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# Three new species of Tubificinae, Oligochaeta, from two plateau lakes in Southwest China

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## Abstract

Three new species of Tubificinae (Naididae, Oligochaeta), *Varichaetadrilus vestibulatus* **n. sp.**, *Aulodrilus apeniatus* **n. sp.**, and *Ilyodrilus mesoprostatus* **n. sp.**, are reported from Fuxian Lake and Xingyun Lake of Yunnan Province, Southwest China. *V. vestibulatus* differs from its allies by possessing modified spermathecal chaetae and thinner cylindrical penial sheaths. *A. apeniatus* is unique in the genus by having no penis. *I. mesoprostatus* is distinguishable from congeners by its prostate glands joining middle portion of atria and having concave, cone-shaped cuticular penial sheaths. Twenty-eight species of freshwater oligochaetes have hitherto been recorded from Yunnan Province, including five endemic species from three plateau lakes.

**Key words:** *Varichaetadrilus*, *Aulodrilus*, *Ilyodrilus*, Tubificinae, taxonomy, new species, Fuxian Lake, Xingyun Lake, Yunnan Province

## Introduction

The study of freshwater oligochaetes in China has a history of about one hundred years. Altogether 117 species, representing 7 families and 47 genera have hitherto been recorded. Previous researches, however, were mainly concentrated in eastern and central China (Wang & Cui 2007). Works from Southwest China such as Yunnan Province were scanty.

The earliest taxonomic work on oligochaetes in Yunnan was done by Liang (1963), who described one aberrant species of branchiobdellidans from Erhai Lake. No further oligochaete record from the province was published until Cui and Wang described a new species, *Potamothrix scleropenis*, from Fuxian Lake in 2005. Several papers on macrozoobenthos containing some faunistic information of Oligochaeta were then published (Nanjing Institute of Geography and Limnology 1990; Yang & Chen 1995; Cui et al. 2008; Cui & Wang 2008). At present, 25 species of oligochaetes belonging to 4 families and 13 genera have been recorded from Yunnan.

During the limnological investigation of Plateau lakes in 2002–2003, a number of oligochaete samples were collected from Fuxian Lake and Xingyun Lake, among which three new species of Tubificinae, *Varichaetadrilus vestibulatus* **n. sp.**, *Aulodrilus apeniatus* **n. sp.**, and *Ilyodrilus mesoprostatus* **n. sp.**, are described herein. Other findings on oligochaetes will be given in a separate paper.

## Materials and methods

Located in the Yunnan-Guizhou Plateau in southwest China, Fuxian Lake and Xingyun Lake originated from a common palaeolake in early Holocene Epoch. They were isolated in the middle Holocene Epoch, but a

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water course, Gehe River, remains, through which Fuxian Lake receives water from Xingyun Lake (Nanjing Institute of Geography and Limnology, 1990). Table 1 presents the main physico-chemical parameters of two lakes. Further characteristics of two plateau lakes were given in two previous accounts (Cui et al. 2007; Cui & Wang, 2008).

**TABLE 1.** Major physico-chemical characteristics of Fuxian Lake and Xingyun Lake (mean±SE).

| Parameter | Fuxian Lake | Xingyun Lake |
|---|---|---|
| Location | N24º17´–24º37´<br>E102º49´–102º57´ | N24º17´–24º23´<br>E102º45´–102º48´ |
| Surface area ($km^2$) | 211 | 35 |
| Water level (m) | 1721 | 1722 |
| Maximum length (km) | 31.5 | 10.5 |
| Maximum width (km) | 11.5 | 5.8 |
| Maximum depth (m) | 155 | 11 |
| Mean depth (m) | 90 | 7.8 |
| Shore development ($D_L$) | 1.72 | 1.64 |
| Catchments area ($km^2$) | 1045 | 378 |
| Water temperature (ºC) | 17.3 | 19.0 |
| Secchi depth (m) | 7.0±0.1 | 1.2±0.1 |
| Conductivity (µm/cm) | 29.2±0.2 | 332±9.8 |
| pH | 8.8±0.04 | 8.9±0.3 |
| Chlorophyll *a* (µg/L) | 2.2±0.1 | 99.8±24.0 |
| Total nitrogen (mg/L) | 0.198±0.003 | 3.922±0.470 |
| Total phosphorus (mg/L) | 0.021±0.001 | 0.310±0.036 |
| Trophic level | Oligotrophic | Eutrophic |
| Type of sediment | Clay or sand | Fine silt |

Data from Nanjing Institute of Geography and Limnology, CAS, 1990; Yang & Chen, 1995; Wang & Dou, 1998.

Sediment samples were collected with a weighted Petersen grab (1/16 $m^2$) and cleaned through a 250 µm sieve. Large worms were sorted manually in a white porcelain dish and the small ones were sorted under a dissecting microscope. All the specimens were preserved in 10% formalin.

