheretima* worms in dorsal view, showing coloration. (A) *P. maculodorsalis*; (B) *P. tigris*; (C) *P. immanis*; (D) *P. longigula*; (E) *P. malindangensis*. Scale bars: A–D, 5 cm; E, 3 cm (from Aspe & James, 2014).

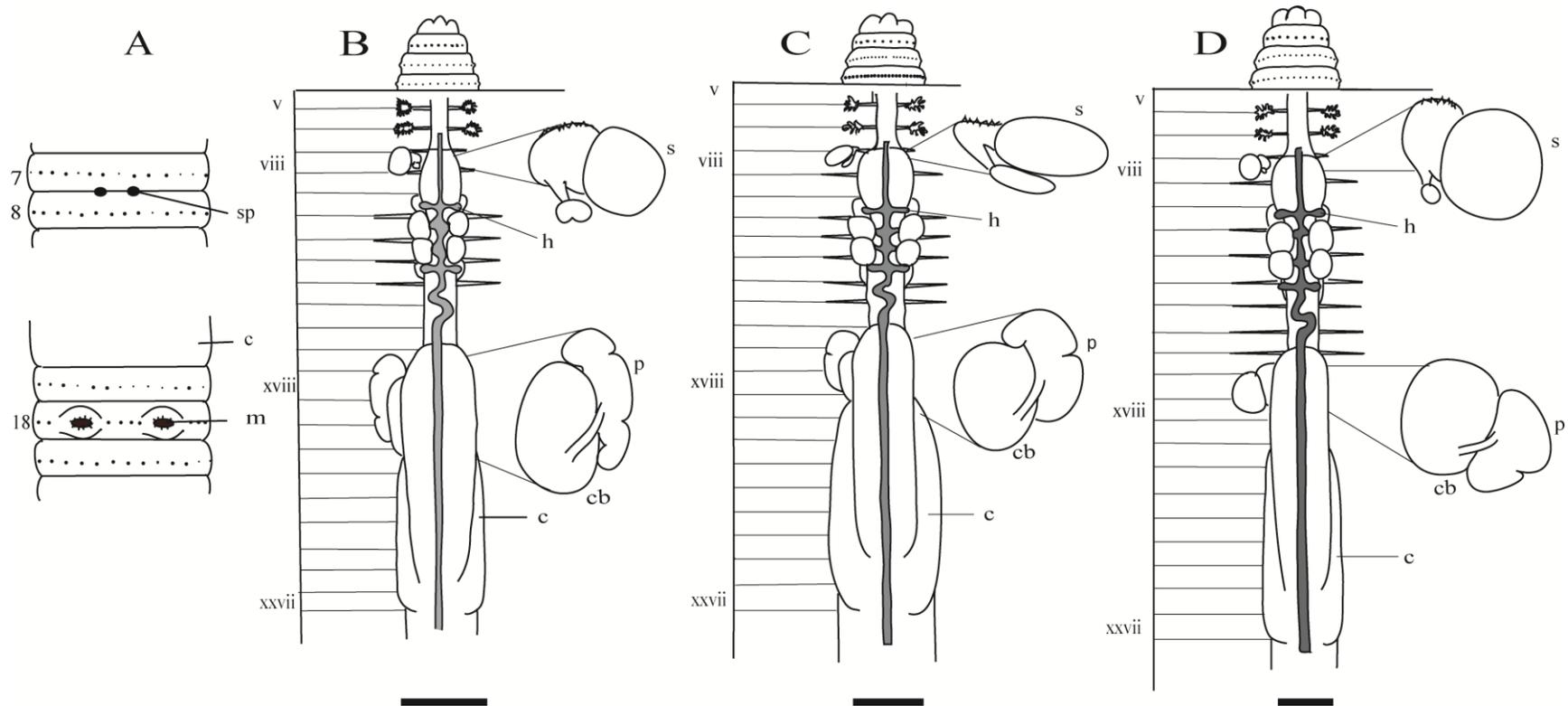


FIGURE 2.3. (A) Diagrammatic ventral view of *P. maculodorsalis* showing the spermathecal pores (sp), clitellum (cl), and male pores (m). (B, C) Schematic drawings of the internal morphology of Philippine *Pheretima* species, in dorsal view: (B) *P. maculodorsalis*; (C) *P. tigris*; (D) *P. immanis*. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: 10 mm (from Aspe & James, 2014).

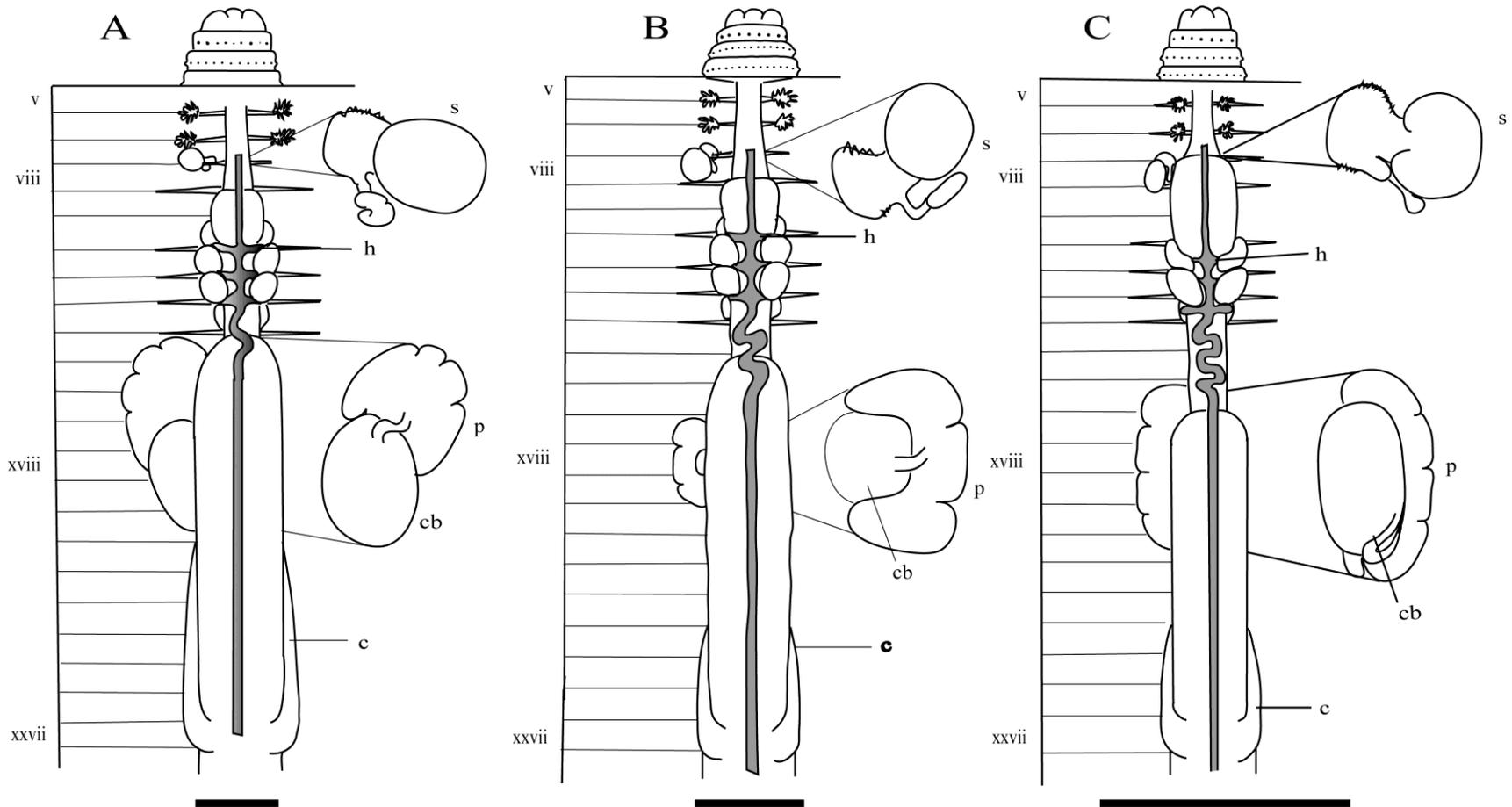


FIGURE 2.4. Schematic drawings of the internal morphology of Philippine *Pheretima* species, in dorsal view: (A) *P. lago*; (B) *P. nunezae*; (C) *P. boniaoi*. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: 10 mm (from Aspe & James, 2014).

