Four new earthworm species of the genus *Amyntas* Kinberg (Oligochaeta: Megascolecidae) from the island of Hainan and Guangdong Province, China

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This paper describes four new species of *morrissi*-group earthworms from Hainan and Guangdong Provinces, China: *Amyntas instabilis* sp. nov., *Amyntas dilatatus* sp. nov., *Amyntas infuscuatus* sp. nov. and *Amyntas qiongzhongensis* sp. nov. All four species have two pairs of spermathecal pores in 5/6–6/7; male pores in XVIII, 0.33 circumference ventrally apart, each on the top of a slightly raised porophore, surrounded by several tiny genital papillae, in a pulvinate pad with three to six circular folds. Characters of the spermathecae, prostate glands and other anatomical features easily distinguish the new species from earthworms previously reported from the *morrissi*-group.

<http://zoobank.org/urn:lsid:zoobank.org:pub:545E9152-C826-44A2-949E-402434D8493D>.

Keywords: *morrissi*-group; new species; genetic distances; cytochrome oxidase subunit I gene; barcode

Introduction

The *morrissi*-group is defined as *Amyntas* with intersegmental spermathecal pores, first spermathecal pores at 5/6, two thecal segments and holandry. Sims and Easton (1972) included 30 species in this group and, since 1972, nine more species from the *morrissi*-group have been reported from China: *Amyntas nanulus* (Chen et al. 1975) (Chen et al. 1975), and *Amyntas parvus* (Chen and Hsu 1977) from mainland China, *Amyntas diaoluomontis* Qiu and Sun, 2009, *Amyntas octopapillatus* Qiu and Sun, 2009, *Amyntas zhangii* Qiu and Sun, 2009, *Amyntas lingshuiensis* Qiu and Sun, 2009, *Amyntas endophilus* Zhao and Qiu, 2013, *Amyntas fluviatilis* Zhao and Sun, 2013, *Amyntas fucatus* Zhao and Jiang, 2013 from Hainan Island of China (Sun et al. 2009; Zhao et al. 2013). *Amyntas piagolensis* Hong and James, 2001, *Amyntas taebaekensis* Hong and James, 2001, *Amyntas naejangensis* Hong and James, 2001, *Amyntas draconis* Hong and James, 2001, *Amyntas assimilis* Hong and Kim, 2002, *Amyntas angulatus* Hong, 2007 and *Amyntas dabudongensis* Hong and James, 2009 have been reported from Korea (Hong and James 2001, 2009; Hong and Kim 2002; Hong 2007).

Earthworms of the *morrissi*-group from the island of Hainan were first reported by Chen Yi (Chen 1938), and included *Amyntas morrissi* (Beddard 1892), *Amyntas puerilis* (Chen 1938), *Amyntas hainanicus* (Chen 1938), *Amyntas oculatus* (Chen

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1938), *Amyntas monoserialis* (Chen 1938) and *Amyntas sinuosus* (Chen 1938). Since then, Sun et al. (2009) have reported four new species while Zhao et al. (2013) reported three new species. In total, 17 species of the *morrisoni*-group have been recorded from Hainan. There are no earlier reports of *morrisoni*-group earthworms from Guangdong Province, which is located just north of Hainan Province and separated from it by Qiongzhou Strait. During the years 2006, 2010 and 2011, we made a broad earthworm collection during field surveys in southern China, and discovered more new species of the *morrisoni*-group.

In this paper, we describe four new species from Xiangtoushan National Nature Reserve of Guangdong, Jianfengling National Nature Reserve and Diaoluoshan National Nature Reserve of Hainan, including *Amyntas instabilis* sp. nov., *Amyntas dilatatus* sp. nov., *Amyntas infuscatus* sp. nov. and *Amyntas qiongzongensis* sp. nov. *Amyntas instabilis* sp. nov. was the only species found in both Hainan and Guangdong Provinces.

Material and methods

The earthworms collected in 2006 were anaesthetized in 10% ethanol solution, and preserved in 10% formalin solution. The earthworms collected in 2010–11 were anaesthetized in 10% ethanol solution, and preserved in 95% ethanol solution. Holotypes and paratypes are deposited in the Shanghai Natural History Museum.

