Four new earthworms of the genus *Amynthas* (Oligochaeta: Megascolecidae) from Mount Emei, Sichuan Province, China

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**ABSTRACT**

Four new species were collected from Mt. Emei, Sichuan Province, China. They are *Amynthas leshanensis* sp. nov., *Amynthas gilvus* sp. nov., *Amynthas retortus* sp. nov. and *Amynthas orbicularis* sp. nov. The first three species could be assigned to the *Amynthas sieboldi* group and the last species could be assigned to the *Amynthas aeruginosus* group according to the definition of Sims and Easton. *A. leshanensis* sp. nov. has the male pore porophore surrounded by a large irregular-shaped pad which extends from 17/18–½ XIX, and the intestinal caeca are complex. *A. gilvus* sp. nov. has each male pore on a slightly raised elliptical glandular flat-top pad, with a pointed extension lateral to each porophore. *Amynthas retortus* sp. nov. has a pair of small, round post-setal genital papillae medial to male porophores on XVII, spermathecal seminal chambers are twisted into zigzags and accessory glands are present on spermathecae and prostate gland areas. *A. orbicularis* sp. nov. has the spermathecal pores near dorso-lateral, complex intestinal caeca complex, three accessory glands with long ducts near the male pores, and the spermathecal seminal chambers are twisted into zigzags. The barcode data (partial sequence of mitochondrial cytochrome c oxidase subunit I) of each species has been submitted to the National Center of Biotechnology Information GenBank.

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**Introduction**

The earthworm fauna of Sichuan (originally transliterated as Szechuan) Province has been investigated by Yi Chen, Gates, Yuanhui Zhong, Ruihua Ding and so on (Chen 1931, 1936, 1946; Gates 1935, 1939; Zhong and Ma 1979; Ding 1985; Zhong 1987). More than 100 earthworm species have been reported from Sichuan Province, which suggests that the ecological environment may be very suitable for earthworm speciation. Specifically, Mt. Emei (originally transliterated as Omei), one of the mountain ranges in Sichuan Province, has a particularly high diversity of earthworms with 23 species known from *Amynthas* (Kinberg) alone (Blakemore 2007).
The 70-million-year-old humid and subtropical Mt. Emei (3099 m above sea level, 29° 34.5′N, 103°21.5′E), is located at the western rim of the Sichuan Basin in the transitional zone between tropical and temperate zonation (Tang and Ohsawa 1997). The parent rocks mainly consist of shale, dolomite, basalt and sandstone (Zhao and Chen 1980). Mt. Emei is widely covered by low-altitudinal evergreen broad-leaved forest, mixed forest of evergreen broad-leaved and coniferous trees at mid-altitude, and coniferous forest near the mountain top (Tang and Ohsawa 1999). The mean annual rainfall of Mt. Emei is 1786 mm at the summit and 1528 mm at the base. The mean temperature is 11°C at 3047 m a.s.l. and 26°C at 447 m a.s.l. (Tang 2006).

This expedition to further sample the earthworm fauna of Mt. Emei was conducted in July of 2011. Twenty-six described species were collected, and descriptions of a further four novel species are reported here.

The earthworms collected were anaesthetised in 10% ethyl-alcohol solution. Type specimens of all four novel species were fixed in 95% alcohol solution, and are deposited at the Shanghai Natural Museum.

*Amynthas leshanensis* Sun and Qiu sp. nov.  
(Figure 1(a–d))

**Type material**

**Holotype.** One clitellate (C-SC201102-02A) specimen: China, Sichuan Province, Mt. Emei (29°35′29″N, 103°17′12″E), 1300 m elevation, woods and bamboo, in dark cinnamon soil, 27 July 2011, J. Sun, J.B. Jiang, X.D. Lei and H.W. Feng coll. Paratypes: A total of two specimens, as follows: One clitellate (C-SC201102-02B): Same data as for holotype. One clitellate (C-SC201102-14): Same data as for holotype.

**Etymology**

This species was named after the collection location – Leshan City, China.

**Diagnosis**

Three pairs of spermathecal pores in 6/7–8/9, 0.4 of circumference ventrally apart; each male pore on an elliptical glandular flat-top pad, surrounded by a large irregular shaped pad which extends from 17/18–½ XIX; intestinal caeca complex with three long and broad finger-shaped sacs; spermathecal diverticulum shorter than main spermathecal axis by 0.42, distal 0.5 dilated into elongated ovoid seminal chamber.