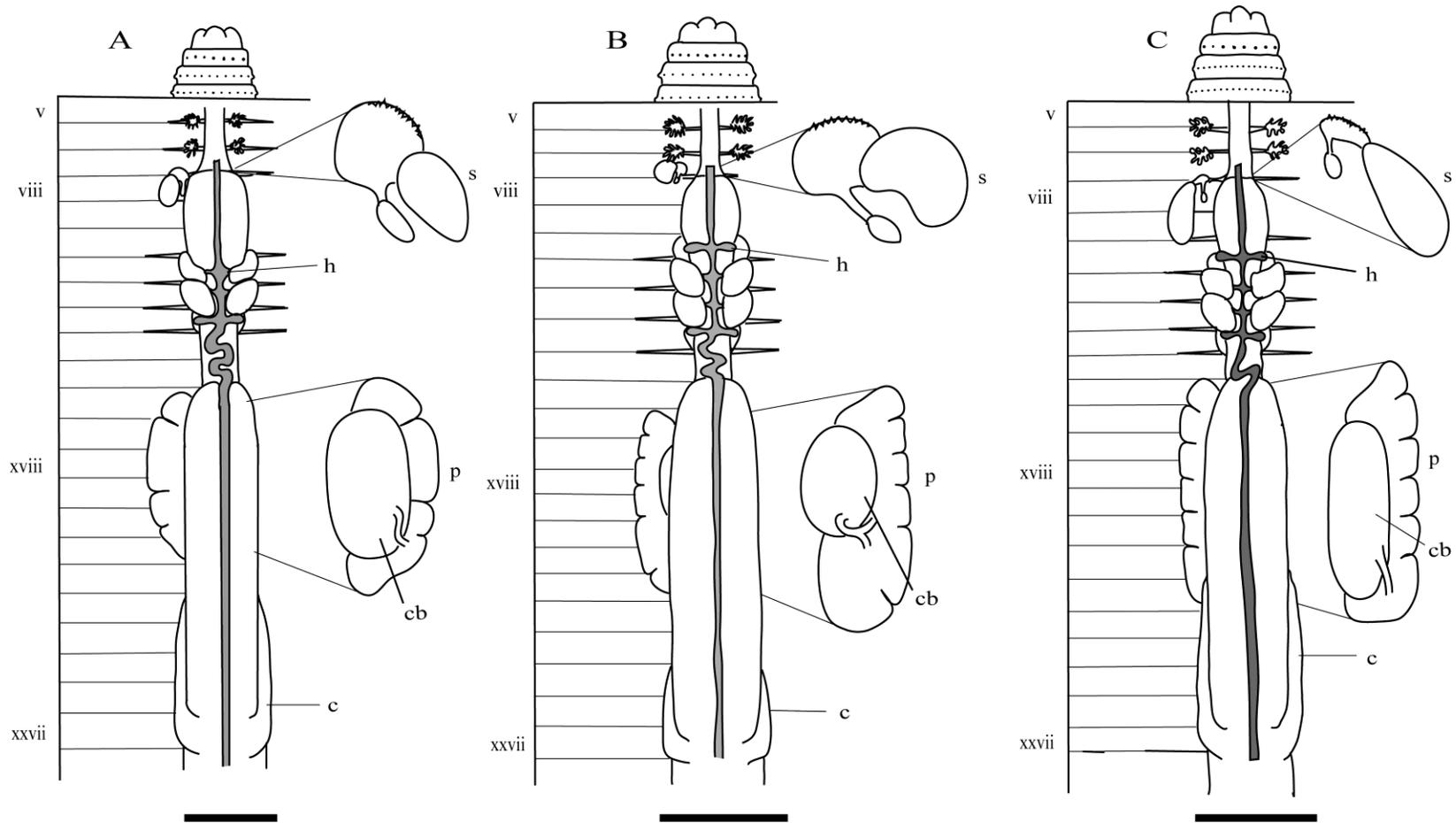


FIGURE 2.5. Schematic drawings of the internal morphology of Philippine *Pheretima* species, in dorsal view: (A) *P. malindangensis*; (B) *P. misamisensis*; (C) *P. wati*; Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: 5 mm (from Aspe & James, 2014).

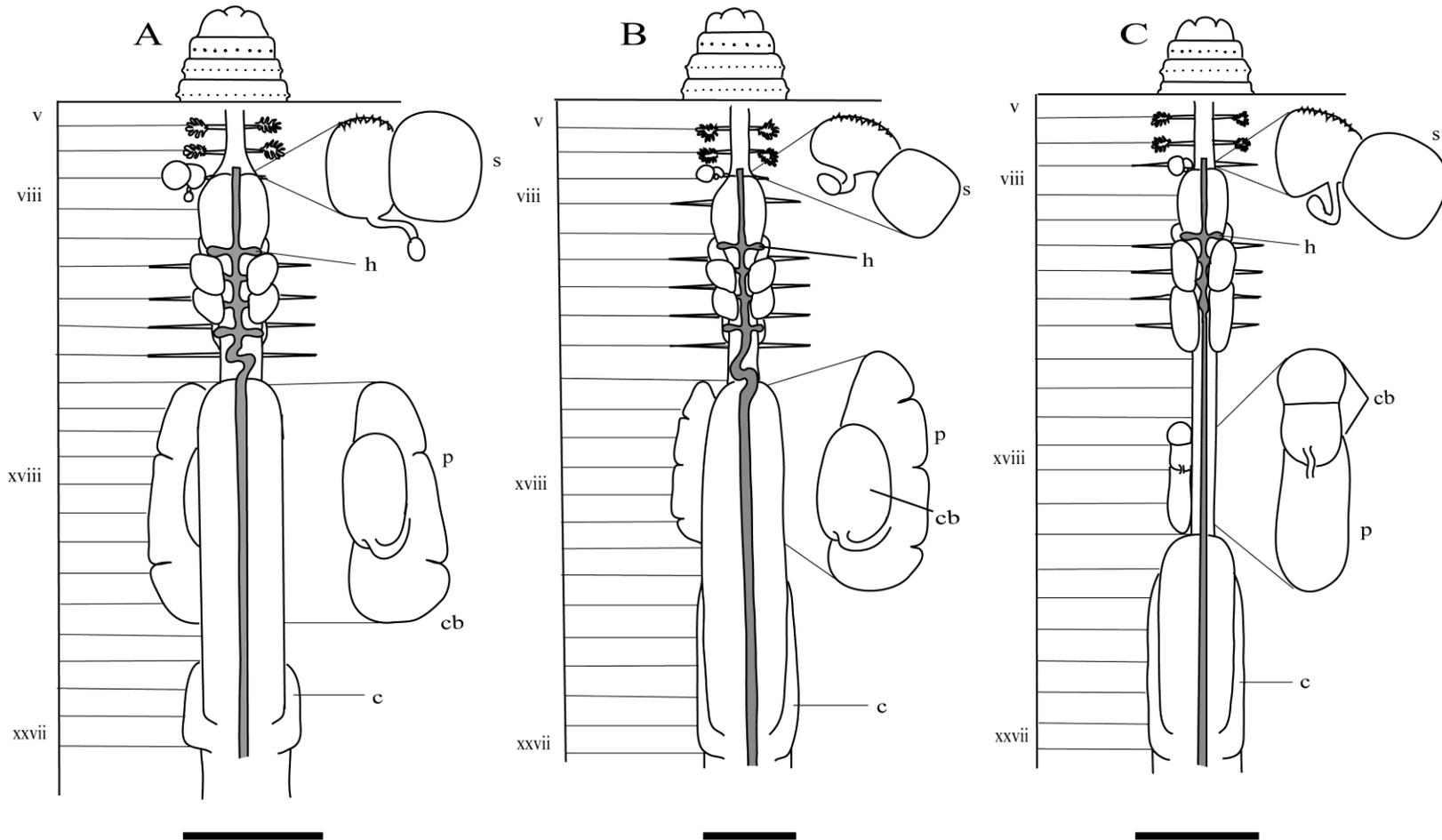


FIGURE 2.6. Schematic drawings of the internal morphology of Philippine *Pheretima* species, in dorsal view: (A) *P. longiprostata*; (B) *P. nolani*; (C) *P. longigula*; Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: 5 mm (from Aspe & James, 2014).

