



## Four species of *Tubifex* Lamarck (Annelida: Oligochaeta: Naididae) from Tibet, China

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### Abstract

Four species of Tubificinae (Oligochaeta: Naididae), *Tubifex laxus* n. sp., *Tubifex gracilentus* n. sp., *Tubifex conicus* He, Wang & Cui, 2012, and *Tubifex tubifex* (Müller, 1774) are reported from Tibet, China. *T. laxus* n. sp. is distinguished from allied species by relatively wide atria. *T. gracilentus* n. sp. differs from congeners in extremely thin and long penial sheaths. *T. conicus* is redefined as a species that lacks spermathecae. *T. tubifex* as redescribed here has slightly hirsute hair chaetae, cuticle rings around the penes and spermathecae with empty ampullae.

**Key words:** Clitellata, Tubificinae, *Tubifex*, new species, taxonomy, Tibet

### Introduction

The Tibetan Plateau is a unique zoogeographical unit. So far, a total of 30 species of aquatic oligochaetes belonging to 3 families (Naididae, Lumbriculidae, Enchytraeidae) and 16 genera have been recorded from this region (Stephenson 1909; Černosvitov 1942; Liang 1963; Liang *et al.* 1979; Liang *et al.* 1998; He *et al.* 2012; Peng *et al.* 2014; Cui *et al.* 2015). Herein, two new species are described and two species are revised from material collected in Tibet, China, which all belong to *Tubifex* Lamarck, 1816.

### Material and methods

Samples were collected with a D-frame dip net and a charged Petersen grap (1/16 m<sup>2</sup>), then cleaned on a 250 µm sieve, and preserved in 10% formalin. Specimens were stained with borax carmine and mounted whole in Canada balsam. After dehydration, desiccation, fixing and gold spraying, the specimens were examined under a Hitachi SU8010 scanning electron microscope (SEM). Measurements were based on permanent mounts, and drawings were made with a camera lucida. The types are deposited in the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS), Wuhan, China.

Abbreviations in the figures: Roman numerals correspond to segment numbers; at = atrium, br = brain, cr = cuticle ring, ed = ejaculatory duct, mb = muscular bulb, mo = mouth, mp = male pore, o = ovary, pe = penis, ph = pharynx, pr = prostate gland, pro = prostomium, ps = penial sheath, sf = sperm funnel, sp=spermatheca, spa=spermathecal ampullae, spp=spermathecal pore, t = testis, vd = vas deferens.

### Results, systematic account

#### Family Naididae

## Subfamily Tubificinae

### *Tubifex laxus* n. sp.

(Figs 1–3)

**Holotype.** IHB XZ20130630j, mature specimen, stained and whole-mounted in Canada balsam.

**Type locality.** Wetland in Cuomujiri Mountain (29°47'46"N, 94°24'53"E), southeast Tibet of China, ca. 3,990 m asl. Sediment silt, with abundant hydrophytes. Water depth 15–20 cm, water temperature 11.5 °C, pH 6.2, dissolved oxygen 9.1 mg/L, and conductivity 19.3 µs/cm. Collected on 30 June 2013 by the authors.

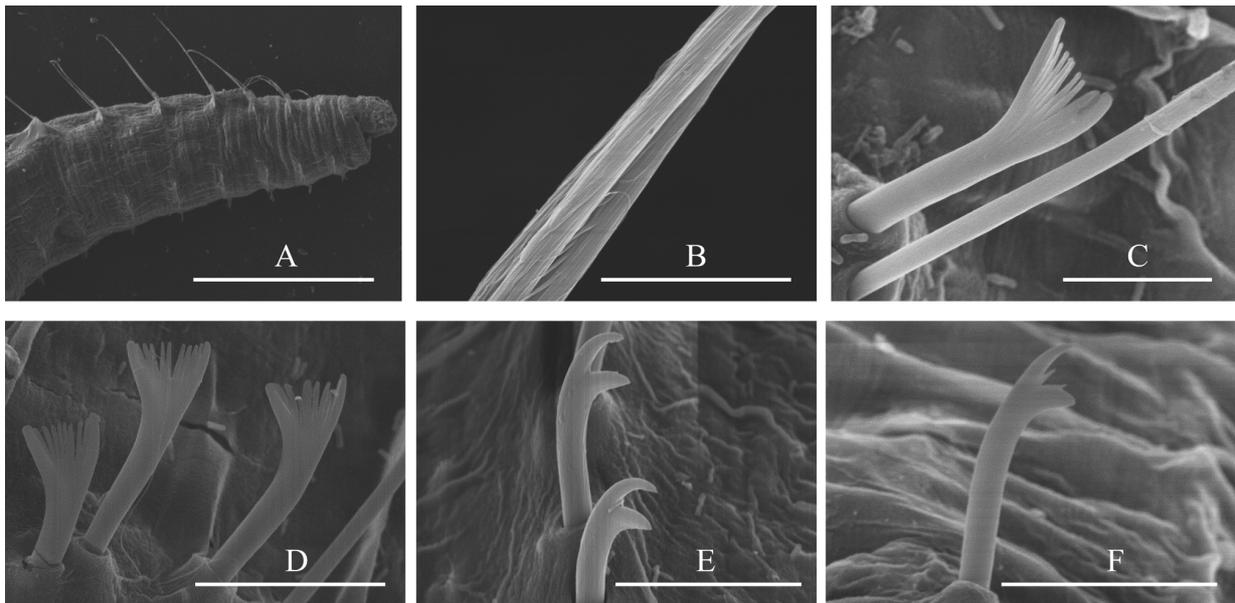
**Paratype.** IHB XZ20130630k, 1 mature specimen whole mounted in Canada balsam, from the type locality.

**Other material.** 1 specimen for SEM, from the type locality.

**Etymology.** The epithet "*laxus*" is Latin for "wide", and refers to the relatively wide atrium of the species.

**Description.** Length 6.8–7.2 mm (holotype: 6.8 mm), width at genital segments X–XI 0.4–0.5 mm (holotype: 0.5 mm). Segments 41–46 (holotype: 46). Prostomium obtuse or conical (Figs. 1A, 2A, 3A: pro). Clitellum inconspicuous, in X–XI. No coelomocytes. Dorsal chaetae (1) 2–3 hairs and 2–5 bifids per bundle. Hairs slightly hirsute, 110–300 µm long, 2–4 µm thick (Fig. 1B, C). Dorsal bifids palmate, 40–90 µm long, 2–4 µm thick, upper and lower tooth subequal, 6–8 µm long, with 6–10 fine intermediate teeth, nodulus distal (Figs. 1C, D, 3D). Ventral chaetae bifid, (3) 4–5 per bundle anteriorly, 2–3 per bundle posteriorly, 60–100 µm long, 2–4 µm thick, upper tooth slightly thinner and longer than lower (4–5 µm/3–5 µm), with (0) 1–2 fine intermediate teeth, nodulus distal (Figs. 1E, F, 3B, C). Ventral chaetae in X–XI present, unmodified. Male pores paired in line with ventral chaetae, in middle to posterior of XI.