Preserved specimens were examined first in temporary glycerine mounts, then stained with borax carmine, dehydrated in an alcohol series, cleared in xylene and mounted in Canada balsam. Measurements of body and chaetae were made from glycerine mounts. Other dimensions were made after the permanent procedures. Drawings were made using a camera lucida. The types and other specimens are deposited in the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS), Wuhan, China.

Abbreviation used in the figures:
Roman numerals = segment number
aa = atrial ampulla
ad = atrial duct
at = atrium
cs = copulatory sac
eo = ectal opening
mu = muscle
pe = penis

pr = prostate gland
ps = penial sheath
sa = spermathecal ampulla
sc = spermathecal chaeta
scs = spermathecal chaeta sac
sd = spermathecal duct
sf = sperm funnel
sm = sperm masses
sv = spermathecal vestibule
sz = spermatozeugmata
vd = vas deferens

## Results

### *Varichaetadrilus vestibulatus* n. sp.

**Holotype:** IHB YAN 20030201a, whole-mounted specimen.

**Type locality:** Northeast of Jianshan (24º36′03″ N, 102º51′14″ E) in Fuxian Lake, eastern Yunnan Province, China; depth 97 m, bottom temperature 13.5°C, dissolved oxygen at bottom 5.6 mg/L, total nitrogen in water 0.193 mg/L, total phosphorus in water 0.018 mg/L, fine clay; 14 Feb 2003, coll. Y. Cui.

**Paratypes:** IHB YAN 20030205a (Paratype-a) ─ 20030205b (Paratype-b), whole-mounted, two mature specimens from east of Lichang (24º32′04″ N, 102º51′43″ E) in Fuxian Lake; depth 113 m, bottom temperature 13.5°C, dissolved oxygen at bottom 5.2 mg/L, total nitrogen in water 0.195 mg/L, total phosphorus in water 0.024 mg/L, fine clay; 14 Feb 2003, coll. Y. Cui.

**Etymology:** The specific name "*vestibulatus*" is Latin for "vestibule", and refers to the pear-shaped vestibule in spermathecal duct.

**Description:** Length 11.3–30.0 mm (Holotype: 30.0 mm), width at XII about 0.6 mm, with 58–140 segments (Holotype: 140). Clitellum inconspicuous.

Dorsal chaetae (Fig. 1A) of II–VII (II–III in paratype-a) bifid only, 3–8 per bundle, 115–140 µm long, 2.5–3.0 µm thick, with upper prong twice as long as and thicker than lower, nodulus distal. Dorsals of VIII–X (IV–IX in paratype-a) 3–5 hairs and 5–8 bifids per bundle; hairs slender and smooth, 250–300 µm long, 2.0 µm thick basally; bifids (Fig. 1C) 110–135 µm long, 2.0–2.5 µm thick, prongs almost parallel, upper one slightly longer than, or as long as lower. Dorsals (Fig. 1D) of XI–XII (X–XI in paratype-a) bifid only, 5–6 per bundle, shorter and thicker than those in II–VII, with upper prong usually curved, and slightly longer than, lower. From XIII (XII in paratype-a) onwards, dorsals 2–5 hairs and 3–6 bifids per bundle, shorter and thinner than those of VIII–X, hairs 200–240 µm long, bifids (Fig. 1A) 100–115 µm long, with prongs similar to those of VIII–X, simple-pointed chaetae (Fig. 1B) sometimes present. Ventral chaetae (Fig. 1E, F) bifid, 6–8 per bundle anteriorly, 100–140 µm long, 2.0–3.0 µm thick, with prongs similar to those of dorsals in II–VII; 3–5 per bundle posteriorly, 90–110 µm long, 1.8–2.0 µm thick, with upper prong longer and thinner than lower. Ventral chaetae absent in IX. Spermathecal chaetae (Fig. 2A, sc; Fig. 2B) one per bundle in mid-X (mid-IX in paratype-a), entally embedded in glandular sacs, about 130 µm long, 4.0 µm thick, ental part curved and ectal part grooved. Penial chaetae (Fig. 1G) 1–2 per bundle in postero-XI (postero-X in paratype-a), 75–82 µm long, 2.8 µm thick, with upper prong slightly longer and thinner than lower, without nodulus. Male pores paired in line with ventral chaetae, posterior to middle of XI (mid-X in paratype-a). Spermathecal pores paired in line with ventral chaetae in mid-X (mid-IX in paratype-a), immediately anterior to spermathecal chaeta.