**Description**

**External characters.** Preserved specimens dark grey on dorsum, lacking pigment on ventrum. Mid-dorsal line pigmented. Dimensions 88–93 mm by 3.0–3.8 mm at clitellum, segments number 95–120. Body cylindrical in cross section, and gradually tapered towards head and tail. Secondary annulations absent. Prostomium ½ epilobous. First dorsal pore in 11/12. Clitellum annular in XIV–XVI, pale, smooth, swollen, no visible setae externally, and dorsal pore usually absent on clitellum. Setae uniformly distributed, dorsal breaks more distinct than ventral, 22–24 at III, 30–35 at V, 28–36 at VIII, 36–42 at XX, 38–44 at XXV; 10 between male pores; 11–14/VII, 11–14/VIII between
spermathecal pores, setal formula $AA = 1–1.2AB$, $ZZ = 2–2.2ZY$. Spermathecal pores three pairs in $6/7–8/9$, intersegmental, about 0.4 of circumference ventrally apart, with tumid epidermis around each spermathecal pore (Figure 1(a)). Genital papillae absent around spermathecal region. Male pores paired in $XVIII$, about 0.33 of circumference ventrally apart, each on a small elliptical glandular flat-top pad, swollen, and surrounded by a larger irregular-shaped pad which extends from $17/18–1/2$ $XIX$. No genital papillae in this region (Figure 1(b)). Female pore single, med-ventral in $XIV$, elliptical, situated in a depression.

**Internal characters.** Septa $5/6–7/8$ and $10/11–12/13$ slightly thickened, $8/9–9/10$ absent. Dorsal blood vessel single, continuous onto pharynx; esophageal hearts four
pairs in X–XIII. Gizzard barrel-like, in VIII–X; intestine beginning to swell in XVI; intestinal caeca complex, with three long and broad finger-shaped sacs, originating in XXVII and extending forward to XXIV (Figure 1(c)). Male sexual system is holandric. Testis sacs two pairs, small, ventral in X and XI, connected with membrane on ventrum. Seminal vesicles paired in XI and XII, developed, broadly connected with each other on ventrum; prostate glands developed, coarsely lobate, in ½ XVI–XXI, ducts U-curved, distal end stouter. No visible accessory glands around base of each duct. Female organs: Ovaries in XIII. Spermathecae paired in VII–IX, about 2.6 mm long; ampulla heart-shaped, with very short duct, about 0.6 mm; diverticulum shorter than main spermathecal axis by 0.42, slender, distal 0.5 dilated into elongated ovoid seminal chamber; no nephridia on spermathecal ducts (Figure 1(d)).

Remarks
Amythus leshanensis sp. nov. keys to the Amythus sieboldi group according to Sims and Easton, 1972 (Sims and Easton 1972). Members of this group can be distinguished from those of the other species of the genus Amythus by three pairs of spermathecal pores intersegmental in 6/7–8/9 and a holandric male sexual system. In many principal respects the present new species is relatively similar to Amythus obscuritoporus (Chen, 1930) and Amythus editus editus (Chen, 1946). Moreover, they share the same collection location – Mt. Emei (Chen 1930, 1946).

The species A. obscuritoporus, which was initially collected in Suzhou City and Nanjing City, Jiangsu Province, China, and subsequently gathered in Wuxi City and Yixing City, Jiangsu Province, Zhejiang Province, Anhui Province and Mt. Emei, Sichuan Province, has several similar characteristics to the new species, as follows: (1) a dark colour on the dorsum and lacking pigment on the ventrum; (2) spermathecal pores three pairs in 6/7–8/9; (3) spermathecal ampulla heart-shaped with the duct shorter than the ampulla; (4) spermathecal diverticulum is usually about half the length of the main spermathecal axis, and the seminal chamber was described as elongated ovoid or bulb-like; (5) the left and right lobe of testis sacs connected ventro-medially in the form of a transverse band. However, the two are chiefly differentiated from each other as follows: (1) the clitellum of the new species present in XIV–XVI, smooth and swollen, but not present in all cases for A. obscuritoporus; (2) even though both have the spermathecal pores in 6/7–8/9, the pores of A. obscuritoporus are too small to recognise, comparing with the new species in which the spermathecal pores are surrounded by a relatively large swollen area; (3) the male pores of the new species are surrounded by a larger irregular-shaped pad, while the male pore region of A. obscuritoporus is an obscure whitish patch and appears vague under the skin, or looks like a small crescent-shaped groove in some cases; (4) the intestinal caeca of the new species is complex, while it is simple for A. obscuritoporus; (5) the prostate glands of the new species are developed, by comparison, but are small or rudimentary in A. obscuritoporus.