The earthworms collected in 2010–11 and preserved in 95% ethanol solution, are suitable for molecular analysis. DNA was extracted from several specimens of *A. instabilis*, *A. qiongzongensis* and *A. morrisoni* (one clitellate, SC201006-03) using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-tek, Norcross, GA, USA). The gene cytochrome oxidase subunit I, (COI), was amplified. Primers used in the research were 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994), or 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TATACTTCTGGGTGTCCGAAGAATCA-3' (Bely and Wray 2004). Sequencing was performed in the Beijing Genomics Institute (Shanghai, China).

Sequences were aligned with Clustal X, and then pairwise distances between these species were calculated using the Kimura two-parameter model of DNA evolution with MEGA 5.

Results

The COI mitochondrial DNA gene, considered as a barcode for earthworm identification (Huang et al. 2007; Novo et al. 2010), is an effective complement to morphological analyses.

Comparisons of COI gene sequences among *A. instabilis* sp. nov., *A. qiongzongensis* sp. nov. and *A. carnosus*, *A. robustus* and *A. triastriatus* (Table 1), which belong to other species-groups and have similar male pores and genital markings and different numbers of spermathecal pores, indicated that the new species greatly differ from these last species. Pairwise distances of COI (Table 2) for *A. instabilis*, *A. qiongzongensis* and other species show very low intraspecific values (0–0.16%), whereas distances between *A. instabilis* and *A. qiongzongensis* were 13.59–13.79%. Among *A. instabilis* and the other three species they were 18.74–21.74%. Among *A. qiongzongensis* and the other three species they were 17.05–19.72%. According to Chang and James (2011), values above 10–15%

Table 1. Species with molecular data used in this study.

Species	Species no.	Locality	Reference	Accession no. of COI in NCBI
<i>Amynthas instabilis</i> 1	C-HN201001-01	China: Hainan	This paper	JX126729
<i>Amynthas instabilis</i> 2	C-HN201101-03	China: Hainan	This paper	JX126727
<i>Amynthas instabilis</i> 3	C-GD201105-03	China: Guangdong	This paper	JX126730
<i>Amynthas qiongzhongensis</i> 1	C-HN201008-01	China: Hainan	This paper	JX126731
<i>A mynthas qiongzhongensis</i> 2	C-HN201114-02	China: Hainan	This paper	JX126728
<i>Amynthascarnosus</i>		Japan: Osaka	(Minamiya 2010, unpublished)	AB542454
<i>Amynthas morrisi</i>	C-SC201006-03	China: Sichuan	This paper	KF021247
<i>Amynthas robustus</i>		China	(Huang et al. 2007)	EF077571
<i>Amynthas triastriatus</i>		China	(Huang et al. 2007)	EF077607

most probably indicate different species. Although the evaluation criterion is uncertain, it is clear that the new species and other species in Table 2 have large genetic divergences. Also, there is little difference between individuals of the same species.

Systematics

Amynthas instabilis Qiu and Jiang sp. nov.
(Figure 1)

Material

Holotype. 1 clitellate (C-HN011-02A): China, Hainan Island, Jianfengling National Nature Reserve (18°43'32" N, 108°53'33"E), 860 m elevation, black sandy soil under shrubbery beside road, 4 July 2006, J.X. Li and W.X. Zhang coll.

Paratypes. 60 clitellates (C-HN011-02B) and 3 clitellates (C-HN011-03)with the same data as for holotype; 1 clitellate (C-HN014-03): China, Hainan Island, Jianfengling National Nature Reserve (18°43'58" N, 108°53'10" E), 890 m elevation, sandy soil under evergreen forest, 4 July 2006, J.P. Qiu and M.B. Bouché colls; 8 clitellates (C-HN015-01): China, Hainan Island, Jianfengling National Nature Reserve (18°43'56" N, 108°53'13" E), 895 m elevation, cinnamon soil under tropical rainforest vegetation, 4 July 2006, J.P. Qiu, M.B. Bouché and X.L. Zhang colls. 2 clitellates (C-HN201001-01): China, Hainan Island, Diaoluoshan National Nature Reserve (18°42'32" N, 109°50'25" E), 631 m elevation, black sandy soil under arbor vegetation, 23 July 2010, J.B. Jiang and Y.Z. Guo coll. 1 clitellate (C-HN201101-03): China, Hainan Island, Diaoluoshan National Nature Reserve (18°44'18" N, 108°52'01" E), 840 m elevation, yellow soil under broadleaved evergreen forest, 23 May 2011, J.P. Qiu, J.B. Jiang, Q. Zhao, D. Cluzeau and W.K.