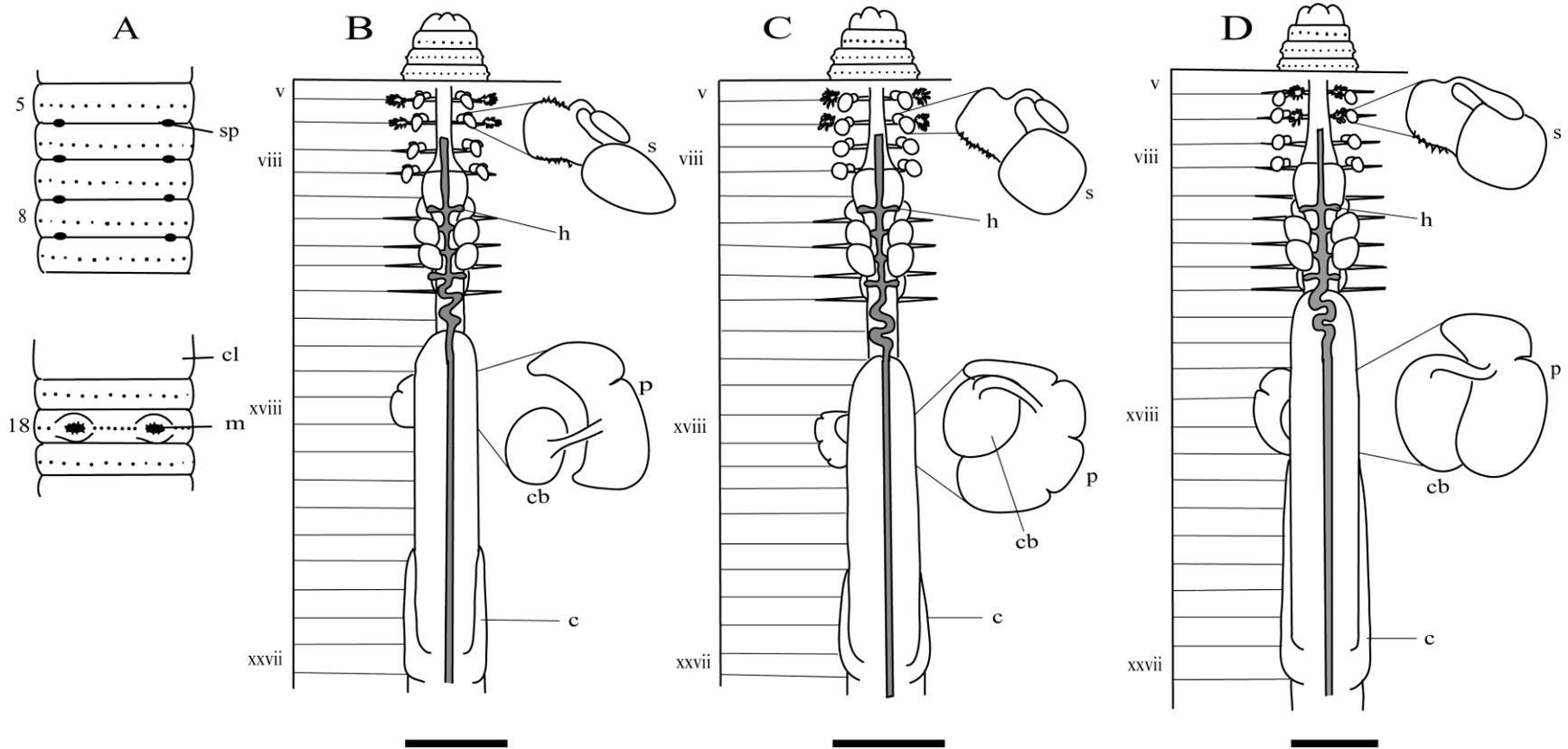


FIGURE 2.7. (A) Diagrammatic ventral view of polythecal *Pheretima adevai*, showing the spermathecal pores (sp), clitellum (cl), and male pores (m). (B–D) Schematic drawings of the internal morphology of Philippine *Pheretima* species, in dorsal view: (B) *P. adevai*; (C) *P. lluchi*; (D) *P. potonganensis*. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: B–D, 5 mm (from Aspe & James, 2014).

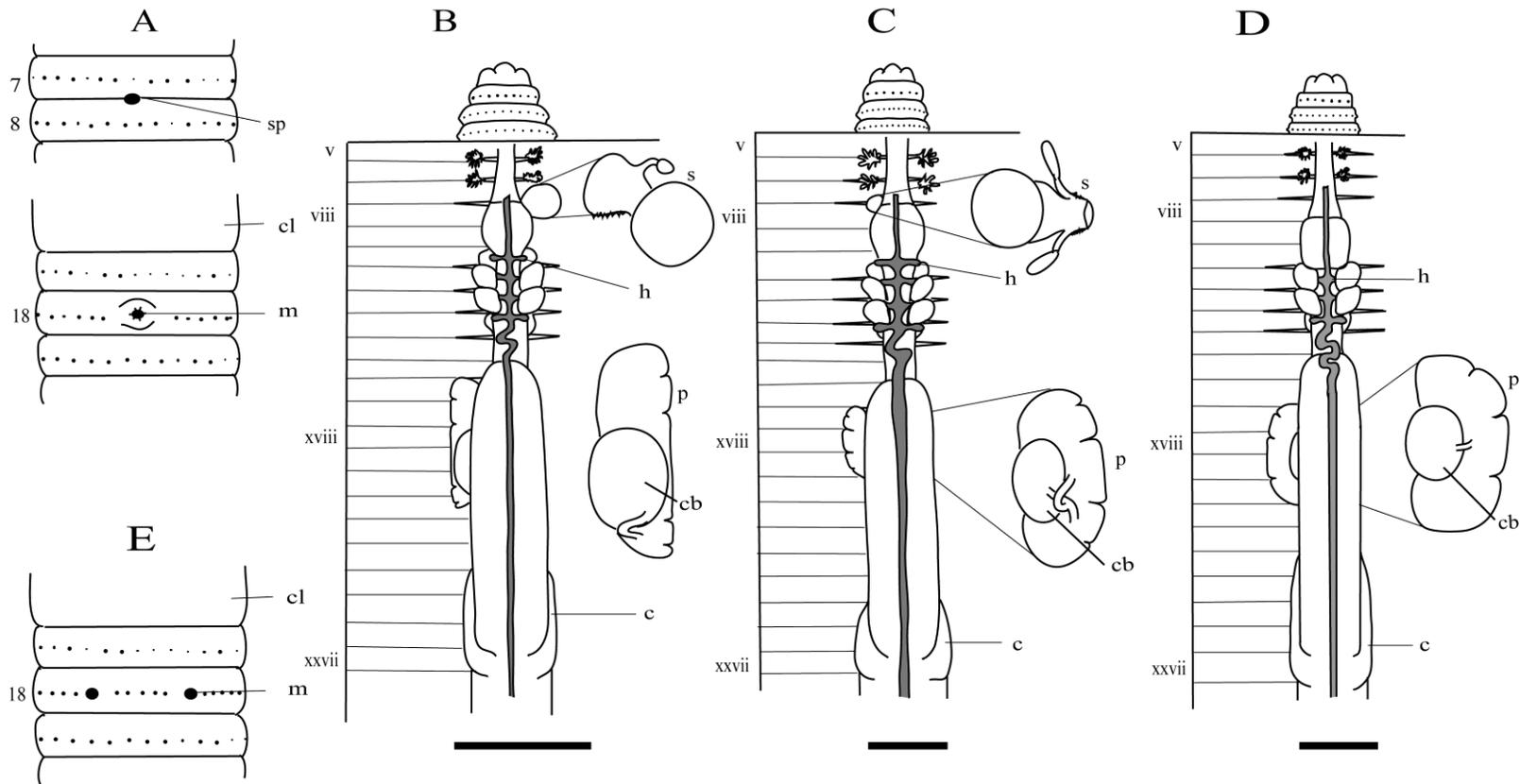


FIGURE 2.8. (A) Diagrammatic ventral view of monothechal *Pheretima vergrandis* showing the single spermathecal pore (sp), clitellum (cl), and single male pore (m). (B–D) Schematic drawings of the internal morphology of Philippine *Pheretima* species: (B) *P. vergrandis*; (C) *P. conceptionensis*; (D) athecate *Pheretima subanensis*. (E) Diagrammatic ventral view showing the clitellum (cl) and a pair of male pores (m), as in *P. conceptionensis* and *P. subanensis*. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: B–D, 5 mm (from Aspe & James, 2014).