Pharyngeal glands in II–III. Chloragogen cells from VI (V in paratype-a) onwards. No coelomocytes. Male genitalia (Fig. 1H) paired in X–XII (IX–XI in paratype-a). Vas deferens (Fig. 1H, vd) up to 35 µm wide, shorter than atrium, although posterior part unclear; entering apical end of atrium. Atrium (Fig. 1H, st)

extending to XII (XI in paratype-a), about 1220 µm long, 40–85 µm wide, tubular and rather homogeneous throughout, with thin outer muscular layer. Prostate glands (Fig. 1D, pr) small, attached to ental portion of atrium by short stalk. Soft part of penis (Fig. 1D, pe) cylindrical, about 75 µm long, 65 µm in diameter, enclosed in copulatory sac; penis surrounded by thin cuticularized, somewhat thimble-shaped sheath (Fig. 1H, ps; Fig. 1I), 80 µm long, 68 µm wide, with 5 µm thick walls. Copulatory sac (Fig. 1H, cs) 95 µm long, 80–100 µm wide, with outer muscular layer 10–20 µm thick.

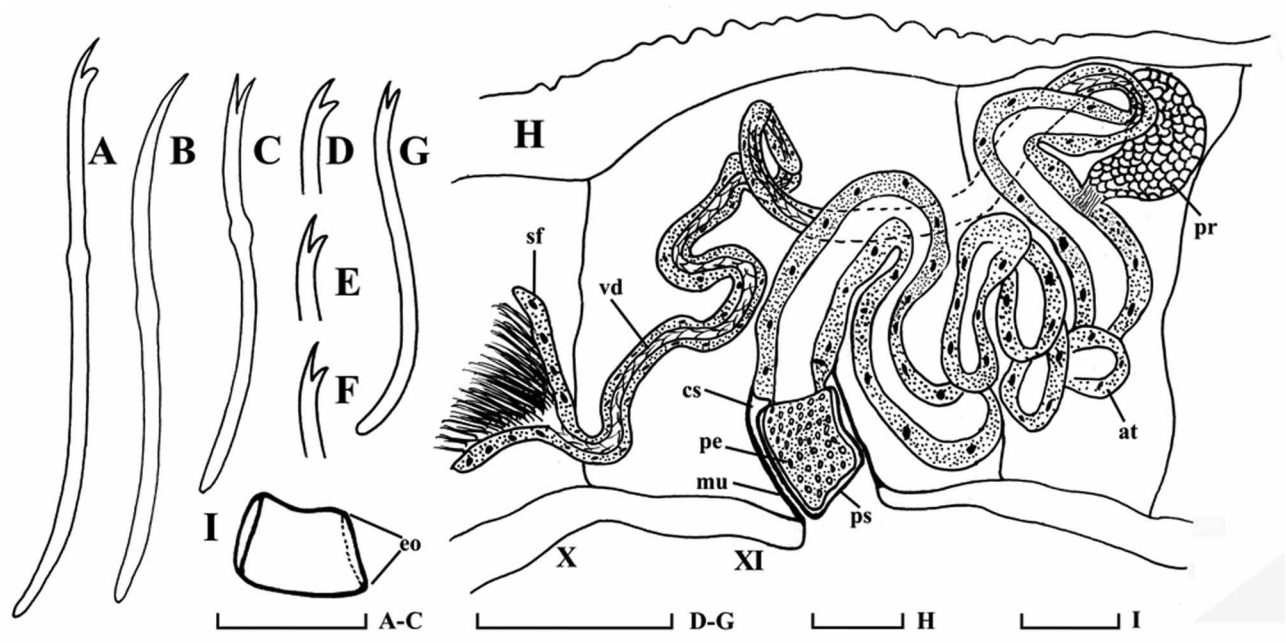



**FIGURE 1.** *Varichaetadrilus vestibulatus*. A. dorsal bifid from III; B. dorsal simple-pointed chaeta from VIII; C. dorsal bifid from VIII; D. distal end of dorsal bifid from X; E–F. distal end of ventral chaetae (XXII, XXXIV, respectively); G. penial chaeta; H. lateral view of male duct in segments X–XII; I. cuticularized penial sheath. A–I illustrated Holotype. Scale bars: A–C, D–G 40 µm; H, I 200 µm.