Table 2. Percentage of pairwise distances obtained for the sequences of COI gene in *Amyntas* spp.

	<i>A.</i> <i>instabilis</i> 1	<i>A.</i> <i>instabilis</i> 2	<i>A.</i> <i>instabilis</i> 3	<i>A.</i> <i>qiongzhongensis</i> 1	<i>A.</i> <i>qiongzhongensis</i> 2	<i>A.</i> <i>carosus</i>	<i>A.</i> <i>morrisi</i>	<i>A.</i> <i>robustus</i>	<i>A.</i> <i>tristriatus</i>
<i>A. instabilis</i> 1									
<i>A. instabilis</i> 2	0.16%								
<i>A. instabilis</i> 3	0	0.16%							
<i>A. qiongzhongensis</i> 1	13.79%	13.59%	13.79%						
<i>A. qiongzhongensis</i> 2	13.79%	13.59%	13.79%	0.00%					
<i>A. carosus</i>	19.42%	19.20%	19.42%	18.92%	18.92%	20.47%			
<i>A. morrisi</i>	21.74%	21.52%	21.74%	19.72%	19.72%	21.09%	20.22%		
<i>A. robustus</i>	20.26%	20.04%	20.26%	17.05%	17.05%	19.60%	20.06%	16.34%	
<i>A. tristriatus</i>	18.95%	18.74%	18.95%	17.24%	17.24%

Figure 1. *Amynthus instabilis* Qiu and Jiang sp. nov. (A) Ventral view of holotype, scale bar 2 mm; (B) spermathecae of holotype, scale bar 1 mm; (C) prostate gland of holotype; (D) male pore region of one paratype.

Zhang coll. 2 clitellates (C-GD201105-03): China, Guangdong Province, Xiangtoushan National Nature Reserve (23°15'38" N, 114°22'31" E), 360 m elevation, yellow soil under broadleaved evergreen forest, 23 October 2011, J.B. Jiang, J. Sun, J.X. Li and X.D. Lei coll.

Ten individuals were dissected and 13 were characterized externally only.

Etymology

The species is named after its characteristic of variable numbers of genital papillae in the male pore region.

Diagnosis

Dimensions 74–125 mm by 2.9–4.0 mm at clitellum, segments 82–145. Setae numbering 30–54 at III, 42–64 at V, 52–62 at VIII, 40–52 at XX, 40–56 at XXV; 0–2 between male pores; 15–22 between spermathecal pores. Spermathecal pores in 5/6–6/7, 0.40 body circumference ventrally apart. Male pores in XVIII, 0.33 body circumference ventrally apart, each on the top of a slightly raised, oval porophore in a pulvinate pad with three to six circular folds. Spermathecae two pairs in VI–VII, ampulla heart-shaped, duct as long as ampulla. Diverticulum half as long as main pouch (duct and ampulla together), terminal one-third dilated into an ovoid chamber.

External characters

Taupe dorsal pigment, ventral pigment lighter present in the pre-clitellum segments of preserved specimens; tan pigment on dorsum, no pigment on ventrum of the post-clitellum segments. Dimensions 74–125 mm by 2.9–4.0 mm at clitellum, segments 82–145; the average values of examined individuals: dimensions 104 mm by 3.3 mm at clitellum, segments 128. Secondary annulations conspicuous in segments XVII–XVIII. Prostomium $\frac{1}{2}$ epilobous. First dorsal pore of all examined individuals in 11/12. Setae numbering 30–54 at III, 42–64 at V, 52–62 at VIII, 40–52 at XX, 40–56 at XXV; 0–2 between male pores; 15–22 between spermathecal pores; setal formula: AA = 1.0–1.3AB, ZZ = 1.0–2.0ZY. Clitellum annular, pinkish or brown, in XIV–XVI, setae invisible externally.

Spermathecal pores two pairs in 5/6–6/7, ventral, eye-like, sometime invisible, milky white porophore in centre, 0.40 circumference ventrally apart from each other. A tiny conical genital papilla before each pore. A similar postsetal genital papilla on midventral line of segment VI, and a pair of presetal genital papillae, 0.2 mm apart from each other, on the anterior border of segment VII.

