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First report of the branchiobdellidan *Holtodrilus truncatus* (Annelida: Clitellata) found on the freshwater atyid shrimp *Neocaridina* sp. from Korea

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The branchiobdellidan species *Holtodrilus truncatus* (Liang, 1963) is reported for the first time in Korea. The genus *Holtodrilus* Gelder and Brinkhurst, 1990 is monotypic within the family Xironodrilidae, and the genus and family of this species are also new to the Korean fauna. The branchiobdellidan specimens were collected from the exoskeletons of the freshwater atyid shrimp, *Neocaridina* sp., sampled from the 'Andeok Valley', Jeju-do Province, South Korea. *Holtodrilus truncatus* is characterized by its terete body form, 7/7 dental formula (with a large medial tooth and three pairs of smaller lateral teeth), and no trunk appendages. In addition, we determined a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 (*CO1*) gene as a DNA barcode marker for *H. truncatus*. The present study is the first record of the branchiobdellidan occurrence on a non-crayfish host (shrimp) in Korea. To date, seven species (four genera in three families) of branchiobdellidans have been reported in the Korean fauna.

Keywords: Branchiobdellida, *CO1*, freshwater shrimp, *Holtodrilus truncatus*, Korea, *Neocaridina*

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Introduction

Branchiobdellidans (Annelida, Clitellata) are ectosymbiotic annelids that live primarily on freshwater crayfishes or atyid shrimps in the Holarctic (Gelder and Brinkhurst, 1990; Gelder, 1999; Niwa *et al*., 2005).

To date, branchiobdellidans in South Korea have included six species, three genera, and two families from only one host, the Korean freshwater crayfish, *Cambaroides similis* (Koelbel, 1892): five species *Branchiobdella kobayashii* Yamaguchi, 1934, *B. orientalis* Yamaguchi, 1934, *Cirrodrilus chosen* (Yamaguchi, 1934), *C. kawamurai* (Yamaguchi, 1934), and *C. suzukii* (Yamaguchi, 1934) in the family Branchiobdellidae; and one species, *Hidejiodrilus koreanus* (Pierantoni, 1912), in the family Bdellodrilidae (Yamaguchi, 1934; Gelder and Brinkhurst, 1990; Subchev *et al*., 1991; Lee *et al*., 2009; Gelder, 2010). As mentioned previously, studies on Korean branchiobdellidans have been focused on a crayfish-based association. Hence, a branchiobdellidan-shrimp association in Korea has not been considered or expected.

The genus *Holtodrilus* Gelder and Brinkhurst, 1990 is monotypic within the family Xironodrilidae Holt, 1986, and has the following diagnostic characteristics: (1) body shape is terete; (2) the ejaculatory is present; (3) the penis is eversible; (4) lateral segmental glands in segments VIII and IX are present; and (5) the number of annuli per segment ranges from three to five (Gelder and Brinkhurst, 1990).

In the present study, we reported the occurrence of this branchiobdellidan species, *Holtodrilus truncatus* (Liang, 1963), for the first time in Korea. In addition, we determined a partial sequence of the mitochondrial cytochrome *c* oxidase subunit 1 (*CO1*) gene as a DNA barcode marker for *H. truncatus*.

Materials and Methods

Sample collection and observation

The branchiobdellidan specimens were collected from the exoskeletons of the freshwater atyid shrimp, *Neocaridina* sp., sampled from the 'Andeok Valley', Je-