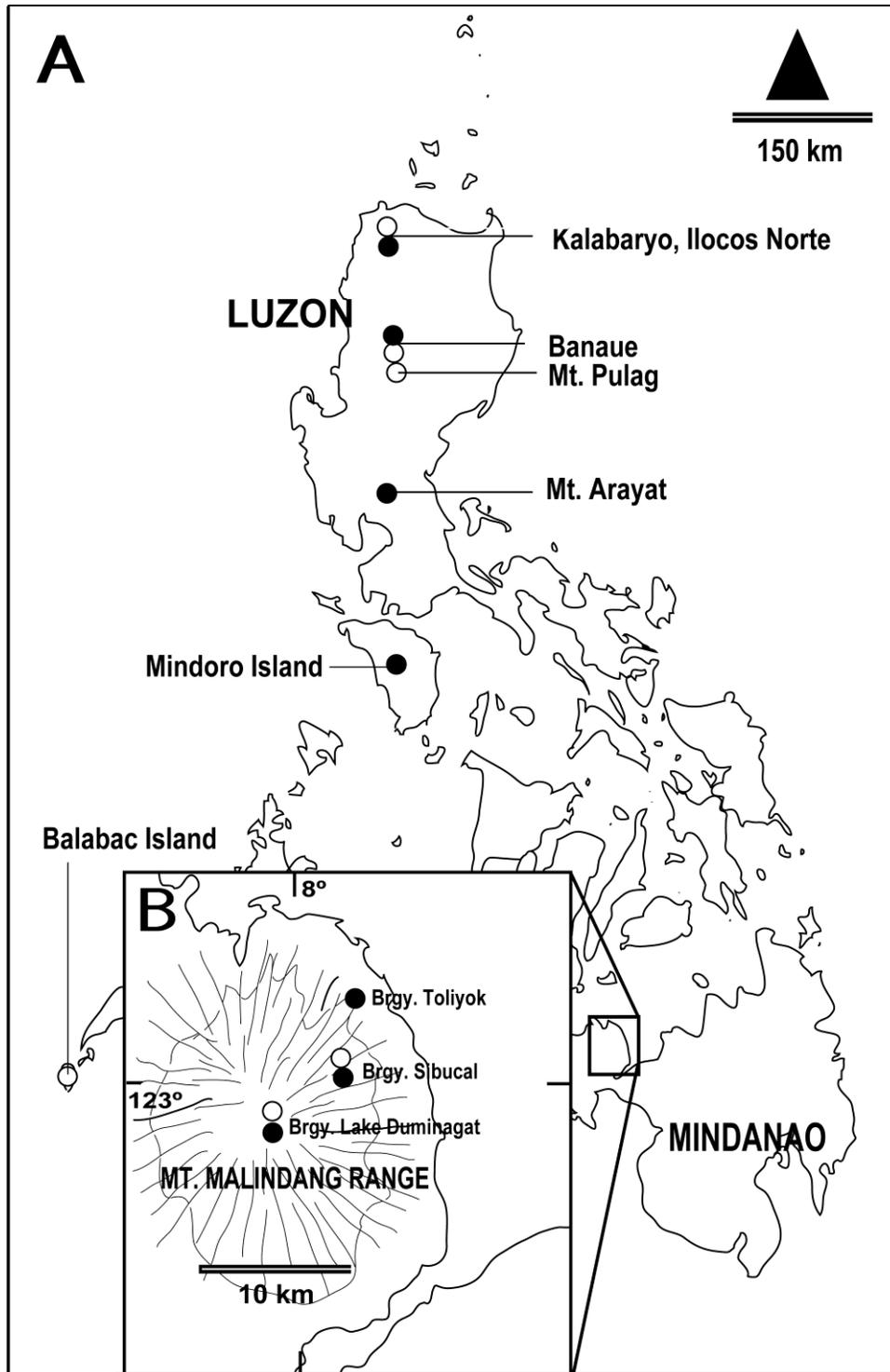


FIGURE 3.1. (A) Map of the Philippines showing localities where species of *Polypheretima* (white dots) and *Pithemera* (black dots) have been collected. (B) Map of Mt. Malindang showing the localities where the *Polypheretima* and *Pithemera* species reported in this study were collected (from Aspe & James, 2015).

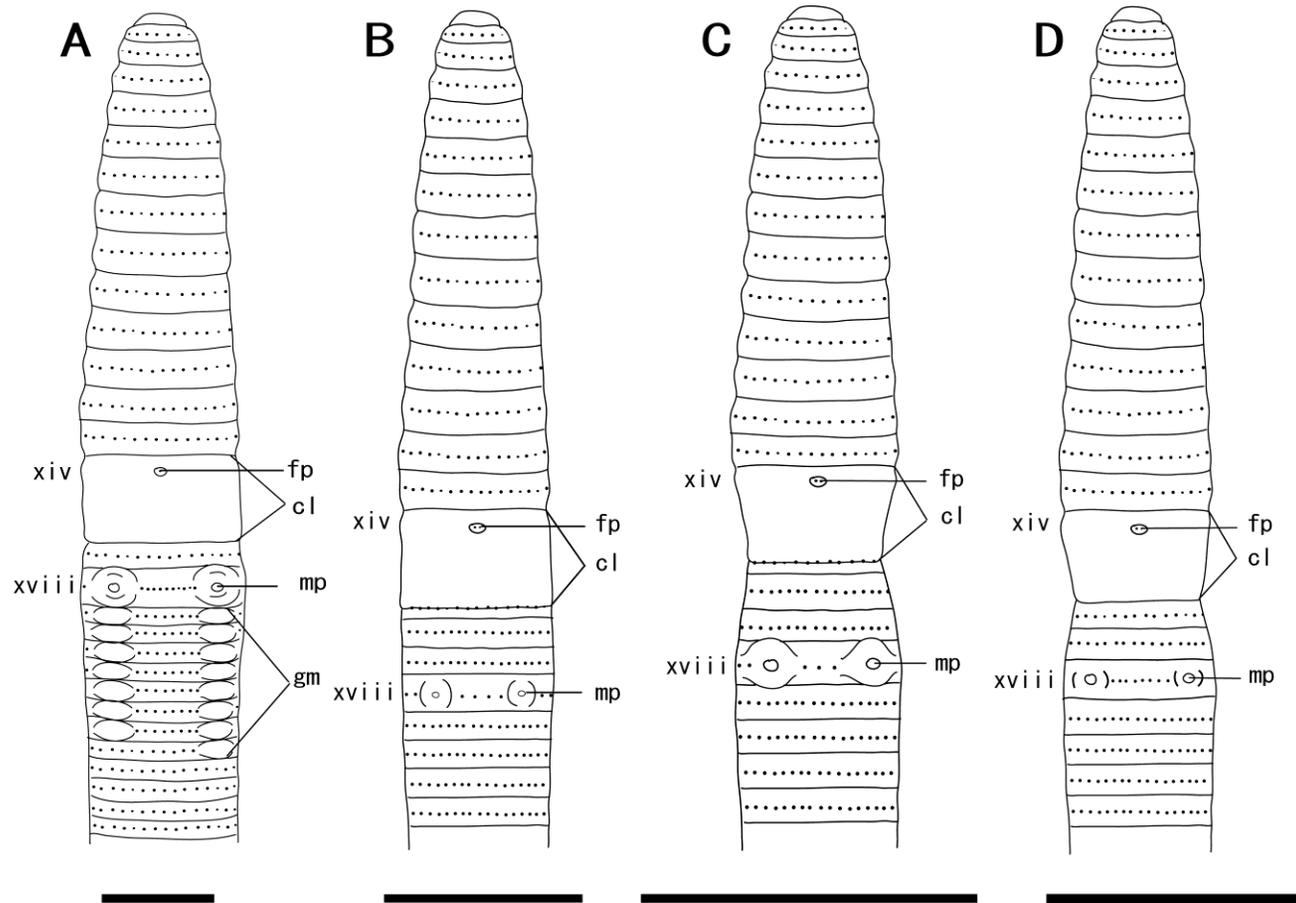


FIGURE 3.2. Schematic drawings of the external antero-ventral area of (A) *Polypheretima mindanaoensis*; (B) *Pithemera malindangensis*; (C) *Pithemera duminagati*; and (D) *Pithemera donvictorianoi*. Abbreviations: fp, female pore; cl, clitellum; mp, male pore; gm, genital markings. Scale bars: 5 mm (from Aspe & James, 2015).

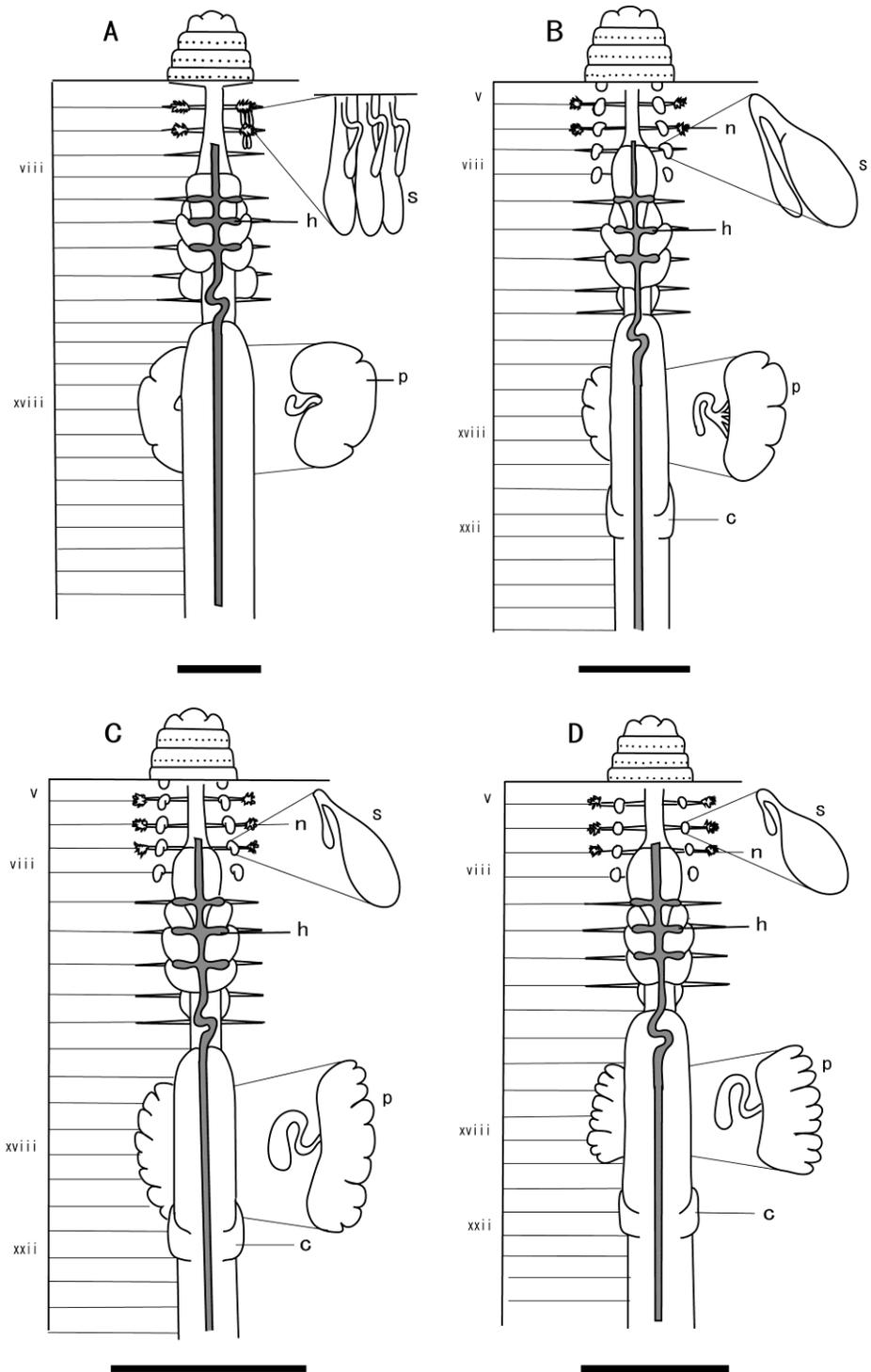


FIGURE 3.3. Schematic drawings of the internal morphology of (A) *Polypheretima mindanaoensis*; (B) *Pithemera malindangensis*; (C) *Pithemera duminagati*; and (D) *Pithemera donvictoriano*. Abbreviations: s, spermatheca; n, dense tufts of nephridia; h, heart; p, prostate gland; c, caecum. Scale bars: 5 mm (from Aspe & James, 2015).