Male pores one pair in XVIII, 0.33 circumference apart ventrally, each on the top of a slightly raised, oval porophore in a pulvinate pad with six circular folds, some paratypes with three circular folds. Two tiny oval genital papillae, present at anterior and posterior of porophore, each male pore is medial to these two tiny papillae (Figure 1A); some paratypes with three tiny oval genital papillae surrounding left male pore (Figure 1D). Genital papillae numbers in male pores region are variable among specimens.

Female pore single in XIV, rounded, milky white.

Internal characters

Septa 4/5–6/7, 10/11 thick and muscular, 11/12 slightly thickened, 8/9–9/10 absent. Gizzard long bucket-shaped, in IX–X. Intestine enlarged distinctly from XVI. Intestinal caeca paired in XXVII, simple, smooth, with a slight indentation on terminal dorsal margin, extending anteriorly to XXIV. Oesophageal hearts in X–XIII.

Ovaries in XIII. Spermathecae two pairs in VI–VII, ampulla heart-shaped, about 2.3 mm long in holotype; ampulla duct slender to stout, as long as ampulla. Diverticulum as long as a half main pouch (duct and ampulla together), slender, terminal one-third dilated into an ovoid chamber (Figure 1B), milky white. No accessory glands observed.

Male sexual system holandric, testis sacs two pairs, in X–XI. Seminal vesicles two pairs, extending in XI–XIII, the posterior pair larger than the anterior one and extending to XIII. Prostate glands developed, inserting in XVIII and extending to XVII and XX, coarsely lobate, prostatic duct U-shaped, slightly thicker at the distal part (Figure 1C). No accessory glands observed.

Remarks

In comparison to the other 30 species of the *morrisi*-group reported from China (19) and other Asian countries (11), *Amyntas instabilis* sp. nov. is closely related to *Amyntas incongruus* (Chen 1933) and *Amyntas tripunctus* (Chen 1946) from China, and to *A. dilatatus* sp. nov., *A. infuscuatus* sp. nov. and *A. qiongzhongensis* sp. nov. These six species share some similarities with similar male pores and genital markings, two pairs of intersegmental spermathecal pores in 5/6–6/7, and simple intestinal caeca. Table 3 illustrates in detail the differences among *A. incongruus* (Chen 1933), *A. tripunctus* (Chen 1946) and the four new species.

Amyntas instabilis sp. nov. differs from *A. tripunctus* (Chen 1946) by the presence of pigment on the dorsum, location of first dorsal pore, setae number in segment VIII, prostate glands and spermathecal characters. *Amyntas instabilis* sp. nov. has tan pigment on the dorsum, first dorsal pore in 11/12, setal number 52–62 at VIII, prostates glands less developed and diverticulum half as long as main pouch. In contrast, *A. tripunctus* has grey pigment on dorsum, first dorsal pore in 12/13, setae number fewer at VIII, more setae between male pores, well-developed prostate glands and a longer diverticulum.

Amyntas instabilis sp. nov. and *A. incongruus* (Chen 1933) can be separated on the basis of spermathecal characters. The ampulla of *A. instabilis* sp. nov. is heart-shaped, a slender to stout duct is as long as ampulla. The ampulla of *A. incongruus* is ovoid or elongate spherical, duct either long and slender, or short stout, terminal three-quarters of diverticulum dilated into a twist chamber. In addition, *A. incongruus* has very small seminal vesicles and accessory glands, with prostate glands absent entirely and in a few cases well developed (Chen 1933).

***Amyntas dilatatus* Qiu and Jiang sp. nov.**
(Figure 2)

Material

Holotype. 1 clitellate (C-HN012-03A): China, Hainan Island, Jianfengling National Nature Reserve (18°43'26" N, 108°53'56" E), 900 m elevation, black

Table 3. Comparison of some species with two pairs of spermathecal pores in 5/6-6/7 of *morrissi*-group of *Amyntas* in China.