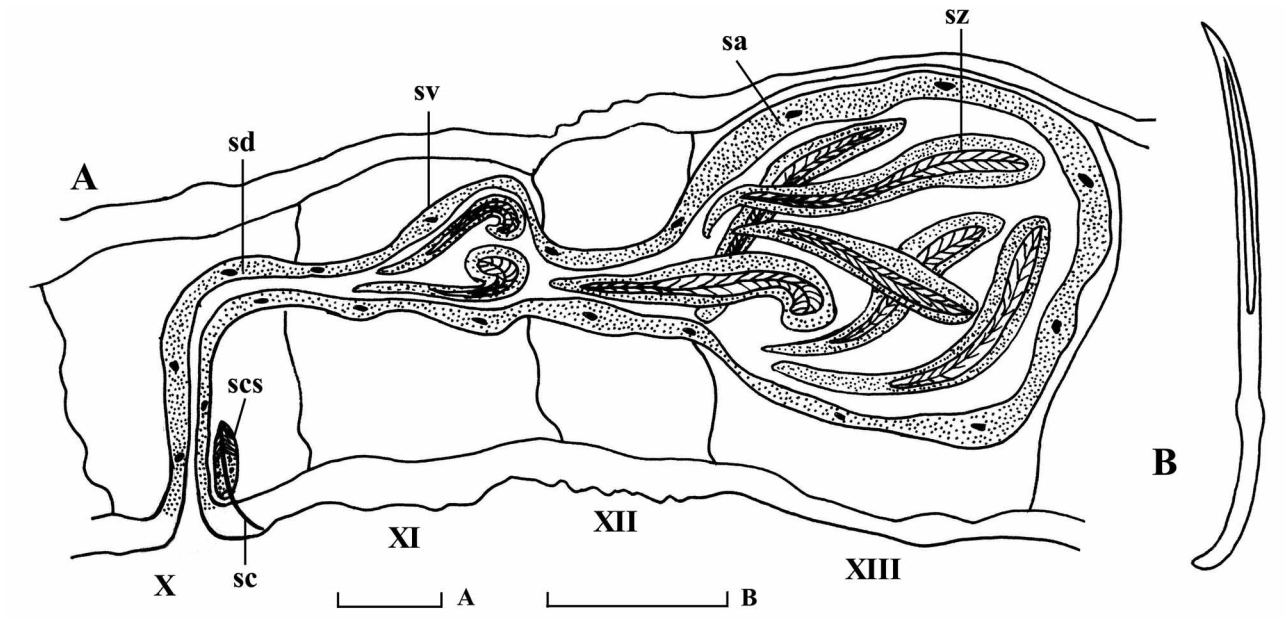



**FIGURE 2.** *Varichaetadrilus vestibulatus.* A. spermatheca; B. spermathecal chaeta. A–B illustrated Holotype. Scale bars: A 200 µm; B 40 µm.

Spermathecae (Fig. 2A) paired in X–XIII (IX–XI in paratype-a). Ampulla (Fig. 2A, sa) up to 600 µm long, maximally 390 µm wide. Duct (Fig. 2A, sd) totally about 950 µm long, tripartite, consisting of: (1) ectalmost part, about 475 µm long, 50–75 µm wide, (2) pear-shaped vestibule (Fig. 2A, sv), about 250 µm long, maximally 120 µm wide, (3) entalmost part, 200 µm long, 63–112 µm wide. Spermatozeugmata (Fig. 2A, sz) about 500–900 µm long, 5–10 of them in ampulla, 1–2 in vestibule.

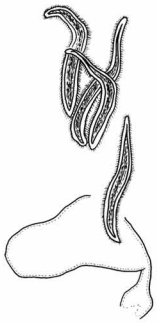
**Distribution and habitat:** Known only from Fuxian Lake, Yunnan Province, China. Freshwater lake, 97–113 m depth, water temperature lower than 14 ºC, fine clay.

**Remarks:** Judging from the long vasa deferentia, long tubular atria each with a small prostate gland, penes with distinct cuticular sheaths, and the atria longer than vasa deferentia, the new species fits the definition of *Varichaetadrilus* Brinkhurst, 1981. Nine species were previously known in the genus, all distributed in the Holarctic region (Timm, 2006).

Among the known members of *Varichaetadrilus*, there are two species with spermathecal vestibules: *V. pacificus* (Brinkhurst, 1981), originally known from Washington, USA (Brinkhurst, 1981), and *V. fulleri* Brinkhurst & Kathman, 1983 recorded only from Kentucky, USA (Brinkhurst & Kathman, 1983). However, *V. vestibulatus* **n. sp.** is easily separated from those two species by the modified spermathecal chaetae and the shape of penial sheaths (Table 2). In addition, the spermathecal duct of the new species has pear-shaped middle vestibule, but *V. pacificus* has cervix-like ental vestibule, and *V. fulleri* has ectal vestibule.

This is the first species of *Varichaetadrilus* recorded from China, and it is the lowest-latitude distributed member hitherto known in the genus.

