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A New Species of the Subterranean Genus *Krenedrilus*Dumnicka (Oligochaeta, Tubificidae) from the Deep Bottom of an Oligotrophic Caldera Lake in Japan

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ABSTRACT—A new tubificid oligochaete, *Krenedrilus towadensis*, is described from deep bottoms of the oligotrophic caldera Lake Towada in northern Japan. It resembles *K. realis* Martínez-Ansemil and Collado, 1996, in the combination of somatic setae, but it is different from all other congeners by the lack of spermathecal setae and epidermal papillae in X, in the structures of penial and supernumerary setae, and in the location of spermathecal pores. The definition of *Krenedrilus* is revised.

Key words: Oligochaeta, Tubificinae, Krenedrilus, caldera Lake Towada, Japan

INTRODUCTION

The freshwater tubificid genus *Krenedrilus* was established by Dumnicka (1983), then redefined by Giani *et al.* (1990). Four species have been described so far, from subterranean habitats in Venezuela (*K. papillatus* Dumnicka, 1983), France (*K. sergei* Giani *et al.*, 1990), and Spain (*K. ibericus* Giani *et al.*, 1990), and a reservoir bottom in Spain (*K. realis* Martínez-Ansemil and Collado, 1996). They exhibit a puzzling combination of characters known from other groups both within and outside the Tubificinae, but they share some apomorphies and are thought to constitute a monophyly within a large complex of plesiomorphic Tubificinae (Giani *et al.*, 1990).

In the course of recent benthological research in a deep, oligotrophic caldera lake, Lake Towada, in northern Honshu, Japan, the author found stenotherm and oxyphilic oligochaete assemblages at profundal bottoms (Ohtaka, 2001). They contained an undescribed species of *Krenedrilus*, in this paper described as a fifth member of this genus.

MATERIALS AND METHODS

Specimens were collected by the author from profundal silty bottoms (40–320 m deep) of Lake Towada, Aomori/Akita Prefecture, Honshu, Japan. The lake basin is composed of a double caldera. The main basin was formed 15,000 years ago and has a broad and flat profundal bottom with a depth of 70–100 m. The secondary basin was formed by an eruption at the southern part of the

FAX. +81-172-39-3369. E-mail: ohtaka@cc.hirosaki-u.ac.jp main basin 6,300 years ago and has a deep bottom of 327 m at the maximum (Hayakawa, 1985; the ages are corrected). The environment of the lake and the sampling procedure were described in Ohtaka (2001). Specimens were fixed in 10% formalin and dehydrated whole in a graded series of ethanol and water solution, cleared in methyl salicylate, and mounted in Canada balsam on microscope slides for examination. Anatomical observations were made on specimens cut serially (sections 10 μm thick) and stained with haematoxylin and eosin. In addition, a detailed observation of setae was made by scanning electron microscopy. Specimens from the type series have been deposited in the Division of Biological Science, Graduate School of Science, Hokkaido University (ZIHU), and National Museum of Natural History (USNM), Smithsonian Institution, Washington D.C., U.S.A.

DESCRIPTION

Family Tubificidae Lamarck Subfamily Tubificinae Eisen Genus *Krenedrilus* Dumnicka

Emended diagnosis. Small, primarily subterranean, freshwater tubificids. Body wall without papillation. Hair and pectinate setae present or absent in dorsal bundles. Ventral somatic setae bifid, or mixed bifid and simple-pointed. Ventral setae of X modified into grooved spermathecal setae or not modified. Ventral setae of XI modified into bifid or hooked penial setae; forked, grooved or simple-pointed supernumerary genital setae also present in XI near male pore. An epidermal papilla present mid-ventrally on spermathecal segment, in most species. Vasa deferentia ciliated, moderately long and entering atria apically. Atria pear-shaped to tubular, not ciliated. Prostate glands medium-sized, entering near entrances of vasa deferentia. Penes present, lacking cuticular sheaths. Spermathecae large with

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conspicuous ectal duct, opening ventrally or dorso-laterally. Spermatozeugmata present or absent.