Character	<i>A. instabilis</i> sp. nov.	<i>A. dilatatus</i> sp. nov.	<i>A. infuscatus</i> sp. nov.	<i>A. qiongzongensis</i> sp. nov.	<i>A. tripunctus</i> (Chen 1946)	<i>A. incongruus</i> (Chen 1933)
Body length (mm)*	74-125	120-130	60-78	81-161	83	130-145
Segment number*	82-145	148-153	130-139	127-159	91	142-160
Pigment on dorsum	tan	light brown	purple or light brown	light brown	grey	grey
Secondary annulations	conspicuous	none	none	conspicuous	conspicuous	none
First dorsal pore	11/12	11/12	12/13	11/12	12/13	11/12
Setae in viii*	52-62	50-64	49-52	50-62	40	48-68
Number of setae between spermathecal pores*	15-22	22-27	-	20-22	22	22-24
Number of setae between male pores*	0-2	0-2	8-10	4-8	12	9-12
Spermathecal pores	0.40 C	0.40 C	0.33 C	0.40 C	5/12 C	0.33 C
Seminal vesicles	the posterior pair larger developed	the anterior pair larger developed	the anterior pair larger developed	the anterior pair larger well developed	large	very small
Prostates glands	invisible	invisible	invisible	invisible	well developed	absent entirely and in few cases well-developed
Accessory glands	invisible	invisible	invisible	invisible	not clearly visible	cluster of gland and stalk
Spermathecae main pouch	ampulla heart-shaped; duct as long as ampulla.	ampulla heart-shaped; duct as long as 2/3 ampulla.	ampulla ovoid; duct twice long as ampulla.	ampulla heart-shaped; duct slightly shorter than ampulla.	fairly large, ampulla heart-shaped; duct longer than ampulla.	ampulla ovoid or elongate spherical; duct either long and slender, or short stout.
Diverticulum	as long as 1/2 main pouch; terminal 1/3 dilated into an ovoid chamber.	slender and short; terminal 9/10 dilated into a swollen chamber.	slightly longer than main pouch; terminal 1/3 dilated into a swollen chamber.	longer than main pouch by 1/5; terminal 2/7 dilated into a swollen chamber.	1/4 longer than main pouch; terminal 1/2 dilated into a cucumber chamber.	longer than main pouch; terminal 3/4 dilated into a twist chamber.

Note: * The values are given from lowest to highest.

Figure 2. *Amynthus dilatatus* Qiu and Jiang sp. nov.; (A) Ventral view of holotype, scale bar 2 mm; (B) spermathecae of holotype, scale bar 1 mm; (C) prostate gland of holotype; (D) male pore region of one paratype.

sandy soil under shrubbery beside road, 4 July 2006, J.X. Li and W.X. Zhang coll.

Paratypes. 1 clitellate (C-HN012-03B) with the same data as for holotype. 7 clitellates (C-HN029-01): China, Hainan Island, Diaoluoshan National Nature Reserve (18°43'31" N, 109°52'01" E), 920 m elevation, black sandy soil under meadow, 7 July 2006, J.P. Qiu, M.B. Bouché, J.X. Li and X.L. Zhang colls.

Both clitellates were externally characterized and dissected.

Etymology

The species is named after the characteristics of the seminal chamber.

Diagnosis

Dimensions 120–130 mm by 2.9–3.2 mm at clitellum, segments 148–153. Setae numbering 46–50 at III, 56–62 at V, 50–64 at VIII, 30–50 at XX, 41–50 at XXV; 0–2 between male pores; 22–27 between spermathecal pores. Spermathecal pores in 5/6–6/7, 0.40 body circumference ventrally apart. Male pores in XVIII, 0.33 body circumference ventrally apart, each on the top of a slightly raised, rounded porophore in a pulvinate pad with three or four circular folds. Spermathecae two pairs in VI–VII, ampulla heart-shaped, duct as long as two-thirds of ampulla. Diverticulum a little longer than the main pouch (duct and ampulla together), seminal chamber swollen.

External characters

Grey dorsal pigment present in the pre-clitellum segments of preserved specimens; light brown pigment on dorsum of the post-clitellum segments; no pigment on ventrum. Dimensions 120–130 mm by 2.9–3.2 mm at clitellum, segments 148–153; the average values of examined individuals: dimensions 125 mm by 3.0 mm at clitellum, segments 150. Prostomium $\frac{1}{2}$ epilobous. First dorsal pore in 11/12. Setae numbering 46–50 at III, 56–62 at V, 50–64 at VIII, 30–50 at XX, 41–50 at XXV; 0–2 between male pores; 22–27 between spermathecal pores; setal formula: AA = 1.0–1.2AB, ZZ = 1.0–1.8ZY. Clitellum annular in XIV–XVI, light brown, swollen; setae invisible externally; gaps in dorsal could be identified clearly.