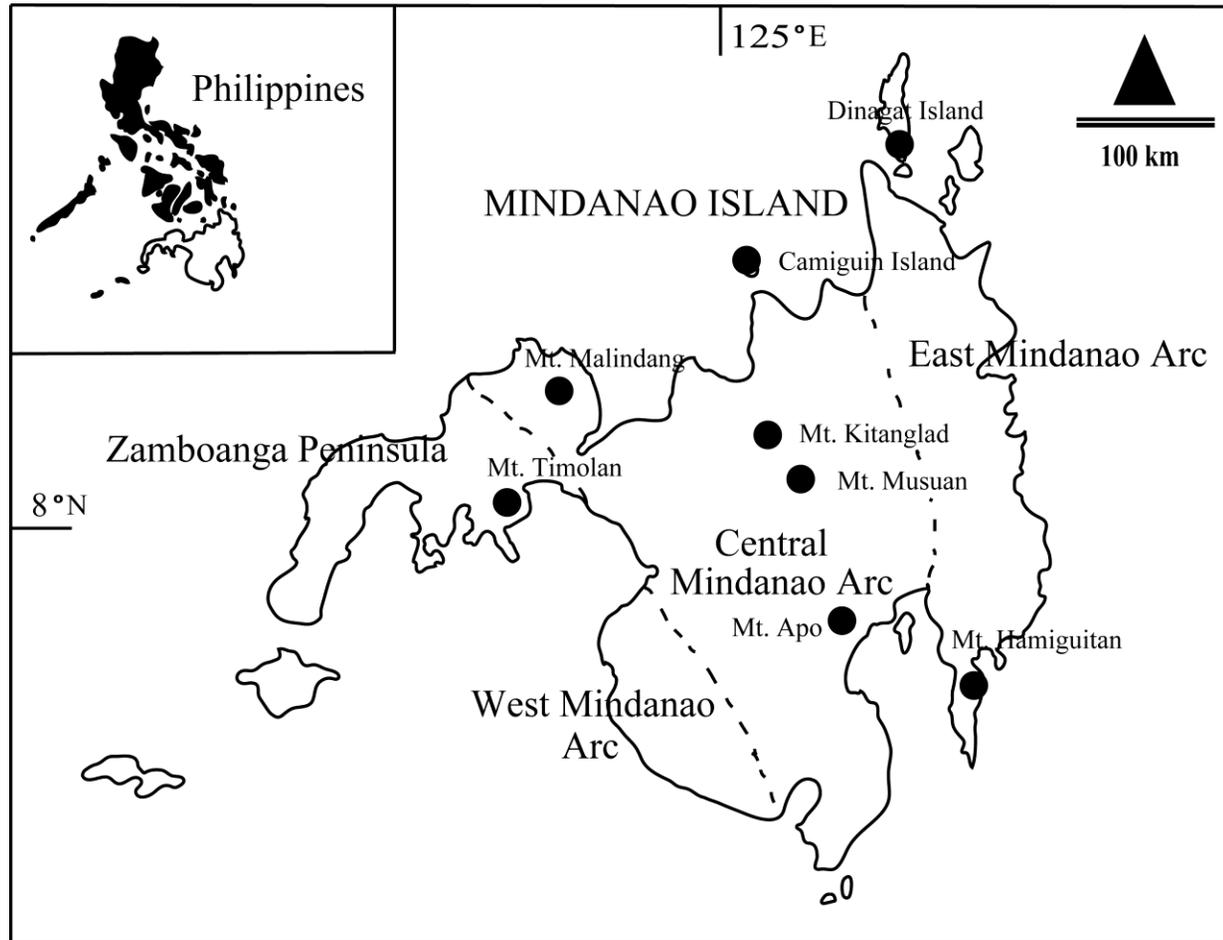


FIGURE 4.1. Map of Mindanao and associated islands. Black circles indicate collecting sites; dashed lines indicate the boundaries among three arc systems and the Zamboanga Peninsula, which coalesced between the Early Miocene and late Pliocene (20–1 Ma) (Aspe & James 2016).



FIGURE 4.2. The mountainous landscapes of Mt. Timpoong at > 1300 m asl (top) and Mt. Apo at > 1500 m asl (bottom), two of the collection sites in Mindanao, Philippines.

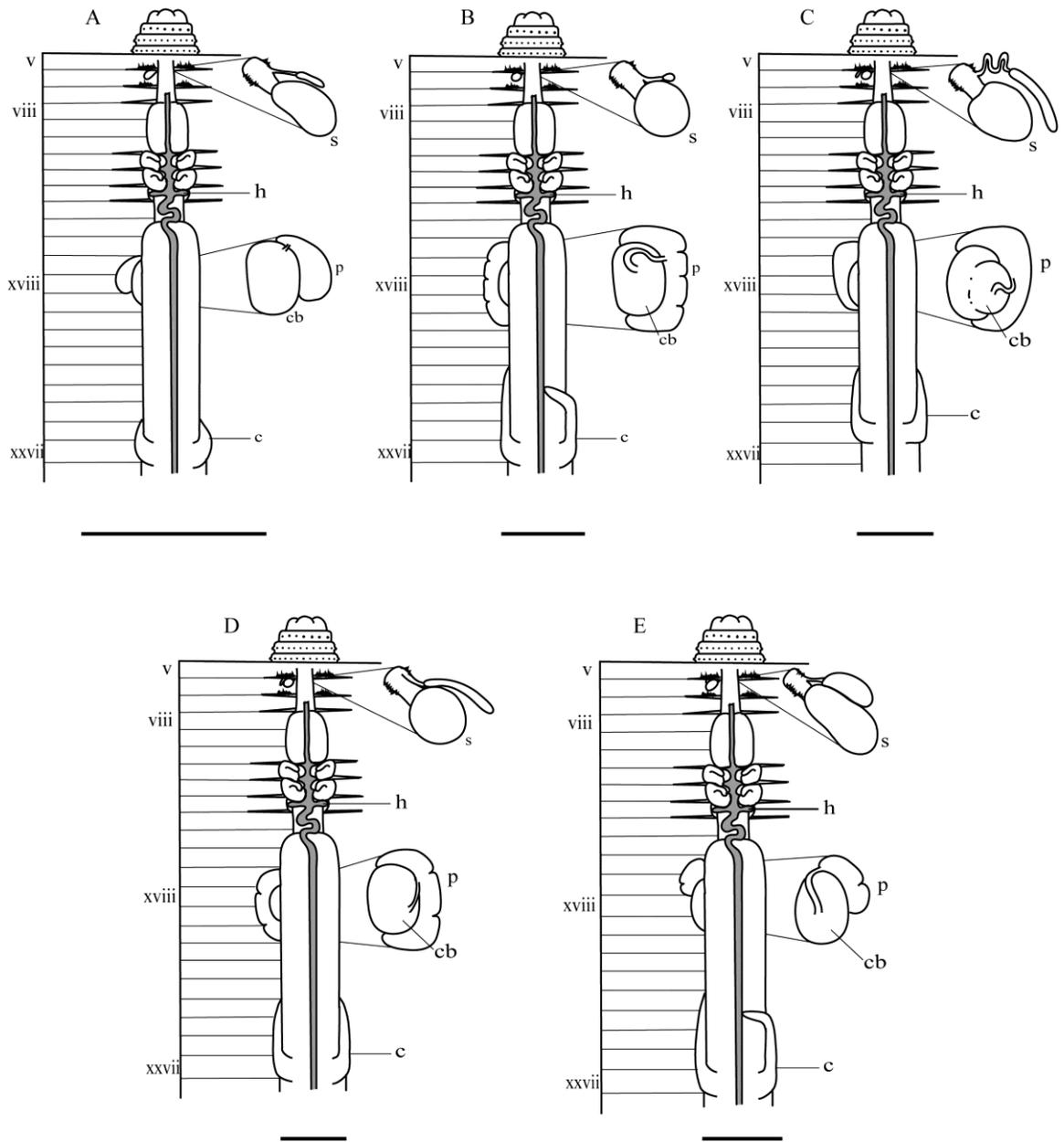


FIGURE 4.3. Schematic dorsal views of the internal morphology of the *Pheretima* (*Pheretima*) *urceolata* group species reported in Chapter 4. **(A)** *P. acia* n. sp.; **(B)** *P. dinagatensis* n. sp.; **(C)** *P. enormis* n. sp.; **(D)** *P. hamiguitanensis* n. sp., with the intestinal origin in xv and prostates in xvii–xx; **(E)** *P. lantapanensis* n. sp. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: A, B, D, E, 5 mm; C, 15 mm (Aspe & James 2016).

