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New species and new records of Japanese Lumbriculidae (Annelida, Clitellata)

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Abstract

Most Japanese records and descriptions of the family Lumbriculidae are from the work of H. Yamaguchi, who described 6 endemic species and one genus, in addition to reporting the cosmopolitan *Lumbriculus variegatus* (Müller, 1774). Yamaguchi's work focused largely on the northern island of Hokkaido; since then, only one new Japanese lumbriculid genus and species has been described, also from Hokkaido. Recent collections from Honshu Island include several species not recorded in earlier literature, and here we report 4 new species of Lumbriculidae, plus a range extension of the Korean *Lamprortus orientalis* Rodriguez, 1994. Of the new species, only *Styloscolex* (*Styloscolex*) *tazawaensis* can be confidently assigned to an accepted genus using current morphological diagnoses. *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp. is provisionally assigned to that Asian genus and subgenus based on the prosoporous male duct, spermathecae in the preatrial segment, and penial sheath—despite differing from congeners in having a single, median atrium in X, and single spermatheca in IX. Two additional species are here attributed to a new genus, *Honshudrilus*, which is distinguished by a combination of characters: spermathecae 2 or more segments anterior to the male pores, non-functional anterior male ducts (with loss of anterior testes), elongate-petiolate atria, penes formed by extruding the atrial duct lining, and spermathecal ducts with vestibules.

Key words: Clitellata, Oligochaeta, Lumbriculidae, taxonomy, Japan

Introduction

The largely Holarctic family Lumbriculidae includes approximately 240 described species and subspecies, most of which are regionally endemic. Excluding the nearly 60 Lake Baikal endemics, only about 25 species have been described from eastern Asia. The Japanese records and descriptions are largely the work of Hideji Yamaguchi (1936a, 1936b, 1937a, 1937b, 1953), who described 6 endemic species, including the monotypic *Hrabea ogumai* Yamaguchi, 1936, and also reported the apparently cosmopolitan *Lumbriculus variegatus* (Müller, 1774). Since then, additional lumbriculid distribution records have been presented by Ohtaka & Nishino (1995), Torii & Ohtaka (2007), Fend & Brinkhurst (2010) and Ohtaka (2014); and a single new species, *Yamaguchia toyensis* Fend & Ohtaka, 2004, has been described.

Most of the lumbriculid work by Yamaguchi was based on collections from the northern island of Hokkaido, with two exceptions: *Lumbriculus multiatriatus* Yamaguchi, 1937 from Sakhalin, Russia (Yamaguchi 1937a), and *Lumbriculus mukoensis* Yamaguchi, 1953 from Hyogo Prefecture, in southern Honshu. *Yamaguchia toyensis* is also known only from Hokkaido. Examination of recent collections from sites on Honshu Island has revealed a number of unusual species belonging to the family Lumbriculidae, and some of these new species are described in this contribution. Additionally, *Lamprortus orientalis* Rodriguez, 1994, described from Korea (Brinkhurst et al. 1994), is represented by new Japanese records.

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Methods

Sampling methods. Specimens examined in the present study were collected from streams, brooks or springs in several localities in Honshu Island, Japan. Bottom substrates in lotic habitats were disturbed to a depth of 10–20 cm, and worms displaced from the sediment were collected in 250–500 µm mesh dip nets; alternatively, bottom sediments from streams and wet soils were repeatedly stirred in water in a tray or bucket, and the suspended worms were collected with a 250 µm screen. Most specimens collected were relaxed in dilute ethanol and fixed in 8–10 % formalin solution for morphological study, or fixed in 90% ethanol for molecular study.

Laboratory methods. Specimens used for morphological study were usually stained in either hematoxylin or borax carmine, partially destained in acidified alcohol, dehydrated in a graded series of ethanol solutions, cleared in methyl salicylate and slide-mounted in Canada balsam. Some larger specimens were sagittally dissected, and where sufficient material was available, sagittal sections were cut at 7 µm and stained with Harris' hematoxylin and eosin Y. Unless otherwise indicated, descriptions of reproductive structures are based on mature specimens, with sperm in the spermathecae, and usually with well-developed eggs. Measurements of chaetae are based on slide-mounted worms, but body length and width measurements were supplemented with unmounted worms in alcohol; unless otherwise noted, measurements of somatic characters were based on specimens with at least partially-developed reproductive pores. Segment number is indicated by Roman numerals, and intersegments by Arabic numerals (as 9/10 to indicate septum between IX and X).

Type specimens are deposited in the National Museum of Nature and Science, Tokyo, Japan (NSMT) and the U.S. National Museum of Natural History, Smithsonian Institution (USNM).

Abbreviations used in the figures:

aa—atrial ampulla
ad—atrial duct
ff—female funnel
fp—female pore
mf—male funnel (mf1—anterior, mf2—posterior)
mp—male pore
ov—ovary
pe—penis
ph—pharynx
pg—pharyngeal gland
pr—prostates
ps—penis sheath
sa—spermathecal ampulla
sd—spermathecal duct
sp—spermathecal pore
sv—spermathecal vestibule
te—testis
vd—vas deferens

Results

Taxonomy: The current list of lumbriculid species known from Japan is given below. In this paper, we discuss new species and a new record for Japan, indicated with an asterisk (*), and the section where described in this paper. Many lumbriculid specimens from the Yamaguchi collection have only recently been rediscovered, and a later contribution will evaluate and supplement descriptions by Yamaguchi (1936a, 1937a, b, 1953), based on original collections and new material.

*_Honshudrilus gudariensis_ n. gen., n. sp. 3.3.1
*_Honshudrilus yuzawaensis_ n. gen., n. sp. 3.3.2
Hrabea ogumai Yamaguchi, 1936
  (Yamaguchi 1936a, 1953; Fend & Ohtaka 2004)
*_Lamprortus orientalis_ Rodriguez, 1994 3.1
  New record
Lumbriculus multiatriatus Yamaguchi, 1937
  (Yamaguchi 1937a, 1953, Cook 1971, Sokolskaya 1972a, Timm & Rodriguez 1994, Timm 1999)
Lumbriculus japonicus Yamaguchi, 1936
  (Yamaguchi 1953, Timm 1997, 1999)
  =_Lumbriculus variegatus japonicus_ Yamaguchi, 1936 (Cook 1971)
Lumbriculus mukoensis Yamaguchi,1953
  (Timm 1997, 1999, Torii & Ohtaka 2007)
  =_Lumbriculus variegatus mukoensis_ Yamaguchi, 1953 (Cook 1971)
Lumbriculus variegatus (Müller, 1774)
  (Yamaguchi 1953)
Rhynchelmis orientalis Yamaguchi, 1936
  (Cook 1971, Kaygorodova & Liventseva 2007, Fend & Brinkhurst 2010, Fend & Lenat 2010)
Styloscolex (_Styloscolex_) _japonicus_ Yamaguchi, 1937
  = _Styloscolex japonicus_ Yamaguchi, 1937 (Yamaguchi 1937b, 1953, Cook 1971, Ohtaka 2014, Sokolskaya 1976, Timm 1997, 1999, Timm & Všivkova 2007, Rodriguez et al. 2014)
*_Styloscolex_ (_Styloscolex_) _tazawaensis_ n. sp. 3.2.1
*_Styloscolex_ (_Neoscolex_) _tatsukoae_ n. sp. 3.2.2
Yamaguchia toyensis Fend & Ohtaka, 2004

3.1 *Lamprortus orientalis* Rodriguez, 1994

(Figure 1)

Material examined: Japan, Hiroshima Prefecture, outlet stream from Haizuka Reservoir, 28 Jan 2010, collected by T. Torii, 5 sagittally dissected and slide mounted. Aomori Prefecture, a spring in Hirosaki, 40.596586N, 140.471261E, elev. 38.8 m, 5 Apr. 2014, 4 mature slide mounts. 9 Apr. 2018, 9 mature slide mounts. All collected by A. Ohtaka.

Supplemental Description: Diameter in segment X 0.85–1.0 mm; length of 1 complete specimen 19 mm; clitellum IX–XV. Chaetae in anterior to middle segments 142–181 µm, nodulus median to slightly distal, 0.33–0.49 from the distal end. Some specimens with a single pair of short, blind lateral blood vessels in posterior segments. Spermathecal ducts tubular, length 140–280 µm in Haizuka streams (Fig. 1C), 250–380 µm in the Hirosaki spring; spermathecal ampullae large, sacciform, extending into adjacent segments (Fig. 1B). Atrium length 590–990 µm in the Haizuka worms and 500–650 µm in Hirosaki specimens; conical penes within spherical, glandular bulbs to about 200 µm diameter.

Remarks: Brinkhurst et al. (1994) justified this monotypic genus "on the basis of the unique plan of the reproductive organs, particularly the possession of a second pair of ovaries, making the taxon hologynous". *Lamprortus* closely resembles the large, Palearctic genus *Lamprodrilus* Michaelsen, 1901 (Michaelsen 1901a) in having elongate atria paired in two segments (X and XI), prosoporous male ducts (without anterior vasa deferentia or testes), posterior vasa deferentia not penetrating the posterior septum, and spermathecae posterior to the male pores. It differs in having two ovarian segments with associated female funnels (in XII and XIII), and in having the single pair of spermathecae opening three segments posterior to the atria, in XIV. In *Lamprodrilus* the spermathecae usually begin in XIII (Cook 1971).