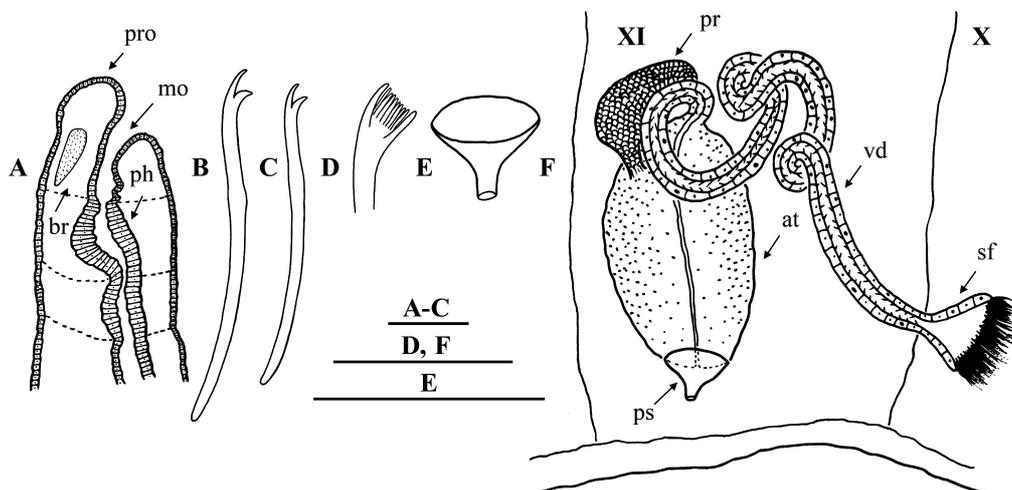
Pharynx in III–IV, 180–200 µm long, 120–150 µm wide (Figs. 2A, 3A: ph). Chloragogen cells from VI onwards. Male genitalia paired in X–XI. Sperm funnel cup-shaped, 20–30 µm long, 55–75 µm wide (Figs. 2C, 3F: sf). Vas deferens 500–600 µm long, about 3 times as long as atrium, uniformly 15 µm wide, internally ciliated along the whole length and entering atrium apically (Figs. 2C, 3F: vd). Atrium ovoid placed vertically in XI, 140–160 µm long, maximally 85–100 µm wide; no ejaculatory duct (Figs. 2B, C, 3F: at). Prostate gland solid, attached to ental portion of atrium by short stalk, 25–35 µm long, 70–85 µm wide (Figs. 2C, 3F: pr). Penis inconspicuous, surrounded by cuticular, symmetrical and funnel-shaped penial sheath. Penial sheath 30–40 µm long, 35–40 µm wide at ental end, length to width ratio nearly 1.0 (Fig. 2D, 3E, F: ps). Testes paired in X, small, immediately behind septum 9/10 (Fig. 2A: t). Seminal vesicles in X–XI, with little developing sperm. Ovaries paired in XI, immediately behind septum 10/11 (Fig. 2A: o). Egg present in XI. Spermathecae absent.



**FIGURE 1.** *Tubifex laxus* n. sp., SEM micrographs. **A.** Lateral view of anterior body. **B.** Hairs in IV. **C.** Hair chaeta and palmate chaeta in XII. **D.** Dorsal palmate chaetae in XII. **E.** Ventral chaetae in IV. **F.** Ventral chaetae in XIII. Scale bars: A. 500 µm; B, C. 10 µm; D–F. 20 µm.



**FIGURE 2.** *Tubifex laxus* n. sp., micrographs from stained whole mounts. **A.** Lateral view of anterior body. **B–D.** Ventrolateral view of male genitalia. Scale bars: A. 200  $\mu$ m; B–D. 50  $\mu$ m.



**FIGURE 3.** *Tubifex laxus* n. sp. **A.** Lateral view of head region. **B.** Ventral chaeta in IV. **C.** Ventral chaeta in a posterior segment. **D.** Distal tip of dorsal palmate chaeta in VIII. **E.** Penial sheath. **F.** Lateral view of male genitalia in X–XI. Scale bars: A. 100  $\mu$ m; B–D. 20  $\mu$ m; E, F. 100  $\mu$ m.

**Remarks.** The new species could be placed in the genera *Tasserkidrilus* Holmquist, 1985 or *Tubifex* (*sensu stricto*) based on: vas deferens at least two times longer than atrium, ejaculatory duct lacking, solid prostate gland attached to ental portion of atrium, and conical penial sheath (Brinkhurst & Jamieson 1971; Holmquist 1983, 1985; Timm 1989). However, in species of *Tasserkidrilus* the ventral chaetae in XI are lacking in mature specimens, the atrium consists of two or three portions of different thickness, and the penial sheath is asymmetrical (Timm 1989).

These characters are inconsistent with those of the new material. Therefore, we discard *Tasserkidrilus* as a possible genus for the new species. Although its vasa deferentia are not bipartite, the new material agrees with the generic diagnosis of *Tubifex* in the following characters: long vas deferens entering atrium apically, ovoid atrium placed vertically in XI with no ejaculatory duct, solid prostate gland attached to ental atrium by a short stalk, and symmetrically funnel-shaped penial sheath (Brinkhurst & Jamieson 1971; He *et al.* 2012). The new species is easily distinguished from allied species by its relatively wide atrium (Brinkhurst & Jamieson 1971; Pickavance & Cook 1971; Hrabě 1981; He *et al.* 2012; Peng *et al.* 2014) (Table 2).

**Distribution and habitat.** Known only from the type locality, Cuomujiri Mountain, southeast Tibet of China. Freshwater.

***Tubifex gracilentus* n. sp.**

(Figs. 4–6)