**TABLE 2.** Comparison of *Varichaetadrilus vestibulatus* **n. sp.** and allied species.

| Species | *V. vestibulatus* n. sp. | *V. fulleri* | *V. pacificus* |
|---|---|---|---|
| Hairs | present from IV or VIII | absent | present |
| Dorsal bifids | bifid or simple-pointed | bifid | pectinate; bifid |
| Spermathecal chaetae | modified | absent | unmodified |
| Penial chaetae | modified | absent | slightly thicker than normal |
| Prostate glands | small | small | moderated |
| Cuticularized penial sheaths |  |  |  |
| Spermathecae |  |  |  |
| Distribution | Yunnan, China | USA | USA |
| References | this study | Brinkhurst & Kathman 1983 | Brinkhurst 1981 |

***Aulodrilus apeniatus* n. sp.**

**Holotype:** IHB YAN 20020813d, whole-mounted specimen.

**Type locality:** About 1 km in front of Gehe River mouth (24º22′58″ N, 102º49′49″ E) in Fuxian Lake, eastern Yunnan, China; depth 41 m, bottom temperature 17.6°C, dissolved oxygen at bottom 8.2 mg/L, total nitrogen in water 0.299 mg/L, total phosphorus in water 0.023 mg/L, fine clay; 8 Aug 2002, coll. Y. Cui & X. Liu.

**Etymology:** The specific name "*apeniatus*" is Latin for "without penis", and refers to the absence of penis in this species.

**Description:** Specimen incomplete, length > 12.8 mm, diameter at XI about 0.4 mm, segments > 80. Clitellum inconspicuous.

Dorsal chaetae 1–4 hairs and 2–4 bifids per bundle; hairs slender and long, without serration, 260–320 µm long anteriorly, and 180–220 µm long in postclitellar segments; bifids (Fig. 3C) pectinate, 80–120 µm long, 2.0–3.0 µm thick, upper prong, slightly longer or as long as, and thinner than lower, with 2–3 thin intermediate teeth. Ventral chaetae (Fig. 3A–B) bifid, 1–4 per bundle, 75–88 µm long, 3.0–3.2 µm thick, with upper prong conspicuously longer and slightly thinner than lower. Ventral chaetae unmodified in X and absent in XI. Male pores paired in line with ventral chaetae, posterior to middle of XI. Spermathecal pores paired in line with ventral chaetae in mid-X.