The present collection represents a new record for Japan. The new specimens appear quite similar to the material described by Rodriguez (in Brinkhurst et al. 1994): positions of genital pores and gonads are as described (Fig. 1A), and basic morphology of reproductive structures appears identical (compare Fig. 1B with Fig. 2 in the original description). Spermathecal ducts (Fig. 1C) were longer than in the original description (reported to 160 µm); however, orientation made length difficult to measure in most specimens (Fig. 1B). Atrium length was not given in the original description, but appears to be about 900 µm in one specimen (Fig.

2 in Brinkhurst et al., 1994), similar to some of the new material. The short, lateral blood vessels seen in posterior segments of some new specimens were difficult to observe. We consider these differences to be minor or subject to preservation and individual variation, and therefore the genus remains monotypic.

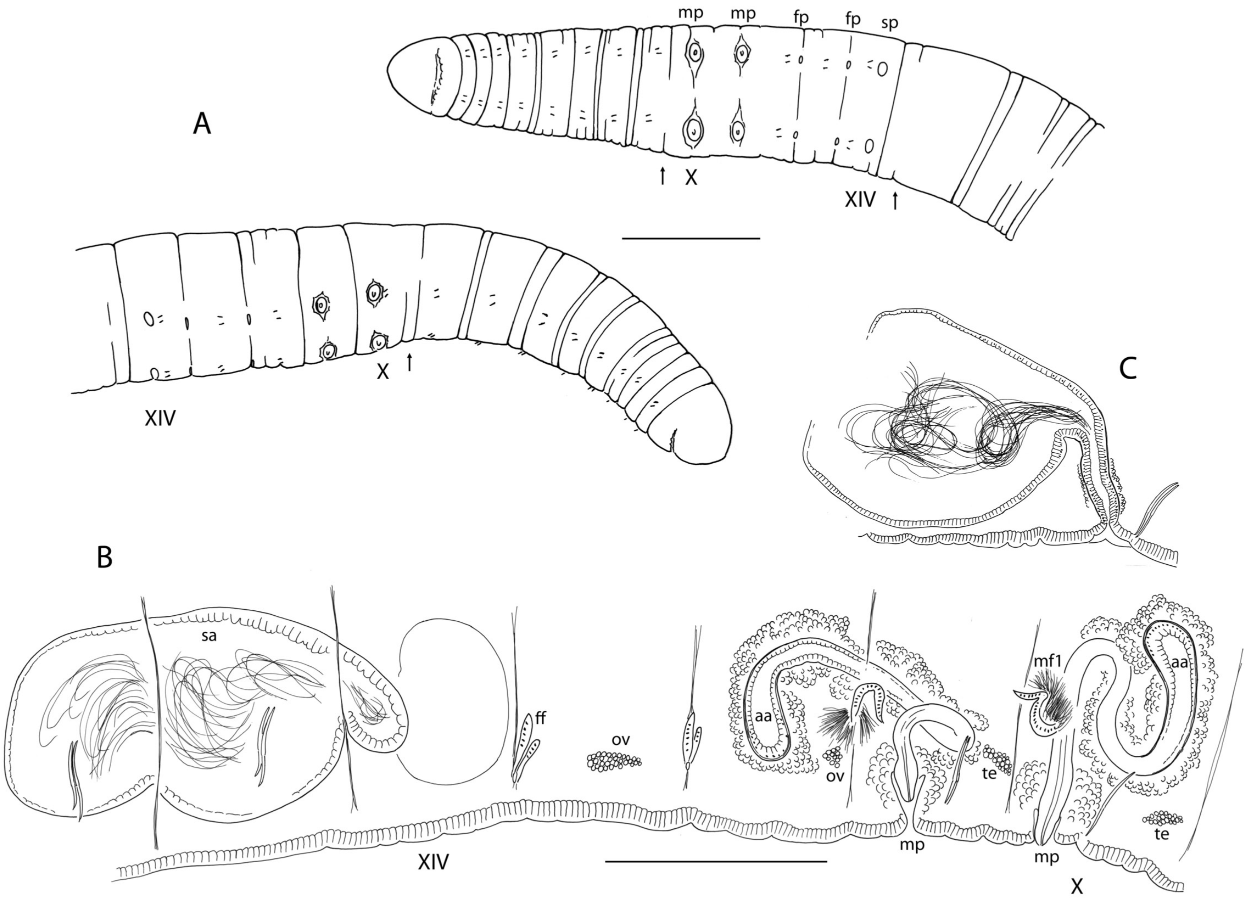

FIGURE 1. *Lamprortus orientalis* Rodriguez 1994. Specimens from Hiroshima Prefecture, outlet stream from Haizuka Reservoir. A. Whole worms, ventral and ventrolateral views, showing locations of genital pores; arrows indicate beginning and end of clitellum. Scale bar = 1 mm. B. Reproductive organs, segments X–XVI, from a dissected worm (vasa deferentia not illustrated); scale bar = 500 µm. C. Spermatheca, lateral view; scale same as (B).

Habitat: The outlet streams from Haizuka-dam in Hiroshima have a gravel bottom with water depth 20–30 cm; filamentous cyanobacteria, mainly *Homoeothrix janthina*, were dominant on cobbles. This cyanobacterium is common in oligotrophic streams in Japan; however, it was not well represented in *L. orientalis* gut contents, which were dominated by filamentous algae, diatoms and fine organic matter. The small stream in Hirosaki lies within an urban landscape; it is spring-fed, with gravel bottom and water depth 10–20 cm; water temperature was 9.2º C and pH 6.2 at the time of collection.

Lamprortus orientalis was described from the Suyong River, Pusan, Korea. The type locality, and other sites within the Suyong drainage, are in areas with agricultural and urban land use, and associated with pollution-tolerant species (Brinkhurst et al. 1994). Occurrence of this species in Hiroshima Prefecture in Honshu, Japan is not surprising considering its proximity to the type locality. The Korean Strait, separating the Korean Peninsula and Honshu Island is relatively recent, dating to about 100,000 ybp in late Diluvial epoch of the Quaternary Era (Ohshima 1990). We suggest that *L. orientalis* may be distributed widely in Honshu Island, based on the additional collections in Aomori Prefecture, located in the northernmost part of Honshu. Additionally, as this species occurs in anthropogenically-modified habitats, its range may have been further increased by human activities (e.g., transfer of plants among drainages).

3.2 *Styloscolex* Michaelsen, 1901

Styloscolex Michaelsen, 1901 (Michaelsen 1901b, Cook 1971, Holmquist 1974, Sokolskaya 1972b, 1978, Timm 1994, Timm & Všivkova 2007)
Styloscolex s. str. Sokolskaya, 1976
Styloscolex (*Styloscolex*) Sokolskaya, 1983
Styloscolex (*Neoscolex*) Sokolskaya, 1976

Remarks: A diagnosis for the genus is given by Cook (1971), and has since then been modified to include two subgenera, *Styloscolex* (*Styloscolex*) Sokolskaya, 1983 (= *Styloscolex* s. str. in Sokolskaya 1976) and *Styloscolex* (*Neoscolex*) Sokolskaya, 1976. *Styloscolex* species are prosoporous, with only one testicular segment. The previously described species all have the reproductive segments shifted anteriad (i.e., testes and male pores in VII or VIII), and are distributed in Lake Baikal and eastern Asia, with one record from northern Alaska (Holmquist 1974). *Styloscolex* (*Styloscolex*) species usually have elongate atria and cuticular penes, and all have an intervening, sterile segment between the testicular and ovarian segments (a character otherwise unknown in the Lumbriculidae). Another remarkable characteristic of the subgenus is that different species have spermathecae in either pre- or post-atrial segments, or both (Sokolskaya 1969, Timm 1994). Two Japanese species conform to *S.* (*Styloscolex*): *Styloscolex japonicus* Yamaguchi, 1937 will be discussed in a future contribution, based on the rediscovered Yamaguchi collection plus several new records from Hokkaido, and a new species is described herein.

The three or four species attributed to *Styloscolex* (*Neoscolex*) by Sokolskaya (1983) resemble *S.* (*Styloscolex*) in having the series of reproductive segments anterior to the usual position, i.e. with male pores VII or VIII instead of X; spermathecae are in the preatrial segment; male ducts are prosoporous, and the short vasa deferentia do not enter the post-atrial segment. They are primarily distinguished from *S.* (*Styloscolex*) by the position of the ovaries, in the post-atrial segment—and thus without an intervening sterile segment. Atrial morphology is variable in this subgenus: atria are sacciform in *S.* (*Neoscolex*) *macer* Sokolskaya, 1976 and in an undescribed species (Sokolskaya 1983), and club-shaped in *S.* (*Neoscolex*) *sokolskajae* Morev, 1978. Only *S.* (*Neoscolex*) *levanidovi* (Sokolskaya, 1977) has the elongate atria and cuticular penes that characterize the nominate subgenus. Members of this subgenus are known only from northeastern Asia; a single Japanese species, described below, is provisionally assigned to this group.