Another species, A. editus editus, also collected in Mt. Emei, Sichuan, is somewhat similar to the new species in that they have a similar density of setae, the spermathecal pores are all located in 6/7–8/9, the male pore papillae occupy more than one segment, the spermathecal ampulla is heart-shaped, the seminal chamber is elongated, and the prostate glands are developed with a U-curved duct. However, they differ in the following main characteristics: (1) the pigment of A. editus editus is pale both dorsally
and ventrally, but just ventrally for the new species; (2) the first two pairs of spermathecal pores of *A. editus editus* are extremely close – one seta interval, compared to the last pair – while the distance is equal in the new species; (3) there is a large lens-shaped papilla placed medio-ventrally in the male pore region of *A. editus editus* but no papillae for the new species; (4) the intestinal caeca is simple for *A. editus editus* but complex for the new species; (5) the spermathecal diverticulum is very long and the ectal portion of duct coiled in *A. editus editus*, compared to a shorter and more slender duct in the new species; (6) the first pair of seminal vesicles in XI are enclosed in testis sacs for *A. editus editus* but are clearly separated for the new species.

Two other recently reported *Amynthas sieboldi*-group species – *Amynthas arenulus* Bantaowong and Panha and *Amynthas longicaeca* Bantaowong and Panha – are somewhat similar to the new species according to the big pad on the male pore region at first glance, and the distance between male pores and spermathecal pores. However, they have many differences. For example: (1) comparing with *A. arenulus* and *A. longicaeca*, the new species has a smaller body size; (2) the spermathecal pores have tumid epidermis for the new species, but not for *A. arenulus* and *A. longicaeca*; (3) the intestinal caeca of the new species are complex; however, they are simple for *A. arenulus* and *A. longicaeca*; (4) the duct of spermathecae is stout for *A. arenulus*, and the diverticulum is very small with ovate knob for *A. longicaeca*, which are both very different from the new species.

The partial cytochrome c oxidase subunit I (COI) sequence of the holotype of *A. leshanensis* sp. nov. has been deposited in the National Center of Biotechnology Information (NCBI) GenBank with accession number KF205474 (specimen C-SC201102-02A).

*Amynthas gilvus* Sun and Qiu sp. nov.  
(Figure 1(e–g))

**Type material**
Holotype: One clitellate (C-SC201102-12A) specimen: China, Sichuan Province, Mt. Emei (29°35′29″N, 103°17′12″E), 1300 m elevation, woods and bamboo, in black cinnamon soil, 27 July 2011, J. Sun, J.B. Jiang, X.D. Lei and H.W. Feng coll. Paratype: One clitellate (C-SC201102-12B): Same data as for holotype.

**Etymology**
The species name *gilvus*, which means light yellow, refers to the pigment of the clitellum.

**Diagnosis**
Three pairs of spermathecal pores in 6/7–8/9; first dorsal pore in 13/14, but also present in 4/5–6/7; each male pore on a slightly raised elliptical glandular flat-top pad, with a pointed extension lateral to each porophore; the first pair of seminal vesicles is enclosed in the second pair of testis sacs; spermathecal diverticulum shorter than main spermathecal axis by 0.61, distal 0.75 dilated into longer chili pepper-shaped seminal chamber.
Description

External characters. Preserved specimens light cinnamon on dorsum, and lacking pigment on ventrum. Mid-dorsal line pigmented. Dimensions 41–59 mm by 2.3–2.8 mm at clitellum, segments number 77–108. Body cylindrical in cross section, and gradually tapered towards head and tail. Secondary annulations absent. Prostomium ½ epilobous. First dorsal pore in 13/14, but also present in 4/5–6/7. Clitellum annular in XIV–XVI, lacking pigment, smooth, swollen, setae not visible on clitellum, the trace of dorsal pores very clear on clitellum, but not forming a hole. Setae uniformly distributed, dorsal breaks more distinct than ventral, 22–24 at III, 24–40 at V, 32–36 at VIII, 22–40 at XX, 30–42 at XXV; 6–7 between male pores; 12–16/VII, 11–15/VIII between spermathecal pores, setal formula AA = 1–1.2AB, ZZ = 1.2–1.8ZY. Spermathecal pores three pairs, in 6/7–8/9, inconspicuous, intersegmental, about 0.33 of circumference ventrally apart, inconspicuous. No genital papillae in this region. Male pores paired in XVIII, 0.33 of circumference ventrally apart; each on a slightly raised elliptical glandular flat-top pad, with a lateral pointed extension of each porophore, without circular ridges surrounding (Figure 1(e)). Genital markings not visible externally. Female pore single, mid-ventral in XIV, elliptical, situated in a depression.

