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Two new earthworm species of the genus *Amynthas* (Oligochaeta: Megascolecidae) from central Taiwan, with comments on some recent species assignments in *Amynthas* and *Metaphire*

HUEI-PING SHEN[1], CHIH-HAN CHANG[2,3] & WEN-JAY CHIH[2]
[1]*Zoology Division, Endemic Species Research Institute, 1 Ming-shen East Road, Jiji, Nantou 552, Taiwan*
[2]*Department of Life Science, National Taiwan University, 1 Roosevelt Road, Section 4, Taipei 106, Taiwan*
[3]*Corresponding author. E-mail: chihhanchang@ntu.edu.tw, Tel: 886-2-33662456, Fax: 886-2-23636837*

Abstract

This study describes two new species of earthworms belonging to the genus *Amynthas* (Oligochaeta: Megascolecidae) from central Taiwan. They are named *Amynthas luridus* **sp. nov.** and *Amynthas ruiyenensis* **sp. nov.** Both species are octothecal with the former found at elevations of 1500–2300 m and the latter at an elevation of 2200 m from the Central Mountain Range. In addition, DNA barcodes are made available for the first time for the following species: *Amynthas catenus* Tsai *et al*., 2001, *Amynthas exiguus aquilonius* Tsai *et al*., 2001, *Amynthas proasacceus* Tsai *et al*., 2001, *Amynthas hohuanmontis* Tsai *et al*., 2002, *Amynthas tessellatus* Shen *et al.*, 2002, *Amynthas fenestrus* Shen *et al.*, 2003, *Amynthas tantulus* Shen *et al.*, 2003, and *Amynthas uvaglandularis* Shen *et al.*, 2003. Furthermore, *Amynthas exiguus* ssp. *aquilonius* Tsai *et al*., 2001 distributed at elevations of 2200–3000 m in the Central Mountain Range is elevated to species level, as *A. aquilonius* Tsai *et al*., 2001. The highest altitude record so far for the exotic *Eukerria saltensis* (Beddard, 1895) in Taiwan, 2200 m above sea level, is documented. Moreover, some recent assignments of species to *Amynthas* and *Metaphire* and synonymies of names are critically discussed. It is argued that idiosyncratic genus concepts, inadequate species comparisons, and unexplained synonymies should be avoided. A hitherto undetected and possibly monophyletic species group of mainly Korean *Amynthas* species in the *A. tokioensis*-group is indicated, characterized by numerous genital papillae around each spermathecal pore and male porophore, large ampullae, long diverticula, large prostate glands, and manicate intestinal caeca. The names *A. bimaculata*, *A. silvatica* and *A. surcata* (Ishizuka, 1999), as well as *A. odaesanensis*, *A. righii, A. fasciiformis,* and *A. sanchongensis* Hong & James, 2001, previously declared as junior synonyms of *A. tappensis* (Ohfuchi, 1935), are revalidated.

Key words: Earthworms, Clitellata, *Amynthas*, new species, Taiwan

Introduction

A total of 19 new species and subspecies of earthworms have been reported from central Taiwan since 1999 (Tsai *et al*. 1999, 2000, 2001, 2002, 2004b, 2007; Shen *et al*. 2002, 2003). Among them, *Metaphire taiwanensis* Tsai *et al*., 2004 with a live body length of 860 mm is the largest earthworm ever found from the island of Taiwan. Tsai *et al*. (2004a) categorized these earthworms into five groups based on their altitudinal distributions. Since then, the categories of Tsai *et al*. (2004a) have been supported and supplemented with additional data (Shen 2018 and this study), and can be updated as follows:

(1) Species widely distributed from the hills to the high mountains, including *Amynthas wulinensis* Tsai *et al*., 2001 at elevations of 850–3200 m, *Amynthas tessellatus* Shen *et al.*, 2002 at elevations of 700–3200 m and *Metaphire bununa* Tsai *et al*., 2000 at elevations of 300–3000 m.
(2) Species distributed in the high mountains, containing *Amynthas hohuanmontis* Tsai *et al*., 2002 occurring at Mt. Hohuan (3000 m) only, *Amynthas catenus* Tsai *et al*., 2001 and *Amynthas exiguus aquilonius* Tsai *et al*., 2001 at elevations of 2200–3000 m, *Amynthas proasacceus* Tsai *et al*., 2001 at elevations of 2100–3000 m, and *Amynthas uvaglandularis* Shen *et al.*, 2003 at elevations of 1800–3000 m.

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(3) Species distributed at elevations of 1800–2300 m, consisting of *Amynthas wangi* Shen *et al.*, 2003 at an elevation of 2300 m only, *Amynthas tantulus* Shen *et al.*, 2003 at elevations of 2200–2300 m, *Amynthas fenestrus* Shen *et al.*, 2003 at elevations of 1800–2300 m, and *M. taiwanensis* at elevations of 1800–2100 m.

(4) Species distributed at elevations of 140–2225 m, comprising *Amynthas tungpuensis* Tsai *et al.*, 1999 at elevations of 140–2225 m [Note: *A. tungpuensis* can reach an elevation up to 2737 m in southern Taiwan (Shen *et al*. 2016)], *Amynthas nanshanensis* Shen *et al.*, 2003 at elevations of 800–1800 m, *Amynthas penpuensis* Shen *et al*., 2003 at elevations of 200–1700 m [Note: *A. penpuensis* can reach an elevation up to 2000 m in southern Taiwan (unpublished data)], and *Amynthas bilineatus* Tsai & Shen, 2007 at an elevation of 1000 m only.

(5) Species distributed at elevations below 1000 m, encompassing *Amynthas binoculatus* Tsai *et al*., 1999 and *Amynthas sexpectatus* Tsai *et al*., 1999.

Also, it is worth mentioning that *A. tessellatus* has two distinct subspecies, *A. t. tessellatus* and *A. t. paucus*, with the former found at elevations of 1000–3200 m and the latter at elevations of 700–1100 m (Shen *et al.* 2002). *A. t. tessellatus* has genital papillae associated with sessile accessory glands, whereas *A. t. paucus* has genital papillae with stalked accessory glands. Apparently, the "backbone" of the island of Taiwan, the Central Mountain Range, provides the evolutionary theater for the speciation of these earthworms. In addition, *A. catenus*, *A. proasacceus*, *A. hohuanmontis* and *A. tungpuensis* have variable numbers of spermathecae, and *A. bilineatus* even lacks spermathecae entirely. Therefore, with the discovery of these species, the limitation of using the number and position of spermathecae as keys to define species-groups in pheretimoid earthworms by Sims and Easton (1972) has become obvious (Shen *et al*. 2012). Furthermore, *A. catenus* and *A. hohuanmontis* were proved to be automictic (meiotic) parthenogens (Shen *et al*. 2012), while the former is polyploid with ploidy levels corresponding to the degree of degeneration of its reproductive organs (Shen *et al*. 2011). All these findings broaden our knowledge of the biodiversity of the earthworm fauna in Taiwan.

Earthworm specimens collected from central Taiwan prior to 2005 were preserved in formalin and not available for DNA sequencing. Fresh specimens were obtained in subsequent collections and DNA barcodes of *A. wulinensis* were reported in Chang *et al*. (2007), those of *A. binoculatus*, *M. bununa* and *M. taiwanensis* in Chang *et al*. (2008), and those of *A. penpuensis* and *A. tungpuensis* in Shen *et al*. (2014) and in Shen *et al*. (2016), respectively. This study describes two new species, *Amynthas luridus* **sp. nov.** and *Amynthas ruiyenensis* **sp. nov.**, from central Taiwan. Both species are octothecal with the former found from elevations of 1500–2300 m and the latter at an elevation of 2200 m from the Central Mountain Range. Except for PCR failure of the type specimen of *A. ruiyenensis*, DNA barcodes (a 658-base-pair section of the mitochondrial cytochrome *c* oxidase subunit I gene) are reported here from type specimens of *A. luridus* and from fresh material of *A. catenus*, *A. exiguus aquilonius*, *A. proasacceus*, *A. hohuanmontis*, *A. tessellatus*, *A. fenestrus*, *A. tantulus*, and *A. uvaglandularis* (Table 1). DNA barcodes of these species are made available for the first time. As for *A. bilineatus*, *A. nanshanensis* and *A. wangi*, no fresh material was acquired.

Amynthas exiguus aquilonius was considered a geographical subspecies north of *Amynthas exiguus exiguus* (Gates, 1930), as compared to another subspecies *Amynthas exiguus austrinus* (Gates, 1932) located to the south of *A. e. exiguus* (Tsai *et al*. 2001). Characters of the three taxa were compared in Tsai *et al*. (2001). Besides the lower setal numbers, much shorter diverticula, and the small, widely paired presetal and/or postsetal papillae in both preclitellar and postclitellar regions which separate *A. e. aquilonius* from the other two taxa (Tsai *et al*. 2001), the position of first dorsal pore in 6/7 of *A. e. aquilonius* also shows considerable difference from that in 12/13 of both *A. e. exiguus* and *A. e. austrinus*. Moreover, as mentioned previously, *A. e. aquilonius* is distributed at elevations of 2200–3000 m in the Central Mountain Range, Taiwan, while *A. e. exiguus* is found from lowlands to about 1219 m [= 4000 feet (Gates 1972)] and *A. e. austrinus* from lowlands to about 335 m [= 1100 feet (Gates 1972)] in Myanmar, Thailand and Vietnam (Gates 1972; Nguyen *et al*. 2016b, 2017). It is evident that *A. e. aquilonius* is originated from within-island speciation. Accordingly, *A. e. aquilonius* should be elevated to species level, namely, *A. aquilonius*.