3.2.1 *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp.
(Figure 2)

Holotype: NSMT-An 522. A dissected worm (anterior segments only), stained with carmine and slide-mounted in Canada balsam.

Type locality: Japan, Akita Prefecture. A spring at Kata, near Lake Tazawa, 39.696440N, 140.662897E, elev. 264 m, 11 May 2008, collected by A. Ohtaka.

Paratype: NSMT-An 523. From the type locality, same collection. One unstained whole mount (anterior segments only) in Canada balsam.

Etymology: Derived from the type locality, Lake Tazawa.

Description: Both specimens incomplete; the longer (paratype) with 47 segments. Diameter 0.46 mm in X, maximum diameter to 0.63 mm. Prostomium rounded, length and width both about 300 µm (Fig. 2A). Epidermis in anterior segments 11–14 µm thick, in clitellum 26–50 µm, and 6–7 µm posteriorly. Clitellum well-developed and glandular VII–XII, cells unordered (not in rows). Secondary annulation may be present as a narrow anterior ring from about IV–VII.

Chaetae simple-pointed, moderately sigmoid, dorsal and ventral pairs similar; length 120–151 µm in anterior region, 122–144 µm posteriorly; thickness 4–5 µm; nodulus approximately 0.37–0.43 from the distal end (Fig. 2E, F). Pharynx moderately thickened in II–IV, without distinct dorsal pad. Pharyngeal glands small in IV (V). Chloragogen tissue covers gut beginning in VI. First nephridia paired on 6/7, the second pair on 11/12 or 12/13; nephridia variable in posterior segments. Blood vessels were not clearly observed.

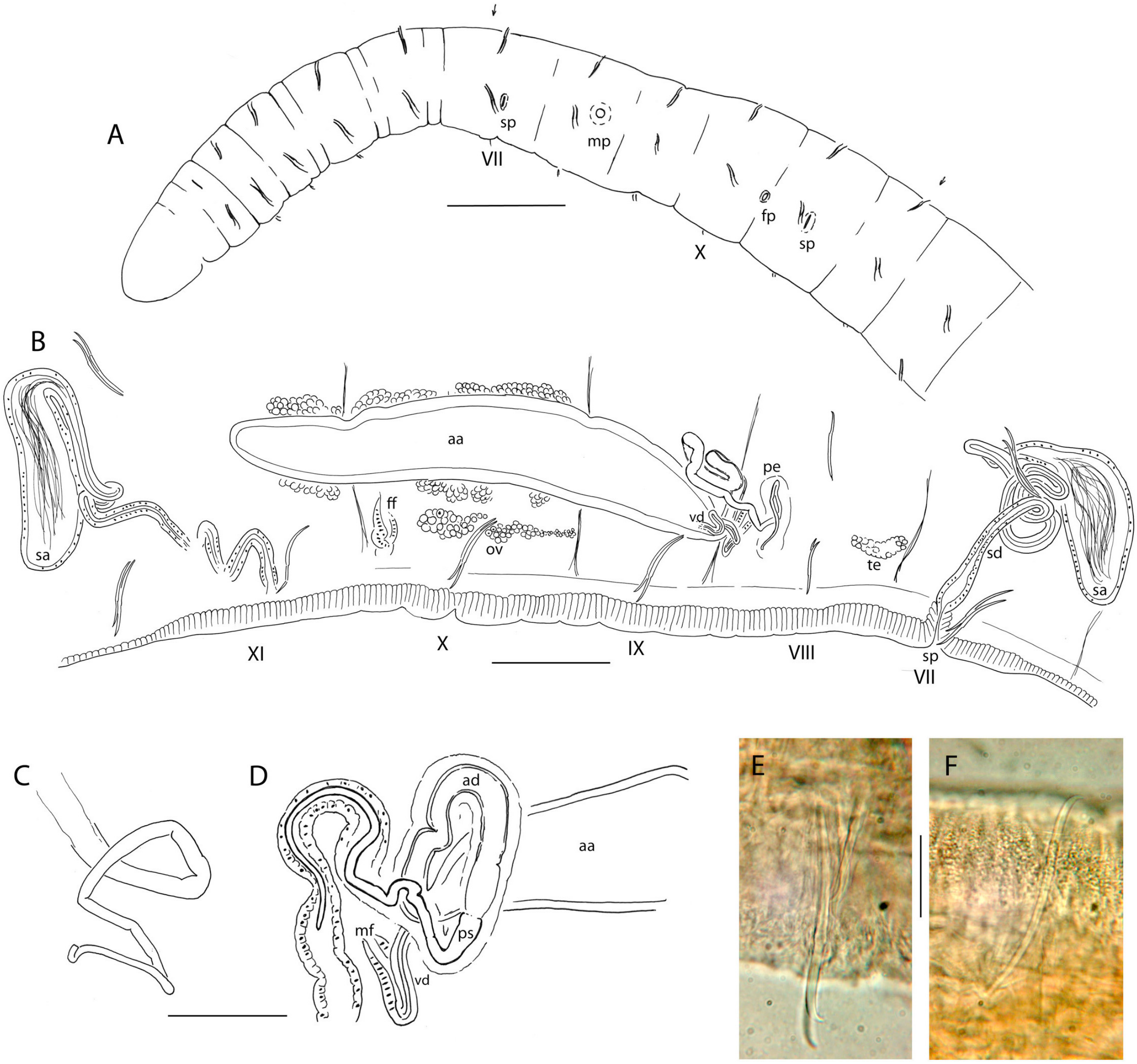

FIGURE 2. *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp. from Akita Prefecture, spring at Kata, near Lake Tazawa. A. Whole worm, ventrolateral view showing location of genital pores, scale bar = 500 µm. B. Reproductive organs in segments VII–XI, from a dissected worm; scale bar = 200 µm. C. Penis sheath, from a whole mount; scale bar = 100 µm. D. Detail of atrial duct, male funnel, penis in sac, from a dissected worm. E. Ventral chaeta in IV; scale bar = 50 µm. F. Dorsal chaeta in XII.

Spermathecal pores are transverse slits, immediately behind ventral chaetae in VII and XI; male pores round, may have small conical papilla, on ventral chaetal line in VIII midway between chaetae and 8/9 (Fig. 2A); female pores on chaetal line at intersegment 10/11. Testes small, in VIII; ovaries in X, may be small or elongate, extending through posterior septum into XI. Egg sacs extending back as far as XV; female funnel 80 µm high.

Spermathecae similar in VII and XI; the ones in XI may extend back into XII. Spermathecal duct ending in a short expansion (to 50 µm wide), the remaining duct tubular, 17–25 µm in diameter, with columnar epithelium; duct may be highly folded, total length 550–700 µm (Fig. 2B). Spermathecal ampulla elongate-sacciform, length 290–390 µm, width 90–100 µm. Ampulla with thin, non-glandular epithelium (8–15 µm); sperm loosely arranged.

Male funnels small and conical, on 8/9 only. Vasa deferentia difficult to see in the available material; apparently do not penetrate the posterior septum; junction with atrium near base (ectal end) of ampulla (Fig. 2B,D). Atrial duct long and convoluted; ectal 420–550 µm forming a penis with a thin, folded cuticular covering ("penial sheath"), sheath diameter about 5 µm at the male pore, expanding to 20–28 µm entally, within a narrow sac (Fig. 2B–D). Remainder (170–190 µm) of duct gradually widens and muscle layer becomes thicker, eventually expanding to form the atrial ampulla. Ampulla elongate-fusiform (almost tubular), extending back to XI or XII; length 850–1000 µm, maximum diameter 75–155 µm; distinct muscle layer 12–22 µm; outer layer of small prostate glands 25–35 µm high; ampulla filled with sperm (Fig. 2B).

Remarks: Despite the limited material available, the new species is clearly attributable to *S.* (*Styloscolex*) by a combination of: prosoporous male ducts with male pores in VIII, a preatrial pair of spermathecae in VII, elongate atria, and ovaries and female funnels in the second postatrial segment (XI). Within the subgenus *Styloscolex*, diagnostic characters of *S. tazawaensis* n. sp. include: male pores paired in VIII, spermathecal pores paired in both VII and XI; atrial duct very long and convoluted; elongate, thin, folded penis sheath; atrial ampulla elongate-fusiform, length about 8x diameter; spermathecal duct tubular, long and folded.

The presence of a second pair of spermathecae in XI is shared only with *Styloscolex tetrathecus* Burow, 1931, described from Lake Baikal by Burov (1931), from northeastern China by Yamaguchi (1940), and from Lake Potatgytkhyn, Koryak upland by Sokolskaya (1978, 1983). The original description of that species is not very detailed; the penes are "long and narrow" and ducts are "distinctly differentiated" from the ampullae (no measurements of these structures were given). However, the very long atrial and spermathecal ducts of *S. tazawaensis* n. sp. seem to differ from both the original description and redescriptions of *S. tetrathecus*; the atrial ampulla is also much longer than the 250 µm given by Sokolskaya (1983) (see Fig. 20 in Sokolskaya 1983 = Fig. 1 in Sokolskaya 1978). Burov's (1931) *S. tetrathecus* description, as well as Sokolskaya's redescription, indicate a much shorter atrial duct, barely passing through the septum into the adjacent segment before widening into the ampulla. Other redescriptions (Yamaguchi 1940, Isossimov 1962) do not provide much detail, focusing on segmental position of reproductive organs. Another widespread species (from Kamchatka and Alaska), *Styloscolex opisthothecus* Sokolskaya, 1969, differs from most congeners in having only post-atrial spermathecae, in XI. The atrial and spermathecal ducts (e.g., Figs. 3–4 in Holmquist 1974) are short, resembling those of *S. tetrathecus*.