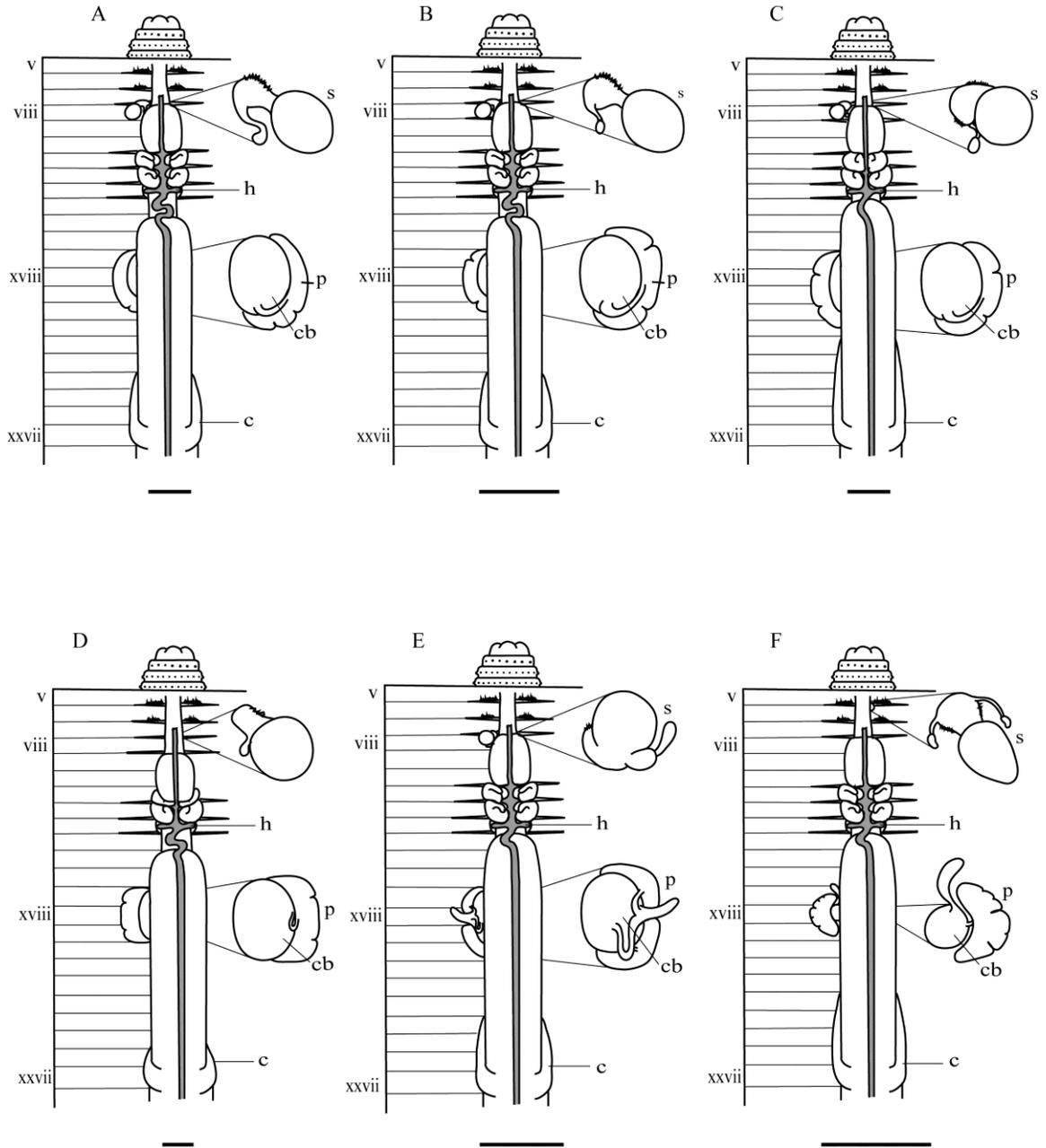


FIGURE 4.4. Schematic dorsal views of the internal morphology of members of the *Pheretima sangirensis* group (**A–D**) and the *Pheretima* (*Parapheretima*) (**E, F**) reported in Chapter 4. (**A**) *P. timpoongensis* n. sp., with intestinal origin in xv, and caeca extending from xxvii–xxiv; (**B**) *P. camiguinensis* n. sp., with prostates in xvii–xxi, and caeca extending from xxvii–xxi; (**C**) *P. sibucalensis* n. sp.; (**D**) *P. apoensis* n. sp. with spermathecae; (**E**) *P. (Paraph.) pandanensis* n. sp.; (**F**) *P. (Paraph.) boaensis* n. sp., with intestinal origin in xiv, prostates in xvii–xix, and caeca extending from xxvii–xxiii. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum. Scale bars: A–F, 5 mm (Aspe & James 2016).

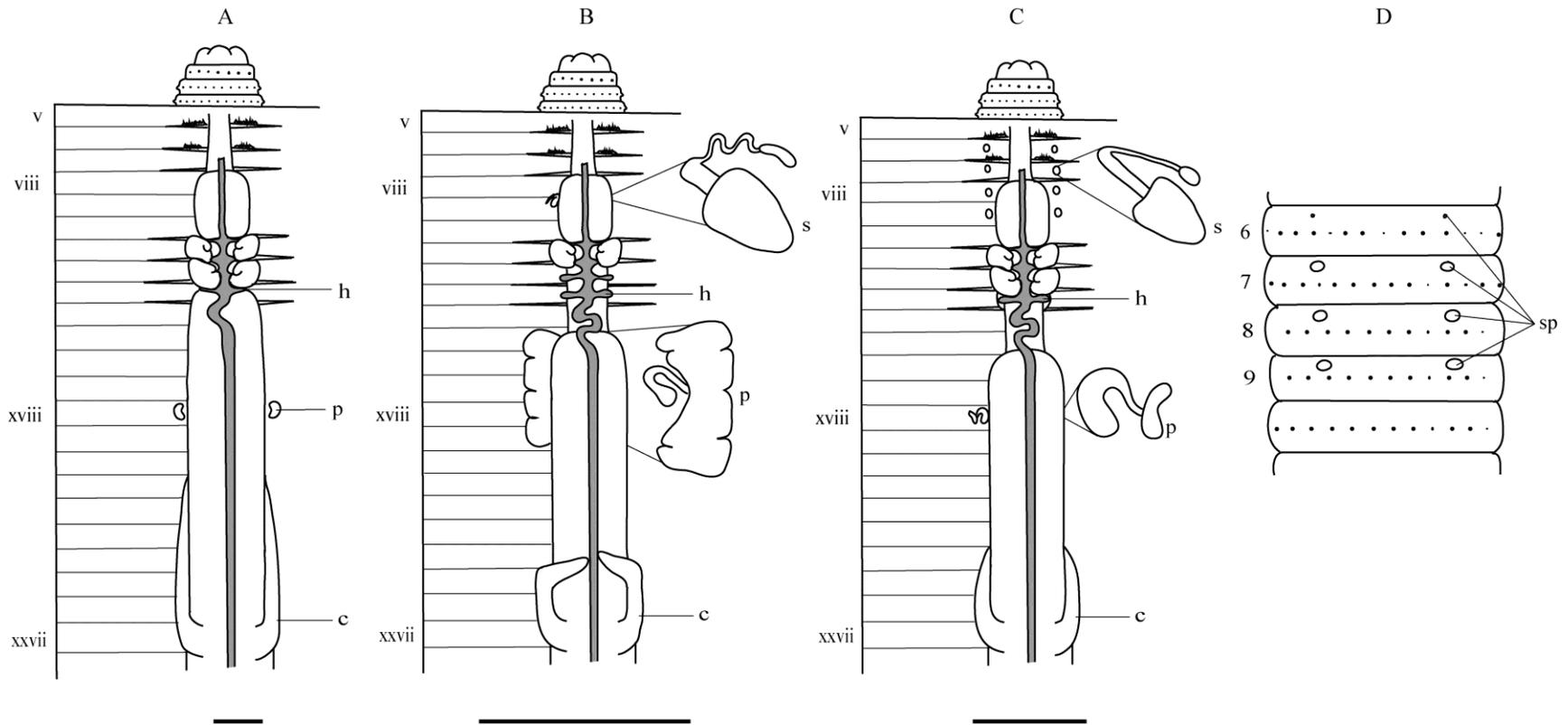


FIGURE 4.5. Schematic dorsal views of the internal morphology of the three *Amynthes* species reported in Chapter 4. **(A)** *A. dinagatensis* n. sp.; **(B)** *A. cagdianoensis* n. sp.; **(C)** *A. talaandigensis* n. sp. Scale bars: A–C: 5 mm. **(D)** Schematic ventral view of polythecal *A. talaandigensis* n. sp., showing the intersegmental spermathecal pores (sp). Abbreviations: s, spermatheca; h, heart; p, prostate gland; c, caecum; sp, spermathecal pores (Aspe & James 2016).