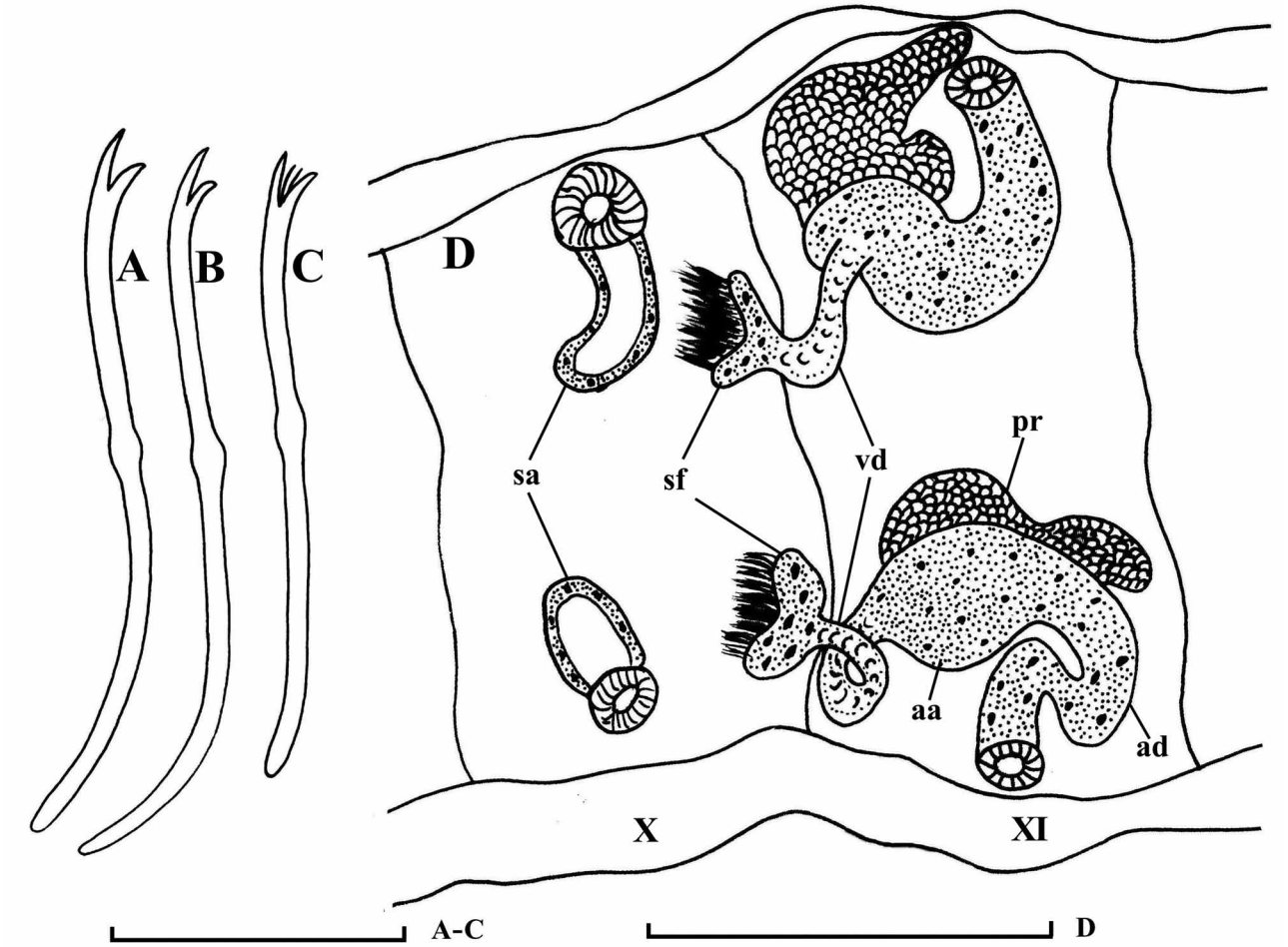



**FIGURE 3.** *Aulodrilus apeniatus*. A–B. ventral chaetae; C. dorsal bifid; D. lateral view of spermathecae and male ducts in segments X–XI. Scale bars: A–C 40 µm; D 200 µm.

Pharyngeal glands in II–III. Chloragogen cells from VI onwards. No coelomocytes. Male genitalia (Fig. 3D) paired. Vas deferens (Fig. 3D, vd) 86–110 µm long, 15–25 µm wide, entering atrium subapically. Atrium (Fig. 3D) club-shaped, transition between ampulla and duct gradual. Atrial ampulla (Fig. 3D, aa) ovoid,

110–120 µm long, 35–68 µm wide, atrial duct (Fig. 3D, ad) 90–160 µm long, 20–35 µm wide. Solid prostate gland (Fig. 3D, pr) large, attached to ental atrium by short stalk. Penis absent.
Spermathecae (Fig. 3D, sa) paired, small, elongated, 50–75 µm long, 35–45 µm wide, with indistinct duct, and without sperm in ampulla.

**Distribution and habitat:** Known only from type locality, Yunnan Province, China. Freshwater lake, 40 m depth, water temperature about 17 ºC, fine clay.

**Remarks:** The short vasa deferentia, the ovoid atria with solid prostate glands, the spermathecae without distinct ducts and the absence of penial chaeta and coelomocytes indicate that the new species fits more closely the definition of *Aulodrilus* Bretscher, 1899 (Brinkhurst & Jamison, 1971) than that of other described genera.

*Aulodrilus apeniatus* **n. sp.** having no penis is a unique feature of *Aulodrilus*. With regard to the simple atria and the dorsal bifids, *A. apeniatus* is similar to *A. pectinatus*, but it differs from the latter by the slender hairs, the pectinate chaetae throughout the body and the absence of penial chaeta.

As a rule, most *Aulodrilus* species have distinctive somatic chaetae, as bifid chaetae with short upper teeth, and posterior end modified into a respiratory organ (Finogenova & Arkhipova, 1994). These features are absent in the present species. The new species is assigned to *Aulodrilus* according to the male genitalia, but the external morphological characteristics. The described specimen of *A. apeniatus* is seemingly a single, unmated animal, and the structure of sexual organs that could be still in a phase of development. So, we gave a provisional home for it, and its systematic placement needs further confirmation from more specimens.

***Ilyodrilus mesoprostatus* n. sp.**

**Holotype:** IHB YAN 20030405n, whole-mounted specimen.

**Type locality:** Xingyun Lake (24°18'01″ N, 102°47'58″ E), eastern Yunnan Province, China; depth 5.0 m, bottom temperature 16.1°C, dissolved oxygen at bottom 7.6 mg/L, total nitrogen in water 2.960 mg/L, total phosphorus in water 0.129 mg/L, fine silt; 7 Apr 2003, coll. Y. Cui.

**Etymology:** "*meso*" and "*prostatus*" are Latin for "middle" and "prostate", respectively. The specific name refers to the prostate glands attaching to the middle part of the atria.

**Description:** Specimen incomplete, length > 4.4 mm, diameter at XI about 0.7 mm, segments > 22. Clitellum inconspicuous.