Of the other *Styloscolex* species, only *Styloscolex hankensis* Timm & Všivkova, 2007, described from Lake Hanka (Russia/China) has been described as having a penis sheath "very long, highly elastic, and doubly folded". The latter has only one spermathecal segment (VII), and as it was described from a single, unmated specimen, it is difficult to compare the spermathecal morphology. As in the new species, *Styloscolex tubulatus* Timm, 1994, from the Magadan Region (eastern Russia) has elongate spermathecal ducts, but has spermathecae only in two post-atrial segments (X and XI). It is further distinguished from the new species by the shorter atrial duct and penes, and by a very large glandular mass surrounding the penial sac.

Habitat: The type locality is a small, spring-fed, muddy *Alnus* swamp in Lake Tazawa basin, Akita Prefecture, Honshu. Water depth was less than 10 cm, and temperature exceeds 20° C in summer. Forest floor vegetation is dominated by the emergent Asian skunk cabbage (*Lysichiton camtschatcensis*). Chironomine and tanypodine chironomids dominated the bottom fauna, and the tubificine oligochaete, *Embolocephalus yamaguchii* (Brinkhurst, 1971) has also been recorded (Ohtaka & Martin 2011). *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp., *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp., and some other lumbriculids were collected by scooping the bottom sediment.

3.2.2 *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp.

(Figure 3)

Holotype: NSMT-An 524. Anteriormost 24 segments, stained with carmine and slide-mounted in Canada balsam.

Type locality: Japan, Akita Prefecture. A spring at Kata, near Lake Tazawa, 39.696440N, 140.662897E, elev. 264 m, 11 May 2008, collected by A. Ohtaka.

Paratype: NSMT-An 525. From the type locality, same collection. One whole mount of anterior segments, in Canada balsam.

Etymology: From Tatsuko, a local girl who became the spirit of Lake Tazawa.

Description: Both specimens incomplete; the longer (paratype) about 12 mm long, with 57 segments; diameter (slide-mounted) 0.4–0.47 mm in X. Prostomium rounded-conical, length about equal to width (Fig. 3A). Clitellum 1/2 IX–XIII, similar thickness to anterior epidermis (10–12 µm), but distinctly glandular.

Chaetae simple-pointed, sigmoid; directed forward in segments anterior to X; length 67–122 µm in anterior segments, 80–98 µm posteriorly; nodulus at 0.36–0.44 from the distal end.

Pharynx in II–III, mostly thickened dorsally; pharyngeal glands in IV–VI. First nephridia on 6/7, with duct terminating in a small, rounded vesicle at the pore, just in front of the chaetae in VII. Next nephridia on 12/13, then in a few segments posteriorly. Ventral blood vessel forms in III; convoluted commissural blood vessels in anterior segments at least to X. No obvious lateral blood vessels or caecae in posterior segments.

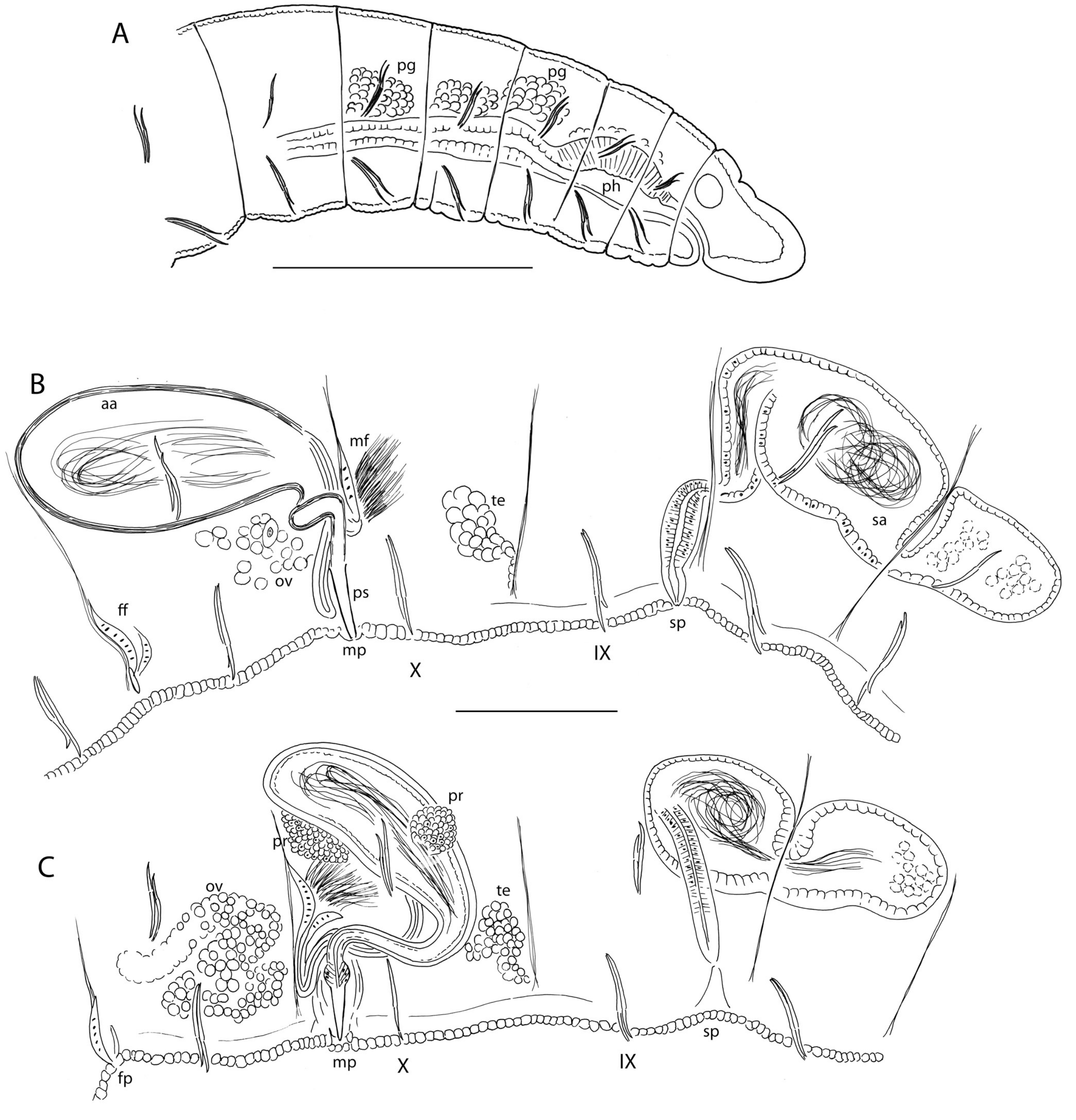

FIGURE 3. *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp. from Akita Prefecture, spring at Kata, near Lake Tazawa. A. Anterior segments, showing pharynx and anteriorly-directed chaetae; scale bar = 500 µm. B. Reproductive organs; scale bar = 200 µm. C. Reproductive organs from another specimen; showing apparent prostate glands.

Spermathecal pore single, midventral in IX, just behind septum 8/9. Male pore single, midventral in X, near 10/11. Female funnels and pores paired near the ventral chaetal line at 11/12. Testes paired in X, sperm sacs IX–XVII; ovaries long, extending to posterior XI, egg sac to XIX. All genital pores simple, slightly recessed, inconspicuous.

Spermathecal duct 130–140 µm long, 30–43 µm wide, formed of thick (to 20 µm), columnar epithelium with indistinct muscle layer and narrow lumen. Duct penetrates 8/9, and ampulla enters VIII. Spermathecal ampulla ovate-sacciform; length 370–480 µm, width 150–170 µm (Fig. 3B,C); irregular epithelium 7–24 µm thick. Sperm loosely distributed, unordered within ampulla.

Male funnels apparently paired on 10/11, may be directed back into XI, within sperm sac. Vas deferens diameter 12–19 µm; not penetrating the posterior septum; joining atrium near base of ampulla. Atrium single in X, petiolate, the atrial duct 120–170 µm long by 25–29 µm diameter, in X or extending into XI; ampulla broadly club-shaped (length 290–360 µm, width 95–170 µm) (Fig. 3B,C). Atrial muscle layer 6–12 µm thick; epithelium thinner than muscle layer, not clearly seen in either specimen. Prostate glands were not clearly seen, but 3–4 faint masses of glandular tissue were apparently associated with the atrial ampullae (Fig. 3C). Atrial duct ends in a narrow-conical penis, 80–95 µm long by 14–18 µm wide; apparently covered by a cuticular sheath, cuticular layer thin and inconspicuous.

Habitat: See above, same locality as *Styloscolex tazwaensis* n. sp.