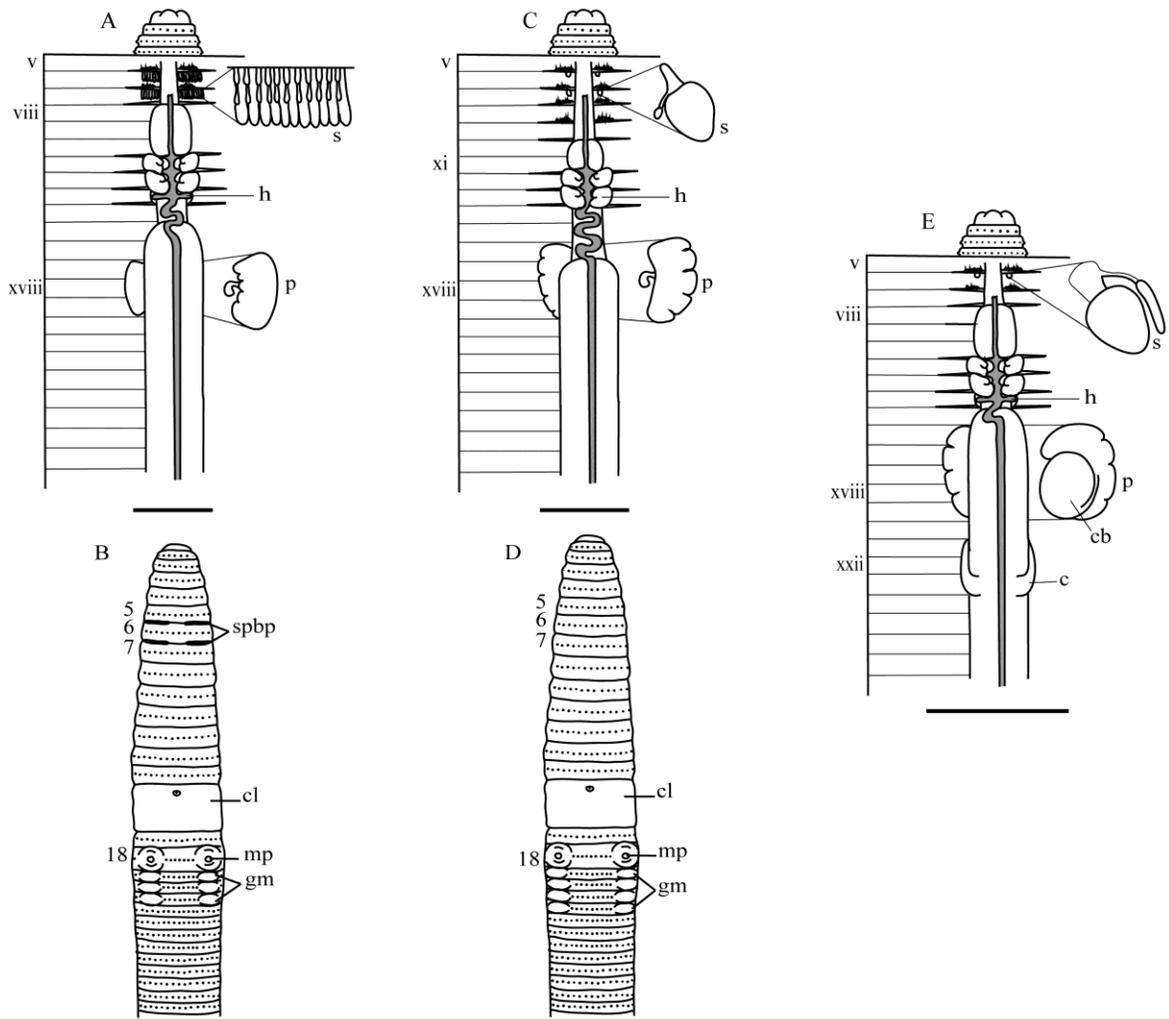


FIGURE 4.6. Schematic views of the internal and external morphology of the *Polypheretima* and *Pithemera* species reported in Chapter 4. **(A, B)** *Polypheretima bukidnonensis*; **(A)** dorsal view of internal morphology; **(B)** external ventral view; **(C, D)** *Polypheretima zamboangensis* n. sp.; **(C)** dorsal view of internal morphology; **(D)** external ventral view; **(E)** *Pithemera nolani* n. sp., dorsal view of internal morphology, with prostates in xv–xix. Abbreviations: s, spermatheca; h, heart; p, prostate gland; cb, copulatory bursa; c, caecum; spbp, spermathecal batteries pores; cl, clitellum, mp, male pores, gm, genital markings. Scale bars: A–E, 5 mm (Aspe & James 2016).

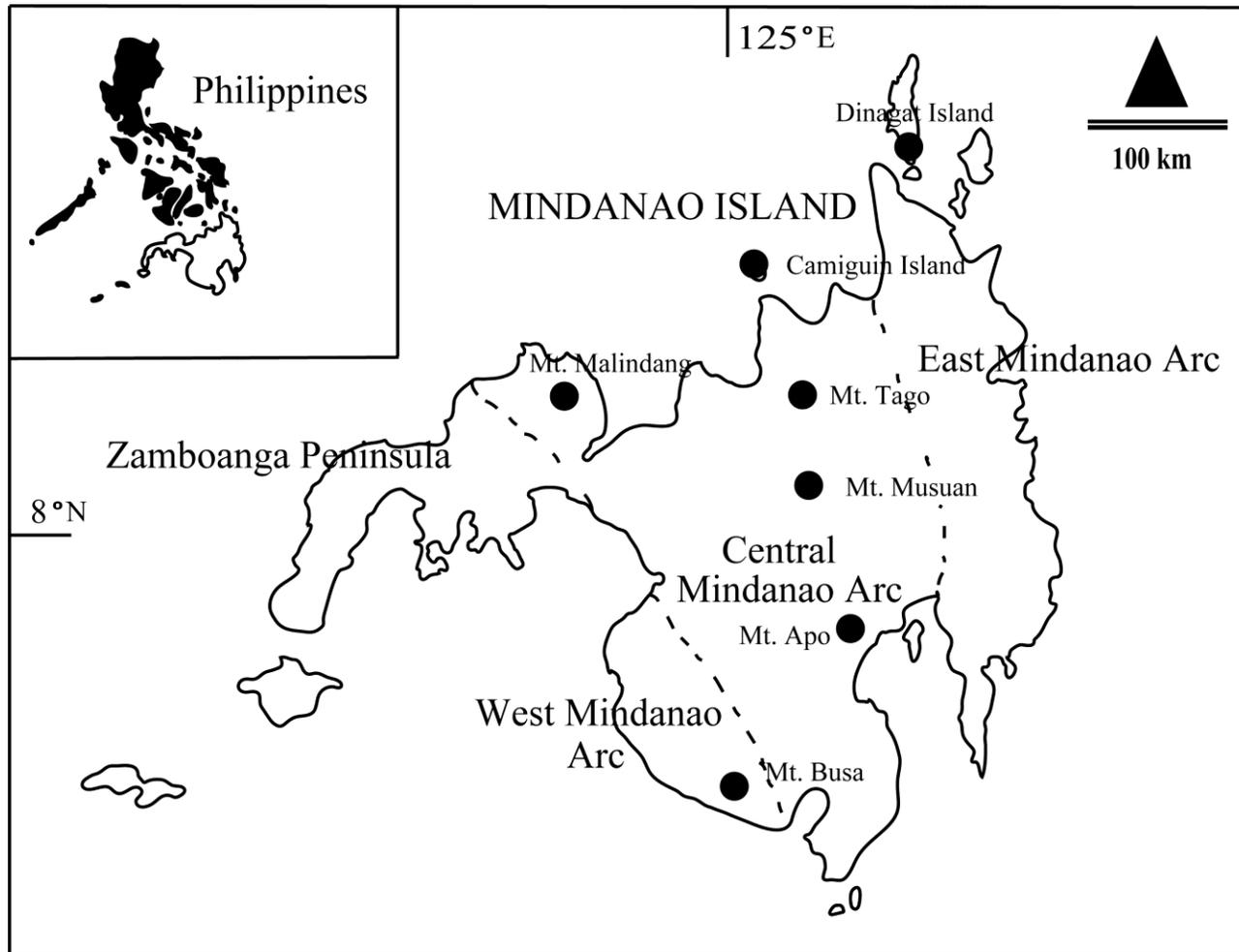


FIGURE 5.1. Map of Mindanao Island showing the seven collecting sites included in the phylogenetic analyses.

