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

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This paper describes four new species of morrisi-group earthworms from Hainan and Guangdong Provinces, China: *Amynthas instabilis* sp. nov, *Amynthas dilatatus* sp. nov, *Amynthas infuscatus* sp. nov. and *Amynthas 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 *morrisi*-group.

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

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

**Introduction**

The *morrisi*-group is defined as *Amynthas* 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 *morrisi*-group have been reported from China: *Amynthas nanulus* (Chen et Yang 1975) (Chen et al. 1975), and *Amynthas parvus* (Chen and Hsu 1977) from mainland China, *Amynthas diaoluomontis* Qiu and Sun, 2009, *Amynthas octopapillatus* Qiu and Sun, 2009, *Amynthas zhangi* Qiu and Sun, 2009, *Amynthas lingshuensis* Qiu and Sun, 2009, *Amynthas endophilus* Zhao and Qiu, 2013, *Amynthas fluviatilis* Zhao and Sun, 2013, *Amynthas fucatus* Zhao and Jiang, 2013 from Hainan Island of China (Sun et al. 2009; Zhao et al. 2013). *Amynthas piagolensis* Hong and James, 2001, *Amynthas taebaekensis* Hong and James, 2001, *Amynthas naejangensis* Hong and James, 2001, *Amynthas draconis* Hong and James, 2001, *Amynthas assimilis* Hong and Kim, 2002, *Amynthas angulatus* Hong, 2007 and *Amynthas dabudongensis* Hong and James, 2009 have been reported from Korea (Hong and James 2001, 2009; Hong and Kim 2002; Hong 2007).

Earthworms of the *morrisi*-group from the island of Hainan were first reported by Chen Yi (Chen 1938), and included *Amynthas morrisi* (Beddard 1892), *Amynthas puerilis* (Chen 1938), *Amynthas hainanicus* (Chen 1938), *Amynthas oculatus* (Chen 1938), and *Amynthas hainanensis* (Chen 1938).
Amynthas monoserialis (Chen 1938) and Amynthas 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 morrisi-group have been recorded from Hainan. There are no earlier reports of morrisi-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 morrisi-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 Amynthas instabilis sp. nov., Amynthas dilatatus sp. nov., Amynthas infuscuatus sp. nov. and Amynthas qiongzhongensis sp. nov. Amynthas 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. qiongzhongensis and A. morrisi (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. qiongzhongensis 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. qiongzhongensis and other species show very low intraspecific values (0–0.16%), whereas distances between A. instabilis and A. qiongzhongensis were 13.59–13.79%. Among A. instabilis and the other three species they were 18.74–21.74%. Among A. qiongzhongensis and the other three species they were 17.05–19.72%. According to Chang and James (2011), values above 10–15%
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, Guangdong, 4 July 2006, J.X. Li and W.X. Zhang colls; 8 clitellates (C-HN2014-03): 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-HN201001-01): 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-HN201101-01): 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 colls. 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 *Amynthas* spp.

|                | *A. instabilis* 1 | *A. instabilis* 2 | *A. instabilis* 3 | *A. qiongzhongensis* 1 | *A. qiongzhongensis* 2 | *A. carnosus* | *A. morrisi* | *A. robustus* | *A. triastriatus* |
|----------------|-------------------|-------------------|-------------------|------------------------|------------------------|----------------|-------------|--------------|------------------|
| *A. instabilis* 1 | 0.16%             |                   |                   |                        |                        |                |             |              |                  |
| *A. instabilis* 2 |                   | 0.16%             |                   |                        |                        |                |             |              |                  |
| *A. instabilis* 3 | 0                 |                   | 0.16%             |                        |                        |                |             |              |                  |
| *A. qiongzhongensis* 1 | 13.79%          | 13.59%            | 13.79%            |                        |                        |                |             |              |                  |
| *A. qiongzhongensis* 2 | 13.79%          | 13.59%            | 13.79%            | 0.00%                  |                        |                |             |              |                  |
| *A. carnosus*    | 19.42%            | 19.20%            | 19.42%            | 18.92%                 | 18.92%                 |                |             |              |                  |
| *A. morrisi*     | 21.74%            | 21.52%            | 21.74%            | 19.72%                 | 19.72%                 | 20.47%         |             |              |                  |
| *A. robustus*    | 20.26%            | 20.04%            | 20.26%            | 17.05%                 | 17.05%                 | 21.09%         | 20.22%      |              |                  |
| *A. triastriatus*| 18.95%            | 18.74%            | 18.95%            | 17.24%                 | 17.24%                 | 19.60%         | 20.06%      | 16.34%       |                  |
Figure 1. *Amythas 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 ½ 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), *Amynthas instabilis* sp. nov. is closely related to *Amynthas incongruus* (Chen 1933) and *Amynthas tripunctus* (Chen 1946) from China, and to *A. dilatatus* sp. nov., *A. infuscatus* 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.

*Amynthas 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. *Amynthas 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.

*Amynthas 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).

*Amynthas 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 *morrisi*-group of *Amynthas* in China.

| Character                                      | A. *instabilis* sp. nov. | A. *dilatatus* sp. nov. | A. *infuscuatus* sp. nov. | A. *qiongzhongensis* sp. nov. | A. *tripunctus* (Chen 1946) | A. *incongruus* (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 | the anterior pair larger | the anterior pair larger   | the anterior pair larger       | large                      | very small                 |
| Prostates glands                                | developed                 | developed                 | developed                  | well developed                 | 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. | slightly longer than main pouch; terminal 9/10 dilated into a swollen chamber. | longer than main pouch by 1/5; terminal 2/7 dilated into a swollen chamber. | longer than main pouch by 1/5; terminal 1/2 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. *Amynthas 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 ½ 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–XI, the pair in XI larger than the other. Prostate glands developed, inserting in XVIII and extending to 1/2XVII 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.

Amynthas infuscatus 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
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 ½ 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. infuscatus* sp. nov. and *A. tripunctus* have similar body length, setae, location of first dorsal pore, seminal vesicles and diverticulum length. However, *A. infuscatus* 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. infuscatus* 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. infuscatus* sp. nov. and *A. instabilis* have no similar spermathecae. The ampulla of *A. infuscatus* is ovoid, smaller than the heart-shaped ampulla of *A. instabilis*, but its ampulla duct and diverticulum are longer.

*Amynthas 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 Qiongzhong, 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 prollobous and ½ epillobous. 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. Amynthas qiongzhongensis 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.

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

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

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