Remarks: The combination of characters is unique within the family: male pore single, midventral behind ventral chaetae in X, just in front of 10/11; spermathecal pore single, midventral, anterior to ventral chaetae in IX, just behind 8/9; atrium single, petiolate, with ovate ampulla; duct ending in conical penis with a thin, cuticular sheath; male ducts prosoporous, vasa deferentia joining the atrium without penetrating the posterior septum. Although some of these characters are unusual, all occur independently in other lumbriculid taxa. The unpaired, median atrium and spermatheca are shared with the Palearctic *Tatriella* Hrabě, 1936 and some Nearctic *Eclipidrilus* Eisen, 1881 [*Eclipidrilus lacustris* (Verrill, 1871) and *Eclipidrilus macphersonae* Fend & Lenat, 2012]. Cuticular penis sheaths are rare in the family; although well-developed and apparently rigid only in some *S.* (*Styloscolex*) species and perhaps in *S.* (*Neoscolex*) *levanidovi*, thin sheaths have also been described in *Sylphella puccoon* Rodriguez et al., 2014, *Eclipidrilus* (*Premnodrilus*) species (Wassell 1984), and in some *Lumbriculus* Grube, 1844 (e.g., Holmquist 1976). The position of the spermathecal pores in the new species, at the anterior margin of IX, is unusual within the family; however, *Eclipidrilus pacificus* Fend, 2005 has (paired) spermathecal pores in the anterior part of IX, and spermathecal pores are somewhat anterior to the chaetae in other taxa, such as *Rhynchelmis* (*Sutroa*) species.

The petiolate atrium and the posterior vas deferens of the new species resemble those of the two *Tatriella* species, both of which have limited distributions in northern and central Europe. However, the new species differs from *Tatriella* in several characters considered to have generic significance: prosoporous, rather than semiprosoporous male ducts; spermathecal pores located in IX (although anteriorly, near the anterior septum), rather than in the posterior part of VIII; and a comparatively long penis, with a weak cuticular sheath.

In addition to a few species having unpaired atria and/or spermathecae, the Nearctic genus *Eclipidrilus* has spermathecae in the pre-atrial segment (IX) and elongate-petiolate atria. *Eclipidrilus* is a diverse genus that has become difficult to define (see discussion in Fend & Lenat 2012), and at least some species have been assigned to different subgenera (Wassell 1984). As in the new species, the posterior vasa deferentia do not form a loop in the post-atrial segment; however, most *Eclipidrilus* species differ in being semiprosoporous (each atrium with anterior and posterior vasa deferentia), with varying degrees of reduction of the anterior duct. *Eclipidrilus* species have been considered to have characteristic atrial morphology (petiolate, with a very elongate ampulla) and histology (muscle layer spiral, cross-hatched or two-layered), and this has been considered diagnostic for the genus (Cook 1971, Sokolskaya 1983, Fend 2005). Although these characters are inconsistently expressed in some species assigned to *Eclipidrilus* (Fend & Lenat 2012), it does not seem appropriate to assign the new species to that genus.

Styloscolex (*Neoscolex*) resembles the new species in having spermathecae in the pre-atrial segment, prosoporous male ducts, and a single species with a cuticular penis sheath. As it also has an eastern Asian distribution, we provisionally assign the new species to that genus and subgenus. The genus *Styloscolex* has been defined in part by a forward shift in reproductive segments (i.e., the testes and atrium in a segment anterior to X); consequently, this tentative attribution is likely to be modified as future collections support more detailed descriptions.

Some characters were not clear in the available material (two whole-mounted, anterior ends), and should be confirmed with additional, sectioned or dissected specimens. In particular, details of the male reproductive organs were obscured by the densely filled sperm sacs. There appeared to be two sperm funnels, although only the uppermost was clearly visible, but only one vas deferens could be clearly seen on either specimen. Paired sperm ducts occur in other lumbriculids having median, unpaired atria, e.g. *Tatriella slovenica* Hrabě, 1936 (cf. Fig. 13 in Hrabě 1939) and *E. pacificus* (cf. Fig. 9B in Fend 2005). The apparent prostate glands were not clearly visible on either specimen, and the structures shown in Fig. 3C may have been unassociated glandular tissue. Absence of prostate glands on the atrial ampulla has only occasionally been reported in lumbriculid descriptions, e.g., in *Eclipidrilus breviatriatus* Fend & Lenat, 2012, and in *Styloscolex baicalensis* Michaelsen, 1901. Prostate glands are present on the atrial duct of in *T. slovenica*, but they are absent from most of the ampulla.

3.3 *Honshudrilus* n. gen.

Type species. *Honshudrilus gudariensis* n. sp.

Diagnosis: (Possible apomorphies underlined.) Small worms without a proboscis. All chaetae simple-pointed. No lateral blood vessels in posterior segments**.** First pair of nephridia in VII (on 6/7). Testes paired in X, ovaries paired in XI. Spermathecal pores on the ventral chaetal line, more than one segment anterior to male pores (in VI or VIII); spermathecal ducts with ectal vestibules. Female pores on ventral chaetal line at 11/12. Male pores behind ventral chaetae in X; penes formed by extruded lining cells of atrial duct. Atria petiolate, with long duct, and elongate ampulla covered with multicellular, petiolate prostate glands. Male ducts semiprosoporous, with nonfunctional anterior vasa deferentia not associated with testes; posterior vasa deferentia penetrate posterior septum, forming a loop in XI.

Included species: *Honshudrilus gudariensis* n. sp. Ohtaka, Fend & Torii, *Honshudrilus yuzawaensis* n. sp. Fend, Ohtaka & Torii.

Etymology: These worms appear to be endemic to the Island of Honshu.

Remarks: Attribution of the two included species to a single genus is somewhat problematic, yet it is reasonably consistent with generic diagnoses outlined by Cook (1968, 1971) and modified by more recent literature. The first major division in Cook's (1971) key separates lumbriculid genera into those with spermathecae opening in segments anterior to the atrial segments vs. spermathecae in the atrial or post-atrial segments. Most species with pre-atrial spermathecae have them in the first pre-atrial segment (Brinkhurst 1989), but (like *Honshudrilus yuzawaensis* n. sp.), they are in the second pre-atrial segment in *Rhynchelmis* Hoffmeister, 1843, *Pseudorhynchelmis* Hrabě, 1982, *Tatriella* Hrabě, 1936, *Pararhynchelmis* Fend & Lenat, 2010, and *Secubelmis* Fend & Gustafson, 2001. A single genus, *Martinidrilus* Fend & Lenat, 2007 has spermathecae opening in either the third or fourth pre-atrial segment. Relative position and number of spermathecae is invariant in many lumbriculid genera, differing most notably within *Styloscolex* (see above), *Rhynchelmis*, *Martinidrilus*, *Lamprodrilus*, and *Lumbriculus*.

Characters distinguishing *Honshudrilus* from similar genera are summarized in Table 1. Possible synapomorphies for the genus, including the penes, the spermathecal vestibules, and the non-functional anterior male ducts, are admittedly weak, as they can vary within other accepted genera. Male pores of both species have "type 2" penes, as defined by Rodriguez & Giani (1994); these temporary structures are formed by extruding the elongate lining cells in the atrial duct (Cook 1967, Fig. 2a; Rodriguez & Giani 1994, Fig. 17F). While spermathecal ducts of both *Honshudrilus* are distinctly widened into "vestibules" at the pores, these structures are morphologically dissimilar in the two species (see below). The nonfunctional anterior male funnels and vasa deferentia seem to be a good apomorphy, as they imply a reduction of the supposedly ancestral semiprosoporous condition for the family (Brinkhurst 1989). Nevertheless, a range of similar, apparently intermediate forms occurs within both *Rhynchelmis* and *Eclipidrilus*.

The two described *Martinidrilus* species, both from southeastern North America, are distinguished from *Honshudrilus* by additional synapomorphies, e.g., spermathecae opening laterally, vasa deferentia joining before entering short atria, and numerous short lateral blood vessels in posterior segments. As none of these unusual characters occurs in either of these two Japanese species, it seems preferable to erect a new genus, rather than compromising the diagnosis of *Martinidrilus*. *Honshudrilus* differs from both described *Martinidrilus* species in having 1) spermathecal pores on the chaetal line, with distinct vestibules, 2) the anterior male duct and funnel are small and nonfunctional, 3) atria have elongate ducts and ampullae, 4) vasa deferentia do not join before joining the atria, 5) posterior lateral blood vessels appear to be entirely lacking, and 6) lining cells in the ectal part of the atrial duct can apparently be extruded, forming a type 2 penis.

TABLE 1. Comparison of *Honshudrilus* n. gen. species with other lumbriculids having similar characters. Underlined characters are considered likely apomorphic within the family; characters with asterisk (*) were considered possible apomorphies for *Pseudorhynchelmis* by Martin & Kaygorodova (2008).