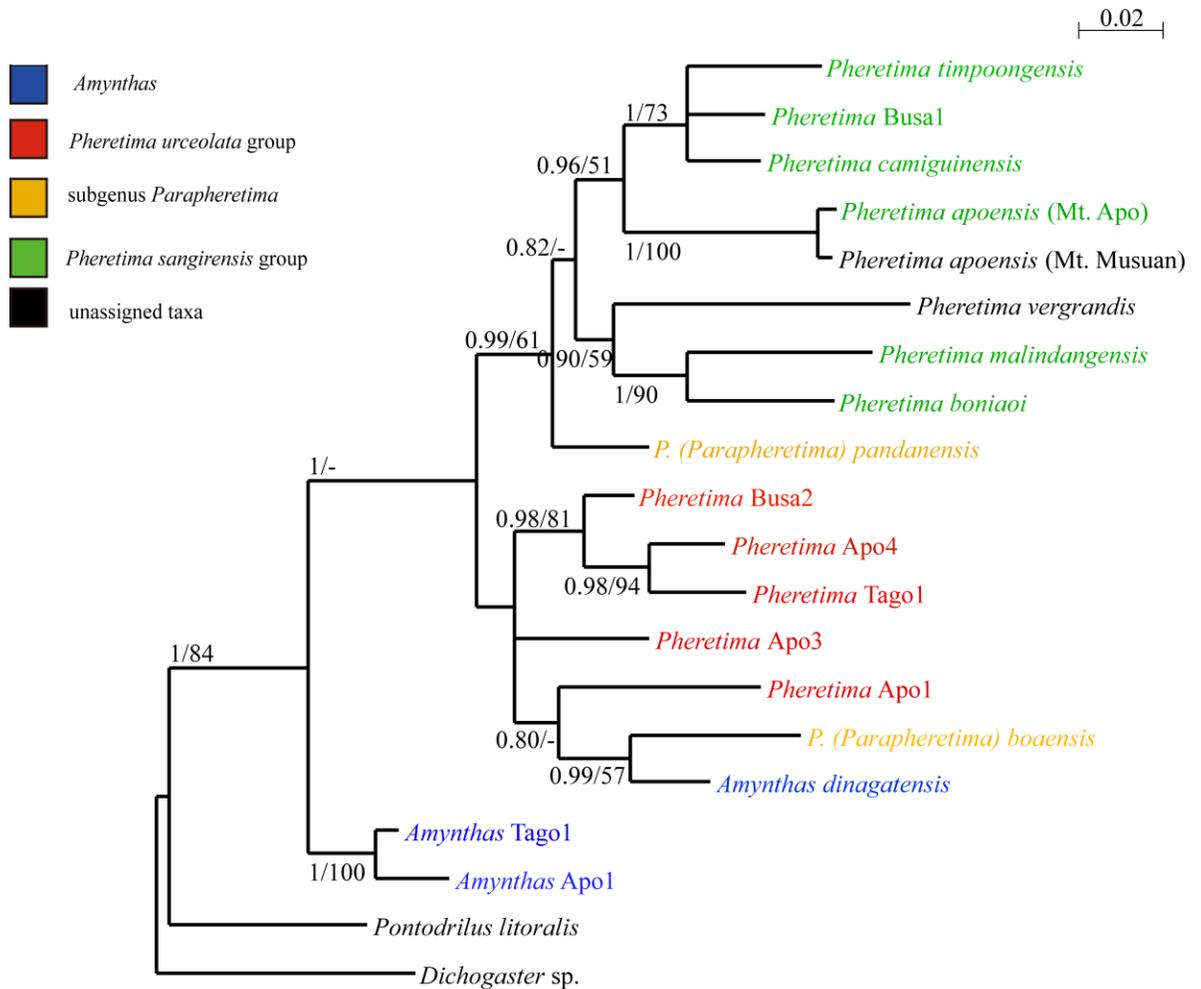


FIGURE 5.2. Tree generated from the combined 16S, COI, 28S and H3 genes showing posterior probability values and bootstrap values. Posterior probability values lower than 0.8 and bootstrap values lower than 50% indicate weak support and are not shown.

APPENDIX

Appendix. Parameters for the concatenated 16S, 28S, COI and H3 genes for maximum-likelihood (ML) analysis using GTR+G+I model.

Gene	Base Frequency	Rate Matrix
16S	A= 0.345397	A-C= 3.595757
	C= 0.193621	A-G= 7.682563
	G= 0.190907	A-T= 4.134709
	T= 0.270075	C-G= 0.083013
		C-T= 14.609200
		G-T= 1.000000
28S	A= 0.191140	A-C= 1.024950
	C= 0.297188	A-G= 0.549549
	G= 0.335872	A-T= 0.406212
	T= 0.175800	C-G= 0.451803
		C-T= 1.887718
		G-T= 1.000000
COI-1	A= 0.252159	A-C= 0.000000
	C= 0.248523	A-G= 1.567707
	G= 0.298522	A-T= 0.000000
	T= 0.200796	C-G= 0.000000
		C-T= 29.392772
		G-T= 1.000000
COI-2	A= 0.124888	A-C= 0.000000
	C= 0.265341	A-G= 0.718013
	G= 0.173524	A-T= 0.000000
	T= 0.436247	C-G= 0.000000
		C-T= 0.000000
		G-T= 1.000000
COI-3	A= 0.502193	A-C= 68.158957
	C= 0.146423	A-G= 670.873380
	G= 0.064287	A-T= 0.000000
	T= 0.287097	C-G= 69.091061
		C-T= 1568.590255
		G-T= 1.000000
H3-1	A= 0.277253	A-C= 0.807557
	C= 0.312833	A-G= 0.000000
	G= 0.318132	A-T= 0.000000
	T= 0.091782	C-G= 0.000000
		C-T= 1.345784
		G-T= 1.000000

continued...

H3-2	A= 0.290879	A-C= 0.000000
	C= 0.254542	A-G= 0.000000
	G= 0.218205	A-T= 0.000000
	T= 0.236374	C-G= 0.000000
		C-T= 0.000000
		G-T= 1.000000
H3-3	A= 0.097697	A-C= 4.846947
	C= 0.367040	A-G= 15.202173
	G= 0.394050	A-T= 8.519325
	T= 0.141212	C-G= 1.906088
		C-T= 8.647881
		G-T= 1.000000

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- Boniao R, Estoista R, de Goede R, Hansel C, Nuñez O, Lumactud R, **Aspe NM**, Poblete M, Roscom B and James SW. 2006. Soil Ecological Diversity and Relevant Interrelationships of Critical Resources in Mt. Malindang, Mis. Occ., Philippines. SEAMEO-SEARCA. Laguna, Philippines.
- Boniao R, Estoista R, Hansel C, de Goede R, Nuñez O, Roscom B, James SW, Lumactud R, Poblete M, and **Aspe NM**. 2006. Assessment of Soil Properties and Ecological Diversity in Mt. Malindang Range, Philippines. Society, Environment and Development: The Mt. Malindang Experience. SEAMEO-SEARCA. Laguna, Philippines.

Presentations:

- **Aspe N.** Earthworm sampling and field identification - Capability Building of Research Assistants, Local Partners, and Researchers on Primary Data Collection for Flora, Fauna and Soil Ecology in Mt. Malindang. Biodiversity Research Programme, SEAMEO-SEARCA. Oral presentation in English. August 2003. Oroquieta City, Philippines
- **Aspe N.** Diversity and distribution of earthworms in Mt. Malindang". 6th Annual National Scientific Convention of the Philippine Society for the Study of Nature. Oral presentation in English. May 2006. Kapalung, Davao del Norte, Philippines

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Papers:

- **Aspe NM**, Nuneza O and Torres MA. 2009. Diversity and Distribution of Earthworms in Mt. Malindang, Philippines. Journal of Nature Studies. 8: 59-67.
- Boniao R, **Aspe NM** and Van Ranst E. 2012. Ecological Properties of the Soil in Mt. Diwata Range, Philippines: A Physico-chemical and Biological Assessment. LAP Lambert Academic Publishing. ISBN 978-3-659-22675-5. 73 pp.

Presentation:

- **Aspe NM**. Perception of the constituents of Iligan City on the concept of urban forestry in the City. Science Seminar, 27th CNSM Science Week Celebration “CNSM Call for Environmental Awareness Curbing Natural Calamities”. Oral presentation in English. February 2012. Mindanao State University, Marawi City, Philippines

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- **Aspe NM** and James SW. 2014. New species of *Pheretima* (Oligochaeta: Megascolecidae) from the Mt. Malindang Range, Mindanao Island, Philippines. *Zootaxa*. 3881: 401-439. <http://dx.doi.org/10.11646/zootaxa.3881.5.1>.
- **Aspe NM** and James SW. 2015. New *Polypheretima* and *Pithemera* (Oligochaeta: Megascolecidae) species from the Mt. Malindang Range, Mindanao Island, Philippines. *Journal of Natural History*. <http://dx.doi.org/10.1080/00222933.2015.1021875>.
- **Aspe NM** and James SW. 2016. New species of *Pheretima*, *Amyntas*, *Polypheretima*, and *Pithemera* (Clitellata: Megascolecidae) from Mindanao and associated islands, Philippines. *Zoological Studies*. 55. [doi:10.6620/ZS.2016.55-08](https://doi.org/10.6620/ZS.2016.55-08).
- **Aspe NM**. 2016. Taxonomy and phylogeny of pheretimoid earthworms (Clitellata: Megascolecidae) from Mindanao and associated islands, Philippines. A PhD Dissertation. Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo City, Japan.
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- **Aspe NM**, Kajihara H, James SW. A molecular phylogenetic study of pheretimoid species (Megascolecidae) in Mindanao Island, Philippines. 6th International Barcode of Life Conference. Oral presentation in English. Aug. 18-21, 2015. University of Guelph, Guelph, Ontario, Canada
- **Aspe NM**, Kajihara H, James SW. A taxonomic and phylogenetic study of earthworms (Clitellata: Annelida) from Mindanao Island, Philippines. A Research meeting on taxonomy of Annelida: Previous works and subjects for further studies in eastern Asia. Oral presentation in English. Nov. 19-20, 2015. The University of Tokyo, Kashiwa City, Chiba, Japan

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