Internal characters. Septa 5/6–7/8 and 10/11–13/14 slightly thickened, 8/9–9/10 absent. Dorsal blood vessel single, continuous onto pharynx; esophageal hearts four pairs in X–XIII. Gizzard barrel-like, in VIII–X; intestine beginning to swell in XV; intestinal caeca simple, originating in XXVII and extending forward to XXIII, horn-shaped sac, surface smooth (Figure 1(f)). Male sexual system is holandric. Testis sacs two pairs, ventral in X and XI, silver, separated ventro-medially. Seminal vesicles paired in XI and XII, separated ventro-medially, anterior pair are enclosed in posterior pair of testis sacs, dorsal lobe of anterior pair is highly developed, extending to X. Prostate glands developed, coarsely lobate, thick, in XVI–XXII, ducts U-curved, distal end stout. No accessory glands observed. Female organs: Ovaries in XIII. Spermathecae paired in VII–IX, about 1.8 mm long; ampulla oval-shaped, with a short duct, about 0.3 mm; diverticulum shorter than main spermathecal axis by 0.61, distal 0.75 dilated into longer chili pepper-shaped seminal chamber; no nephridia on spermathecal ducts (Figure 1(g)).

Remarks

Amynthas gilvus sp. nov. also keys to the Amynthas sieboldi group (Sims and Easton 1972). This new species is close to two species – Amynthas jaoi (Chen 1946) and Amynthas loti (Chen and Hsü).

Amynthas gilvus sp. nov. resembles A. jaoi in such characters as: (1) the body size is relatively small; (2) the spermathecal pores are three pairs in 6/7–8/9 with the distance about 0.33 of circumference ventrally apart; (3) the simple intestinal caeca; (4) the spermathecal duct is short; (5) the prostates are developed. However, they differ as follows: (1) the pigment of the new species is not as dark as that of A. jaoi; (2) the number of setae per segment for the new species is lower than in A. jaoi; (3) the first dorsal pore of the new species is 13/14 but the pores in 4/5–6/7 are present, in contrast with the first dorsal pore in 12/13 for A. jaoi; (4) the male pore porophores have the lateral points but are without other genital markings in the male pore region for the new.
species; in contrast the male porophore is a single round papilla and with an accompanying postsetal genital marking for *A. jaoi*; (5) the diverticulum of the new species is clearly shorter than the main spermathecal axis; however it is longer than the main pouch for *A. jaoi*; (6) the first pair of seminal vesicles is enclosed in the second pair of testis sacs for the new species, while this is not the case for *A. jaoi*; (7) the prostatic duct is unusual and shows a double U-curve shape for *A. jaoi*, while it is a more typical, simple U-curve shape for the new species; (8) there are no observed accessory glands for the new species, but inconspicuous and sessile accessory glands are present in *A. jaoi*.

Considering the decolouring effect of anhydrous ethanol, *A. gilvus* sp. nov. and *A. loti* have the similar pigment, even though the pigment is described as light cinnamon on the dorsum for the new species but red-brown on the dorsum for *A. loti*; the density of setae is similar for the two species; the spermathecal pores are located in 6/7–8/9 for both species; and they both have a simple intestinal caeca. Nevertheless, these two species can be distinguished on the following main aspects: (1) the distance between the two spermathecal pores of *A. loti* is bigger than in the new species, and two pairs of genital papillae are present on VII and VIII for *A. loti* but absent for the new species; (2) the male pore porophore is cone-like and surrounded by 3–4 circles in *A. loti*, but it is a slightly raised elliptical glandular flat-top pad accompanied by a tail in the new one; (3) the septa in 8/9–9/10 is membranous in *A. loti*, but absent in the new species; (4) the seminal chamber of *A. loti* is ovoid-like but long pepper-shaped in the new species; (5) there is not a developed prostate gland in *A. loti*, but it is developed in the new species; (6) the ball-like accessory gland is present on the spermathecal region in *A. loti*, but there is no accessory gland in the new species; (7) the first pair of seminal vesicles are enclosed in the second pair of testis sacs of the new species, but this is not the case for *A. loti*.

The partial COI sequence of the holotype of *A. gilvus* sp. nov. has been deposited in the NCBI GenBank with accession number KF205478 (specimen C-SC201102-12A).

**Amynthas retortus** Sun and Jiang sp. nov.
(Figure 2(a–d))

**Type material**
Holotype: One clitellate (C-SC201102-15A) specimen: China, Sichuan Province, Mt. Emei (29°35′29″N, 103°17′12″E), 1300 m elevation, woods and bamboo, in black cinnamon soil, 27 July 2011, J. Sun, J.B. Jiang, X.D. Lei and H.W. Feng coll. Paratype: One clitellate (C-SC201102-15B): Same data as for holotype.