**Holotype.** IHB XZ20150511a, mature specimen, stained and whole-mounted in Canada balsam.

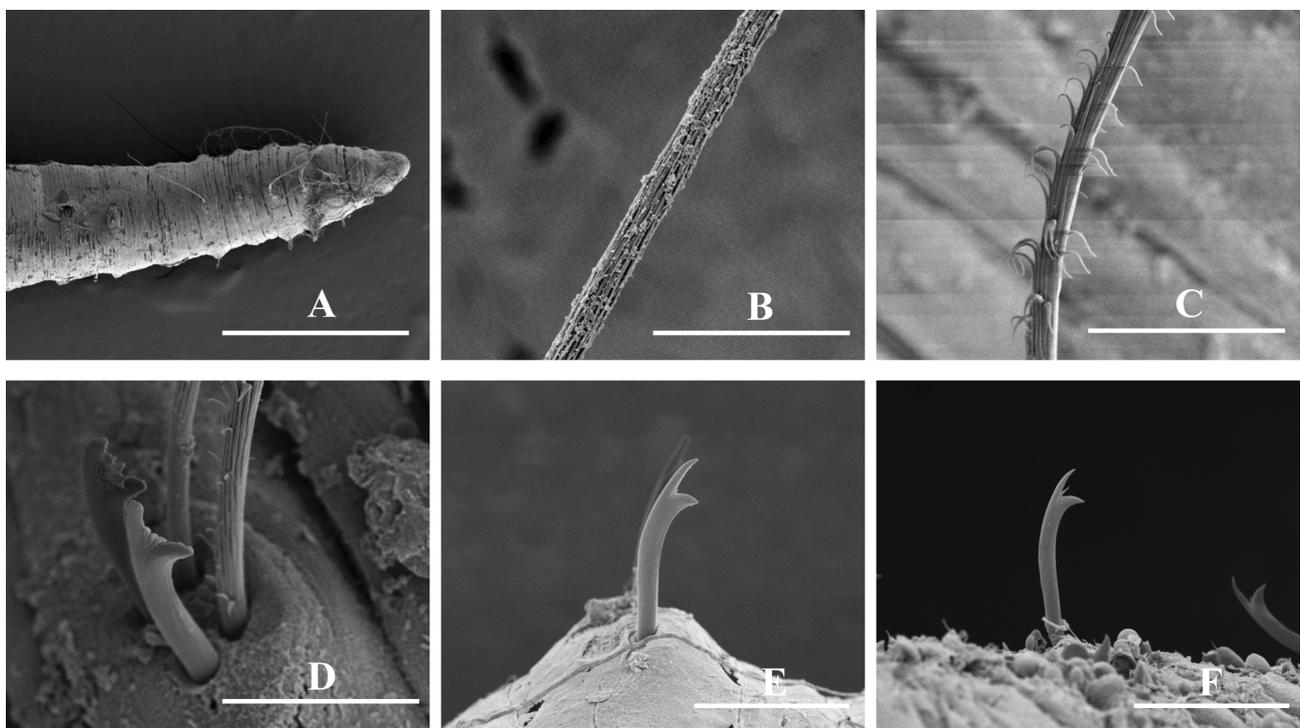
**Type locality.** Juemu River (29°39'23"N, 94°17'12"E), southeast Tibet of China, ca. 3,290 m asl. Sediment silt. Water depth 40–60 cm, water temperature 6.4°C, pH 6.2, dissolved oxygen 8.8 mg/L, and conductivity 12.3 µs/cm. Collected on 11 May 2015 by the authors.

**Paratype.** IHB XZ20150511b, 1 mature specimen, stained and whole-mounted in Canada balsam, from the type locality.

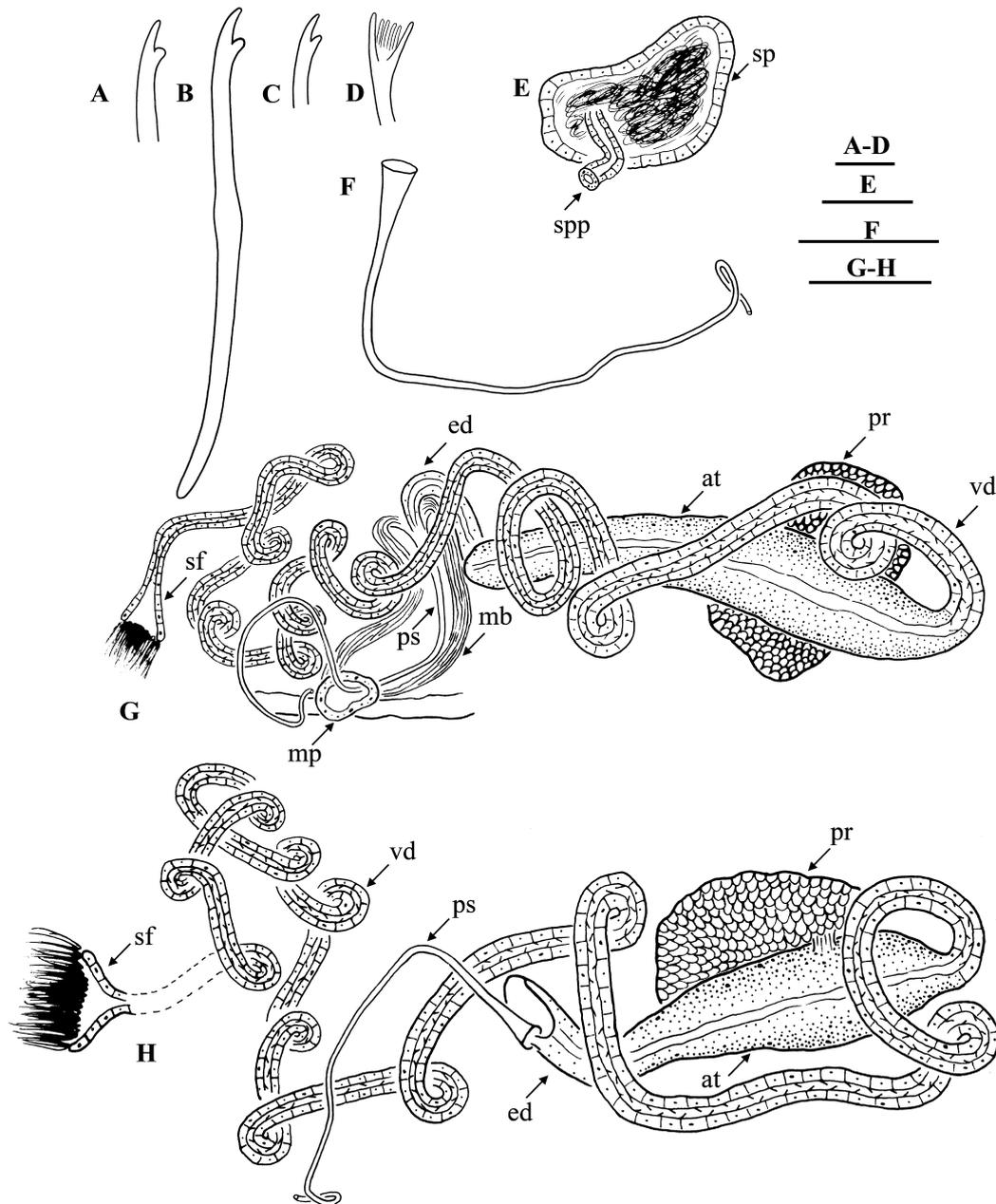
**Other material.** 1 specimen for SEM, from the type locality; 1 mature specimen whole-mounted in Canada balsam, from Trigu Co (Co means lake in Tibet).

**Etymology.** The epithet “*gracilentus*” is Latin for “slender and thin”, and refers to the thin and long penial sheath of this species.

