# Two New Species of Terrestrial Earthworms of the Genus *Amynthas* Kinberg, 1867 (Clitellata, Oligochaeta, Megascolecidae) from Northern Laos

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**ABSTRACT.**— This paper describes two new species of terrestrial earthworms belonging to the genus *Amynthas* from northern Laos; *A. panhai* **sp. nov.** and *A. oudomxayensis* **sp. nov.**, are described based on external morphology and anatomy. They were determined to be new species after comparison with the other members of their respective species groups based on their original descriptions and some reference materials from Biozentrum Grindel und Zoologisches Museum, Hamburg, and Chulalongkorn University, Museum of Zoology, Thailand. However, they differ from all other known species of *Amynthas* by a combination of the following characters: male field and genital marking pattern, shape of spermathecae, and prostate gland and duct.

KEYWORDS: Amynthas, earthworm, new species, Laos, taxonomy

#### INTRODUCTION

Amynthas is a genus consisting of more than 600 species of terrestrial earthworms in the family Megascolecidae. Central and Southeast Asian regions are recognized centers of biodiversity for this group of worms, as many of its members are native to this area; many species have been recorded recently and more are still being discovered (Aspe and James, 2016, 2017; Bantaowong et al., 2011, 2014, 2015, 2020; Beddard, 1900; Blakemore, 2011; Gates, 1972; Hong and James, 2004, 2008; James, 2004; Nguyen et al., 2020, 2022; Reynolds, 2009; Sims and Easton, 1972; Stephenson, 1931; Sun, 2013). Among Southeast Asian countries, Laos has the greatest species diversity of this genus, with more than 50 Amynthas species currently known (Hong, 2007, 2008, 2010, 2017, 2019; Hong et al., 2008, 2014a, b, 2018; Thai and Samphon, 1988, 1989, 1990a, b, 1991a, b).

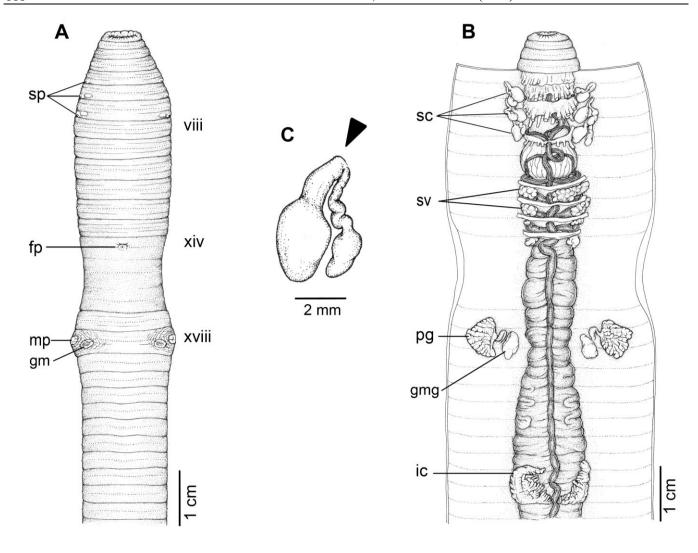
In April 2015, while examining earthworm specimens in the invertebrate collection of the Biology Department, Faculty of Science, National University of Laos, Vientiane, we found fifteen unidentified worm specimens belonging to the genus *Amynthas* which had been collected from northern Laos. After carefully examining these specimens under a stereomicroscope and comparing them with type material of closely related species, we concluded that these species were new to science.

#### MATERIALS AND METHODS

Specimens of terrestrial earthworms in the third author's collection from northern Laos were recently examined. The worms were killed in 30% (v/v) ethanol, photographed, transferred to 4% (w/v) formalin for approximately 12 hours, and then transferred to 70% (v/v) ethanol for longer term preservation and subsequent morphological studies. Animal procedures were approved by the Institutional Animal Care and Use Committee of Khon Khaen University (IACUC-KKU-32-65).