**Etymology**
The species name comes from the twisted shape of the spermathecal diverticulum.

**Diagnosis**
Three pairs of spermathecal pores in 6/7–8/9; each male pore on an oval-shaped pad with an elliptical glandular flat-top pad in the centre, surrounded by three circular ridges inside and five circular ridges outside, a pair of small round genital papillae medial to two male porophores postsetally on XVII; two bud-like accessory glands observed medial to each prostatic duct; spermathecal diverticulum shorter than main spermathecal axis by 0.17,
Figure 2. (a) *Amynthas retortus* Sun and Jiang sp. nov., holotype, spermathecal pore region; (b) *A. retortus* sp. nov., male pore region; (c) *A. retortus* sp. nov., intestinal caecum; (d) *A. retortus* sp. nov., spermathecae. (e) *Amynthas orbicularis* Sun and Jiang sp. nov., holotype, dorsal view of spermathecal pores; (f) *A. orbicularis* sp. nov., male pore region; (g) *A. orbicularis* sp. nov., intestinal caecum; (h) *A. orbicularis* sp. nov., prostate gland region; (i) *A. orbicularis* sp. nov., spermathecae.
distal 0.5 dilated and twisted into a zigzag-shaped seminal chamber; genital markings beneath the left spermathecae on VIII and IX and the right spermathecae on IX.

**Description**

**External characters.** Preserved specimens light brown on dorsum, and lacking pigment on ventrum. Mid-dorsal line pigmented. Dimensions 36–37 mm by 3.0–3.6 mm at clitellum, segments number 61–74. Body cylindrical in cross section, gradually tapered towards head and tail. Secondary annulations absent. Prostomium ½ epilobous. First dorsal pore in 13/14. Clitellum annular in XIV–XVI, lacking pigment, smooth, no visible setae externally, and dorsal pore absent on clitellum. Setae uniformly distributed, dorsal breaks more distinct than ventral, 20–24 at III, 36–39 at V, 40 at VIII, 40–48 at XX, 36–42 at XXV; 2–5 between male pores; 6–7/VII, 6/VIII between spermathecal pores, setal formula AA = 1.2–1.4AB, ZZ = 1.6–1.8ZY. Spermathecal pores three pairs, in 6/7–8/9, intersegmental, cannot be seen externally, one tiny genital papilla on VII and two similar papillae on VIII (Figure 2(a)). Male pores paired in XVIII, a little less than 0.33 of circumference ventrally apart, each on an oval-shaped pad with an elliptical glandular flat-top pad in the centre, surrounded by five circular ridges inside and three circular ridges outside. One small round genital papilla medial to each male porophore, and postsetally on XVII (Figure 2(b)). Female pore single, mid-ventral in XVI.

**Internal characters.** Septa 5/6–7/8 and 10/11–11/12 slightly thickened, 8/9–9/10 absent. Dorsal blood vessel single, continuous onto pharynx; esophageal hearts four pairs in X–XIII, developed. Gizzard barrel-like, in VIII–X; intestine beginning to swell in XV; intestinal caeca simple, originating in XXVII and extending forward to XXIII, fingershaped sac, smooth on ventral and dorsal margin (Figure 2(c)). Male sexual system is holandric. Testis sacs two pairs, ventral in X and XI. Seminal vesicles paired in XI and XII, developed, each is enclosed in the front of testis sacs, separated ventro-medially. Prostate glands developed, coarsely lobate, botryoidal, in XVI–XXII, ducts short and stout. Two bud-like accessory glands observed medial to each prostatic duct root. Female organs: Ovaries in XIII. Spermathecae paired in VII–IX, about 1.5 mm long; ampulla ball-shaped, with long duct as long as 0.67 ampulla; diverticulum shorter than main spermathecal axis by 0.17, slender, distal 0.5 dilated and twisted into a zigzag shaped seminal chamber; no nephridia on spermathecal ducts, stalked genital marking beneath the left spermathecae on VIII and IX and the right spermathecae on IX (Figure 2(d)).

**Remarks**

*Amynthas retortus* sp. nov. keys to the *Amynthas sieboldi* group (Sims and Easton 1972). The new species appears similar to *Amynthas cruratus* (Chen, 1946) in the characters of the pigment, the density of setae, the location of spermathecal pores, intestinal caeca shape and prostate gland. But it is distinct when the following characteristics are regarded: the body size, the arrangement of male pores and male pore region genital papillae, spermathecal diverticulum chamber, seminal vesicles and accessory glands aspect (Table 1).