Dorsal chaetae 2–5 hairs and 2–4 bifids per bundle, hairs slender and smooth, 250–350 µm long, 2.0 µm thick basally; bifids (Fig. 4C) 100–125 µm long, 2.5–3.0 µm thick, with upper prong longer and thicker than lower. Dorsal chaetae absent in XI. Ventral chaetae (Fig. 4A–B) bifid, 2–4 per bundle, 100–120 µm long, 2.5–3.0 µm thick, with upper prong longer and thinner than lower. Spermathecal chaetae unmodified in X. Penial chaetae absent in XI. Male pores paired in line with ventral chaetae in mid-XI. Spermathecal pores paired in line with ventral chaetae in mid-X.

Pharyngeal glands in II–III. Chloragogen cells from VI onwards. No coelomocytes. Male genitalia (Fig. 4D) paired. Vas deferens (Fig. 4D, vd) short and broad, 240–360 µm long, 36–46 µm wide, entering atrium apically. Atrial ampulla (Fig. 4D, aa) somewhat spindle-shaped, 280–320 µm long, 58–108 µm wide. Prostate gland (Fig. 4D, pr) large, attached to middle portion of atrium by short stalk. Atrial duct (Fig. 4D, ed) curved, about 54 µm long, 25–36 µm wide. Soft part of penis (Fig. 4D, pe) cylindrical, about 32 µm long, 20 µm diameter, enclosed in copulatory sac; penis surrounded by thin cuticularized, truncated-cone shaped sheath (Fig. 4D, ps; Fig. 4E), 116 µm long, 40–80 µm wide, one side of the ectal opening (Fig. 4E, eo) curved upwards. Copulatory sac (Fig. 4D, cs) 64 µm long, 40–50 µm wide.

Spermathecal ampullae (Fig. 4D, sa) oval to round, 105–125 µm in diameter, with sperm masses (Fig. 4D, sm) in lumina, ducts (Fig. 4D, sd) 160–250 µm long, 35–58 µm wide.

**Distribution and habitat:** Known only from type locality, Yunnan Province, China. Freshwater lake, 5.0 m depth, water temperature 16 ºC, fine silt.

**Remarks:** The genus *Ilyodrilus* consists of the type species, *I. perrieri* Eisen, 1879, together with *I. templetoni* (Southern, 1909) and the dubious entities *I. frantzi* Brinkhurst, 1965 and *I. fragilis* Eisen, 1879. The principal characteristics and the distribution of congeners are shown in Table 3.

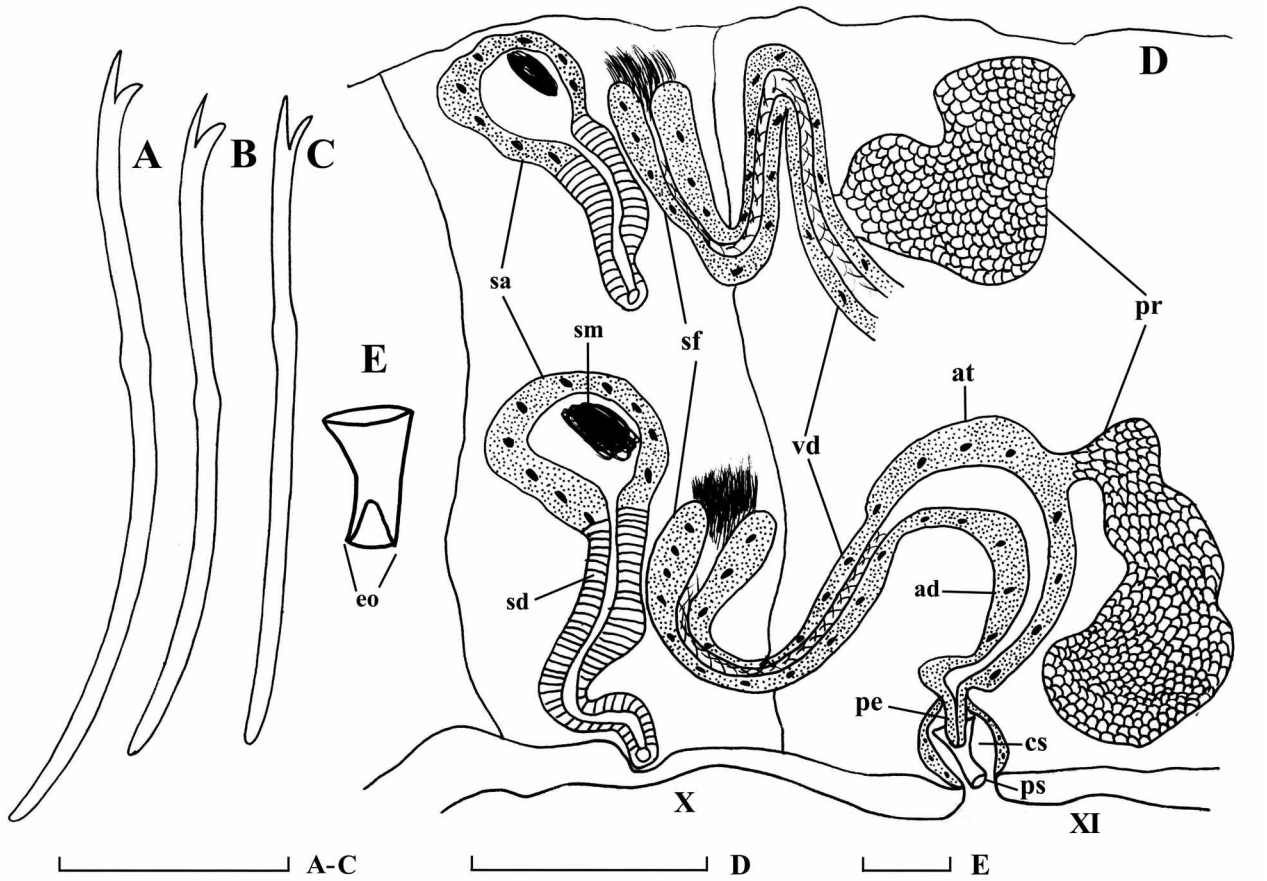
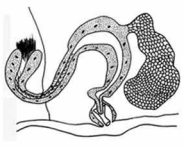
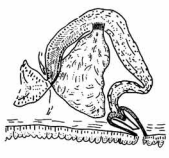