Spermathecal pores two pairs in 5/6–6/7, ventral, eye-like, 0.40 circumference ventrally apart from each other. One or two tiny conical genital papillae before each pore. Three similar presetal genital papillae irregularly present on posterior border of segments VII.

Male pores one pair in XVIII, 0.33 circumference ventrally apart from each other, each on the top of a slightly raised, rounded porophore in a pulvinate pad with three or four circular folds. each male pore is medial to these two tiny papillae (Figure 2A), some paratypes with four or five tiny oval genital papillae surrounding each male pore (Figure 2D). In holotype, a pair of tiny conical genital papillae present on anterior border of segments XVIII, 0.2 mm apart from each other.

Female pore single in XIV, ovoid, milky white.

Internal characters

Septa 7/8–8/9 thick and muscular, 10/11–11/12 slightly thickened, 8/9–9/10 absent. Gizzard long bucket-shaped, in IX–X. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, simple, smooth, with two large indentation on dorsal and ventral margin, extending anteriorly to XXIV. Oesophageal hearts in X–XIII.

Ovaries in XIII. Spermathecae two pairs in VI–VII, ampulla heart-shaped, about 1.7 mm long in holotype; ampulla duct slender to stout, as long as two-thirds of ampulla. Diverticulum about 2.2 mm long, slender and short; seminal chamber swollen, about 2.0 mm (Figure 2B). No accessory glands observed.

Male sexual system holandric, testis sacs two pairs, in X–XI. Seminal vesicles two pairs, extending in X–XII, the pair in XI larger than the other. Prostate glands developed, inserting in XVIII and extending to 1/2XXVII and XX, coarsely lobate composed of three major lobes. Prostatic duct U-shaped, conspicuously curved at the distal part (Figure 2C). No accessory glands observed.

Remarks

The present species is obviously different from the other species in Table 3 because of the long diverticulum with a swollen seminal chamber. In addition, *A. dilatatus* sp. nov. differs from *A. tripunctus* (Chen 1946) in the features of body length, segment number, setae number between male pores, location of first dorsal pore and prostate glands.

***Amyntas infuscuatus* Jiang and Sun sp. nov.**
(Figure 3)

Material

Holotype. 1 clitellate (C-HN020-02A): China, Hainan Island, Jianfengling National Nature Reserve (18°44'45" N, 108°50'32" E), 1020 m elevation, cinnamon soil under tropical rainforest vegetation, 5 July 2006, J.P. Qiu, M.B. Bouché, J.X. Li and X.L. Zhang colls.

Paratypes. 2 clitellates (C-HN020-02B) with the same data as for holotype.

All three clitellates were externally characterized and dissected.

Etymology

The species is named after its dark pigment.

Diagnosis

Dimensions 60–78 mm by 1.4–1.6 mm at clitellum, segments 130–139. Setae numbering 46–48 at III, 56–60 at V, 49–52 at VIII, 42–46 at XX, 44–48 at XXV, 8–10 between male pores. Spermathecal pores two pairs in 5/6–6/7, 0.33 circumference ventrally apart from each other. Male pores in XVIII, 0.33 body circumference ventrally apart, each on the top of a slightly raised, oval porophore in a pulvinate

Figure 3. *Amynthus infuscuatus* Jiang and Sun sp. nov. (A) Ventral view of holotype, scale bar 2 mm; (B) spermathecae of holotype, scale bar 1 mm; (C) prostate gland of holotype.

pad with three to four circular folds. Spermathecae two pairs in VI–VII, ampulla ovoid, duct twice as long as ampulla. Diverticulum slightly longer than main pouch, slender, terminal one-third dilated into a swollen chamber.