Type-species. Krenedrilus papillatus Dumnicka, 1983

Krenedrilus towadensis sp. nov. (Figs.1–4) Tubificidae gen. sp. Ohtaka, 2001, p. 89.

Type series. Holotype, ZIHU-2051, a whole-mounted specimen from a bottom in the western basin of Lake Towada at 40 m depth (40°25'21.1"N, 140°52'21.0"E), 6 June

1997. Paratypes, ZIHU-2052, a whole-mounted specimen from the western basin of Lake Towada at 98 m depth (40°27'48.6"N, 140°52'08.0"E), 15 June 1999, ZIHU-2053, a sagittally sectioned specimen from the type locality, date as for holotype; USNM-1020936, a whole-mounted specimen from the type locality, date as for holotype.

Other material. 11 mature specimens from profundal bottoms of Lake Towada at 40–320 m depth, 6 June 1997, 16 June, 9 Sep. 1998, 15 June 1999. Two immature speci-

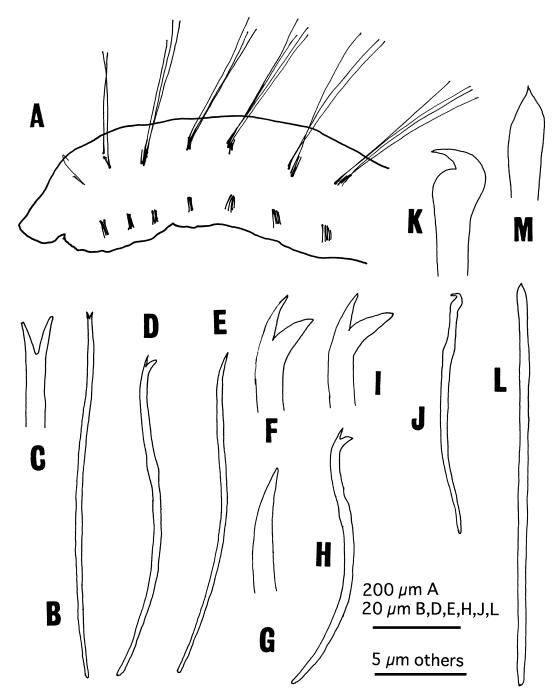


Fig. 1. Krenedrilus towadensis sp. nov. (A) anterior part of body; (B-M) setae: (B) dorsal crotchet in IX; (C) the same, distal end; (D and E) ventral setae in an anterior segment; (F and G) the same, distal ends; (H) ventral seta in a posterior segment; (I) the same, distal end; (J) penial seta; (K) the same, distal end; (L) supernumerary genital seta; (M) the same, distal end.

mens from a gravelly shore at Wainai, Lake Towada, water temperature 1.5°C, 23 May 1999.

Etymology. The specific epithet is derived from the only known locality, Lake Towada, northern Honshu, Japan.

Description. In mature and living state, body transpar-

ent, 3–8 mm long, 0.20–0.35 mm wide in middle segments, not coiling up when stimulated. Segments 45–60. Body naked without pigments. Prostomium conical (Fig. 1A), 80 μm long, 110–130 μm wide at base. Clitellum extending over X-XII, weakly developed. Male pores paired, located ventrally in the posterior part of XI, behind ventraral setae