**FIGURE 4.** *Ilyodrilus mesoprostatus*. A–B. ventral chaetae; C. dorsal bifid; D. lateral view of spermathecae and male ducts in segments X–XI (lower one is right); E. cuticularized penial sheath. Scale bars: A–C 40 µm; D 200 µm; E 80 µm.

**TABLE 3.** Comparison of *Ilyodrilus mesoprostatus* **n. sp.** with its congeners.

| Species | *I. mesoprostatus* n. sp. | *I. perrieri* | *I. templetoni* | *I. frantzi* | *I. fragilis* |
|---|---|---|---|---|---|
| Hairs | present | absent | present or absent | absent or present | present |
| Dorsal bifids | bifid | pectinate | pectinate or bifid | bifid | pectinate |
| Male ducts |  |  |  |  |  |
| Cuticularized penial sheaths |  |  |  |  |  |
| Spermathecae | Present, with sperm masses | Present, but spermatozeugmata unknown | Present, with spermatozeugmata; or absent | Present, with spermatozeugmata | Present, but spermatozegmata unknown |
| Distribution | Yunnan, China | California, British Colombia | Europe, Asia, N. America, S. Africa | Western USA | California |
| References | This study | Eisen 1879; Brinkhurst & Jamison 1971; Holmquist 1985 | Brinkhurst 1965; Hrabe 1966; Brinkhurst & Jamison 1971; Wang 2002; Ohtaka & Nishino 2006 | Brinkhurst 1965, 1978; Brinkhurst & Jamison 1971; Holmquist 1985 | Eisen 1879; Brinkhurst 1965; Brinkhurst & Jamison 1971 |

*Ilyodrilus mesoprostatus* **n. sp.** is distinguishable from congeners mainly in the respect that attachments of prostate glands are situated at middle part of the atria, while those of previously described species are all situated near the ental part of the atria (Table 3). The penial sheaths of different species are dissimilar. Despite the undeveloped type specimens, *I. perrieri* from California has more or less tubular, cuticular penial sheaths (Holmquist 1985). *I. fragilis* has thin sheaths (Eisen 1879; Brinkhurst 1978). The cosmopolitan *I. templetoni* has long conical tapering distally sheath with irregular opening (Brinkhurst 1965; Hrabě 1966) and *I. frantzi* has thin, truncated cone-shaped cuticular sheath (Brinkhurst 1965). The new species has truncated-cone shaped sheath, with ectal opening curved upwards at one side (Table 3).

The presence of spermatozeugmata is regarded a diagnostic character of the genus *Ilyodrilus* (Brinkhurst & Jamison 1971). Although spermatozeugmata are absent in the present new species, it was assigned to this genus according to the structure of male genitalia. Spermatozeugmata are in fact not always present in *Ilyodrilus*. For instance, they were not mentioned in previous descriptions of *I. perrieri* and *I. fragilis* (Eisen, 1879; Holmquist 1985; Brinkhurst, 1965; Brinkhurst & Jamison 1971). Some specimens of *I. templetoni* have been described without spermathecae at all (Brinkhurst & Jamison 1971, Wang 2002), while for *I. frantzi*, the presence of spermatozeugmata was confirmed (Holmquist 1985). In the new species, *I. mesoprostatus*, the sperm were massed. So, the genus *Ilyodrilus* Eisen, 1879, needs a revision in the future.

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