Twenty species were added to the *Amynthas sieboldi* group after 1972. They are *Amynthas heterogens* (Chen and Hsu), *Amynthas contingens* (Zhong and Ma 1979),
Table 1. Comparison between *Amynthas retortus* sp. nov. and *A. cruratus* (Chen 1946) according to some main characteristics.

|                         | *A. retortus* sp. nov. | *A. cruratus* (Chen 1946) | *A. sacculus* Qiu and Wang, 1993 | *A. tungpuensis* Tsai, Shen and Tsai, 1999 |
|-------------------------|------------------------|---------------------------|---------------------------------|------------------------------------------|
| Dimensions              | 36–37 mm * 3.0–3.6 mm in 95% ethanol | 90 mm * 3.5 mm in formalin | 101–124 mm * 3–4 mm in formalin | 142–160 mm * 4.5–5.3 mm in formalin |
| Pigment                 | Light brown dorsally, no pigment ventrally | Light chocolate dorsally, pale ventrally | – – | Violet brown on clitellum, light gray on ventrum |
| Setae numbering         | 20–24/III, 36–39/V, 40/VIII, 40–48/XX, 36–42/XXV | 26/III, 30–32/VI, 29–40/IX, 35/XXV | 18–26/III, 24–28/V, 32–35/VIII, 34–44/XX, 31–46/XXV | 6/7–8/9 |
| Spermathecal pores      | 6/7–8/9 | 6/7–8/9 | Paired papillae on ventral sides of VII, VIII and IX or VII and VIII or only VIII | 6/7–8/9 |
| Genital papillae in spermatic pore region | Tiny papilla, one on VII and two on VIII | Two stumpy cushions laterally projected on ventral side of X on each of which are found from 5 to 13 small papillae. The glandular cushions are continuous ventromedially | A genital papilla immediately anterior to setal line in the medial position between the male pores in XVII, an additional papilla at the same location in XVII for some specimens | Two transverse rows pre-setal in VII and IX, numbering 4–11 for each row, an additional pair of post-setal papillae present at the ventro-lateral part of VIII in some specimens |
| Male pores              | XVIII, < 0.33 C | XIX, 0.33 C | XVIII, 0.33 C | XVIII, 0.26–0.29 C |
| Genital papillae in male pore region | A pair of small round genital papillae medial to two male porophores, postsetally on XVII | Similar papillae, two or three in each group on each side of XVII and XVIII | Two small papillae surround each male pore; paired papillae on ventral sides of XVII, XVIII and XIX, but sometimes lacking | A genital papilla immediately anterior to setal line in the medial position between the male pores in XVII, an additional papilla at the same location in XVII for some specimens |
| Intestinal caeca        | Simple | Simple | Simple | Simple |
| Spermatheca diverticulum | < main spermathecal axis by 0.17 | Nearly as long as main pouch | < main pouch | – – |
| Seminal chamber         | Twisted into zigzag | Usually crooked | Round or long-round | Peach-shaped |
| Seminal vesicles        | Each enclosed in the front of testis sacs | Ordinary | Well developed, separate with testis sacs | A small dorsal lobe |
| Prostate glands         | Developed | Well developed | Developed | Developed |
| Accessory glands in spermaticheca region | Stalked genital marking beneath the left spermathecae on VIII and IX and the right spermathecae on IX | No | Long-sac shaped with smooth surface, well developed | No |
| Accessory glands in prostate gland region | Two bud-shaped accessory glands, medial to each prostatic duct root | Stalked, each corresponding to respective papilla externally, gland conical or spherical | Long-sac shaped with smooth surface, well developed | No |
*Amynthas daulis daulis* (Zhong and Ma 1979), *Amynthas daulis fanjinmontis* Qiu, 1992, *Amynthas saccatus* Qiu and Wang, *Amynthas binoculatus* Tsai, Shen and Tsai, *Amynthas sexpectatus* Tsai, Shen and Tsai, *Amynthas tayalis* Tsai, Shen and Tsai, *Amynthas tungpuensis* Tsai, Shen and Tsai, *Amynthas moakensis* Hong and Kim, *Amynthas fenestrus* Shen, Tsai and Tsai, *Amynthas tantulus* Shen and Tsai, *Amynthas carnosus lichenensis* Wang and Qiu, *Amynthas huangi* James, Shih and Chang, *Amynthas monsoonus* James, Shih and Chang, *Amynthas hongyehensis* Tsai, Shen and Tsai, *Amynthas apapilatus* Zhao and Qiu, *A. arenulus*, *Amynthas dongyinensis* Shen, Chang and Chih and *A. longicaeca*. So, the other two similar species – *A. saccatus* and *A. tungpuensis* – were also put into Table 1. *A. saccatus* and the new species were both collected in Southwest China, and they both have concentric ridges on the male pore region. Moreover, the genital papillae always appear in the male pore and spermathecal pore region. However, they have some differences in the characteristics of the body size, the spermathecal seminal chamber and so on. *A. tungpuensis* and *A. retortus* sp. nov. have similar characters in male pore porophores and intestinal caeca, but the genital papilla of spermathecal pore and male pore region, the accessory glands, the shape of spermathecae and body size are very different. The partial COI sequence of the holotype of *A. retortus* sp. nov. has been deposited in the NCBI GenBank with accession number KF205479 (specimen C-SC201102-15A).