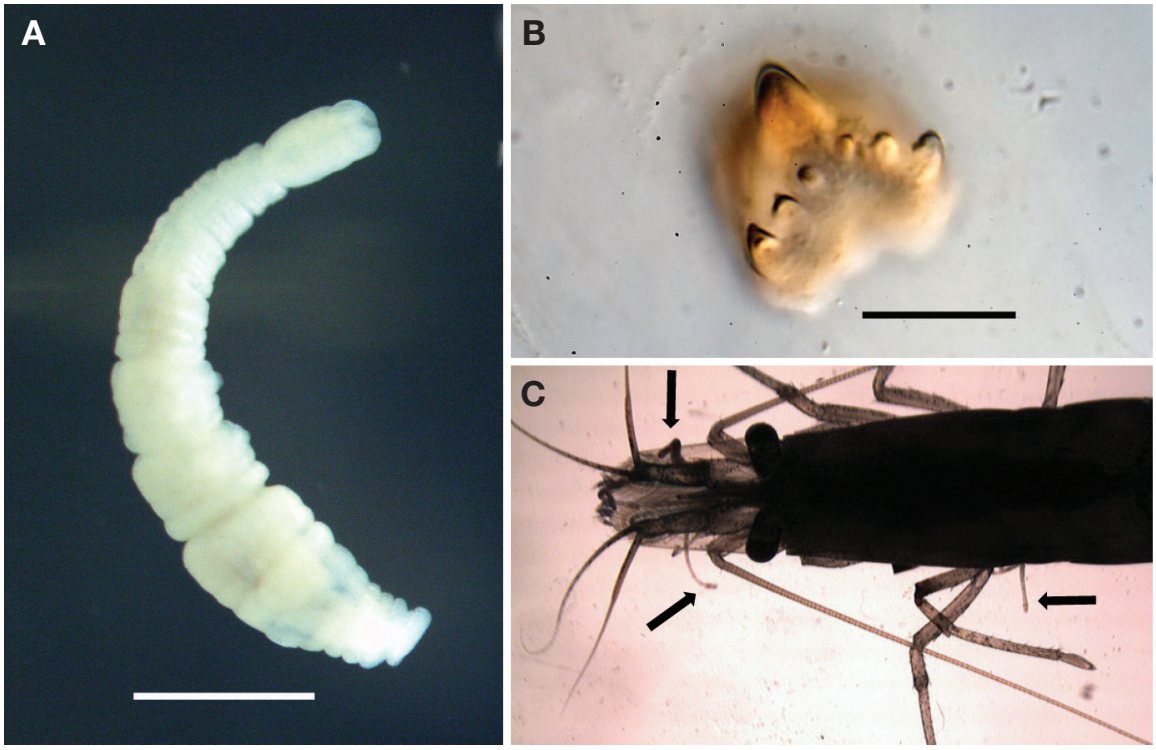

Fig. 1. *Holtodrilus truncatus* (Liang, 1963) from the 'Andeok Valley', Jeju-do Province, South Korea. A. whole body (preserved); B. each jaw with a large median tooth and three pairs of small lateral teeth (3-1-3/3-1-3); C. live specimens of *H. truncatus* (marked with arrows) on the body surface of the freshwater atyid shrimp, *Neocaridina* sp. Scale bars: A = 0.5 mm, B = 20 μm.

ju-do Province, South Korea. The shrimp were captured with a hand-net from the stream, and were preserved directly in 95% ethyl alcohol at the collection site. In the laboratory, the branchiobdellidans were separated from the shrimp under a stereo-microscope (Olympus, SZX12) and were stored at −20°C until DNA extraction. All examined specimens were deposited in the Inha University and the National Institute of Biological Resources (NIBR), South Korea.

DNA sequencing

Genomic DNA was extracted from a whole body specimen using the LaboPass^{TM} Tissue genomic DNA Isolation Kit Mini (Cosmo Genetech Co., Seoul, Korea) according to the manufacturer's instructions. The partial *COI* gene of the mitochondrial DNA was amplified by polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR amplification was conducted under the following conditions: 2 min at 94°C, 45 cycles at 95°C for 20 s, 48°C for 20 s, and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were purified using the LaboPass^{TM} PCR Purification Kit (Cosmo Genetech Co., Seoul, Korea) and were sequenced with an ABI3100 automated sequencer (Perkin Elmer, Foster City, CA, USA).