The exotic earthworm *Eukerria saltensis* (Beddard, 1895) was first collected from Nantou County, central Taiwan in 1999 (Shen *et al*. 2008). It was recorded from an elevation of 228 m from Yunlin County, southwestern Taiwan and an elevation of 1770 m from the Taipingshan National Forest Recreation Area, Ilan County, northeastern Taiwan as well (Shen *et al*. 2008). *E. saltensis* was found at an elevation of 2200 m from Ruiyen Creek Nature Reserve, Nantou County in 2009 (unpublished data). So far, it is the highest altitude record for this species in Taiwan.

Compared with the exotic *Pontoscolex corethrurus* (Müller, 1856) dominant in Taiwan from coastal plains to an elevation up to about 1000 m, *E. saltensis* seems to be even more adapted to the diverse local climate and environment here in Taiwan.

TABLE 1. GenBank accession numbers of COI sequences of specimens reported in this study.

Species	Locality	Voucher no.	GenBank accession no.
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY003	MK251475
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY004	MK251476
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY005	MK251477
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY006	MK251478
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY007	MK251479
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY008	MK251480
Amynthas aquilonius Tsai *et al*., 2001	Ruiyen Creek Nature Reserve, Nantou County[2]	RY009	MK251481
Amynthas catenus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	Cat1	MK251482
Amynthas catenus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	Cat2	MK251483
Amynthas catenus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	Cat5	MK251484
Amynthas catenus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	Cat6	MK251485
Amynthas catenus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	Cat8	MK251486
Amynthyas fenestrus Shen *et al*., 2003	Ruiyen Creek Nature Reserve, Nantou County[1]	RY010	MK251487
Amynthyas fenestrus Shen *et al*., 2003	Ruiyen Creek Nature Reserve, Nantou County[1]	RY011	MK251488
Amynthas hohuanmontis Tsai *et al*., 2002	Mt. Hohuan, borders of Hualien and Nantou counties[1]	HH1	MK251489
Amynthas hohuanmontis Tsai *et al*., 2002	Mt. Hohuan, borders of Hualien and Nantou counties[1]	HH2	MK251490
Amynthas hohuanmontis Tsai *et al*., 2002	Mt. Hohuan, borders of Hualien and Nantou counties[1]	HH7	MK251491
Amynthas hohuanmontis Tsai *et al*., 2002	Mt. Hohuan, borders of Hualien and Nantou counties[1]	HH9	MK251492
Amynthas luridus **sp. nov.**	Ruiyen Creek Nature Reserve, Nantou County[1]	RY018	MK251502
Amynthas luridus **sp. nov.**	Ruiyen Creek Nature Reserve, Nantou County[1]	RY020	MK251503
Amynthas luridus **sp. nov.**	Ruiyen Creek Nature Reserve, Nantou County[1]	RY021	MK251504
Amynthas proasacceus Tsai *et al*., 2001	Mt. Hohuan, borders of Hualien and Nantou counties[1]	pro	MK251495
Amynthas proasacceus Tsai *et al*., 2001	Road 8, Nantou County	East434	MK251496
Amynthas tantulus Shen *et al*., 2003	Ruiyen Creek Nature Reserve, Nantou County[1]	RY012	MK251497
Amynthas tantulus Shen *et al*., 2003	Ruiyen Creek Nature Reserve, Nantou County[1]	RY013	MK251498
Amynthas tessellatus Shen *et al*., 2002	Mt. Hohuan, borders of Hualien and Nantou counties	tes5	MK251499
Amynthas tessellatus Shen *et al*., 2002	Mt. Hohuan (East Peak), borders of Hualien and Nantou counties	tes6	MK251500
Amynthas tessellatus Shen *et al*., 2002	Sunlinksea Forest Recreation Area, Nantou County	tes7	MK251501
Amynthas uvaglandularis Shen *et al*., 2003	Meifong, Nantou County	uva2	MK251493
Amynthas uvaglandularis Shen *et al*., 2003	Mt. Hohuan, borders of Hualien and Nantou counties	uva5	MK251494

[1]Type locality.

[2]Paratype locality.

Material and methods

Earthworms were collected during 2005–2016 from central Taiwan. They were anesthetized in 10% ethyl alcohol and then preserved in 95% ethyl alcohol, and investigated with a stereoscopic microscope and dissected dorsally. A subset of the specimens of *A. aquilonius*, *A. catenus*, *A. fenestrus*, *A. hohuanmontis*, *A. proasacceus*, *A. tantulus*, *A. tessellatus*, *A. uvaglandularis* and the two new species were selected for molecular analysis (Table 1). Muscle tissues were taken from the posterior 10–20 segments of these specimens and then preserved in 95% ethyl alcohol at -20 °C. DNA extraction was conducted using the Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotec, Pingtung, Taiwan). Polymerase chain reaction for COI was carried out using the primers LCO1490 and HCO2198 (Folmer *et al*. 1994) in a 50-µl total volume with 1 cycle at 94 °C for 1 min, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 54 °C, and extension for 50 s at 72 °C, with a final extension at 72 °C for 10 min. Sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, V3.1 and analyzed on an ABI 3730 XL DNA analyzer (Applied Biosystems, CA, USA). The sequences obtained are available under GenBank accession numbers MK251475–MK251504. COI sequences of *Amynthas hongyehensis* Tsai & Shen, 2010, *Amynthas amis* Shen, 2012 and *Amynthas dinghuensis* Shen & Chih, 2016 were retrieved from GenBank. All analyses were done using *A. corticis* as outgroup. COI sequences were analyzed following Chang & Chen (2005). Briefly, sequences were aligned using the default settings of ClustalX 2.0 (Larkin *et al*. 2007). Neighbor-joining analyses were conducted using Kimura's two-parameter model (Kimura 1980) in MEGA7 (Kumar *et al*. 2016) with 1000 bootstraps to evaluate the robustness of clades. All the vouchers and earthworm specimens are deposited at the Taiwan Endemic Species Research Institute (TESRI), Jiji, Nantou, Taiwan. Catalogue numbers are given in the species section, together with voucher numbers of barcoded specimens (Table 1). Note that each voucher number refers to one specimen only, whereas TESRI catalogue numbers may refer to more than one specimen.

Systematics

Family Megascolecidae

***Amynthas luridus* Shen & Chang, sp. nov.**
(Figure 1)

Holotype: Clitellate (mature) specimen (97 mm in total length, dissected), from roadside ditches in Ruiyen Creek Nature Reserve, Nantou County, 24°06'47.99"N, 121°10'10.93"E, 1526 m asl, 15 April 2009, H.-P. Chen (TESRI-O-H-54; RY021).

Paratype: 1 aclitellate, from roadside slopes in Ruiyen Creek Nature Reserve, Nantou County, 24°06'35.85"N, 121°11'20.19"E, 2198 m asl, 26 March 2009, T.-J. Lin & D.-H. Chen (TESRI-O-P-48; RY020).

Other material. 1 clitellate (dissected) and 5 aclitellates (one dissected) from Mt. Beidongyen, Nantou County, 1800 m asl, 8 December 1999, C.-F. Tsai, S.-C. Tsai, P.-H. Ho & H.-P. Shen (TESRI-O-1999-29-6); 1 aclitellate from Mt. Beidongyen, Nantou County, 24°04'59.05"N, 121°08'12.18"E, 1817 m asl, 19 August 2005, T.-L. Huang, I.-M. Hsiao & H.-P. Shen (TESRI-O-2005-9-3); 1 clitellate (RY019, PCR failed) from roadside ditches in Ruiyen Creek Nature Reserve, Nantou County, 24°06'14.29"N, 121°10'34.01"E, 2195 m asl, 25 March 2009, T.-J. Lin & D.-H. Chen (TESRI-O-2009-6-5); 1 clitellate (RY018) from ditches around forestry station in Ruiyen Creek Nature Reserve, Nantou County, 24°06'29.76"N, 121°11'24.07"E, 2308 m asl, 25 March 2009, M.-H. Shen (TESRI-O-2009-7).

Diagnosis. Length (clitellates) 83–107 mm. Segments numbering 94–132. Setae 45–59 in VII, 49–65 in XX, 9–13 between male pores. First dorsal pore in 12/13. Clitellum XIV–XVI. Spermathecal pores four pairs in 5/6–8/9, 0.28–0.32 body circumference ventrally apart. Male pores 0.26–0.27 body circumference ventrally apart in XVIII, each on a round porophore about 0.5 mm in diameter, with an anterior and/or a posterior genital papillae. Additional papillae often widely paired in postsetal XVII or occasionally paired in presetal XIX. Spermathecae four pairs in VI–IX. Seminal vesicles vestigial, two pairs in XI and XII. Prostate glands lobed, paired in XVII–XIX. Prostatic ducts small, short, C-shaped in XVIII. Accessory glands sessile or stalked, corresponding to external genital papillae.