**Description.** Length 6.4–6.7 mm (Holotype: 6.7 mm), width at genital segments X–XI 0.5–0.6 mm (holotype: 0.6 mm). Segments 32–34 (holotype: 32). Prostomium obtuse or conical (Fig. 4A). Clitellum inconspicuous. No coelomocytes.



**FIGURE 4.** *Tubifex gracilentus* n. sp., SEM micrographs. **A.** Lateral view of anterior body. **B.** Hair in IV. **C.** Hair in XII. **D.** Dorsal pectinate chaeta in XII. **E.** Ventral chaeta in IV. **F.** Ventral chaeta in XIII. Scale bars: A. 500 µm; B–F. 20 µm.



**FIGURE 5.** *Tubifex gracilentus* n. sp. **A.** Ventral chaeta in II. **B.** Ventral chaeta in IV. **C.** Ventral chaeta in a posterior segment. **D.** Distal tip of dorsal pectinate chaeta in VIII. **E.** Spermatheca. **F.** Penial sheath. **G.** Male genitalia in X–XII, dorsal view. **H.** Male genitalia in X–XII, lateral view. Scale bars: A–D. 20  $\mu$ m; E–H. 100  $\mu$ m.

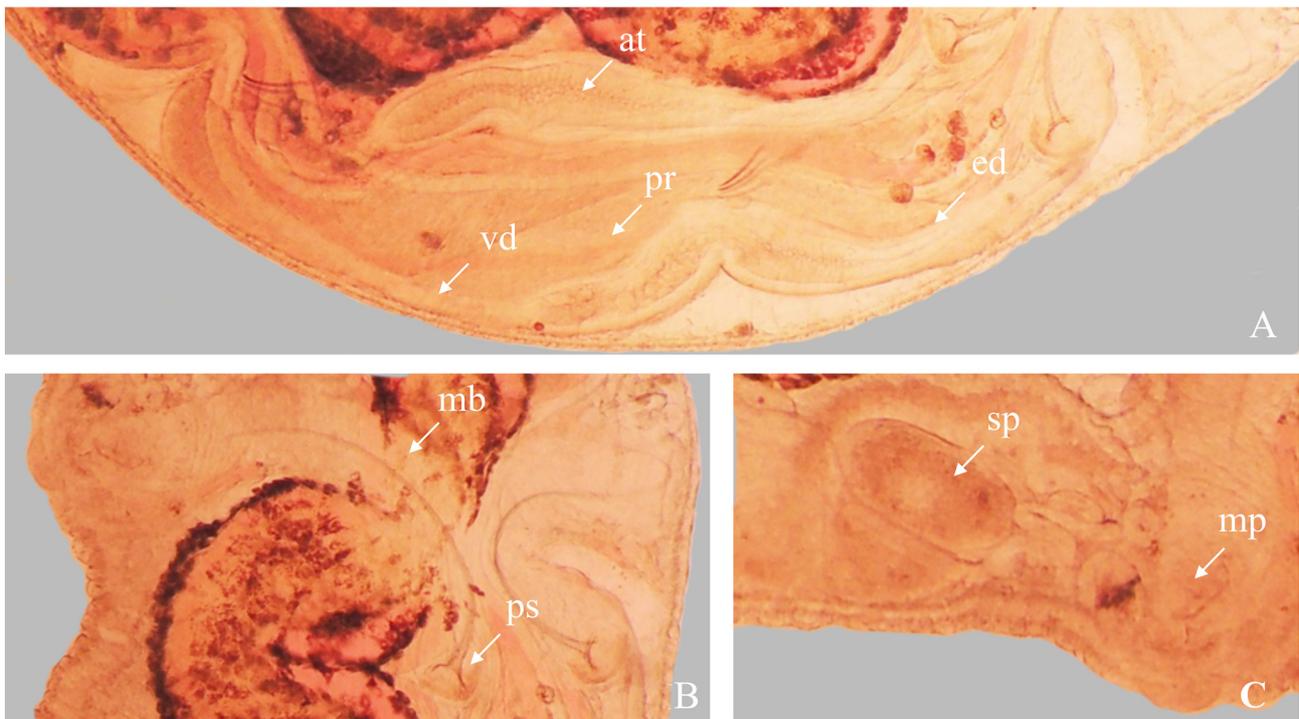
Dorsal chaetae (0) 1–2 hairs and 1–4 bifids per bundle. Hairs smooth anteriorly (Fig. 4B), serrate posteriorly (Fig. 4C), 120–300  $\mu$ m long, 2–3  $\mu$ m thick. Dorsal bifids pectinate, 50–100  $\mu$ m long, 1.5–2.5  $\mu$ m thick, upper and lower tooth subequal, 6–8  $\mu$ m long, with 2–3 (4) fine intermediate teeth, nodulus distal (Figs. 4D, 5D). Ventral chaetae bifid, (2) 4–6 per bundle anteriorly, 2–3 per bundle posteriorly, 60–160  $\mu$ m long, 2–3  $\mu$ m thick, upper tooth twice as long as lower (4  $\mu$ m/2  $\mu$ m), with (0) 1–2 fine intermediate teeth, nodulus distal (Figs. 4E,F, 5A–C). Ventral chaetae in X–XI present, unmodified. Male pores (Figs. 5G, 6C: mp) paired in line with ventral chaetae in anterior of X. Spermathecal pores (Fig. 5E: spp) paired in line with ventral chaetae, middle to posterior of XI.

Chloragogen cells from VI onwards. Male genitalia paired in X–XII. Sperm funnel cup-shaped, 30–40  $\mu$ m long, 40–60  $\mu$ m wide (Fig. 5G, H: sf). Vas deferens long and irregularly coiled, 1800–2000  $\mu$ m long, at least 3 times as long as atrium, anteriorly 14–16  $\mu$ m wide, posteriorly 28  $\mu$ m wide, ciliated throughout and entering atrium apically (Figs. 5G, H, 6A: vd). Atrium spindle-shaped, 560–640  $\mu$ m long, maximally 80–100  $\mu$ m wide (Figs. 5G,

H, 6A: at). Ejaculatory duct 100–120  $\mu\text{m}$  long, 20–32  $\mu\text{m}$  wide, entering muscular bulb (Fig. 5G, H, 6A: ed; Fig. 5G: mb). Prostate gland large, attached to ental portion of atrium, 120–140  $\mu\text{m}$  long, 80–100  $\mu\text{m}$  wide (Fig. 5G, H, 6A: pr). Penis inconspicuous, surrounded by cuticular, symmetrical and funnel-shaped penial sheath. Penial sheath extremely thin, long and sinuous, 32–36  $\mu\text{m}$  wide at ental end, 3–5  $\mu\text{m}$  wide at thin and prolonged ectal portions, 520–540  $\mu\text{m}$  long, length to width ratio nearly 15–16 (Figs. 5G, H, 6B: ps). Testes paired in X, immediately behind septum 9/10. Ovaries paired in XI, immediately behind septum 10/11. Spermathecae paired in X, ampullae oblong, 200–280  $\mu\text{m}$  long, 140–250  $\mu\text{m}$  wide; duct 100–120  $\mu\text{m}$  long, 24–28  $\mu\text{m}$  wide; sperm random in lumina, no spermatozeugmata (Figs. 5E, 6C: sp).