Systematic Accounts

Class Clitellata Linnaeus, 1740
Order Branchiobdellida Holt, 1965
거머리지렁이목 (신칭)
Family Xironodrilidae Holt, 1986
새우거머리지렁이과 (신칭)
Genus *Holtodrilus* Gelder and Brinkhurst, 1990
새우거머리지렁이속 (신칭)

***Holtodrilus truncatus* (Liang, 1963)**
새우거머리지렁이 (신칭) (Fig. 1)
Stephanodrilus truncatus Liang, 1963: 565-567, fig. 4; Liu, 1984: 353-354.
Holtodrilus truncatus Gelder and Brinkhurst, 1990: 1320-132, fig. 3; Niwa *et al.*, 2005: 685-686, fig. 1; Niwa and Ohtaka, 2006: 182-185, fig. 2; Ohtaka, 2007: 486-487, fig. 1; Ohtaka and Chen, 2010: 100-102, fig. 2; Ohtaka *et al.*, 2012: 1547-1554, fig. 1; Niwa *et al.*, 2014: 80-84, figs. 2-3.

Material examined. Korea, Jeju-do Province, Seogwi-po-si, Andeok-myeon, Gamsan-ri (Andeok Valley) (33°15′ N, 126°21′ E), 23 Jun 2010, D.H. Ahn; 23 Aug 2015, D.H. Ahn; 18 May 2016, D.H. Ahn.
Diagnosis. Body terete, transparent, less than 2 mm long.

Diameter of head about the same as segment II. No peristomial lobes (Fig. 1A). Both jaws similar in shape and size, yellowish, triangular, dental formula 7-7 (with a large median tooth and three pairs of small lateral teeth [3-1-3/3-1-3]) (Fig. 1B). 3-5 annuli per segment. No trunk appendages. Clitellum inconspicuous. Diameter of posterior sucker about the same as head region. Spermatheca located in segment V, not bifurcated. Glandular atrium (spermiducal gland) tubular, bent posteriorly to the short ejaculatory duct. Vasa deferentia linked into the spermiducal gland. No prostate gland. Paired testes located in segment V and VI. Both muscular bursa and cone-shaped penis eversible.

Host. This species was found on the exoskeletons of freshwater shrimp, *Neocaridina* sp. (Decapoda: Caridea: Atyidae).

Habitat. Ectosymbiont, living on the host's exoskeleton (Fig. 1C).

Distribution. Korea (this study), China (Henan, Guangdong, Anhui, and Zhejiang Provinces), Japan (Sugo River, Hyogo Prefecture; Miyakojima Island, Okinawa Prefecture), northwestern Taiwan.

Depository. NIBRIV0000668071, NIBRIV0000668072, NIBRIV0000668073.

Molecular characteristics. The partial sequence of the mitochondrial *CO1* gene for *H. truncatus* was 674 bp long. The sequence was determined and registered for the first time for this genus at the NCBI GenBank database (accession number: KX683299). The sequence did not contain any insertions or deletions. No frame shift was detected during amino acid translation with the invertebrate mitochondrial genetic code.

Remarks. *Holtodrilus truncatus* is the sole species in the genus *Holtodrilus* (family Xironodrilidae). This species was originally described and placed in the genus *Stephanodrilus* by Liang (1963) based on the Chinese shrimp *Neocaridina denticulata sinensis* (Kemp, 1918) in Henan Province, China. Since then, *H. truncatus* was also found on other atyid genera such as *Paratya* (Tanaka *et al*., 2016) and *Caridina* (Fujita *et al*., 2010; Ohtaka and Chen, 2010; Tanaka *et al*., 2016).

This paper reports the first record of *H. truncatus* from South Korea, and the genus and family of this species are also new to the Korean fauna. Moreover, this is the first record of a branchiobdellidan from a non-crayfish host (shrimp), unlike those previously known from crayfish in Korea.

To date, seven species (four genera in three families) of branchiobdellidans have been reported in the Korean fauna. Further studies are needed to determine if the Korean population of *H. truncatus* was native or introduced, and if *H. truncatus* was found from other atyid shrimps in Korea (such as *Paratya compressa*, *Caridina multidentata*, and *C. leucosticta*).

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