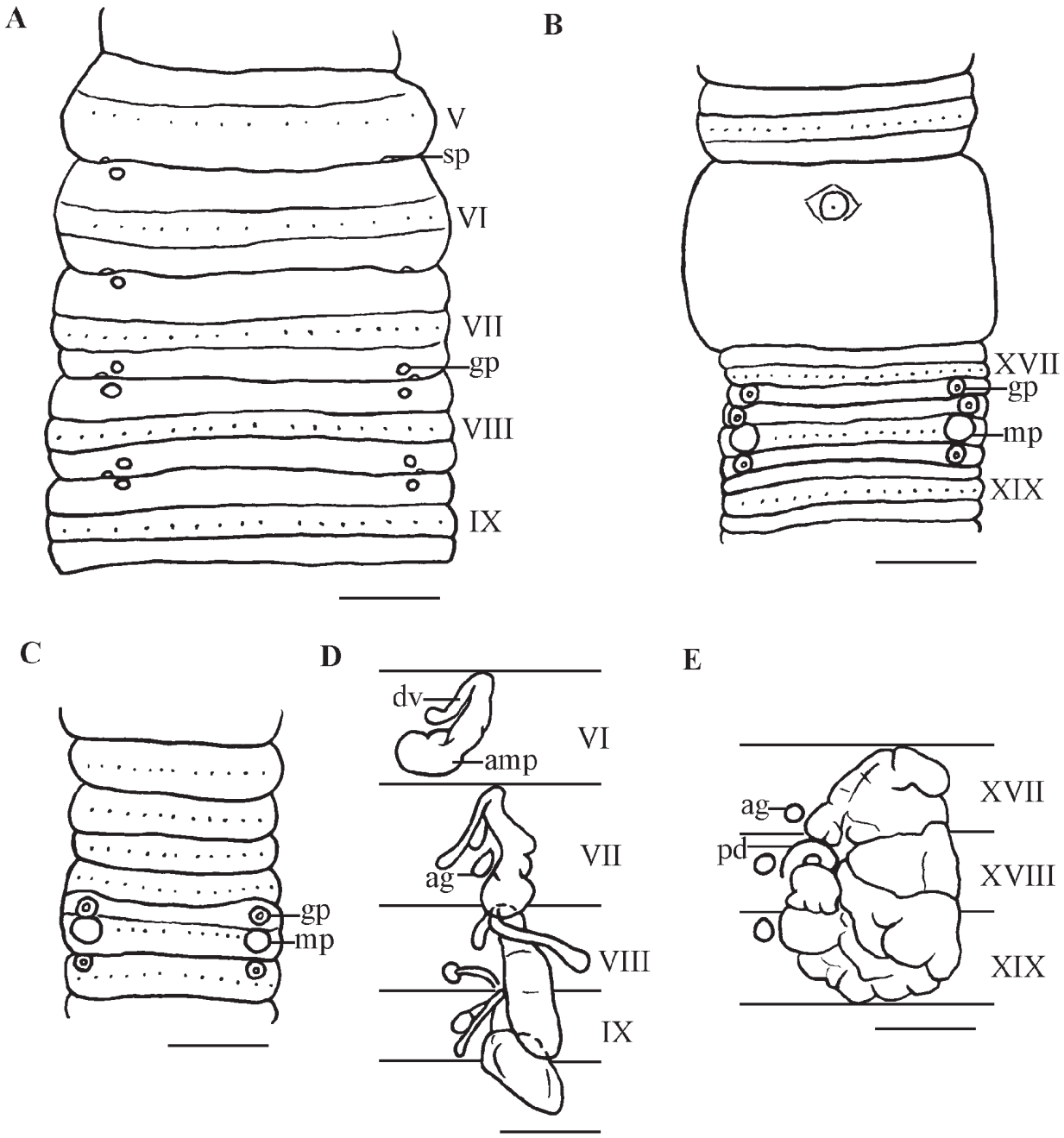

FIGURE 1. *Amynthas luridus* **sp. nov. A.** Ventral view of spermathecal pore region of Holotype (TESRI-O-H-54) (sp, spermathecal pore; gp, genital papilla). **B.** Ventral view of male pore region of holotype (mp, male porophore). **C.** Ventral view of male pore region of paratype. **D.** Dorsal view of right spermathecae of holotype (amp, ampulla; dv, diverticulum; ag, accessory gland). **E.** Dorsal view of right prostate gland of holotype (pd, prostatic duct). Scale bars = 1 mm.

Description. External: Length (clitellates) 83–107 mm, weight 0.98–1.54 g in 95% ethanol. Segments numbering 94–132. Clitellum XIV–XVI, setae and dorsal pores absent, 2.83–4.22 mm in length and 3.47–4.38 mm in width. Prostomium epilobous. Three annuli (secondary segments) per segment in VI–XIII. Setal number 45–59 in VII, 49–65 in XX, and 9–13 between male pores in XVIII. First dorsal pore in 12/13. Spermathecal pores four pairs in intersegmental furrows of 5/6–8/9, distance between paired pores 0.28–0.32 body circumference ventrally apart, each pore small, often with a small papilla posteromedial and another anteromedial to each pore, papilla 0.25–0.3 mm in diameter (Fig. 1A). No other genital papillae in the preclitellar region. Female pore single, mid-ventral in XIV. Male pores inconspicuous, paired in XVIII, 0.26–0.27 body circumference ventrally apart, each situated on a round porophore about 0.5 mm in diameter. Genital papillae anterior and/or posterior to male pores, paired, in

line with and smaller than male porophore. Additional papillae often widely paired in line with male porophore in postsetal XVII or occasionally paired in presetal XIX. For all 11 specimens examined, male pores with anterior and posterior papillae for two specimens and one of them (RY021) with an additional pair in postsetal XVII (Fig. 1B); male pores with anterior papillae for two specimens and one of them (RY020) with an additional pair in presetal XIX (Fig. 1C); male pores with posterior papillae for seven specimens and five of them with an additional pair in postsetal XVII. Each papilla round, with a slightly concave center, about 0.35 mm in diameter. Genital papillae absent in the mid-ventral region. Live worms white. Preserved specimens white and slightly darker on clitellum.

Internal: Septa 5/6–7/8 thick, 8/9/10 absent, 10/11 thick, 11/12–13/14 muscular. Nephridial tufts on anterior faces of 5/6/7. Gizzard large, round in VIII–X. Intestine enlarged from XVI. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXIV or XXIII. Esophageal hearts paired in XI–XIII. Spermathecae four pairs in VI–IX (Fig. 1D), ampulla elongated oval-shaped, surface wrinkled, 1.26–1.45 mm long and 0.6–0.84 mm wide, spermathecal duct stout, 0.3–0.6 mm in length. Diverticulum stalk slender, seminal chamber rudimentary or absent, not iridescent, 0.8–1.32 mm in total length. Accessory glands sessile or stalked, 0.3–0.5 mm in total length, each corresponding to external genital papilla. Holandric. Testes small, round, two pairs in ventrally joined sacs in X and XI. Seminal vesicles vestigial, two pairs in XI and XII, occupying less than half of the segmental compartment. Prostate glands paired in XVII–XIX (Fig. 1E), wrinkled and lobed. Prostatic ducts short, small, C-shaped in XVIII. Accessory glands round, sessile, about 0.3 mm in diameter, corresponding to external genital papillae.

DNA barcodes. GenBank accession numbers MK251504 (RY021, holotype), MK251503 (RY020, paratype) and MK251502 (RY018) (Table 1).

Etymology. The name *luridus* refers to the white, pale body color of this species.

Remarks. The postclitellar genital papilla arrangement of *Amynthas luridus* **sp. nov.** looks somewhat similar to that of *A. aquilonius* from central Taiwan, *Amynthas hongyehensis* Tsai & Shen, 2010 and *Amynthas amis* Shen, 2012 from eastern Taiwan, and *Amynthas dinghuensis* Shen & Chih, 2016 from southwestern Taiwan. *A. aquilonius* has postclitellar papillae widely paired in line with male porophore in presetal and postsetal XVII–XX (Tsai *et al*. 2001); *A. hongyehensis* has postclitellar papillae widely paired in line with male porophore in presetal XVIII–XX (Tsai *et al*. 2010; Shen 2012); *A. amis* occasionally has a papilla antero-medial to each male porophore (Shen 2012); *A. dinghuensis* has postclitellar papillae widely paired in line with male porophore in postsetal XVII and presetal XIX (Shen *et al*. 2016). These species are octothecal with four pairs of spermathecae in VI–IX (for an exception in *A. hongyehensis* see below). Their characters are compared in Table 2. All four species are morphologically distinguishable from *A. luridus*: *A. aquilonius* is much smaller, and has fewer segments, lower setal number, anteriorly placed first dorsal pore in 6/7, large seminal vesicles and large prostate glands (Tsai *et al*. 2001); *A. hongyehensis* is much larger, and has variable numbers of spermathecae from three pairs in VII–IX to four pairs in VI–IX with normal, iridescent diverticula, and large testes, large seminal vesicles and large prostate glands (Tsai *et al*. 2010; Shen 2012); *A. amis* has fewer setae, preclitellar genital papillae but no postclitellar papillae, spermathecae with normal, iridescent diverticula, and large testes, large seminal vesicles and large prostate glands (Shen 2012); *A. dinghuensis* is much smaller, and has fewer setae, spermathecae with normal, iridescent diverticula, and large testes, large seminal vesicles and large prostate glands (Shen *et al*. 2016). All these species are also genetically distinct (Fig. 2). Additionally, the GenBank accession number of the holotype of *A. amis* reported in Shen (2012) was erroneous and should be corrected to JX290409.