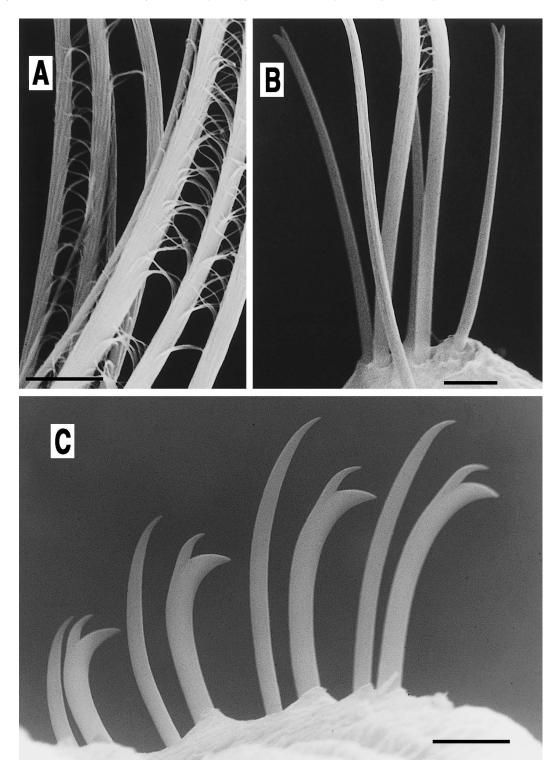


Fig. 2. SEM photomicrographs of setae in *Krenedrilus towadensis* sp. nov. (A) dorsal hair setae in an anterior segment; (B) dorsal crotchets and hair setae in an anterior segment; (C) ventral setae in V. Scale bars, 5 μm.

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and on the same line. Spermathecal pores opening dorso-laterally in X.

Dorsal setal bundles consisting of long hairs and short crotchets (Fig. 1A). Hair setae serrated (Fig. 2A), 3-5 per bundle, 144–520 μ m long anteriorly, 1-3 per bundle, 245–360 μ m long posteriorly. Dorsal crotchets (Fig. 1B) slender, straight or weakly bayonet-shaped, 2-4 per bundle, 66–86 μ m long anteriorly, 1-3 per bundle, 56–70 μ m long posteriorly; distal end bifid with equal, divergent teeth (Figs. 1C, 2B). Nodulus of dorsal crotchets hardly detected, but weak nodulus sometimes found at 1/3 from distal end. Ventral setal bundles in anterior half of body consisting of 3-6 bifid (Fig. 1D) and 3-6 single-pointed setae (Figs. 1E, G) arranged alternately (Fig. 2C), both types 56–70 μ m long. Bifid setae in anterior segments with distal nodulus and with

upper tooth almost as long as but thinner than lower (Fig. 1F). Single-pointed setae devoid of nodulus and thinner than bifid setae. Single-pointed setae fewer posteriorly, and between XX and XXX, ventral bundles gradually reduced to 3-6 bifid setae, 50–66 μm long, in which nodulus situated distally and upper tooth shorter and thinner than lower (Figs. 1H, I

Penial setae in XI, 3-10 per bundle, 52–64 μm long, straight with a distal nodulus (Fig. 1J), distal end clubshaped with a pointed hook (Fig. 1K). Supernumerary genital setae present in XI, 1 (occasionally 2) per "bundle," 80–94 μm long, thick and straight or gently curved basally without nodulus (Fig. 1L). Distal end of supernumerary genital setae a little swollen with a pointed tip (Fig. 1M). No spermathecal seta.

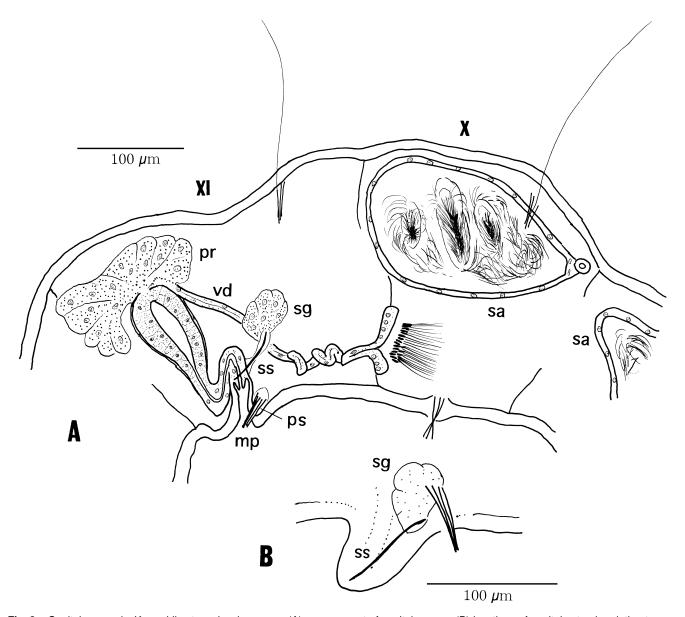


Fig. 3. Genital organs in *Krenedrilus towadensis* sp. nov. (A) arrangement of genital organs; (B) locations of genital setae in relation to protruded penis, right side anterior. Abbreviations in the figure: at, atrium; pr, prostate gland; ps, penial seta; sg, setal gland; sa, spermathecal ampulla; ss, supernumerary genital seta; vd, vas deferens.