External characters

Purple dorsal pigment before segment VIII of preserved specimens; light brown pigment on dorsum after segment VIII, no pigment on ventrum. Dimensions 60–78 mm by 1.4–1.6 mm at clitellum, segments 130–139; the average values of examined individuals: dimensions 68 mm by 1.5 mm at clitellum, segments 134. Prostomium $\frac{1}{2}$ epilobous. First dorsal pore in 12/13. Setae numbering 46–48 at III, 56–60 at V, 49–52 at VIII, 42–46 at XX, 44–48 at XXV; 8–10 between male pores; setal formula: AA = 1.0–1.2AB, ZZ = 1.0–1.2ZY. Clitellum annular, orange colour, in XIV–XVI, setae visible externally.

Spermathecal pores two pairs in 5/6–6/7, ventral, eye-like, 0.33 circumference ventrally apart from each other.

Male pores one pair in XVIII, 0.33 circumference ventrally apart from each other, each on the top of a slightly raised, oval porophore in a pulvinate pad with three or four circular folds. Two rounded genital papillae, flat-topped, present at inner ridge of porophore, anteromedial one larger than the other (Figure 3A).

Female pore single in XIV, oval.

Internal characters

Septa 6/7–7/8 thick and muscular, 10/11–12/13 slightly thickened, 8/9–9/10 absent. Gizzard long bucket-shaped, in IX–X. Intestine enlarged distinctly from XVI. Intestinal caeca paired in XXVII, simple, smooth, brown, extending anteriorly about to XXIV. Oesophageal hearts in X–XIII.

Ovaries in XIII. Spermathecae two pairs in VI–VII, ampulla ovoid, about 2.5 mm long in holotype; duct slender, twice as long as ampulla. Diverticulum slightly longer than main pouch, slender, terminal one-third dilated into a swollen chamber (Figure 3B), milky white. No accessory glands observed.

Male sexual system holandric, testis sacs two pairs, in X–XI. Seminal vesicles two pairs, extending in X–XII, the anterior pair larger. Testis sacs and seminal vesicles separated from each other on ventrum. Prostate glands developed, inserting in XVIII and extending to XVI and half of XX, coarsely lobate composed of two major lobes. Prostatic duct U-shaped, slender (Figure 3C). No accessory glands observed.

Remarks

Both *A. infuscuatus* sp. nov. and *A. tripunctus* have similar body length, setae, location of first dorsal pore, seminal vesicles and diverticulum length. However, *A. infuscuatus* sp. nov. is distinguished from *A. tripunctus* by its segment number, ampulla shape and seminal chamber length.

In comparison to *A. incongruus*, spermathecae of *A. infuscuatus* sp. nov. is quite different. Ampulla is heart-shaped, duct slightly shorter than ampulla, and terminal two-sevenths of diverticulum dilated into a swollen seminal chamber. Moreover, body length and first dorsal pore are also distinctive characters.

Although both have terminal third dilated into seminal chamber, *A. infuscuatus* sp. nov. and *A. instabilis* have no similar spermathecae. The ampulla of *A. infuscuatus* is ovoid, smaller than the heart-shaped ampulla of *A. instabilis*, but its ampulla duct and diverticulum are longer.

***Amyntas qiongzhongensis* Jiang and Zhao sp. nov.**
(Figure 4)

Material

Holotype. 1 clitellate (C-HN022-02A): China, Hainan Island, Diaoluoshan National Nature Reserve (18°43'30" N, 109°52'07" E), 920 m elevation, brown sandy soil under shrubbery, 6 July 2006, J.P. Qiu, M.B. Bouché, J.X. Li and X.L. Zhang colls.

Paratypes. 9 clitellates (C-HN022-02B) with the same data as for holotype. 2 clitellates (C-HN201008-01), 2 clitellates (C-HN201008-02): China, Hainan Island, Diaoluoshan National Nature Reserve (18°43'35" N, 109°52'02" E), 934 m elevation, black soil under palm trees, 24 July 2010, J.B. Jiang and Y.Z. Guo coll. 1 clitellate (C-HN201012-02): China, Hainan Island, Diaoluoshan National Nature Reserve (18°43'15" N, 109°52'18" E), 933 m elevation, black soil under scrubs, 24 July 2010, J.B. Jiang and Y.Z. Guo coll. 1 clitellate (C-HN201114-02): China, Hainan Island, Limushan Nature Reserve, Quling Mountain (19°13'40" N, 109°44'15" E), 739 m elevation, 27 May 2011, J.P. Qiu, J.B. Jiang, Q. Zhao, D. Cluzeau and W.K. Zhang coll.