The genital papilla arrangement in the male pore region of *A. luridus* is also similar to that of *Amynthas oyamai* (Ohfuchi, 1937) from northern Japan, *Amynthas morii* (Kobayashi, 1938) from Korea, *Amynthas dangi* (Thai, 1984) from Vietnam, and *Amynthas nametensis* Hong *et al*., 2018 from Laos. *A. oyamai* has a papilla antero-medial to each male porophore (Ohfuchi 1937); *A. morii* has a papilla anterior to each male porophore (Kobayashi 1938b); *A. dangi* has papillae widely paired in line with male porophore in presetal XVIII–XIX and a papilla medial to each male pore (Thai 1984; Nguyen *et al*. 2016a); *A. nametensis* has papillae widely paired in line with male porophore in presetal XVIII–XIX (Hong *et al*. 2018). Like *A. luridus*, *A. oyamai* and *A. morii* are also octothecal and have small or little developed seminal vesicles and small, short prostatic duct (Ohfuchi 1937; Kobayashi 1938a, b). However, their characters of larger body size, lower setal numbers, absence of postclitellar genital papillae in XVII and XIX, and absent or poorly developed prostate glands are different from those of *A. luridus* (Table 2). As to *A. dangi*, its characters of large body size (length > 300 mm), much higher setal numbers (59 setae in segment II and more than 100 setae per segment after segment VIII) and two pairs of spermathecal pores in 7/8/9 (Thai 1984; Nguyen *et al*. 2016a) clearly separate it from *A. luridus*. The body size and setal numbers of *A. nametensis* are close to those of

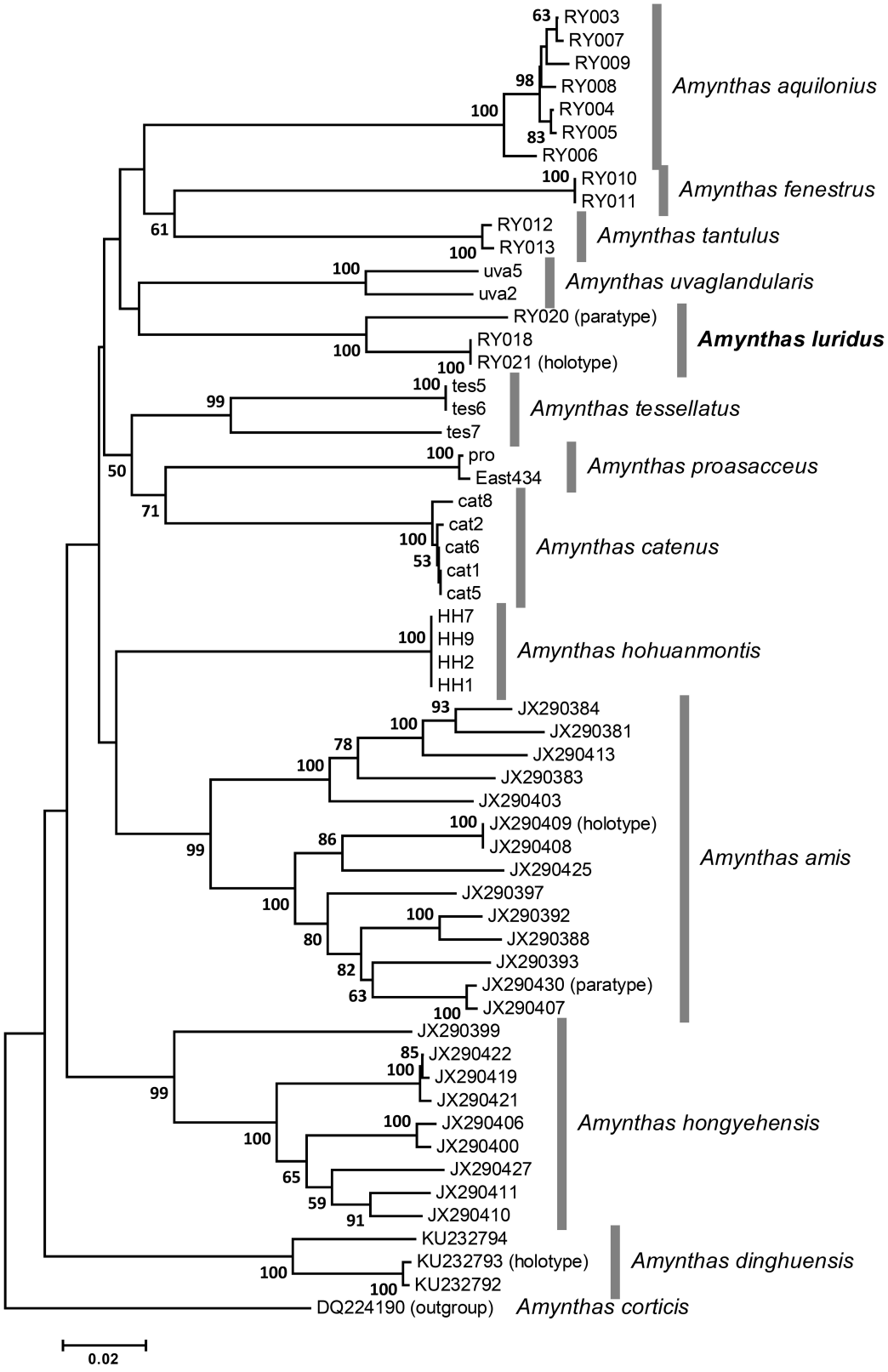

FIGURE 2. Neighbor-joining tree of *Amynthas luridus* and representatives of *Amynthas aquilonius* Tsai *et al*., 2001, *Amynthas catenus* Tsai *et al*., 2001, *Amynthas proasacceus* Tsai *et al*., 2001, *Amynthas hohuanmontis* Tsai *et al*., 2002, *Amynthas tessellatus* Shen *et al*., 2002, *Amynthas fenestrus* Shen *et al*., 2003, *Amynthas tantulus* Shen *et al*., 2003, *Amynthas uvaglandularis* Shen *et al*., 2003, *Amynthas hongyehensis* Tsai & Shen, 2010, *Amynthas amis* Shen, 2012, and *Amynthas dinghuensis* Shen & Chih, 2016 from Taiwan. The tree is based on COI gene and Kimura's two-parameter model, and rooted using *Amynthas corticis*. Specimens from this study and sequences retrieved from GenBank are shown by their voucher numbers and GenBank accession numbers, respectively. Type materials are indicated in parentheses. Numbers around nodes are bootstrap values above 50.

TABLE 2. A comparison of characters among *Amynthas luridus* **sp. nov.**, *A. aquilonius* Tsai *et al*., 2001, *A. hongyehensis* Tsai & Shen, 2010, *A. amis* Shen, 2012, *A. dinghuensis* Shen & Chih, 2016, *A. oyamai* (Ohfuchi, 1937), and *A. morii* (Kobayashi, 1938).

	Amynthas luridus **sp. nov.**	*Amynthas aquilonius* (Tsai *et al*. 2001)	*Amynthas hongyehensis* (Tsai *et al*. 2010; Shen 2012)	*Amynthas amis* (Shen 2012)	*Amynthas dinghuensis* (Shen *et al*. 2016)	*Amynthas oyamai* (Ohfuchi 1937)	*Amynthas morii* (Kobayashi 1938b)
Locality	central Taiwan	central Taiwan	eastern Taiwan	eastern Taiwan	southwestern Taiwan	Japan	Korea
Elevation (m asl)	1500–2300	2200–3000	250–1350	245–1500	1486–1664	–	–
Length (mm)	83–107	39–63	129–197	53–183	41–52	217–278 (162–185[1])	115–118
Clitellum width (mm)	3.47–4.38	1.9–2.6	4.48–7.17	2.83–5.37	1.78–1.98	6–7 (8[1])	4.5
Segments	94–132	70–84	85–138	77–115	75–87	116–123 (126–130[1])	101–103
First dorsal pore	12/13	6/7	11/12	11/12 or 10/11	11/12 or 12/13	12/13	11/12
Setal number							
VII	45–59	26–35	46–73	28–42	26–35	38–40 (37–39[1])	32–33 (VI)
XX	49–65	28–38	59–82	40–48	31–34	43–44 (47–48[1])	49–52
between male pores	9–13	5–9	10–18	8–12	6–8	8–9 (7–10[1])	13–14
Genital papillae							
preclitellar	absent or one posteromedial and one anteromedial to each spermathecal pore	widely paired presetal and postsetal in VII–IX	absent	single median, or one pair closely or widely paired presetal in VII–IX	absent to two pairs widely paired presetal in VIII–IX	absent to three pairs presetal in VII–IX[1]	1–2 pairs presetal in each segment of VII–IX, arranged oblique to the setal line and medial to each spermathecal pore
postclitellar	one anterior and/or one posterior to each male pore; widely paired in postsetal XVII or occasionally paired in presetal XIX	widely paired presetal and postsetal in XVII–XX	widely paired presetal in XVIII–XX	absent or one anteromedial to each male pore	widely paired in postsetal XVII and presetal XIX	absent or one anteromedial to each male pore	absent or one anterior to each male pore
Hearts	XI–XIII	XI–XIII	XI–XIII	XI–XIII	XI–XIII	XI–XIII[1]	X–XIII

.....continued on the next page

TABLE 2. (Continued)

	Amynthas luridus **sp. nov.**	*Amynthas aquilonius* (Tsai *et al*. 2001)	*Amynthas hongyehensis* (Tsai *et al*. 2010; Shen 2012)	*Amynthas amis* (Shen 2012)	*Amynthas dinghuensis* (Shen *et al*. 2016)	*Amynthas oyamai* (Ohfuchi 1937)	*Amynthas morii* (Kobayashi 1938b)
Spermathecae	VI–IX, diverticulum vestigial, not iridescent	VI–IX, diverticulum short, vestigial	VII–IX or VI–IX, diverticulum with normal, iridescent seminal chamber	VI–IX, diverticulum with normal, iridescent seminal chamber	VI–IX, diverticulum with normal, iridescent seminal chamber	VI–IX, small, diverticulum absent	VI–IX, small ampulla and long diverticulum
Testes	small	small	large	large	large	–	moderate in size
Seminal vesicles	vestigial	large	large	large	large	less developed	small
Prostate glands	XVII–XIX	large in XVI–XX	large in XVI–XX	XVI–XX	XVI–XX	absent or poorly developed	absent
Prostatic ducts	small, short, C-shaped in XVIII	large, C-shaped	long, U-shaped in XVII–XVIII	C-shaped in XVIII or U-shaped in XVII–XVIII	S-shaped in XVIII	short, C- or S-shaped in XVIII[1]	small, short
Accessory glands	sessile or stalked	stalked	sessile	short-stalked in spermathecal region	stalked	–	large, stalked in spermathecal region

[1]Data from Kobayashi (1938a).