**Amynthas orbicularis** Sun and Jiang sp. nov.  
(Figure 2(e–i))

**Type material**  
Holotype: One clitellate (C-SC201102-19A) specimen: China, Sichuan Province, Mt. Emei (29°35′29″N, 103°17′12″E), 1300 m elevation, woods and bamboo, in black cinnamon soil, 27 July 2011, J. Sun, J.B. Jiang, X.D. Lei and H.W. Feng coll. Paratypes: A total of 10 specimens, as follows: Nine clitellate (C-SC201102-19B): Same data as for holotype. One clitellate (C-SC201102-21): Same data as for holotype.

**Etymology**  
This is a descriptive name deriving from the dome-like male pore porophore.

**Diagnosis**  
Two pairs of spermathecal pores in 7/8–8/9, slightly dorso-lateral; male pore porophore dome-like, glandular between two male pores; intestinal caeca complex with three slim and long finger-shaped sacs on dorsal margin; spermathecal diverticulum shorter than main spermathecal axis by 0.5, distal 0.5 twisted into zigzag seminal chamber, silver; three long stalked accessory glands medial to each prostatic duct root.

**Description**  
**External characters.** Preserved specimens red and brown on dorsum, lacking pigment on ventrum. Mid-dorsal line pigmented. Dimensions 65–89 mm by 3.8–4.2 mm at
clitellum. Segment number 72–112. Body cylindrical in cross section, gradually tapered towards head and tail. Prostomium ½ epilobous. First dorsal pore in 12/13. Clitellum annular XIV–XVI, ring-shaped, no pigment, smooth, swollen; setae cannot be seen externally in clitellum, dorsal pores absent on clitellum. Setae uniformly distributed, dorsal breaks more distinct than ventral, 20–28 at III, 38–42 at V, 36–46 at VIII, 36–38 at XX, 36–40 at XXV; 9–10 between male pores; 20–28/VIII between spermathecal pores, setal formula AA = 1.0–1.2AB, ZZ = 1.2–1.8ZY. Spermathecal pores two pairs, in 7/8–8/9, intersegmental, slightly dorso-lateral (Figure 2(e)). No genital papillae in this region. Male pores paired in XVIII, a little more than 0.33 circumference ventrally apart, each on a pad with a dome-like porophore in the centre, surrounded by 6–7 concentric ridges which are angular outside, skin between two male pores glandular. No genital papillae in this region (Figure 2(f)). Female pore single, mid-ventral in XIV, elliptical.

**Internal characters.** Septa 6/7 thickened, 5/6, 7/8 and 10/11–12/13 slightly thickened, 8/9–9/10 absent. Dorsal blood vessel single, continuous onto pharynx; esophageal hearts four pairs in X–XIII, developed. Gizzard barrel-like, in VIII–X; intestine beginning to swell in XV; intestinal caeca complex, with three slim and long finger-shaped sacs on dorsal margin, smooth on ventral margin, originating in XXVII and extending forward to ½ XXIII (Figure 2(g)). Male sexual system is holandric. Testis sacs two pairs, ventral in X and XI, anterior pair separated ventro-medially, posterior pair silver, connected ventro-medially. Seminal vesicles paired in XI and XII, developed. Prostate glands developed, coarsely lobate, composed of two main lobes upper and lower section, respectively, the left lobe in XVI–½ XXIII, the right lobe in XVI–XXII, the distal end of prostatic duct muscular and club-shaped (Figure 2(h)). Three long stalked accessory glands medial to each prostatic duct attachment to body wall in XVIII, corresponding with the tumid area outside. Female organs: Ovaries in XIII. Spermathecae paired in VIII–IX, the spermathecae in IX more developed than in VIII, about 2.1 mm long in IX; ampulla irregular mass-shaped with a gradual slender distal duct as long as 0.33 ampulla; diverticulum shorter than main spermathecal axis by 0.5, distal 0.5 twisted into zigzag shaped seminal chamber, silver; no nephridia on spermathecal ducts, genital marking not visible (Figure 2(i)).