The descriptions of each species were made during observation under an Olympus SZX16 stereoscopic microscope. Drawings were made of the body segments, external characters and internal organs, as mentioned above, and are shown in Figures 1 and 3 for the new species. The number of segments and the body width and length were measured in both full adults and juveniles; measured data are presented as the range (min-max) and mean  $\pm$  one standard deviation. Holotype and paratype specimens were deposited in the Chulalongkorn University Museum of Zoology, Bangkok, Thailand (CUMZ) and the Biology Department, Faculty of Science, National University of Laos, Vientiane (BDNUL).

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



**FIGURE 1.** External and internal morphology of holotype (CUMZ 3832) of *Amynthas panhai* sp. nov. **A.** External ventral view, **B.** internal dorsal view, and **C.** spermatheca; black arrow indicates the connection of the spermatheca and spermathecal pore.

#### **RESULTS**

#### **Taxonomy**

#### Family Megascolecidae Rosa, 1891

#### Genus Amynthas Kinberg, 1867

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

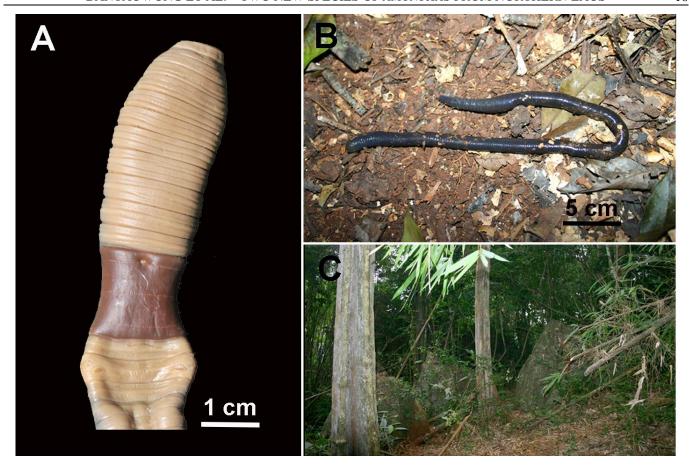
#### Amvnthas panhai sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:6D3B511B-426B-43FE-A599-51DC54C1C3FF (Figs 1, 2)

**Description of Holotype.** Dimensions: 376 mm by 17.0 mm at segment ix, 19.2 mm at segment xviii, 14.3 mm at clitellum; body cylindrical with 117 segments.

Number of annuli (secondary segmentation) per segment three in v-xiii. Setae regularly distributed around segmental equators, numbering 95 at vii, 135 at xx, 39 between male pore, setae formula AA:AB:ZZ:ZY =2:1:1.5:1 at xii. Single female pore at xiv. Prostomium epibolic. First dorsal pore at 13/14. Clitellum annuli xiv–xvi with setae on ventral site.

Male pores paired laterally on ventral of xviii, within shallow circular invagination of body wall, 0.40 circumference ventrally apart, distance between male pores 19 mm. Genital markings paired, ellipsoid shape, oriented transversely, and flattened on top; they are situated on the inner side of the male pores, and posterior to the setal line, as is shown in Figure 1A. Spermathecal pores 3 pairs at 5/6–7/8, slit-like, 0.48 body circumference, distance between spermathecal pores 24 mm. Segment vii bears a pair of genital markings which are slightly ellipsoidal, near the



**FIGURE 2.** Photographs of holotype (CUMZ 3832) of *Amynthas panhai* sp. nov. **A.** specimen showing the coloration of the ventral side after preservation with 4% (w/v) formalin and in 70% (v/v) ethanol, **B.** living specimen from the type locality showing coloration and, **C.** type locality of *Amynthas panhai* sp. nov.

spermathecal pores, and another one on the left-hand side on vi.

Septa, 5/6–7/8 very thickened; 8/9–9/10 aborted, 10/11–13/14 very thickened. Gizzard large behind 7/8. Intestinal origin in xv, no lymph glands observed. Typhlosole simple, lamelliform, poorly developed. Intestinal caeca paired at xxvii extending forward to xxv, simple with (incised margins) serrated form on ventral and dorsal margin. Hearts esophageal in x-xiii.

Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Pseudovesicles in xiii and xiv. Prostate glands racemose, paired xviii, large extending from xvii-xviii. Prostatic ducts loosely coiled in U-shape. Large sessile genital marking glands are in xviii corresponding to each external genital marking.

Ovaries paired in xiii. Spermathecae 3 pairs are situated in segments vi, vii, viii. Ampulla ovoid elongate in shape, and continuous with the duct which is of the same length as the ampulla. Diverticulum slender with elongated heart-shaped seminal chamber, the distal half of its stalk loosely coiled.

**Type locality.**— Evergreen forest, Sam Tai District, Houaphanh Province, Laos, 19°59'9.09"N, 104°51' 31"E, 1280 meters elevation (10<sup>th</sup> December 2008).

**Etymology.**— This species is named in honor of Prof. Dr. Somsak Panha who is the patron and driver of Thai earthworm taxonomy.

**Type Materials.**— Holotype: One adult (CUMZ 3832); Laos, Houaphanh Province, deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ).

**Diagnosis.**– Three pairs of spermathecal pores at 5/6–7/8, slit-like. Female pore mid-ventral on xiv. Male pores paired, extremely large, alate on xviii. Spermathecae large ovate ampulla, diverticulum with straight stalk and long zig-zag terminal chamber. Genital markings paired close to the male pores at xviii, each with sessile genital marking glands. Testis sacs paired in x and xi. Seminal vesicles paired in xi, xii. Prostate glands paired in xviii-xviii.

Remarks.— This new species is a huge earthworm, with body length of 376 mm and width of 17.0 mm, and was found in Houaphanh Province. Previously, 14 species had been recorded from this area (Hong, 2010, Hong et al., 2014a, 2014b, 2018), but most are small to medium in size, ranging in length from 40 to 153 mm. Comparing the new species with regional species in the gracilis group, namely A. defecta (Gates, 1930), A. gracilis (Kinberg, 1867), A. inclara (Gates, 1932), A munglonganus (Thai & Tran, 1986) and A. munglongoides Nguyen, Lam & Nguyen, 2016, it can be distinguished from the first three species by the larger body (376 mm by 17.0 mm); A. defecta, A. gracilis and A. inclara range in length from 68-254 mm and in width from 3-10 mm. The present species appears to be closely related to A. munglonganus and A. munglongoides from Vietnam based on its large size, three pairs of spermathecal pores in 5/6-7/8, genital marking at spermathecal pores and male pore region. However, Amynthas munglonganus differs from the new species by the shape of the male pores, sessile genital markings corresponding to each external genital marking in gland present in v, vi and viii and spermathecal ampulla duct short. Amynthas munglongoides can be distinguished from the new species in having first dorsal pore in 11/12, three pairs of genital markings at male pore, ampulla large with very short diverticulum.

#### Amynthas oudomxayensis sp. nov.

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(Fig. 3)

**Description of Holotype.**— Dimensions: 313 mm by 15.0 mm at segment ix, 12.0 mm at segment xviii, 10.0 mm at clitellum; body cylindrical with 142 segments. Number of annuli (secondary segmentation) per segment three in vi-xiii. Setae regularly distributed around segmental equators, numbering 61 at vii, 91 at xx, 21 between male pores, setae formula AA:AB:ZZ:ZY =1:1:1 at xii. Single female pore at xiv. Prostomium epibolic. First dorsal pore at 12/13. Clitellum annuli xiv—xvi with setae on ventral site.

Male pores paired in xviii, within shallow circular invagination of body wall, 0.24 circumference ventrally apart, distance between male pores 10 mm. Genital marking two pairs, ellipsoidal shape transversely and flattened on top, and situated on intersegment 17/18 and 18/19 in line with male pores, and posterior to the setal line. Spermathecal pores 2 pairs at 7/8–8/9, slit-like, 0.35 circumference ventrally apart, distance between spermathecal pores 14 mm.

Septa, 5/6–7/8 very thickened; 8/9–9/10 aborted, 10/11–13/14 very thickened. Gizzard large behind 7/8. Intestinal origin in xv, no lymph glands observed.

Typhlosole simple, lamelliform, poorly developed. Intestinal caeca paired at xxvii extending forward to xxiii, simple. Hearts esophageal in x-xiii.

Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Prostate glands well developed, paired in xviii, large extending from xvi-xxii. Prostatic ducts hairpin. Large sessile genital marking glands are in xviii corresponding to each external genital marking.

Ovaries paired in xiii. Spermathecae 2 pairs, situated in segments viii and ix. Ampulla heart shaped or pear shaped with a short stalk. Diverticulum long slender, becoming gradually narrow toward the terminal end, with several alternating incomplete deep circular grooves on surface at proximal half of straight stalk, terminal chamber with elongated knob.

**Variation.**— The holotype measures 313 mm in body length with 142 segments; the fourteen paratypes range in size from 167–204 mm (179.6±10.7 mm) body length with 135–141 segments.

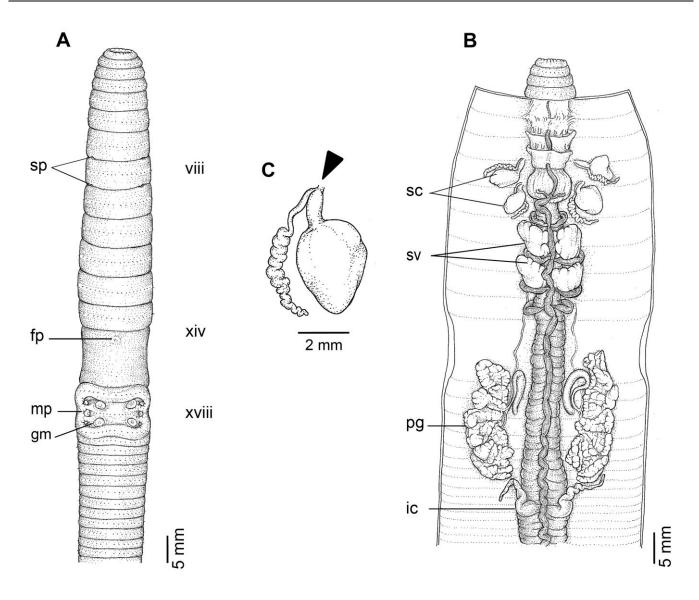
**Type locality.**— Hauy Sang Kham village, Pak Beng District, Oudomxay Province, Laos. 20°02'23.0"N 101°05'46.1"E, 1550 meters elevation.

**Etymology.**— This species was named after its type locality, Oudomxay Province.

**Type Materials.**— Holotype: One adult (BDNUL 0054) Laos, Oudomxay Province, and 14 paratypes (juvenile) are deposited in the Biology Department, Faculty of Sciences, National University of Laos, Vientiane.

**Diagnosis.**— Two pairs spermathecal pores at 7/8–8/9, slit-like. Female pore mid-ventral on xiv. Male pores paired, within shallow circular invagination on xviii. Spermathecae heart-shaped ampulla, diverticulum long zig-zag with elongated terminal chamber. Genital markings two pairs, ellipsoidal shape at 17/18 and 18/19. Testis sacs paired in x and xi. Seminal vesicles paired in xi, xii. Prostate glands paired in xvi-xxii.

**Remarks.**— Amynthas oudomxayensis sp. nov. is a quadrithecate earthworm belonging to the aeruginosus species group in Sims and Easton (1972), which consists of more than 50 species and subspecies. Among these, the taxa that have body width more than 10 mm and present genital marking near the male pore include A. bonthainensis (Benham, 1896), A. dangi (Thai, 1984) and A. jampeana fumigata Michaelsen, 1899. The new species can be separated easily from the first of these by the male pore region; the male pore of



**FIGURE 3.** External and internal morphology of holotype (BDNUL 0054) of *Amynthas oudomxayensis* sp. nov. **A.** External ventral view, **B.** internal dorsal view, and **C.** spermatheca; black arrow indicates the connection of the spermatheca and spermathecal pore.