A. luridus, but *A. nametensis* is quadrithecal with two pairs of spermathecal pores in 7/8/9. Furthermore, *A. nametensis* has globular ampulla, diverticulum coiled into irregular ovate block, and prostate glands extending from XVII to XXIII with long, stout, coiled ducts (Hong *et al*. 2018), whereas *A. luridus* has elongated oval-shaped ampulla, diverticulum with a slender stalk and a seminal chamber rudimentary or absent, and prostate glands in XVII–XIX with short, small, C-shaped prostatic ducts.

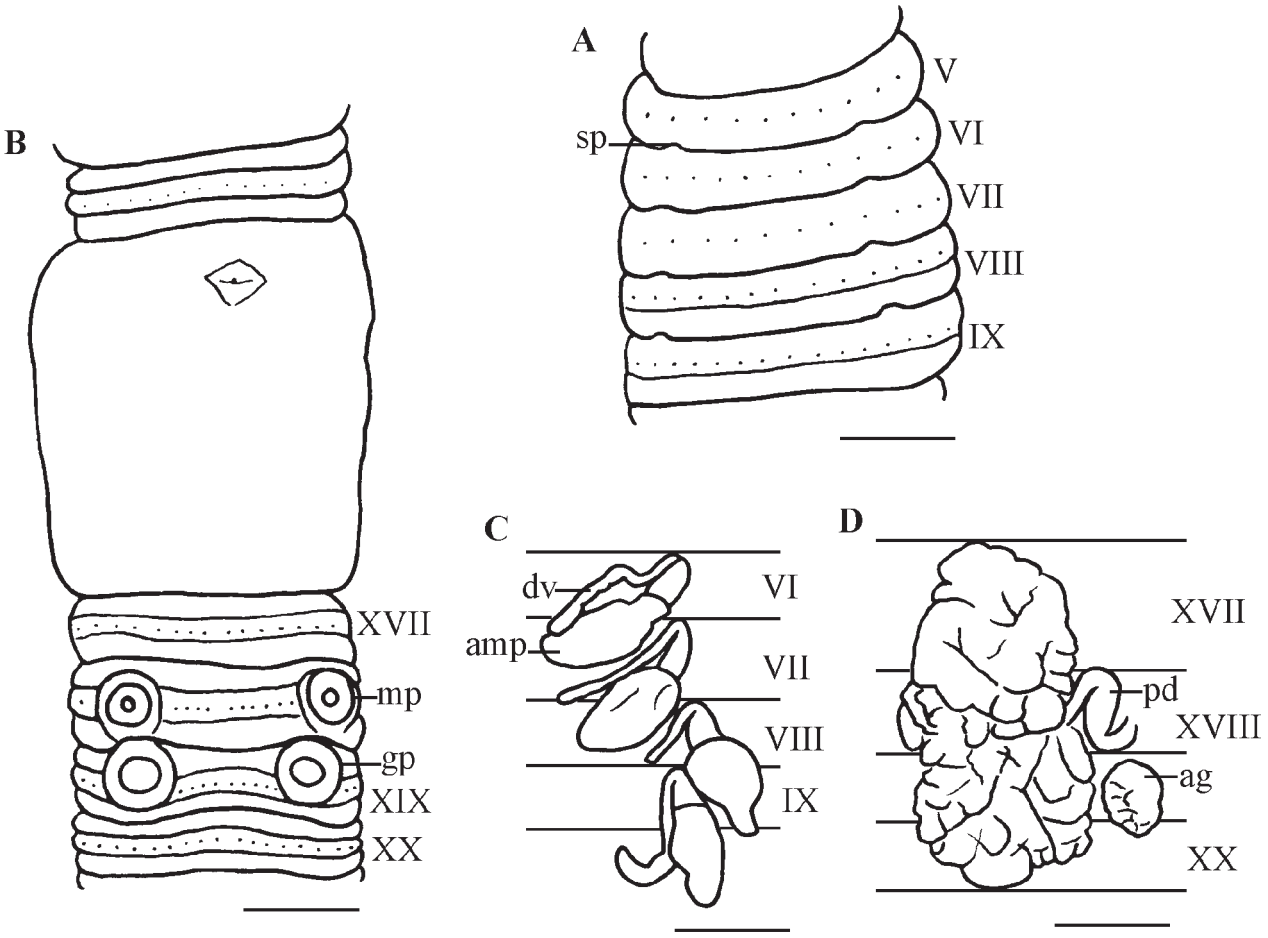

FIGURE 3. *Amynthas ruiyenensis* **sp. nov.** Holotype (TESRI-O-H-55). **A.** Ventral view of spermathecal pore region (sp, spermathecal pore). **B.** Ventral view of male pore region (mp, male porophore; gp, genital papilla). **C.** Dorsal view of right spermathecae (amp, ampulla; dv, diverticulum). **D.** Dorsal view of left prostate gland (pd, prostatic duct; ag, accessory gland). Scale bars = 1 mm.

***Amynthas ruiyenensis* Shen, sp. nov.**
(Figure 3)

Holotype: Clitellate (mature) specimen (97 mm in total length, amputated, dissected), from roadside ditches in Ruiyen Creek Nature Reserve, Nantou County, 24°06'14.29"N, 121°10'34.01"E, 2195 m asl, 2 July 2009, T.-J. Lin & J.-H. Wu (TESRI-O-H-55; RY023, PCR failed).

Diagnosis. Length 97 mm (holotype). Segments numbering 111. Setae 65 in VII, 59 in XX, and 10 between male pores. First dorsal pore in 12/13. Clitellum XIV–XVI. Spermathecal pores four pairs in 5/6–8/9, 0.24–0.25 body circumference ventrally apart. Male pores 0.25 body circumference ventrally apart in XVIII, each on a round

porophore about 0.67 mm in diameter. Genital papillae large, one pair in presetal XIX. Spermathecae four pairs in VI–IX. Seminal vesicles small to large, two pairs in XI and XII. Prostate glands lobed, paired in XVII–XX. Prostatic ducts S-shaped in XVIII. Accessory glands large, sessile, paired in XIX, corresponding to external genital papillae.

Description. External: Length (clitellate) 97 mm, weight 0.52 g in 95% ethanol. Segments numbering 111. Clitellum XIV–XVI, setae and dorsal pores absent, 4.63 mm in length and 3.59 mm in width. Prostomium epilobous. Two annuli (secondary segments) per segment in VIII–IX and three annuli per segment in X–XIII. Setae minute, vague, number 65 in VII, 59 in XX and 10 between male pores in XVIII. First dorsal pore in 12/13. Spermathecal pores four pairs in intersegmental furrows of 5/6–8/9, distance between paired pores 0.24–0.25 body circumference ventrally apart, (Fig. 3A). Genital papillae absent in the preclitellar region. Female pore single, mid-ventral in XIV. Male pores inconspicuous, paired in XVIII, 0.25 body circumference ventrally apart, each situated on a round porophore about 0.67 mm in diameter, surrounded by a circular skin fold (Fig. 3B). One pair of large genital papillae in presetal XIX (Fig. 3B), separated by 9 setae, each papilla about 1.06 mm in diameter. Live worms white. Preserved specimens white and pale brown on clitellum.

Internal: Septa 5/6–7/8 thick, 8/9/10 absent, 10/11–13/14 thick. Nephridial tufts dense, bush-like mass on anterior faces of 5/6/7. Gizzard large, round in VIII–X. Intestine enlarged from XVI. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXV or XXIII. Esophageal hearts paired in XI–XIII. Spermathecae four pairs in VI–IX (Fig. 3C), ampulla elongated oval-shaped, 1.04–1.33 mm long and 0.7–0.86 mm wide, spermathecal duct stout, 0.44–0.56 mm in length. Diverticulum vestigial or long, tubular, 0.6–1.79 mm in total length, stalk slender, seminal chamber normal, iridescent to absent. Holandric. Testes large, oval, two pairs in ventrally joined sacs in X and XI. Seminal vesicles follicular, cotton-like, two pairs in XI and XII, variable in size: large one occupying an entire segmental compartment and small one about half of the segmental compartment. Prostate glands paired in XVII–XX (Fig. 3D), wrinkled and lobed. Prostatic ducts enlarged in the middle, S-shaped in XVIII. Accessory glands large, sessile, paired in XIX, 0.65 mm in width and 0.86 mm in length, corresponding to external genital papillae (Fig. 3D).