**Remarks.** The main identification characteristics of long and winding vas deferens divided into thin and thick sections, long atrium gradually tapering towards distal portion, large prostate gland and elongate penial sheath suggest that the present species belongs to the genus *Tubifex* (Brinkhurst & Jamieson 1971; Holmquist 1985; Juget *et al.* 2006). *Tubifex tubifex* (Müller, 1774) resembles this species in pectinate dorsal chaetae, long and winding vas deferens divided into thin and thick sections, large prostates, and in atria with a long ejaculatory duct, but differs from it in a cylindrical penis covered with a short and tub-shaped cuticle ring. Furthermore, spermathecae are absent, or present with spermatozeugmata, or present with empty ampullae. *T. gracilentus* n. sp. is easily distinguished from its congeners by the extremely thin and long funnel-shaped penial sheath (Table 2).

**Distribution and habitat.** Known from the type locality, Cuomujiri Lake and Trigu Co. Freshwater.



**FIGURE 6.** *Tubifex gracilentus* n. sp., micrographs from live worms. **A.** Ventral view of male genitalia in X–XII. **B.** Penial sheath and muscular bulb. **C.** Spermatheca and male pore.

### *Tubifex conicus* He, Wang & Cui, 2012

(Figs 7–8)

*Tubifex conicus* He, Wang & Cui, 2012: 160–162, Fig. 1.

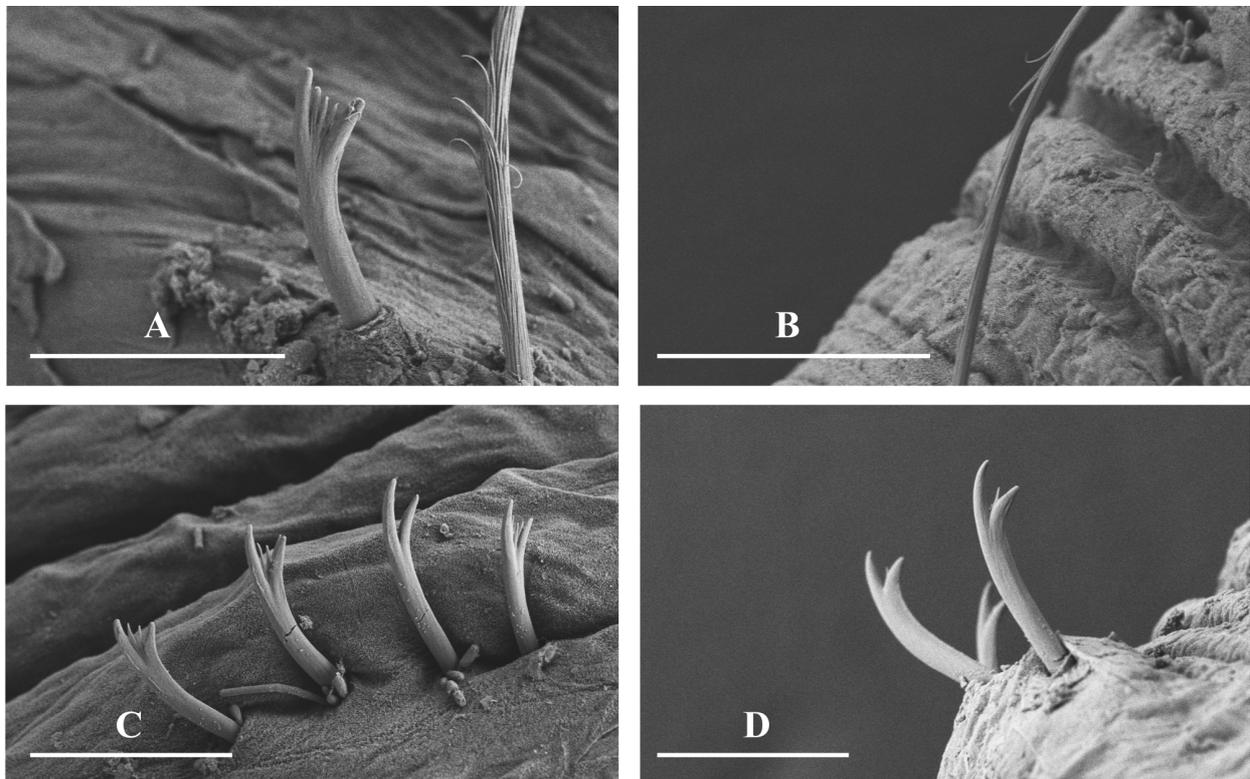
**New material.** IHB XZ20150602 a–e, 5 mature specimens, whole-mounted in Canada balsam, from Gyaring Co, preserved in IHB, CAS. Gyaring Co (30°58' 43"N, 88°28'04"E), a lake located in northern Tibet of China, ca. 4,648–4,659 m asl. Water depth 11–37 m, water temperature 6.3–8.9°C, pH 6.3–8.9, dissolved oxygen 7.3–7.7 mg/L, conductivity 323–329  $\mu\text{s}/\text{cm}$ . Collected by Yongde Cui and Baoqiang Wang on 2 June 2015.

**Description.** Length 6.1–11.6 mm, width at genital segments X–XI 0.28–0.42 mm. Segments 38–65. Prostomium obtuse. Clitellum inconspicuous. No coelomocytes. Dorsal chaetae (0) 1 hair and 1–4 bifids per bundle. Hairs serrate, posteriorly generally absent (Fig. 7A, B). Dorsal bifids pectinate, upper and lower tooth subequal, with 2–3 fine intermediate teeth (Fig. 7A). Ventral chaetae bifid, 3–4 per bundle anteriorly, 2 per bundle posteriorly, upper tooth slightly longer than lower, with (0) 1–2 fine intermediate teeth (Fig. 7C, D). Ventral chaetae in X present, unmodified. Ventral chaetae in XI absent. Male pores paired in line with ventral chaetae, middle to posterior of XI.