	Honshudrilus gudariensis	*Honshudrilus yuzawaensis*	*Pseudorhynchelmis* (Baikal spp.)	*Martinidrilus*	*Eclipidrilus frigidus*
spermathecae relative to atrial segment	4 segments anterior (VI)	2 segments anterior (VIII, usually)	2 segments anterior (VIII)	2–4 segments anterior (VII–VIII or VI)	1 segment anterior (IX)
spermathecal duct	small vestibule	large vestibule	1 species with vestibule or bulb	not modified	large vestibule
spermathecal position in segment	paired, on chaetal line	paired, on chaetal line	paired, on (or slightly inside) chaetal line	paired, lateral or dorsolateral	paired, on chaetal line
testes	1 pair, in X	1 pair, in X	2 pairs, IX, X	2 pairs, IX, X	2 pairs, IX–X
anterior male duct	non-functional, reduced	non-functional, reduced	functional	functional	usually functional, reduced
male duct enters post-atrial segment	yes	yes	yes	yes	no
vas deferens structure	thin, tubular	thin, tubular	*widened entally (usually)	thin, tubular; joining before atrium	thin, tubular
atrium shape	elongate-petiolate	elongate-petiolate	ovate to elongate-tubular	short-ovate	elongate-petiolate
atrial musculature	thin	thin	thin	thin	thick, spiral
atrial epithelium	somewhat thickened	somewhat thickened	*thick and glandular	somewhat thickened	thin
penes	type 2	type 2	*type 1 (usually)	small papilla	large type 2 (in sac)
modified genital chaetae	none	none	spermathecal, penial, or none	none	none
posterior lateral blood vessels	none	none	none	many short lateral vessels	2 pairs, branched
distribution	Japan, Honshu	Japan, Honshu	Lake Baikal	Southeastern North America	Western North America

An alternative classification would place *Honshudrilus* in *Pseudorhynchelmis*, a group of taxa largely restricted to Lake Baikal (with one exception in European groundwater); *Pseudorhynchelmis* species also have atria in X and spermathecae are always two segments anteriad, in VIII. Atria vary from ovate to elongate in the different species; where the atrium is elongate, it is tubular, rather than petiolate, and both vasa deferentia are functional. Martin & Kaygorodova (2008) discussed the difficulty of defining consistently-expressed apomorphies for *Pseudorhynchelmis*, but suggested (1) ental widening of the vasa deferentia, (2) thick-glandular atrial epithelium, and (3) pendant penes in sacs ("type 1" penes, as defined by Rodriguez & Giani 1994) as possibly diagnostic. *Pseudorhynchelmis alyonae* (Martin et al., 1998) also has an elaborate spermathecal duct, but the large expansion at the spermathecal pore appears to be a solid "bulb", rather than a deep vestibule. Additionally, genital chaetae occur in several species. As *Honshudrilus* species lack all of these diagnostic characters, and also have non-functional anterior male ducts (not associated with testes), it does not seem appropriate to combine them with *Pseudorhynchelmis* at this point.

Several *Rhynchelmis* species have vestigial or nonfunctional anterior male funnels and ducts, but these differ from *Honshudrilus* species in most other respects. For example, *Rhynchelmis* species are large, robust worms, usually with a proboscis, and well-developed lateral blood vessels; atria are usually tubular; spermathecae usually join the gut; and even the expanded spermathecal ducts of *Rhynchelmis* (*Sutroa*) species are glandular "bulbs", rather than vestibules.

3.3.1 *Honshudrilus gudariensis* Ohtaka, Fend & Torii

(Figure 4)

Holotype: NSMT-An 526. A whole worm, stained with hematoxylin, slide-mounted in Canada balsam. Collected 1 March 2017 by A. Ohtaka.

Type locality: Japan: Aomori Prefecture, spring-fed Gudari-numa Stream in Hakkōda Mountains, Komagome 40.669844N, 140.952302E, elev. 589 m.

Paratypes: All from the type locality. NSMT-An 527–529, 17 March 2017, 1 dissected and slide mounted. 11 February 2016, 1 whole mount. 4 June 2014, 1 sagittally sectioned on slide. USNM 1610892-1610894, 4 June 2014, 1 whole mount. 17 March 2017, 2 whole mounts in Canada balsam.

Other material: Type locality, collected by A. Ohtaka, 19 April 2014, 2 whole mounts. 4 June 2014, 4 whole mounts. 10 August 2014, 5 whole mounts. 31 August 2014, 1 whole mount. 2 November 2014, 3 whole mounts. 17 March 2017, 2 whole mounts and 1 dissected on slide. 2 November 2014, 4 sagittal sections. Akita Prefecture, a spring-fed brook at O-mori, near Lake Tazawa, 39.719979N, 140.697021E, with gravel bottom, depth 5–10 cm, 20 September, 2015, 1 whole mount. Takamatsu, Yuzawa-shi, Yamagata, 1 May 2013, collected by T. Torii, 1 whole mount.

Etymology: From the type locality, Gudari-numa.

Description: Body whitish in color without pigment. Length of preserved worms 22 to 32 mm; maximum diameter around X, 0.28 to 0.36 mm; 64 to 75 segments. Prostomium rounded or conical, as long as or slightly shorter than wide (Fig. 4A). Secondary segmentation typically a narrow anterior ring in IV–VIII, otherwise inconspicuous throughout the body. Epidermis 8–16 μm thick in anterior segments. Clitellum inconspicuous, with slightly thickened (13–20 μm) and glandular epidermis, usually from beginning of X to end of XII. Circular muscle of body wall 2–4 μm thick, and longitudinal muscles 10–16 μm thick.

Chaetae paired, in 4 bundles in each segment from II. Chaetae sigmoid and simple-pointed, with nodulus at ⅓ from the distal end, shape and size not different between dorsal and ventral bundles (Fig. 4B). Chaetae 80 to 98 μm long in anterior segments and 72 to 88 μm in posterior segments.

Pharynx thickened dorsally in II–III (sometimes IV), pharyngeal glands developed in IV–VI. Chloragogen begins in VII. First pair of nephridia on 6/7; nephridia absent in X–XIII, occurring irregularly in middle and posterior segments. Main dorsal and ventral vessels prominent, without secondary longitudinal vessels. Commissural blood vessels prominent in II, III, IV; blind lateral vessels not observed in posterior segments.

One pair of male pores on the chaetal line in X, posterior to ventral chaetae, within a 60–70 μm ring of slightly thickened epidermis (Fig. 4C,D). One pair of spermathecal pores on the chaetal line in VI, posterior to ventral chaetae. Female pores inconspicuous, on chaetal lines on intersegment 11/12; female funnels small, about 50 μm high. Paired testes on anterior septa in X, usually extending to mid-segment. Ovaries in XI, usually extending to posterior septum. Sperm sacs may extend anteriorly into IX and posteriorly as far as XVI; egg sacs extend as far back as XVIII. Two pairs male funnels, on 9/10 and 10/11; the posterior pair functional (with sperm), large (to 90–100 μm high) and extending forward into X; the anterior pair smaller (to 50–60 μm high) and non-functional (Fig. 4C). Anterior vasa deferentia nonfunctional, lumen not obviously ciliated, 8–12 μm in diameter; posterior vasa deferentia about 350 μm long, 14–20 μm thick throughout their length, with ciliated lumen 8–11μm wide, forming a short loop in the postatrial segment before entering X; both anterior and posterior vasa deferentia follow atrial duct and join basal part of atrial ampulla (Fig. 4C). Atrial ampulla pyriform or elongate-ovate in shape, 110–150 μm long and 55–75 μm wide; irregularly transverse outer muscle layer 3–7 μm thick; lumen to 30 μm wide. Epithelium of ampulla appears to vary with stage of development: in some specimens it is cuboidal, 5–10 μm thick (Fig. 4F); in other specimens it is thicker (12–20 μm), granular, with cell boundaries obscure (Fig. 4G). Atrial ampulla loosely covered with non-glandular cells and 6 to 12 petiolate prostate glands 32–50 µm high (Fig. 4H). Atrial duct long and thick, well marked off from ampulla, 300–350 µm long and 40–50 µm wide; duct epithelium less glandular than in ampulla, 10–15 µm thick and lumen 3–8 µm wide. Terminal ⅓ of atrial duct slightly thicker than ental part, projecting vertically within body; epithelial cells in this section usually columnar; near the pore they are directed outward, and may project out of the body, forming a penis (Fig. 4E).