Etymology. The name *ruiyenensis* is given to this species with reference to its type locality in Ruiyen Creek Nature Reserve, Nantou, central Taiwan.

Remarks. *Amynthas ruiyenensis* **sp. nov.** is akin to *A. dinghuensis* from southwestern Taiwan in having four pairs of spermathecae in VI–IX and a pair of genital papillae in presetal XIX. However, the much smaller body size and genital papillae, lower segment and setal numbers, and stalked accessory glands of *A. dinghuensis* distinguish it from *A. ruiyenensis*. *Amynthas longicauliculatus* (Gates, 1931) from Burma, *Amynthas murayamai* (Kobayashi, 1938) from Korea and *Amynthas shimaensis* (Goto & Hatai, 1899) from Japan are also octothecal and have large, paired postclitellar genital markings. Compared with *A. ruiyenensis*, *A. longicauliculatus* is apparently much larger with body length of 140–244 mm and width of 7–10 mm, and has higher segment number (137–140) and three pairs of genital markings on 18/19, 19/20 and 20/21 (Gates 1931). The position and size of genital papillae of *A. ruiyenensis* appear like those of *A. murayamai*, but the latter has higher segment number (137–139), lower setal numbers (42 in IX and 52 in XX) and wider spacing between paired spermathecal pores and male pores with about 0.45 and 0.3 ventral circumference, respectively (Kobayashi 1938b). In addition, the reproductive organs of *A. ruiyenensis* and *A. murayamai* vary greatly: *A. murayamai* has small, equal-sized seminal vesicles, relatively large pseudovesicles in XIII, small prostate glands consisting of two main lobes in XVII–XVIII, thin prostatic ducts looped in a C- or L-shape, no accessory glands corresponding to external genital markings, and spermathecae with large diverticula (Kobayashi 1938b). As to *A. shimaensis*, it is a large earthworm with a body length of 205 mm, a width of 7 mm, and 163 segments (Goto & Hatai 1899). Its genital papillae are also larger with a diameter of about 2.5 mm and located in postsetal XIX rather than presetal XIX (Goto & Hatai 1899).

Apart from all the aforementioned species, *Amynthas omodeoi* Zhao & Qiu, 2009 and *Amynthas edwardsi* Zhao & Qiu, 2009 from Hainan Island, China also have large, paired postclitellar genital papillae (Zhao *et al*. 2009). However, both species are sexthecal with three pairs of spermathecae in VI–VIII and have coiled diverticula. *A. omodeoi* has much lower setal numbers with 26–27 in V and 30–36 in XX, papillae paired in 18/19, large prostate glands in XVII–XXIII, U-shaped prostatic ducts, and long stalked accessory glands (Zhao *et al*. 2009). Like *A. ruiyenensis*, *A. edwardsi* also has paired papillae in presetal XIX, but it is also similar to *A. omodeoi* regarding the much lower setal numbers with 32–34 in V and 32–33 in XX, large prostate glands in 1/2XVI–XXIV and U-shaped prostatic ducts. *A. ruiyenensis* can be easily distinguished from *A. omodeoi* and *A. edwardsi* by these disparate features.

On some recent assignments of species to *Amynthas* and *Metaphire*

According to Sims and Easton (1972, p. 200), the only character that distinguishes *Amynthas* from *Metaphire* is the absence of copulatory pouches in the former and presence of copulatory pouches in the latter. Even though this distinction was based on phenetics and both genera are now believed to be polyphyletic, maintaining the two genera is a convenient practicality as it helps to provide an overview over a vast number of species in this group of pheretimoids.

However, Hong *et al*. (2018), in their most recent descriptions of nine new *Amynthas* species from Laos, described "copulatory pouches" for the following species: *Amynthas hoauykanangensis*, *Amynthas fleischmani*, *Amynthas antethecus* and *Amynthas elenabondae*. Also, the term "secondary male pores" was mentioned for *A. fleischmani*, *A. antethecus* and *A. elenabondae* in the text, and the "transverse sphincter-like slit" of *A. fleischmani*, "opening surrounded by tubercular wrinkled epidermal ring" of *A. antethecus* and "crescent openings" of *A. elenabondae* described by the authors actually are openings of copulatory pouches (= secondary male pores). The reason for placing these species in *Amynthas* as stated by Hong *et al*. (2018, p. 247) is as follows: "It is simply because all other characters agree closely with other species unequivocally assignable to *Amynthas*, and to the *aeruginosus* group within that genus". However, there is no further explanation regarding what all the other characters are and how closely these characters agree with the other 68 species and subspecies of the *aeruginosus*-group listed in Sims and Easton (1972). The generic assignments are arbitrary, as they do not respect the criteria that hitherto all authors (Hong and co-authors included) have adhered to, and a phylogenetic framework of the pheretimoid group, which might justify such a decision, is lacking.

James *et al*. (2005, p. 1013) cite Gates (1975, p. 7) and state that they support following the suggestion of Gates (1975) and support restricting *Metaphire* to species with well-developed copulatory pouches protruding into the coelom. By this criterion, many of the species listed under *Metaphire* in Sims and Easton (1972) would have to be transferred to *Amynthas*, such as the two peregrine species, *Metaphire californica* (Kinberg, 1867) and *Metaphire houlleti* (Perrier, 1872), *Metaphire bianensis bianensis* (Stephenson, 1931) from Vietnam, *Metaphire fordi* (Michaelsen, 1934) from Sarawak, Malaysia, *Metaphire flavarundoida* (Chen, 1935) from Hong Kong, *Metaphire ignobilis* (Gates, 1935) from Sichuan, central China, and *Metaphire riukiuensis* (Ohfuchi, 1957) from the southern part of the Ryukyu Archipelago, Japan. Yet Gates' (1975, p. 7) statement only shows his concern about the oversimplified classification by Sims and Easton (1972) without better defining the various status of male pores. Gates uses the genus *Pheretima* for all the pheretimoid earthworms in all his publications and has never accepted the revision by Sims and Easton (1972). How come Gates' (1975, p. 7) statement become the suggestion of restricting *Metaphire* to species with well-developed copulatory pouches protruding into the coelom? This restricted understanding of copulatory pouches has become a source of taxonomic confusion and even errors. For instance, in James *et al*. (2005), *Amynthas huangi* was compared with *Amynthas taipeiensis* (Tsai, 1964), while it should have been compared with *M. houlleti* listed in the *houlleti*-group of the genus *Metaphire* in Sims and Easton (1972) (see Table 3). The male pores of *A. huangi* were described as on small knobs visible under hoods covering male pore openings (James *et al*. 2005, p. 1014). The so-called "small knobs" and "male pore openings" should be penes and secondary male pores (= openings of copulatory pouches), respectively. In fact, *A. huangi* is indeed a junior synonym of *M. houlleti* as pointed out by Tsai *et al*. (2009). Other synonyms as indicated in Tsai *et al*. (2009) are as follows: *Amynthas chaishanensis* James *et al*., 2005 synonymous with *Metaphire formosae* (Michaelsen, 1922), *Amynthas kaopingensis* synonymous with *Metaphire paiwanna* Tsai *et al*., 2000, *Amynthas ailiaoensis* synonymous with *Metaphire feijani* Chang and Chen, 2004. Likewise, Hong and James (2013) compared *Amynthas chiakensis* with species of the *tokioensis*-group of the genus *Amynthas*, while it should have been compared with *Metaphire soulensis* (Kobayashi, 1938) listed in the *glandularis*-group of the genus *Metaphire* in Sims and Easton (1972) as shown by Blakemore *et al*. (2015) (see also Table 4). However, the higher setal numbers and presence of hearts in segment X in *A. chiakensis* maintain both species as separate species (Table 4).

As stated in Sims and Easton (1972, p. 211), in comparison with species of allied genera, the members of the genus *Amynthas* can be readily recognized by the absence of copulatory pouches. Consequently, when the term "copulatory pouch" is used, species possessing this structure should not be placed under *Amynthas* no matter it is intramural copulatory pouch, intracoelomic copulatory pouch or well-characterized copulatory pouch. Nevertheless, what mentioned in the last part of the discussion section in James *et al*. (2005), "Two newly discovered *Amynthas* from the Philippines...have a different and unique structure of the prostatic duct, which is modified to form a sheath over a

small penis visible through the outer secondary male pores", is obviously in contradiction to Sims and Easton (1972) since secondary male pore is the opening of copulatory pouch, not to mention penis!

Although James *et al*. (2005), Hong and James (2009, 2013) and Hong *et al*. (2018) cited Sims and Easton (1972) in their papers and compared their species with species listed in certain species groups classified by Sims and Easton (1972), they actually followed James *et al*.'s (2005) concept, which is more restricted than the one in Sims and Easton (1972). This is corroborated by the fact that among all the new earthworm species published by these authors from Korea to Laos during 2001–2018, no *Metaphire* species was reported. Since these authors considered their new species to belong to *Amynthas*, no comparison with other *Metaphire* species listed in Sims and Easton (1972) was made. Therefore, readers as well as the original authors need to be aware that species regarded as *Amynthas* based on James *et al*.'s (2005) definition might resemble species assigned to *Metaphire* in Sims and Easton (1972). To conclude, no matter how a copulatory pouch is defined, and no matter to which of the two genera a new species is assigned, species comparisons should always be made with the most similar species in both *Amynthas* and *Metaphire* to avoid possible synonyms.