Coelomocytes absent. Pharyngeal glands in II through IV. Chloragogen cells from VI on. Oesophageal wall thickened in VIII and IX (about 20 $\mu m)$ (Fig. 4A); epithelial cells ciliated and not granulated, and not different in structure from those in neighboring segments. Seminal vesicles extending to VII anteriorly, to XIV posteriorly. Egg sac extending backwards to XIV.

Male genitalia (Fig. 3A) paired. One pair of testes attached behind septum 9/10; one pair of ovaries attached behind septum 10/11. Sperm funnel small, thick and asymmetrical, located in ventral part of septum 10/11. Vas deferens more than two times longer than atrium, ciliated, 11–15 μm wide, coiled in ental half of the course and entering atrium somewhat subapically. Atrium large, ovoid to subcylindrical in shape, 80–120 μm long, maximally 40–50 μm wide, with thick (9–13 μm), non-ciliated and granulated inner epithelium and thin (less than 3 μm) outer layer of muscles (Fig. 4B). Atrium prolonged into a long, non-granulated ejaculatory duct, 14–16 μm thick and folded. Prostate gland a lit-

tle larger than atrium, connected to ental end of atrium with broad base, close to entrance of vas deferens. Small protrusible penis set in a deep and narrow penial pouch opening ventrally in XI. Penial setae arranged close together, at frontal edge of male pore. Supernumerary genital seta located dorsal to penial setae (Figs. 3A–B, 4C), enclosed in a small tubular sac. A large and compact setal gland associated with dorsal end of each supernumerary genital seta, $56~\mu m$ long by $32~\mu m$ wide, its structure similar to that of prostate gland. When penes are protruded, supernumerary genital setae stick out along with these, while penial setae retain at frontal base of penis (Fig. 3B).

Spermathecae paired. Spermathecal ampullae large and ovoid (150–200 μ m long, 80–110 μ m wide) with thin wall, occupying dorsal part of X (one ampulla often penetrates deeply into IX); spermathecal ducts short and bulb-shaped, 20–40 μ m long, opening dorso-laterally on setal lines behind septum 9/10. Some to several ovoid to spindle-shaped spermatozeugmata in spermathecal ampulla, along

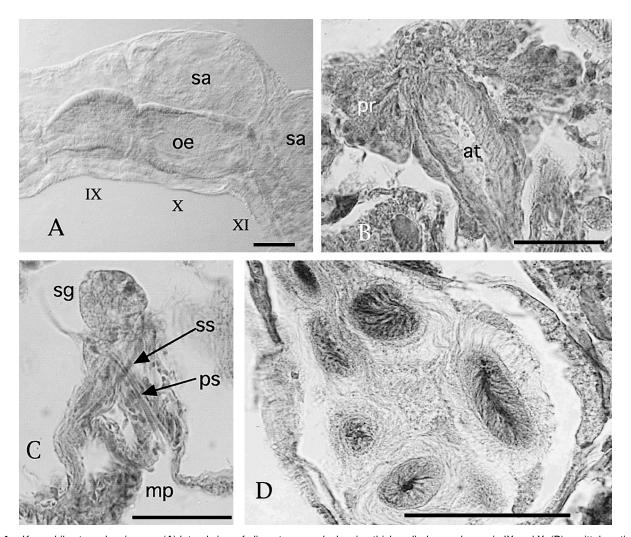


Fig. 4. Krenedrilus towadensis n. sp. (A) lateral view of alimentary canal, showing thick-walled oesophagus in IX and X; (B) sagittal section of atrium with prostate gland; (C) sagittal section of male pore, showing penial and supernumerary genital setal arrangements, right side anterior; (D) spermathecal ampulla with spermatozeugmata and free spermatozoids. Abbreviations in the figure: at, atrium; mp, male pore; oe, oesophagus; pr, prostate gland; ps, penial seta; sg, setal gland; sa, spermathecal ampulla; ss, supernumerary genital seta. Scale bars, 50 μm.