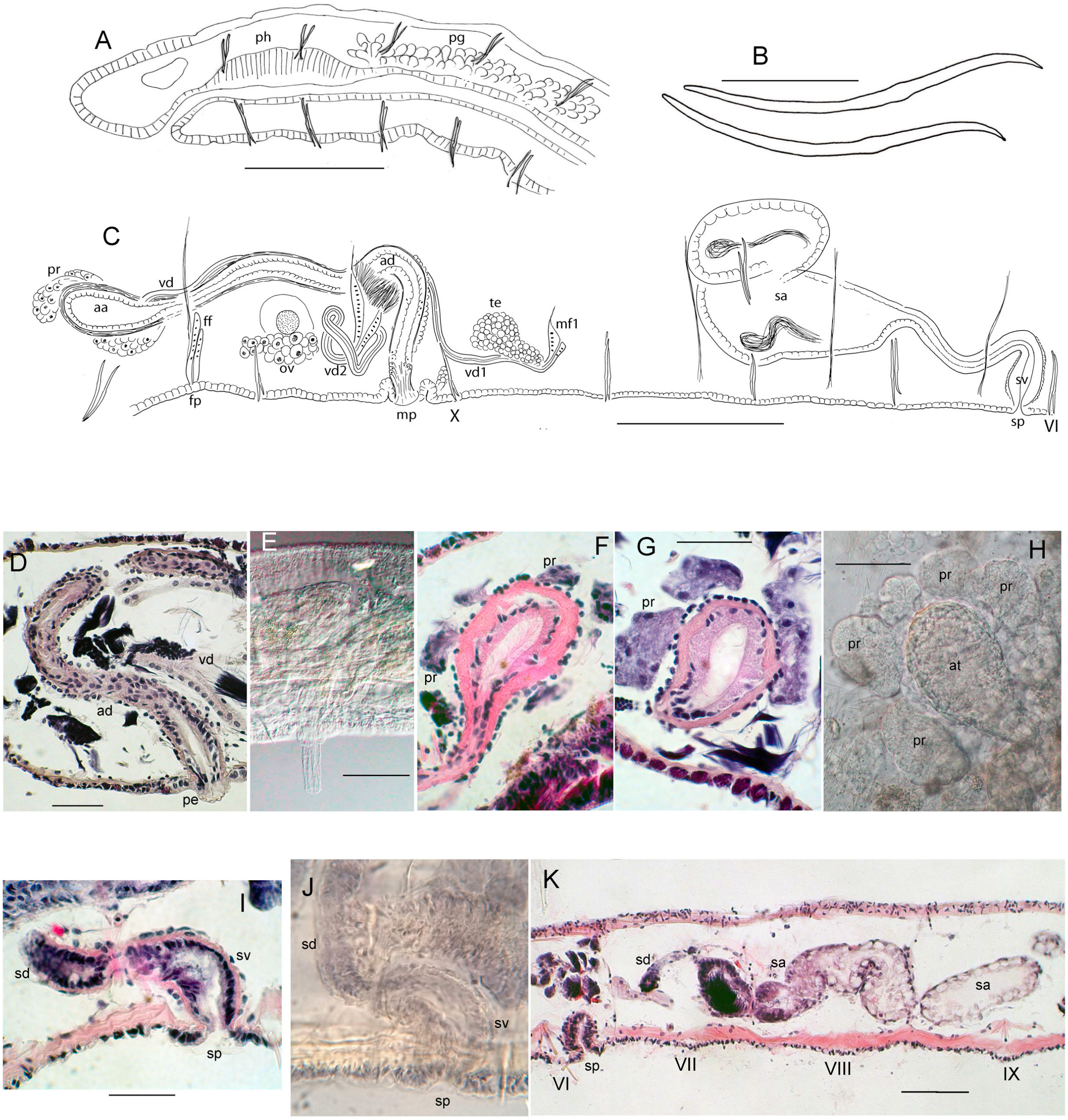

FIGURE 4. *Honshudrilus gudariensis* n. sp. from Aomori Prefecture, spring-fed Gudari-numa Stream in Hakkōda Mountains, Komagome. A. Anterior segments, showing pharynx; scale bar = 200 µm. B. Paired chaetae; scale bar = 20 µm. C. Reproductive organs in segments VI–XII, from a dissected worm; scale bar = 200 µm. D. Atrial duct with partially-extruded penis, from a sagittal section; scale = 50 µm. E. An extruded penis; scale = 100 µm. F, G. Atrial ampullae with thin and thick epithelium, from sectioned worms; scale = 50 µm. H. Atrial ampulla with prostates; scale = 50 µm. I, J. Ectal end of spermathecal duct with vestibule; (I) is from a section; scale = 50 µm. K. Spermatheca, with ampulla extending into IX, from a section; sperm is concentrated in ectal part of ampulla, ental part with vacuoles in epithelium; scale = 100 µm.

Spermathecal ducts 260–400 µm long, opening ventrally between chaetal bundle and posterior septa in VI; ectal part of duct widened into a vestibule, 60–90 x 35–50 µm, with thin but distinct muscle layer (Fig.

4I,J); ental part of duct tubular, 130–210 x 20–26 µm, nearly filled by densely-packed, columnar epithelium. Spermathecal ampulla elongate-sacciform, 200–350 µm long, 50–105 µm wide, extending backward to VII–IX; about the ectal ⅓ thin-walled, densely filled with unordered sperm; the remainder with thicker, vacuolated epithelium and very sparse sperm (Fig. 4K); vacuoles may be sorptive, apparently containing some sperm.

Remarks: The anterior position of spermathecae in VI, four segments anterior to the atria in X is shared only with *Martinidrilus carolinensis* Fend & Lenat, 2007. As stated above, the two genera differ in morphology of atria and vasa deferentia, and in the lateral position of spermathecal pores.

The spermathecal ampulla was relatively small, and not differentiated in one specimen; instead, the entire sac was thin-walled, and sperm was distributed throughout. The specimen also lacked mature eggs in the egg sacs, suggesting that the worm had recently mated, and was at an earlier stage of development. The large, vacuolar cells in the ental part of the ampulla of mature specimens suggest sperm sorption, which occurs commonly in the family (see Černosvitov 1930, Cook 1975, Fend & Lenat 2012).

Histology of the atrial ampulla is quite variable, and appearance of the epithelial layer probably varies with stage of development. A thick, granular layer likely represents secretions of the prostate glands. Similar variation has been noted in other lumbriculid species (Timm 1998).

Gut contents were dominated by pennate and centric diatoms along with detritus. The material resembled the bottom deposit in the habitat, Gudari-numa Stream, suggesting the detritivorous nature of this species.

Habitat: Spring-fed Gudari-numa Stream is located in the Hakkōda Mountains, Aomori Prefecture, Honshu. Water temperature is around 7° C throughout the year (Baba and Ohtaka 2017). The substrate consists of gravels and sand, with clusters of submerged *Ranunculus nipponicus*. The bottom fauna is numerically dominated by diamesine chironomids; other cool-water or stygobiont invertebrates include flatworms, nerillid polychaetes, bathynellaceans, and harpacticoid copepods. An endemic, subterranean amphipod, *Pseudocrangonyx gudariensis* Tomikawa & Sato, 2016 was also described from the site (Tomikawa et al. 2016). Dominant oligochaetaes were the lumbriculids *H. gudariensis*, *Lumbriculus* sp. and another species in an uncertain genus. A single specimen was collected at the type locality for *H. yuzawaensis* n. sp. (see below) and another from a spring-fed brook near Lake Tazawa in Akita Prefecture.

3.3.2 *Honshudrilus yuzawaensis* n. sp.

(Figure 5)

Holotype: NSMT-An 530. A whole worm, not stained, slide-mounted in Canada balsam.

Type locality: Japan: Akita Prefecture: a mountain stream in Takamatsu, Yuzawa, 38.976783N, 140.538141E, elev. 540 m, 1 May 2013. Collected by T. Torii.

Paratypes: NSMT-An 531–533. From the type locality, same collection. 1 whole mount, on same slide as holotype. 2 whole mounts, both on a different slide. USNM 1610895. 1 whole mount.

Other material: From the type locality, same collection. 3 whole mounts on 1 slide.

Etymology: From the type locality.

Description: Length of 3 slide-mounted worms 14.1–16.3 mm; 59–64 segments; maximum diameter 0.30–0.42 mm; prostomium rounded-conical, length about equal to width; secondary annulation IV–VII. Chaetae simple-pointed, sigmoid; length 75–109 µm in anterior segments, nodulus 0.33–0.43 from the distal end (Fig. 5D). Epidermis 7–12 µm thick in anterior segments, 12–18 µm in clitellum; clitellum X–XII(XIII). Longitudinal muscle 10–12 µm thick anteriorly.

Pharynx thickened dorsally in II–III, pharyngeal glands in IV–VI. Gut filled with fine particulate organic matter. First nephridia on 6/7. Brain deeply bilobed. Commissural blood vessels in preclitellar segments, lateral blood vessels absent posteriorly.

Both male and spermathecal pores on ventral chaetal line. Spermathecal pores inconspicuous, usually paired behind ventral chaetae in VIII, rarely also in VI or IX (Fig. 5B). Male pores paired in X (Fig. 5A,B), midway between chaetae and posterior septum within a 90 µm, slightly concave ring of thickened epidermis, most with a small papilla, but cylindrical penis is extruded to 135 µm in 1 specimen (Fig. 5C,F). Testes medium-size, in X; ovaries in XI, extending to mid-segment. Female funnel 70–100 µm high, pore intersegmental at 11/12. Sperm sacs to XVI, mature eggs to XVIII.