TABLE 3. Evidence that *Amynthas huangi* James *et al*., 2005 is a junior synonym of *Metaphire houlleti* (Perrier, 1872), based on specimens of the latter species from the same region (Shen *et al*. 2005).

	M. houlleti (Perrier, 1872)[1]	*A. huangi* James *et al*., 2005
Locality	Pingtung, southern Taiwan	Pingtung, southern Taiwan
Elevation (m asl)	200–300	391
Number of specimens	6	1
Body length (mm)	107–118	70
Segment number	86–102	101
Clitellum width (mm)	2.39–3.6	3.2
First dorsal pore	9/10	12/13
Setal number		
VII	30–36	38
XIV (clitellum)	about 40 setal pits	absent in text, present in Fig. 2E
XV (clitellum)	about 41 setal pits	absent in text, present in Fig. 2E
XVI (clitellum)	about 42 setal pits	absent in text, present in Fig. 2E
XX	50–52	–
XXV	–	48
between male pores	9–10	10
Copulatory pouch	C-shaped opening in the inner side	C-shaped opening in the inner side, covered with hood
Spermathecal pores	3 pairs (6/7–8/9), deep slits	3 pairs (6/7–8/9), deep slits
Spermathecae	ampulla oval, large; diverticulum with a slender stalk and an enlarged, greatly coiled end	ampulla ovoid, large; diverticulum with a slender stalk and an ovate mass of tightly folded tubular chamber
Genital papillae		
preclitellar	absent	absent
postclitellar	absent	absent
Preclitellar accessory glands	stalked, connecting to spermathecal ducts	stalked, next to spermathecal ducts
Hearts	4 pairs in X–XIII	4 pairs in X–XIII
Intestine enlarged from	XV	XV
Intestinal caeca	XXVII–XXIV	XXVII–XXIV
Testes	X, XI	X, XI
Seminal vesicles	small, XI, XII	small, XI, XII
Prostate glands	large, XVI–XXII or XVI–XXIII	large in XVIII
Accessory glands in XVIII	white patch, sessile	large, sessile mass

[1]Data from Shen *et al*. (2005).

TABLE 4. A comparison of characters between *Metaphire soulensis* and *Amynthas chiakensis*. Data from the original descriptions.

	M. soulensis (Kobayashi, 1938)	*A. chiakensis* Hong & James, 2013
Locality	Korea	Korea
Number of specimens	13	7
Body length (mm)	51–86	70–82
Segment number	83–95	93–94
Greatest diameter (mm)	5	5.5
First dorsal pore	12/13	12/13 or 11/12
Prostomium	epilobous	epilobous
Setal number		
VII	41–47	54–63
XX	44–45	59–71
XXX	46–53	–
between male pores	15–16	18–22
Genital papillae		
preclitellar	absent	absent
postclitellar	absent	absent
Male pores	each surrounded by 3–6 small papillae on top of a protruded disc which can be wholly everted or withdrawn into the body cavity	each surrounded by small circular papillae arranged in C-shaped array
Spermathecal pores	6/7/8, about 4/11 (= 0.36) body circumference ventrally apart	6/7/8, 0.25–0.34 ventral circumference apart
Spermathecae	vestigial without diverticulum	ampulla ovate with a long diverticulum
Hearts	3 pairs in XI–XIII	4 pairs in X–XIII
Intestine enlarged from	XV	XV
Intestinal caeca	XXVII–XXIII, complicated with 5 or 6 finger-shaped secondary caeca with the dorsalmost longest and gradually shorter for more ventral ones	XXVII–XXIV, manicate with 4–5 small finger-shaped lobes decreasing in size ventrally
Testes	small in X and XI	X, XI
Seminal vesicles	each moderate in size with a large dorsal lobe in XI and XII	small with dorsal lobes in XI and XII
Prostate glands	absent	XVI–XXII
Accessory gland	3–6 large, whitish	small, stalked

On a hitherto undetected species group in *Amynthas*

One of the species mentioned above, *A. chiakensis* Hong & James, 2013, was compared with *Amynthas sonjaesiki* Hong & James, 2009 and also with *Amynthas sanchongensis* Hong & James, 2001 in the primary literature (Hong & James 2013). However, comparisons between the latter two species, *A. sonjaesiki* and *A. sanchongensis*, have never been made. Actually, the two species are fairly similar to each other (see comparisons in Table 5). It seems that the only difference is the higher number of genital papillae in *A. sonjaesiki*.

Meanwhile, *A. sanchongensis* was suggested as a junior synonym of *A. tappensis* (Ohfuchi, 1935) (Blakemore 2012; Blakemore *et al*. 2015). Three further species, described by Hong and James (2001), had also been synonymized with *A. tappensis*, namely *Amynthas odaesanensis*, *Amynthas righii,* and *Amynthas fasciiformis* (Blakemore 2003, 2012). Together with *Amynthas gucheonensis* (Song & Paik, 1970) and *Amynthas bangtaesan* and *Amynthas seoraksan* described by Blakemore *et al*. (2015), these species share the following characters: (1) two pairs of spermathecal pores in intersegmental furrows of 6/7/8, (2) numerous genital papillae around each spermathecal pore and male porophore, (3) large ampullae, (4) long diverticula, (5) large prostate glands, and (6) manicate intestinal caeca (Table 5). The synonymies of these species proposed by Blakemore (2003, 2012) and Blakemore *et al*. (2015) are rejected here (see also Table 5). In spite of the morphological similarities, comparisons among these new spe-

TABLE 5. A comparison of characters among *Amynthas sanchongensis, Amynthas sonjaesiki*, *Amynthas tappensis*, *Amynthas gucheonensis*, *Amynthas odaesanensis*, *Amynthas righii*, *Amynthas fasciiformis*, *Amynthas bangtaesan bangtaesan*, *Amynthas bangtaesan confinius*, *Amynthas seoraksan seoraksan*, and *Amynthas seoraksan iti*. Data from the original descriptions.

	A. sanchongensis Hong & James, 2001	*A. sonjaesiki* Hong & James, 2009	*A. tappensis* (Ohfuchi, 1935)
Locality	Korea	Korea	northern Japan
Number of specimens	32	6	32
Body length (mm)	88–121	92–118	131–208
Segment number	78–95	71–97	84–115
Diameter (mm)	5.2–7.1	5.3–7.0	5–8
First dorsal pore	12/13	12/13	12/13
Prostomium	epilobous	epilobous	–
Setal number			
VII	53	51	39–40
XX	48	49	55–64
between male pores	14–18	14–20	16–25
Male pores	each on a large, conical, protuberant porophore with 4–5 small, presetal papillae medial to it	each on a large, oval, protuberant porophore with 14–28 small, presetal papillae medial to it	each deeply caved on a round porophore ringed with papillae together with an oblong-shaped patch containing 7–10 small, presetal papillae medial to it
Spermathecal pores	6/7/8, flanked anteriorly and posteriorly by tumid lips; each lip bearing 0–6 small papillae	6/7/8, surrounded by thickened lips; 8–16 small papillae posteromedial to each pore	6/7/8, 4–11 small papillae posteromedial to each pore
Spermathecae	ampulla large, diverticulum slender, cayenne pepper-shaped	ampulla large, diverticulum with a long, cayenne pepper-shaped seminal chamber	ampulla large, ovoid or sac-like, diverticulum long, coiled
Hearts	4 pairs in X–XIII	4 pairs in X–XIII	last in XIII
Intestine enlarged from	XV	XV	XV
Intestinal caeca	XXVII–XXIII, manicate with 5 or 6 finger-shaped lobes	XXVII–XXIII, manicate with 7 or 8 finger-shaped lobes	XXVII–XXIII, manicate with 5 finger-shaped lobes
Testes	X, XI	X, XI	–
Seminal vesicles	middle size, well-developed in XI and XII	large in XI and XII	well-developed in XI and XII
Prostate glands	large in XVI–XXI	large in XVI–XX	large in XVI–XX
Prostatic ducts	long, looped	long, thick	looped, strongly developed
Accessory gland	small, stalked	stalked	short-stalked

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TABLE 5. (Continued)

	A. gucheonensis (Song & Paik, 1970)	*A. odaesanensis* Hong & James, 2001	*A. righii* Hong & James, 2001
Locality	Korea	Korea	Korea
Number of specimens	55	4	65
Body length (mm)	103–147	73–77	49–95
Segment number	83–119	81–84	57–96
Diameter (mm)	5.5–8	2.9–3.4	4.5–4.7
First dorsal pore	12/13	12/13	12/13
Prostomium	epilobous	epilobous	epilobous
Setal number			
VII	59–79	60	49
XX	65–82	64	48
between male pores	16–28	13–15	16
Male pores	each on a circular-shaped disc with an oblong-shaped patch containing 11–19 small, presetal papillae medial to it	each on an ovate to circular pad with 11–15 small, presetal papillae and about 6 postsetal papillae medial to it	each on an oval pad with 1–33 small, presetal and postsetal papillae medial to it
Spermathecal pores	6/7/8, 3–10 small papillae posteromedial to each pore	6/7/8, 5–8 small papillae posteromedial to each pore	6/7/8, 9–15 small papillae posteromedial to each pore
Spermathecae	ampulla large, diverticulum slender with a rather long, sausage-shaped seminal chamber	ampulla large, diverticulum slender, cayenne pepper-shaped	ampulla large, diverticulum slender with a long, sausage-shaped seminal chamber
Hearts	3 pairs in XI–XIII	3 pairs in XI–XIII	3 pairs in XI–XIII
Intestine enlarged from	XV	XV	XV
Intestinal caeca	XXVII–XXIII, manicate with 5–7 finger-shaped lobes	XXVII–XXIV, manicate with 5 finger-shaped lobes	XXVII–XXV, manicate with 5 or 6 finger-shaped lobes
Testes	X, XI	X, XI	X, XI
Seminal vesicles	well-developed in XI and XII	XI, XII	well-developed in XI and XII
Prostate glands	well-developed in XVI–XX, XXI	large in XVII–XX	large in XVII–XX
Prostatic ducts	U-shaped loop	short, thick	short, thick
Accessory gland	short-stalked	small, stalked	small, stalked