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Table 1. Comparison of taxonomic characters in the five Krenedrilus species.

species	papillatus	ibericus	sergei	realis	towadensis sp. nov.
references	Dumnicka (1983) Giani <i>et al.</i> (1990)	Giani <i>et al.</i> (1990)	Giani <i>et al.</i> (1990)	Martínez-Ansemil and Collado (1996)	present study
dorsal setae	hair and pectinate	bifid	hair and pectinate	hair and bifid	hair and bifid
ventral setae	bifid and simple-pointed	bifid	bifid and simple-pointed	bifid and simple-pointed	bifid and simple-pointed
spermathecal setae in X	present	present	presnt	present	absent
penial setae	bifid	bifid	bifid	bifid	hooked
supernumerary genital setae	forked or grooved	grooved	grooved	grooved	not grooved
location of supernumerary genital setae	dorsal to penial setae	behind penial setae	behind penial setae	in front of penial setae	dorsal to penial setae
epidermal papillae in X	present	present	present	present	absent
atrial form	tubular	subcylindrical	pear-shaped	pear-shaped	subcylindrical
penes	cone-shaped	protrusible	small, protrusible	protrusible	protrusible
spermathecal ampullae	confined in X	confined in X	confined in X	in X and XI	in X and IX
spermathecal openings	ventral	ventral	ventral	ventral	dorso-lateral
sperm in spermathecae	spermatozeugmata	masses	globular masses	compact masses	spermatozeugmata
oesoghagus	widened in VIII	thick-walled in VII/VIII	not modified	not modified	thick-walled in VIII-IX
coelomocytes	?	present	present	present	absent
locality	Venezuela	Spain	France	Spain	Japan
habitat	slow-running spring	hyporheic flow of	stream into cave	bottom of reservoir	lake profundal
		stream			

with free spermatozoids (Fig. 4D).

Distribution and habitat. Widely distributed in the profundal zone of Lake Towada, from 40–320 m depth, with densities of up to 726 m⁻² (Ohtaka, 2001; as Tubificidae gen. sp.). The bottom sediment is composed of fine silt. The species was also collected at gravel shores of the lake, but only in winter (water temperature 1.5°C).

Remarks. The present species can be regarded as belonging to the genus Krenedrilus, as it has mixed genital setae (penial setae and supernumerary genital seta) in XI, which is thought to be a synapomorphic trait in the genus (Giani et al., 1990). However, it differs much from the four congeners described so far in the genus (see Introduction) as follows. All other species possess unpaired epidermal papillae in X, and grooved (or forked in K. papillatus; Giani et al., 1990) spermathecal setae in X (Dumnicka, 1983; Giani et al., 1990; Martínez-Ansemil and Collado, 1996). Moreover, in K. towadensis the spermathecal ampullae are confined to X (in K. realis they can penetrate into XI) and the spermathecal pores are located ventrally. On the other hand, in the present species, the ventral setae of X are not modified, and there are no epidermal papillae in the segment, but the spermathecal ampullae often penetrate into IX and the spermathecal pores open dorso-laterally (Table 1). The penial setae have hooked ends and the supernumerary genital setae are not grooved-two characters that also separate this species from all congeners, which have bifid penial setae and grooved supernumerary genital setae. The combination and structures of the somatic setae in this species most resemble those of K. realis, in that dorsal and ventral bundles are composed of hair and bifid setae, and bifid and simple-pointed setae, respectively. A similar arrangement in the ventral bundles is also encountered in *K. papillatus* and *K. sergei*.