Six individuals were dissected and 10 individuals were characterized externally only.

Etymology

The species is named after its type locality (Li-Miao Autonomous County of Qiongzong, Hainan).

Diagnosis

Dimensions 81–161 mm by 3.4–4.6 mm at clitellum, segments 127–159. Setae numbering 42–46 at III, 48–64 at V, 50–62 at VIII, 40–54 at XX, 40–80 at XXV; 4–8 between male pores; 21–26 between spermathecal pores. Spermathecal pores in 5/6–6/7, 0.40 body circumference ventrally apart. Male pores in XVIII, 0.33 body circumference ventrally apart, each on the top of a slightly raised, oval porophore in a pulvinate pad with three to five circular folds. Spermathecae two pairs in VI–VII, ampulla heart-shaped, duct slightly shorter than ampulla. Diverticulum is longer than the main pouch by one-fifth, terminal two-sevenths dilated into a swollen chamber.

External characters

Grey dorsal pigment present in the pre-clitellum segments of preserved specimens; light brown pigment on dorsum of the post-clitellum segments; no pigment on ventrum. Dimensions 81–161 mm by 3.4–4.6 mm at clitellum, segments 127–159; the average values of examined individuals: dimensions 125 mm by 3.9 mm at clitellum, segments 147. Secondary annulations conspicuous in pre-clitellum segments. Prostomium combined prolobous and $\frac{1}{2}$ epilobous. First dorsal pore in 11/12. Setae numbering 42–46 at III, 48–64 at V, 50–62 at VIII, 40–54 at XX, 40–80 at XXV; 4–8 between male pores; 21–26 between spermathecal pores; setal formula: AA = 1.0–1.5AB, ZZ = 1.0–2.0ZY. Clitellum annular, purple or light brown colour, in XIV–XVI, setae invisible externally.

Figure 4. *Amyntas qiongzongensis* Jiang and Zhao sp. nov. (A) Ventral view of holotype, scale bar 2 mm; (B) spermathecae of holotype, scale bar 1 mm; (C) prostate gland of holotype.

Spermathecal pores two pairs in 5/6–6/7, ventral, 0.40 circumference apart from each other. A couple of antrorse genital papillae surround each spermathecal pore. A pair of tiny, collapse-topped, presetal genital papillae present on anterior border of segments VII.

Male pores one pair in XVIII, 0.33 circumference ventrally apart from each other, each on the top of a slightly raised, oval porophore in a pulvinate pad with three to five circular folds. Four or five genital papillae tiny, collapse-topped, surrounding each male pore, present on the porophore (Figure 4A).

Female pore single in XIV, rounded, milky white.

Internal characters

Septa 5/6–7/8 thick and muscular, 10/11–12/13 slightly thickened, 8/9–9/10 absent. Gizzard short bucket-shaped, in 1/2VIII–IX. Intestine enlarged distinctly from XVI. Intestinal caeca paired in XXVII, simple, smooth, extending anteriorly to XXIV. Oesophageal hearts in X–XIII.

Ovaries in XIII. Spermathecae two pairs in VI–VII, ampulla heart-shaped, about 2.8 mm long in holotype; stout duct slightly shorter than ampulla. Diverticulum is longer than the main pouch by one-fifth, slender, terminal two-sevenths dilated into a swollen chamber (Figure 4B), milky white. No accessory glands observed.

Male sexual system holandric, testis sacs two pairs, in X–XI. Seminal vesicles two pairs, extending in XI–XII, the anterior pair well developed. Prostate glands well developed, inserting in XVIII and extending to 1/2XV and XXIII, coarsely lobate composed of several major lobes. Prostatic duct U-shaped, slender, conspicuously curved at the distal part (Figure 4C). No accessory glands observed.

Remarks

The new species is similar to *A. tripunctus* in having similar body length, body width, prostate glands and ampulla shape, but it has a shorter seminal chamber, while the terminal half of the diverticulum of *A. tripunctus* is dilated into a cucumber-shaped chamber.

Amyntas qiongzhongensis sp. nov. differs greatly from *A. incongruus* by the characteristics of the accessory glands and spermathecae.

Amyntas qiongzhongensis sp. nov. differs from *A. instabilis* sp. nov. in the features of spermathecae, prostate glands and other characters.

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