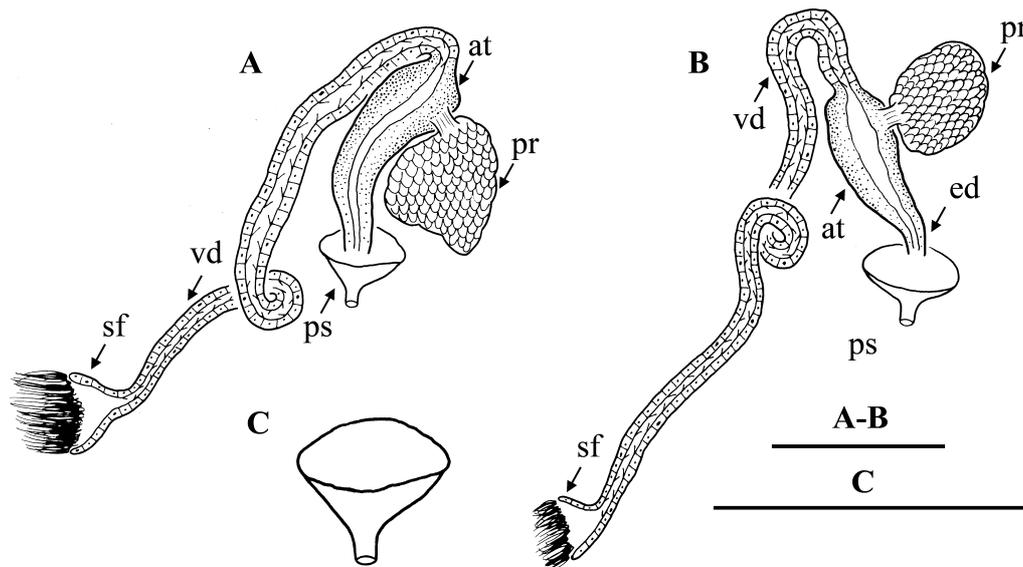
Chloragogen cells from VI onwards. Male genitalia paired in X–XI. Sperm funnel cup-shaped, 20  $\mu\text{m}$  long, 40  $\mu\text{m}$  wide (Fig. 8A,B: sf). Vas deferens 400–560  $\mu\text{m}$  long, 12–15  $\mu\text{m}$  wide, nearly 3–4 times as long as atrium, ciliated throughout and entering atrium apically (Fig. 8A, B: vd). Atrium spindle-shaped, 120–160  $\mu\text{m}$  long, maximally 32–40  $\mu\text{m}$  wide (Fig. 8A, B: at). Ejaculatory duct present. Prostate gland solid, 80–84  $\mu\text{m}$  long, 60–64  $\mu\text{m}$  wide, attached to ental portion of atrium (Fig. 8A, B: pr). Penis inconspicuous, surrounded by cuticular, symmetrical and funnel-shaped penial sheath. Penial sheath 36–40  $\mu\text{m}$  long, 48–56  $\mu\text{m}$  wide at ental end (Fig. 8A, B, C: ps). Testes paired in X, immediately behind septum 9/10. Ovaries paired in XI, immediately behind septum 10/11. Spermathecae absent.

**Remarks.** The main difference of our redescription from *T. conicus* as originally described (He *et al.* 2012) is the absence of spermathecae. However, after our reinvestigation of the type series (holotype and paratypes), we are sure that the testes of *T. conicus* were mistaken for spermathecae in the original description. Further slight differences between the descriptions concern thickness of atrial epithelium (thicker in the redescription) and penial sheaths (thicker in the original description). They may relate to the stage of maturity or to different modes of interpretation of observations. The size and shape of atria in the original observation and this research are about the same. Penial sheaths appear to have a thick cuticle laterally in the holotype, but when comparing type series and our material, we found no significant differences in penial sheath thickness.

**Distribution and habitat.** Known from Yamdrok Yumco (Lake Yamzho Yumco), Gyaring Co, Chargut Co, Uruni Co, Chikui Co, Amdo Tsonak Co, Tibet, China. All these lakes are located at above 4,000 m asl in Tibet. Gyaring Co, the locality of present redescribed materials, is about 300 km from the holotype locality of *T. conicus*, Yamdrok Yumco. Freshwater and brackish water.



**FIGURE 7.** *Tubifex conicus* He, Cui & Wang, 2012, SEM micrographs. **A.** Dorsal chaetae in VII. **B.** Hair chaeta in a posterior segment. **C.** Ventral chaetae in II. **D.** Ventral chaetae in a posterior segment. Scale bars: A–D. 20  $\mu\text{m}$ .



**FIGURE 8.** *Tubifex conicus* He, Cui & Wang, 2012. **A, B.** Male genitalia, lateral view. **C.** Penial sheath. Scale bars: A–C. 100  $\mu$ m.

***Tubifex tubifex* (Müller, 1774)**

(Figs 9–10, table 1)

*Lumbricus tubifex* Müller, 1774: 27.

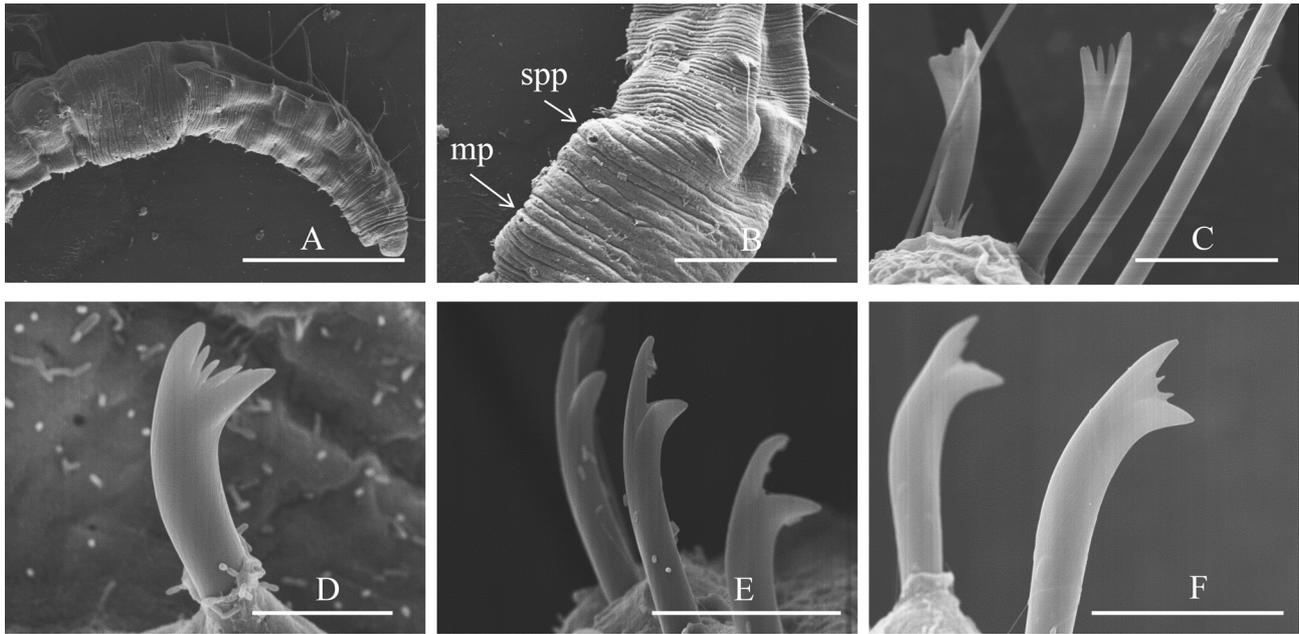
*Tubifex tubifex* Müller: Liang 1962: 20–22; Brinkhurst 1965: 123–124; Brinkhurst 1966: 145; Brinkhurst & Jamieson 1971: 453–456; Brinkhurst & Marchese 1987: 3154–3155; Holmquist 1983: 189–192; Timm 2006: 32–37; Marotta *et al.* 2009: 181–187.