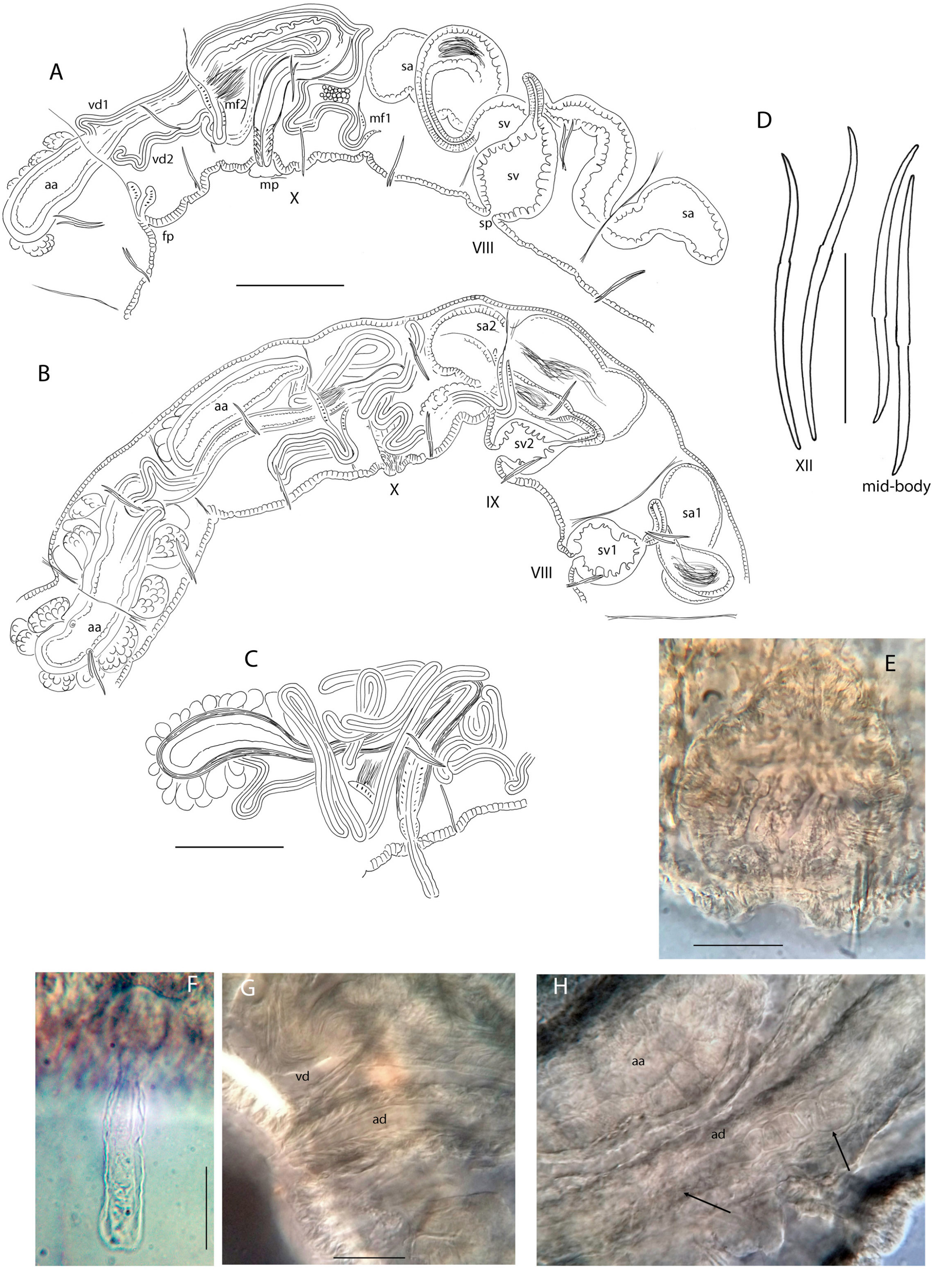

FIGURE 5. *Honshudrilus yuzawaensis* n. sp. from Akita Prefecture, mountain stream in Takamatsu, Yuzawa. A, B. Reproductive organs, segments VIII–XII or XIII, from whole-mounted worms; (A) has the normal spermathecal configuration; (B) with an additional spermatheca in IX; scale = 200 µm. C. Detail of atrium (with extruded penis) and vasa deferentia; scale = 200 µm. D. Dorsal (left) and ventral chaetal pairs; scale = 50 µm. E. Spermathecal vestibule; scale = 50 µm. F. Extruded penis; scale = 50 µm. G. Male pore and duct, penis not extruded; scale = 50 µm. H. Detail of atrial duct, with detached lining indicated by arrows, same scale as G.

Ectal end of spermatheca with a large (to 200 x 140 µm), ovoid or turnip-shaped vestibule (Fig. 5A,B,E), lined with thick, folded epithelium, apparently with a thin, cuticular lining in some specimens. Vestibule joins a tubular duct, 125–170 µm long, diameter 20–26 µm; duct with columnar epithelium and narrow lumen. Spermathecal ampulla sacciform, commonly extending into adjacent segments; thin-walled (4–10 µm) in mated specimens; sperm loosely dispersed throughout, but denser at ectal end; ampulla length 300–400 µm, diameter to 170 µm (Fig. 5A,B).

Male funnels on 9/10 and 10/11 (semiprosoporous), the anterior pair small and nonfunctional (60 µm high), the posterior pair larger (100 µm), with sperm (Fig. 5A,B). Vasa deferentia ciliated (or only posterior ciliated), both anterior and posterior pairs long and winding in X, following the atria posteriorly within sperm sacs; diameter of anterior pair 18–24 µm, posterior (functional) pair 22–26 µm. Posterior vasa deferentia penetrate the posterior septum, forming a large loop in XI; they approach and apparently join the atrial ampulla near the midpoint.

Atrial duct very long and narrow, total length 610–830 µm, extending back into XI or XII. About the ectal 200 µm of the duct is oriented vertically, relatively straight and tubular, diameter 40–50 µm (Fig. 5A,G); entally, diameter varies from about 50 µm down to about 20 µm, narrowest near junction with ampulla. Near the male pore, the atrial duct has elongated lining cells, which may be extruded as a type 2 penis (Fig. 5F,G); the remainder of duct with thick epithelium and a diffuse muscle layer; internally, the duct may be lined with a (cuticular?) tubule, which may be straight or somewhat folded (Fig. 5H). Ental end of the atrial duct expands to form the well-defined atrial ampulla.

Atrial ampulla usually extending back into XI or XII; elongate (length 230–330 µm, diameter 90–130 µm). Ampullar epithelium thick (to 25 µm), surrounded by a dense muscle layer 10–12 µm thick. Prostate glands 10 or more multicellular bundles 50–80 µm high.

Habitat: The type locality is a small stream in Akita Prefecture, Honshu. Water depth was less than 20 cm and temperature around 20° C in summer, or 2° C in winter. The substrate consists of gravels and sand, covered with diatoms and the cyanobacteria *Homoeothrix janthina*. Nemouridae and Chironominae dominated the bottom fauna, and the naidine oligochaete, *Piguetiella denticulata* Liang & Xie, 1997 has also been recorded.

Remarks: Location of spermathecae may be variable. Of the 8 specimens, one had spermathecae paired in VIII and single in VI; another had paired spermathecae in both VIII and IX; the remaining 6 had one pair in VIII only (cf. Fig. 5A). The very large spermathecal vestibule is unusual within the Lumbriculidae, and the apparently folded lining suggests that it can be expanded or even everted.

A cuticular lining of the atrial duct has also been described (Fend 2005) in *Eclipidrilus frigidus* Eisen, 1881 and *Eclipidrilus palustris* (Smith, 1900); in both of these species the cuticular layer appears coiled within the duct, but straightens when the penis is extruded. A similar cuticular lining within the atrial duct of *Uktena riparia* Fend et al., 2015 appears to have a different function: spermatophores are formed by the detached lining, and apparently trasferred to the spermatheca of the partner.

Development of the (non-functional) anterior vasa deferentia appears to vary; in most specimens they are distinctly narrower than the posterior vasa, and in some specimens they do not appear to be ciliated. Nevertheless, the anterior male funnels are always smaller than the posterior ones, and lack sperm. The junction of the long and winding vasa deferentia with the atrial ampulla was difficult to see in the available material.

Discussion

Most Japanese lumbriculid descriptions have been the legacy of Yamaguchi (1936a, b; 1937a, b, 1953), and were based largely on collections from Hokkaido. Thus it is not surprising that the fauna of Honshu (and perhaps other Japanese islands) includes undiscovered taxa. The traditional generic diagnoses, outlined by Cook (1971) and based largely on number, relative positions, and morphology of reproductive organs, have been generally upheld by both the morphology-based cladistic analysis of Brinkhurst (1989) and by the more limited molecular analyses to date (e.g., Kaygorodova et al. 2007, Zhou et al. 2010). Cook's morphology-based taxonomy was based largely on well-studied regions, particularly Europe, eastern Russia, and parts of North America. Most subsequently described genera have been from less-studied areas, or from subterranean habitats (Cook 1975, Rodriguez & Giani 1987).

With the exception of *Styloscolex tazawaensis* n. sp., taxonomic affinities of the new species discussed here are not clear, and they cannot confidently be assigned to existing genera defined on the basis of species from other regions. As material is limited, we have attempted a conservative solution, erecting a single genus (*Honshudrilus*) for two rather different species, provisionally assigning *S. tatsukoae* n. sp. to the closest matching taxon *Styloscolex* (*Neoscolex*), and postponing the formal descriptions of two additional species until sufficient material is collected. In addition to eventually clarifying these issues, it seems likely that further sampling will show that the lumbriculid fauna of Japan is not only diverse, but represents undescribed lineages. This may be comparable to the situation in southeastern North America, where several genera have only recently been described, based on recent collections (Fend & Lenat 2007, 2010; Rodriguez et al. 2014; Fend et al. 2015).

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