**Remarks**

*Amynthas orbicularis* sp. nov. keys to the *Amynthas aeruginosus* group (Sims and Easton 1972). This group is diagnosed by two pairs of spermathecal pores intersegmental in 7/8–8/9 and a holandric male sexual system. *Amynthas omeimontis* (Chen 1931), collected initially in Mt. Emel, Sichuan Province, China, and then reported in Mt. Fanjing, Guizhou Province (Qiu 1987), China, is similar with the new species in the following main characteristics: (1) some stalked accessory glands present near the distal end of prostate gland ducts; (2) two pairs of spermathecal pores in 7/8–8/9; (3) complex intestinal caeca with several finger-shaped sacs; (4) the spermathecal ampulla duct not longer than half the length of ampulla, and the diverticulum seminal chamber twisted into zigzag type. However, these two species have some differences. First, abundant papillae are present in XVIII and XI in *A. omeimontis*, but there are no papillae in the new species except a glandular region between two male pores. Second, the distance between the two spermathecal pores is about 0.42
body circumference in *A. omeimontis*, while it is slightly more than 0.5 of circumference ventrally apart in the new species. Third, the male apertures of *A. omeimontis* are in a shallow depression, but the male pores of the new species are on pads with a dome-like porophore in the centre and surrounded by 6–7 concentric ridges. Finally, both pairs of testis sacs are connected medially with their fellow in *A. omeimontis*, but the anterior pair of testis sacs is separated ventro-medially, and the posterior pair connected ventro-medially, in the new species. Accordingly, these two species can be distinguished clearly.

Four recently reported species in the *A. aeruginosus* group are *Amynthas banlaensis* Hong, 2010, *Amynthas khamlai* Hong, 2010, *Amynthas phadeangensis* Hong, 2010 and *Amynthas naphopensis* Hong, 2010, all from Laos (Hong 2010). The morphological characters of these species were thoroughly compared with the new species; however, they are very different considering the long distance between the two spermathecal pores, lacking papillae on the male pores region and zigzag-shaped diverticulum seminal chamber for the new species. In contrast, four species from Laos have the distance between the two spermathecal pores shorter than half the circumference, some genital markings present on the male pore region, and the shape of the diverticulum seminal chamber not as complex as that of new species.

The partial COI sequence of the holotype of *A. orbicularis* sp. nov. has been deposited in the NCBI GenBank with accession number KF205481 (specimen C-SC201102-19A).

**Discussion**

Even though the morphological characteristics are enough to prove that the above four species are certainly new, the barcodes were determined to enhance the conclusion, and the average evolutionary divergences were calculated (Table 2). All of the species which are discussed above are included except those that are unavailable – *A. obscuritoporus, A. editus editus, A. jaoi* and *A. cruratus*. The accession numbers of the barcodes used for *A. loti* and *A. omeimontis* are EF077592 and KF205480, respectively. The number of base substitutions per site from between sequences is shown. Standard error estimate (s) are shown above the diagonal. Analyses were conducted using the Jukes-Cantor model. The analysis involved six nucleotide sequences. Codon positions included were 1st + 2nd + 3rd. All positions containing gaps and missing data were eliminated. There were a total of 596 positions in the final data set. Evolutionary analyses were conducted in MEGA5.

**Table 2.** Estimates of evolutionary divergence between sequences.

|                | *A. leshanensis* | *A. gilvus* | *A. loti* | *A. orbicularis* | *A. omeimontis* | *A. retortus* |
|----------------|------------------|-------------|-----------|------------------|-----------------|-------------|
| *A. leshanensis* | 0.020            | 0.020       | 0.022     | 0.021            | 0.022           |             |
| *A. gilvus*     | 0.171            | 0.021       | 0.022     | 0.021            | 0.021           |             |
| *A. loti*       | 0.188            | 0.192       | 0.232     | 0.014            | 0.020           |             |
| *A. orbicularis*| 0.230            | 0.199       | 0.230     | 0.116            |                | 0.020       |
| *A. omeimontis* | 0.216            | 0.201       | 0.230     | 0.116            | 0.020           |             |
| *A. retortus*   | 0.214            | 0.216       | 0.201     | 0.186            | 0.188           |             |

*A. = Amynthas.*
The average evolutionary divergence is 0.199 according to these species, and the data arranged from 0.116 through 0.232, which is good evidence that these species could be separated clearly. However, *A. orbicularis* sp. nov. and *A. omeimontis* have the closest relationship according to the data (0.116), which suggests that these two species may share a recent ancestor and are in the process of separating into two species.

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