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TABLE 5. (Continued)

	A. fasciiformis Hong & James, 2001	*A. bangtaesan bangtaesan* Blakemore, 2015	*A. bangtaesan confinius* Blakemore, 2015
Locality	Korea	Korea	Korea
Number of specimens	25	1	2
Body length (mm)	62–87	63	62–80
Segment number	68–92	83	89
Diameter (mm)	4.3–4.5	–	–
First dorsal pore	12/13	13/14	12/13
Prostomium	epilobous	epilobous	epilobous
Setal number			
VII	47	50–60	50–60
XX	48	50–60	50–60
between male pores	16–17	16	12
Male pores	each on a small, round porophore with a small, postsetal papilla and 16–25 very small, presetal papillae medial to it	each on a small, round porophore with 6 or 7 small, presetal papillae and 8–10 small, postsetal papillae medial to it	each on a round porophore ringed with papillae together with an oblong-shaped patch containing about a dozen small, presetal papillae medial to it
Spermathecal pores	6/7/8, 9–13 small papillae posteromedial to each pore	6/7/8, 6 or 7 small papillae posteromedial to each pore	6/7/8, 6 or 7 small papillae posteromedial to each pore
Spermathecae	ampulla large, diverticulum with a long, cayenne pepper-shaped seminal chamber	ampulla saccular with clavate diverticulum	ampulla deflated with clavate diverticulum
Hearts	4 pairs in X–XIII	–	–
Intestine enlarged from	XV	–	–
Intestinal caeca	XXVII–XXIII, manicate with 5 finger-shaped lobes	manicate in XXVII	manicate in XXVII
Testes	X, XI	–	–
Seminal vesicles	small in XI and XII	large in XI and XII	large in XI and XII
Prostate glands	large in XVI–XX	racemose	racemose
Prostatic ducts	thick	straight, muscular	U-shaped, muscular
Accessory gland	stalked or sessile	stalked	stalked

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TABLE 5. (Continued)

	A. seoraksan seoraksan Blakemore, 2015	*A. seoraksan iti* Blakemore, 2015
Locality	Korea	Korea
Number of specimens	1	6
Body length (mm)	80	111–120
Segment number	86	–
Diameter (mm)	–	–
First dorsal pore	12/13	12/13
Prostomium	epilobous	epilobous
Setal number		
VII	40–50	50–60
XX	40–50	50–60
between male pores	21	–
Male pores	each on a round porophore with 6 or 7 small, presetal papillae medial to it	each on a round porophore with about a dozen small, presetal papillae medial to it
Spermathecal pores	6/7/8, 1 or 2 small papillae posteromedial to each pore	6/7/8, 2–6 small papillae posteromedial to each pore
Spermathecae	ampulla saccular with clavate diverticulum	ampulla deflated with clavate diverticulum
Hearts	–	–
Intestine enlarged from	–	–
Intestinal caeca	manicate in XXVII	manicate in XXVII
Testes	–	–
Seminal vesicles	large in XI and XII	large in XI and XII
Prostate glands	racemose	racemose
Prostatic ducts	bent, muscular	muscular
Accessory gland	stalked	stalked

cies have been lacking since their publication. This is resulted majorly from inadequate literature review. The brief and oversimplified original descriptions of *A. bangtaesan* and *A. seoraksan* together with their subspecies rendered detailed comparisons with the above-mentioned species difficult, since these species were subtly differentiated. Blakemore *et al*. (2015) compared *A. bangtaesan* and *A. seoraksan* morphologically with *A. gucheonensis* only. However, *A. bangtaesan* should have been compared with *A. odaesanensis* and *A. righii*, since all three species are much closer in body size and setal numbers, and in having small, postsetal papillae medial to each male porophore and prostatic ducts which are not looped (Table 5). As to *A. seoraksan*, it has a higher morphological affinity with *A. sanchongensis* than with *A. gucheonensis* with regard to body size, setal numbers, and numbers of papillae medial to each spermathecal pore and each male porophore (Table 5). Again, it should be emphasized that species comparisons should always be made with the most similar species to avoid possible synonyms. All the taxa listed in Table 5 are considered to be valid here. Comparisons with new species similar to this group and published hereafter can follow Table 5. Among this group of earthworms, *A. tappensis* is from Japan and it deviates from all the other taxa from Korea in terms of larger body size and long, coiled diverticula. Perhaps the Korean earthworms in this group speciated from the territory of Korea and are monophyletic just like the *Metaphire formosae* species-group from Taiwan (Chang *et al*. 2008, 2014). It would be interesting to resolve the phylogenetic relationships among these earthworms.

Three Japanese species, *Amynthas bimaculata* (Ishizuka, 1999), *Amynthas silvatica* (Ishizuka, 1999) and *Amynthas surcata* (Ishizuka, 1999), were also synonymized with *A. tappensis* by Blakemore (2003, 2012). Like *A. tappensis*, the former two species have long and coiled diverticula, whereas each diverticulum of *A. surcata* is with a slender stalk and a long, sausage-shaped seminal chamber (Table 6). In further comparisons with *A. tappensis*, both *A. bimaculata* and *A. silvatica* are much smaller, have higher setal number in VII and lower setal number in XX (Table 6). Moreover, *A. bimaculata* has paired patches of papillae in presetal XIX, while *A. silvatica* has papillae drastically reduced in numbers and distributed in presetal XVII and XIX (Table 6). The synonymies of these species are also rejected. *A. bimaculata* and *A. silvatica* are excluded from the aforementioned group of Korea species on account of their long and coiled diverticula. *A. surcata* is also ruled out since it has very low numbers of genital papillae in the spermathecal and the male pore regions and large, long-stalked accessory glands (Table 6).

TABLE 6. A comparison of characters among *Amynthas tappensis, Amynthas bimaculata*, *Amynthas silvatica*, and *Amynthas surcata*.

	A. tappensis (Ohfuchi, 1935)	*A. bimaculata* (Ishizuka, 1999)	*A. silvatica* (Ishizuka, 1999)	*A. surcata* (Ishizuka, 1999)
Locality	northen Japan	Tokyo, Japan	Tokyo, Japan	Tokyo, Japan
Number of specimens	32	12	9	40
Body length (mm)	131–208	40–70	60–80	60–125
Segment number	84–115	80–90	69–93	54–99
Diameter (mm)	5–8	3–4.5	4–5.8	4–5
First dorsal pore	12/13	12/13	11/12	12/13
Setal number				
VII	39–40	44–48	50–54	47–48
XX	55–64	44–50	50–58	51–56
between male pores	16–25	about 12	about 18	about 15
Male pores	each deeply caved on a round porophore ringed with papillae together with an oblong-shaped patch containing 7–10 small, presetal papillae medial to it	each simple on a round porophore with an oblong-shaped patch containing about 12 small, presetal papillae medial to it	each simple on a round porophore with 4 presetal papillae medial to it	each simple on a round porophore with 2 presetal papillae medial to it
Spermathecal pores	6/7/8, 4–11 small papillae posteromedial to each pore	6/7/8, 2 papillae posteromedial to each pore	6/7/8, 1 papilla posteromedial to each pore on 7/8	6/7/8, 1 papilla posteromedial to each pore on 7/8

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TABLE 6. (Continued)

	A. tappensis (Ohfuchi, 1935)	*A. bimaculata* (Ishizuka, 1999)	*A. silvatica* (Ishizuka, 1999)	*A. surcata* (Ishizuka, 1999)
Genital papillae				
XVII	absent	absent	2 presetal papillae on each side, in line with male pore papillae	absent
XIX	absent	an oblong-shaped patch containing about 6 small, presetal papillae on each side, in line with male pore papillae	2 presetal papillae on each side, in line with male pore papillae	absent
Spermathecae	ampulla large, ovoid or sac-like, diverticulum long, coiled	ampulla large, shovel-shaped, diverticulum long, coiled	ampulla large, shovel-shaped, diverticulum long, coiled	ampulla large, shovel-shaped, diverticulum slender with a long, sausage-shaped seminal chamber
Intestine enlarged from	XV	XV	XV	XV
Intestinal caeca	XXVII–XXIII, manicate with 5 finger-shaped lobes	XXVII–XXIII, manicate with 5–7 finger-shaped lobes	XXVII–XXIV, manicate with 4–6 finger-shaped lobes	XXVII–XXIII, manicate with 4–6 finger-shaped lobes
Seminal vesicles	well-developed in XI and XII	large in XI and XII	large in XI and XII	large in XI and XII
Prostate glands	large in XVI–XX	large in XVI–XXII	large in XVII–XXII	large in XVI–XX
Prostatic ducts	looped, strongly developed	looped, stout	–	C-shaped
Accessory gland	short-stalked	stalked	stalked	large, long-stalked

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