For further synonymies and citations of the name see Brinkhurst & Jamieson (1971).

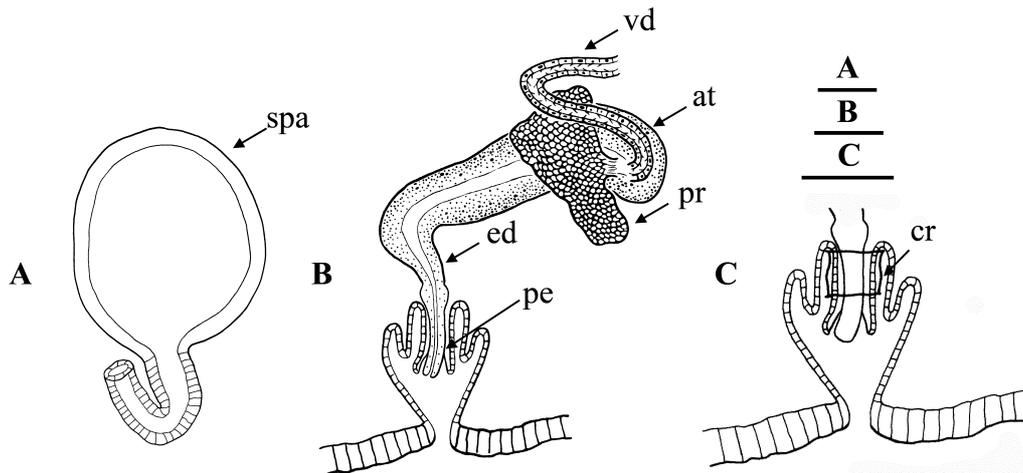
**New material.** IHB XZ20130630 m–r, 6 mature specimens, whole-mounted in Canada balsam. 1 mature specimen, processed for Scanning Electron Microscopy. Collected from one river in Cuomujiri Mountain (29°45'54" N, 94°24'25" E) of southern Tibet, China., ca. 3,537 m asl. Substrate type silt, hydrophytes abundant. Water depth 30–100 cm, water temperature 11.1°C, pH 7.0, dissolved oxygen 9.0 mg/L, conductivity 16.5  $\mu$ s/cm. Collected on 30 June 2013 by the authors.

**Description.** Length 14–21 mm, width at genital segments X–XI 1.0–1.2 mm. Segments 76–84. Prostomium obtuse (Fig. 9A). Clitellum inconspicuous. No coelomocytes. Dorsal chaetae 1–2 hairs and 2–3 bifids per bundle. Hairs slightly hirsute, 250–550  $\mu$ m long, 2–4  $\mu$ m thick (Fig. 9C). Dorsal bifids pectinate, 100–150  $\mu$ m long, 2–4  $\mu$ m thick, two short outer teeth nearly equal, 7.5  $\mu$ m long, with 1–4 fine intermediate teeth, nodulus distal (Fig. 9D). Ventral chaetae bifid, anteriorly 3–5 per bundle, posteriorly 2–3 per bundle, 55–150  $\mu$ m long, 3–5  $\mu$ m thick, anterior upper tooth slightly thinner than and nearly 2 times as long as lower (10  $\mu$ m/6  $\mu$ m), posterior upper tooth as long as lower (7  $\mu$ m/6  $\mu$ m), with (0) 1–3 fine intermediate teeth, nodulus distal (Fig. 9E–F). Ventral chaetae in X and XI present, unmodified. Spermathecal pores paired in line with lateral line, in anterior of X (Figs. 9B: spp). Male pores paired in line with ventral chaetae, middle to posterior of XI (Fig. 9B: mp).

Chloragogen cells beginning in VI onwards. Male genitalia generally paired in X–XI, in one specimen shifted to VI–VII. Sperm funnel cup-shaped. Vas deferens long and irregularly coiled, anteriorly 30  $\mu$ m wide, posteriorly 50  $\mu$ m wide, ciliated throughout and entering atrium subapically (Fig. 10B: vd). Atrium comma-shaped, 500–600  $\mu$ m long, maximally 120–125  $\mu$ m wide, with long ejaculatory duct gradually becoming narrower toward the ectal end, and enlarged into a characteristic subspherical widening at the base of penis (Fig. 10B: at). Large compact prostate gland with stalk-like attachment connected to ental atrium subapically near the vas deferens, 150–200  $\mu$ m long, 200–250  $\mu$ m wide (Fig. 10B: pr). Penis cylindrical (Fig. 10B: pe), 150–175  $\mu$ m long, covered with a short, tub-shaped, flexible (= non-chitinous), cuticle ring. Testes paired in X, immediately behind septum 9/10. Ovaries paired in XI, immediately behind septum 10/11. Spermathecae generally paired in X (One specimen shifted to VI), with ampullae partially pressed into XI, 500–625  $\mu$ m long, 250–330  $\mu$ m wide; duct 150–225  $\mu$ m long; sperm not observed in lumina (Fig. 10A: spa).



**FIGURE 9.** *Tubifex tubifex* (Müller, 1774), specimens from Tibet, SEM micrographs. **A.** Lateral view of anterior body. **B.** Spermathecal pore in X and male pore in XI. **C.** Hairs and pectinate chaetae in V. **D.** Dorsal pectinate chaeta in XII. **E.** Ventral chaetae in III. **F.** Ventral chaetae in XVI. Scale bars: A. 1000 µm; B. 500 µm; D. 10 µm; C, E, F. 20 µm.



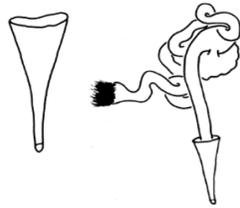
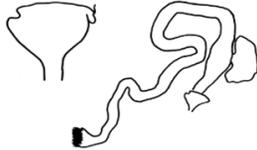
**FIGURE 10.** *Tubifex tubifex* (Müller, 1774), specimens from Tibet. **A.** Spermatheca. **B–C.** Lateral view of male genitalia in segment XI. Scale bar: A–C. 100 µm.