The present species has a thick-walled oesophagus in VIII-IX. Similar modifications were found in different segments of *K. papillatus* and *K. ibericus* (Giani *et al.*, 1990), but not in *K. sergei* (Giani *et al.*, 1990) and *K. realis* (Martínez-Ansemil and Collado, 1996). Oesophageal modifications occur in three subfamilies in the Tubificidae (Limnodriloidinae, Rhyacodrilinae, and Tubificinae) as well as in some naidids and enchytraeids, but complex structures such as diverticulate and barrel-shaped modifications are limited to Limnodriloidinae, and there invariably restricted to segment IX (Gustavsson and Erséus, 1999). Gustavsson and Erséus (1999) concluded that different types of oesophageal modifications could evolve independently even in the subfamily Limnodriloidinae. Thick-walled modifications found in Rhyacodrilinae and Tubificinae could also evolve independently.

DISCUSSION

All Krenedrilus species so far known resemble each other in the basic structure of their male ducts. They have relatively small funnels, long and evenly thick vasa deferentia entering atria subapically, pear-shaped to tubular atria with thick and granulated inner epithelia, single and solid prostate glands attached to atria apically, prolonged ejaculatory ducts and simple penes. Such structures are principally of the tubificine types, but, as Giani et al. (1990) already demonstrated, they are also shared by several "aberrant" species outside the Tubificinae. A similar situation is true also for the modified genital setae as noted by Giani et al. (1990). Modified spermathecal setae and an unpaired

epidermal papilla in X were once regarded as outapomorphies for the genus *Krenedrilus* (Giani *et al.*, 1990), but they are not shared by the present species. Only the combination of penial and supernumerary genital setae in XI remains as a possible synapomorphic trait of the species of this genus within Tubificinae. The mid-ventral epidermal papillae in X found in other congeners are suggested to represent an "anchorage system" during copulation, being used in combination with the penial setae in XI (Cuadrado and Martínez-Ansemil, 2001). The "anchorage system" can also be related to the ventral location of the spermathecal pores in the congeners. The absence of ventral epidermal papillae and the dorso-lateral location of the spermathecal pores in the present species imply a copulatory behavior in the present species that is different from that of the congeners.

The genus *Krenedrilus* is stygobiont, found from subterranean or phreatic waters (Giani *et al.*, 1990; Giani *et al.*, 2001). Habitats of localities of the previously described species are: a spring flow (*K. papillatus*; Dumnicka, 1983), a stream flowing into a cave (*K. sergei*; Giani *et al.*, 1990), the hyporheic flow of a stream (*K. ibericus*; Giani *et al.*, 1990), and the bottom of a clean water reservoir (*K. realis*; Martínez-Amsemil and Collado, 1996). The present species was found mainly in a profundal habitat in a young oligotrophic caldera lake. It is demonstrated that zoobenthic communities in the Lake Towada profundal are primarily composed of stenothern and oxyphilic upper-riverine and/or groundwater species (oligochaetes, tricladids, acarina, and copepods; Ohtaka *et al.*, 1999; Ohtaka, 2001), and the present species may also be primarily subterranean.

Many subterranean oligochaetes are small and interstitial meiobenthic animals, and often they possess characters implying phyletic affinities to marine lineage (Erséus, 1992; Sambugar *et al.*, 1999; Giani *et al.*, 2001). The shapes of the distal ends of the genital setae in the present species also seem to suggest some relationship with the mainly marine subfamily Phallodrilinae. That is, the clubbed and hooked penial setae in the present species are also seen in some phallodriline genera (*Peosidrilus* Erséus, *Bermudrilus* Erséus, *Adelodrilus* Cook), and the single-pointed distal end of the supernumerary genital setae also resembles those of the penial setae in *Uniporodrilus* Erséus and *Peosidrilus* (Erséus, 1992).

Each of the five species included in *Krenedrilus* are only known from its type-locality. The large and discontinuous distribution of the genus could explain the variations observed in some of the characteristics of the five species of the genus. These variations are also a warning that the genus may not be monophyletic; the future discovery of

additional species could allow a reappraisal of these taxa, to confirm its validity or to split it in two or more distinct lineages.

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