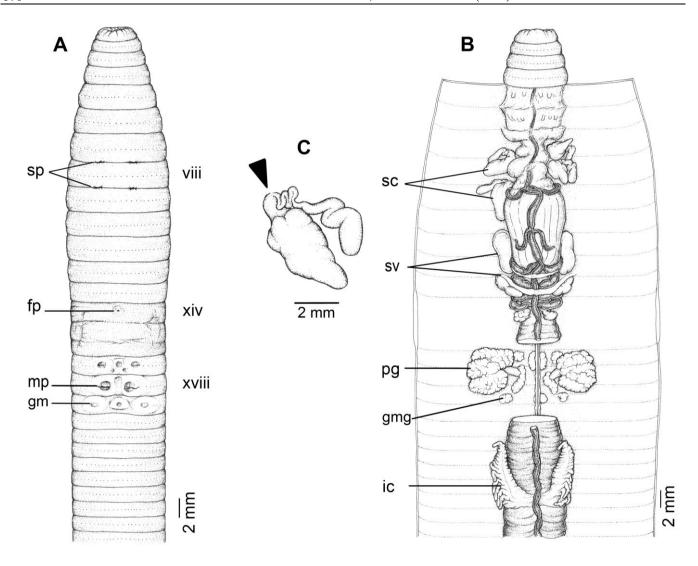
A. bonthainensis is a transverse slit on a round swollen area and there are papilla genital markings on xvii, xix, xx. Moreover, A. bonthainensis has a compact prostate gland with short duct. This new species is fairly similar to A. dangi from Vietnam and A. jampeana fumigata from Celebes due to its large size, and having genital markings at the male pore region. However, Amynthas dangi differs from the new species in having a genital marking close to the male pore and an additional three pairs of genital markings on ventral xviii and xix, and the ampulla duct is almost absent, whereas A. jampeana fumigata is distinguished by 0.07 body circumference with distance between male pores 2.5 mm and genital markings in xvii and xix; in contrast, the new species exhibits 0.24 body circumference with distance between male pores 10 mm and genital markings on intersegments 17/18 and 18/19.

## Amynthas jampeana fumigata Michaelsen, 1899 (Fig. 4)

Amynthas jampeana fumigata Michaelsen, 1899: 224 Type locality: Celebes, Indonesia. Sims and Easton, 1972: 234.

**Material examined.**— Syntype ZMH (V 5175; Fig. 4): one adult in 70% ethanol.

**Remarks.**– Amynthas jampeana fumigata differs from A. oudomxayensis sp. nov. by having genital markings in xvii and xix corresponding with genital marking glands, and serrate intestinal caeca and elongate sac of spermathecae. The distance between male pores is 2.5 mm with 0.07 body circumference.



**FIGURE 4.** External and internal morphology of syntype (ZMH V 5175) of *Amynthas jampeana fumigata*. **A.** External ventral view, **B.** internal dorsal view and, **C.** spermatheca; black arrow indicates the connection of the spermatheca and spermathecal pore.

#### DISCUSSION

Of the 96 species of earthworms currently known to exist in Laos, more than half are in the Oriental genus Amynthas Kinberg, 1867 of the family Megascolecidae, and most of these were recently discovered (Hong, 2008, 2010, 2017, 2019; Hong et al., 2008, 2014a, b, 2018). Members of the genus Amynthas are terrestrial earthworms, occurring in natural forests, agriculture fields, paddy fields, residential areas or even near rivers, such as A. mekongianus (Cognetti, 1922). Mostly, individuals are small to medium in size, ranging in length of 40-150 mm and width of 2-10 mm, but two new species A. panhai sp. nov. and A. oudomxayensis sp. nov. are significantly larger (376 mm by 17 mm and 313 mm by 15.0 mm, respectively). Indeed, A. panhai sp. nov. is the largest terrestrial earthworm that has been recorded from northern Laos and adjacent areas. However, only one specimen of *A. panhai* new species has been collected so far, suggesting that it is rare and has a distribution restricted to the northern part of Laos. The destruction of the natural forest due to human activities can threaten the habitat of these earthworms; therefore, the protection of remaining natural habitat is vital to conserve and protect the biodiversity of *Amynthas*. Further studies, employing molecular techniques and ecological analysis are strongly recommended to provide further understanding of the relationships among the *Amynthas* species of Laos.

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