**Remarks.** *T. tubifex* is one of the most widely distributed species in the world, and has been redescribed over and over again (Liang 1962; Brinkhurst & Jamieson 1971; Brinkhurst & Marchese 1987; Hrabě 1981; Holmquist 1983; Timm 2006; Marotta *et al.* 2009). The morphological accounts of this cosmopolitan species show some differences, mainly in the hair chaetae, the penial sheath and the spermatheca (Table 1). Some of these differences, however, may be due to different observation techniques or modes of interpretation: Under SEM-observation, the hair chaetae of *T. tubifex* from Tibet are slightly hirsute. Other authors, using light-microscopy, could not determine whether the hair chaetae of *T. tubifex* from other areas were serrate or not (Liang 1962; Brinkhurst & Jamieson 1971; Brinkhurst & Marchese 1987; Hrabě 1981). Furthermore, we identified a cuticle ring around the penis of *T. tubifex* from Tibet, whereas other authors, referring to the same structure, describe a penial sheath (Brinkhurst 1965; Brinkhurst & Jamieson 1971; Brinkhurst & Marchese 1987; Hrabě 1981). The cuticle ring in specimens from Tibet is flexible and non-chitinous—we observed contractions in living specimens—and it shows clearly *in vivo* but is difficult to see or seemingly absent in material that has been stained with borax carmine and mounted whole in Canada balsam. We therefore agree with Holmquist (1983) and Timm (2006) that *T. tubifex* has no penial sheaths proper.

**Table 1.** Comparison of *Tubifex tubifex* from southern Tibet and other areas

Area	Tibet, China	Sungari River, China	North America	Slovakia	Denmark	Netherlands	Italy
Hair	slightly hirsute	serrate	serrate or not	serrate	serrate or not	smooth	serrate
Penial cuticle	cuticle ring	no description	penial sheath	penial sheath	cuticle ring	cuticle ring	muscular sheath
Spermatheca	present without spermatzozeugmata	present without spermatzozeugmata	absent or present with spermatzozeugmata	no description			
Reference	present research	Liang 1962	Brinkhurst & Jamieson 1971	Hrabě 1981	Holmquist 1983	Timm 2006	Marotta <i>et al.</i> 2009

**Table 2.** Comparison of *Tubifex* species with funnel-shaped penial sheaths

Species	<i>Tubifex laxus</i> n. sp.	<i>Tubifex gracilentus</i> n. sp.	<i>Tubifex montanus</i> Kowalewski, 1919	<i>Tubifex conicus</i> He, Cui & Wang, 2012	<i>Tubifex newfei</i> Pickavance & Cook, 1971
Hair chaetae	slightly hirsute	smooth anteriorly, serrate posteriorly	smooth	serrate	smooth
Dorsal bifid chaeta	palmate	pectinate	palmate	pectinate	pectinate
Body length (mm)	6.8–7.2	6.4–6.7	10–12	4.6–5.2	25–30
Atrium length (µm)	100–140	560–640	343	140	470–490
Atrium width (µm)	85–100	80–100	72	44	80–120
Atrium length/Atrium width	1.5	6.4–7.0	4.8	3.2	3.9–6.1
Penial sheath length (µm)	30–40	520–540	172	45	60–70
Penial sheath length-width ratio	1.0	15–16	2.4	1.0	1.0
Drawing of male duct	See Fig. 3	See Fig. 5			
Spermathecae	absent	present	absent	present	present
Distribution	Cuomujiri Mountain, southern Tibet, China	Juemu River, Cuomujiri Lake, Trigu Co	Tatra Mountains, Europe; Cuomujiri Mountain, southern Tibet, China	Yamdruk Yumco, Gyaring Co, Chargut Co, Uruni Co, Chikui Co, Amdo Tsonak Co, Tibet, China.	Newfoundland, Canada
References	present research	present research	Hrabě 1981; Brinkhurst & Jamieson 1971; Peng <i>et al.</i> 2014	He <i>et al.</i> 2012; present research	Pickavance & Cook 1971

In the material of *T. tubifex* from Tibet, spermathecae are generally paired in X with no sperm observed in lumina, which is different from *T. tubifex* from other areas that usually have paired spermathecae with spermatozeugmata, or only one spermatheca with spermatozeugmata, or no spermatheca at all (Brinkhurst & Jamieson 1971; Hrabě 1981; Holmquist 1983; Timm 2006).

**Distribution and habitat.** Cosmopolitan. Freshwater.

## Discussion

With the two new species described here, there are now five species in the genus *Tubifex* with funnel-shaped penial sheaths: *T. laxus* n. sp., *T. gracilentus* n. sp. and *T. conicus* only from Tibet, China, *Tubifex newfei* Pickavance & Cook, 1971 only from Canada (Pickavance & Cook 1971), *Tubifex montanus* Kowalewski, 1919 both from Europe and Tibet, China (Hrabě 1981; Peng *et al.* 2014). A comparison of these species is given in Table 2. The penial sheaths of *T. laxus* n. sp., *T. conicus* and *T. newfei* are similar in shape and size. However, *T. laxus* n. sp. is different from the latter two species by its ovoid atrium and palmate dorsal chaetae. *T. gracilentus* n. sp. has extremely thin and long penial sheaths with especially thin and prolonged ectal portions, which is obviously distinguished from other species. *T. montanus*, which was once dubiously included into *Tasserkidrilus* by Holmquist (1985) and re-allocated in *Tubifex* by Timm (1989), has funnel-shaped penial sheaths with thin and prolonged ectal portion. Therefore, it just resembles the former four species in the shape of the penial sheaths but differs from them clearly in the size of the penial sheaths. Absence of spermathecae in *T. laxus* n. sp. is also common in *T. montanus* and *T. conicus*. However, *T. laxus* n. sp. is obviously different from *T. montanus* and *T. conicus* in having ovoid atrium with no ejaculatory duct. Considering the chaetae, *T. laxus* n. sp. and *T. montanus* both have palmate dorsal chaetae, which is clearly different from other freshwater *Tubifex* species.

*T. tubifex* still has several problems in its taxonomic identification due to a great variability of morphological characters (Holmquist 1983). Holmquist's revision in 1985 has met with different levels of acceptance among the specialists (Juguet *et al.*, 2006). Recently, research based on molecular phylogenetic analysis suggested that cryptic species exist in *T. tubifex sensu lato* (Crottini *et al.* 2008; Marotta *et al.* 2014).

To date, approximately 22 valid species have been described in *Tubifex*, of which five species have been recorded in China (Sokol'kaya 1961; Brinkhurst & Jamieson 1971; Pickavance & Cook 1971; Chekanovskaya 1975; Erséus 1976; Hrabě 1981; He *et al.* 2012; Marotta *et al.* 2014; Peng *et al.* 2014; Timm 2017). The systematics of the genus *Tubifex* is still controversial for having no clear character catalogue that distinguishes it from other genera within Tubificinae (Juguet *et al.* 2006; He *et al.* 2012). For three species (*T. laxus*, *T. conicus* and *T. montanus*) from Tibet, absence of spermathecae is a specific character which suggests that these species may belong to one new taxonomical group. Indeed, *Tubifex* is in need of revision, which should be based on a comprehensive study using morphological and molecular data.

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