

New Record of *Kellicottia bostoniensis* and Redescription of Two Freshwater Rotifers from Korea (Rotifera: Monogononta)

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ABSTRACT

In this study, we identified three monogonont rotifers from South Korea: *Kellicottia bostoniensis* (Rousselet, 1908), *Trichocerca tenuior* (Gosse, 1886), and *Lepadella triptera* (Ehrenberg, 1830). The distribution records of *K. bostoniensis* were mainly located in the Nearctic, Neotropic and Western Palearctic regions. After Japan, this is the second record of it in Asia. *Trichocerca tenuior* and *Lepadella triptera* have already been recorded in Korea, but the data of two species were insufficient in previous study. Here, we describe the morphological characteristics of the three species and the trophi structures of *K. bostoniensis* and *T. tenuior*. This study is the first to characterize the trophi structure of *K. bostoniensis*, observed using a scanning electron microscope. In addition, we have determined the partial cytochrome *c* oxidase subunit 1 (*COI*) and 18S rRNA gene sequences of *T. tenuior* and *L. triptera* for their DNA barcodes.

Keywords: biodiversity, East Asia, monogonont rotifers, SEM, taxonomy

INTRODUCTION

Research on rotifers in Korea was started by Hada (1936), who collected 17 species from Lake Seo-ho in Suwon-si, Gyeonggi-do, South Korea. So far, 305 species have been recorded in Korea (National Institute of Biological Resources and Ministry of Environment, 2020) and of these, 195 are monogonont rotifers. According to the National Species List of Korea (2020), one *Kellicottia* (Cho et al., 1978), 20 *Trichocerca* (Hada, 1936; Yamamoto, 1953; Song, 1989) and 11 *Lepadella* (Hada, 1936; Yamamoto, 1953; Chung et al., 1991; Kim et al., 1991) species have been recorded in South Korea.

In this study, we identified three monogonont rotifers from South Korea, *Kellicottia bostoniensis* (Rousselet, 1908), *Trichocerca tenuior* (Gosse, 1886), and *Lepadella triptera* (Ehrenberg, 1830). *Kellicottia bostoniensis* is indigenous to North America and is distributed in the Nearctic, Neotropic and Western Palearctic region: United States, Canada, Mexico, Brazil, Argentina and several European countries (Rousselet, 1908; Leentvaar, 1961; Arnemo et al., 1968; Eloranta, 1988; Balvay, 1994; De Paggi, 2002; Landa et al., 2002; Nandini et al., 2008; Zhdanova and Dobrynin, 2011). After Japan, this is the second record of it in Asia (Sudzuki and Kawakita,

1999).

The genus *Trichocerca* Lamarck, 1801 is a species-rich taxon in the monogonont rotifers. It comprises 77 species and inhabits various environments such as fresh, brackish, and marine water (Segers, 2007; Jersabek and Leitner, 2013). The morphology of the trophi and the lorica are the main characteristics for taxonomical analysis. The morphological similarity of lorica in *Trichocerca* species is not always correlated with the trophi; that is, some exhibit similar lorica features but different trophi structure, and vice-versa. This peculiarity makes the genus *Trichocerca* a species-rich group (Segers, 2003).

The genus *Lepadella* Bory de St. Vincent, 1826, like the genus *Trichocerca*, is also a species-rich taxon. *Lepadella triptera* is a cosmopolitan species that has been recorded in three East Asian countries: China, Japan, and Mongolia (Yamamoto, 1950, 1952; Zhuge et al., 1998; Jersabek, 2010). Its high median keel is a distinguishing feature. According to Koste and Voigt (1978), the shape of the lorica in dorsal view is rhombus to circular. The *L. triptera* population, collected so far in East Asia, has oval to circular lorica shape.

In Korea, *T. tenuior* and *L. triptera* have already been recorded by Cho (1993). However, in previous study, the mor-

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phological description and the collecting site of two species were not clearly recorded. Here we provide the morphological characteristics and molecular data of Korean population of *T. tenuior* and *L. triptera*.

MATERIALS AND METHODS

Three monogonont rotifers were collected from three different freshwater ponds and reservoirs using a 120 µm plankton net. Collected rotifers were transferred to the laboratory in a living or formalin-preserved state. The living rotifers were isolated and divided in to two subsamples for observing living and preserved specimen. Without narcotization, rotifers were fixed using formalin (final conc. 4%) and were transferred to glycerol (final conc. 100%) for microscopic observation. All specimens were observed using a stereo microscope (SZX12; Olympus, Japan) and an optical microscope (DM2500; Leica, Germany). Preparation of trophi for light and scanning electron microscope (SEM) was done following De Smet (1998) using commercial sodium hypochlorite solution (Yuhanclorox, Korea). Scanning electron microscopy was performed using Hitachi SEM model SU8010 (Hitachi, Japan), at 10–15 kV accelerating voltage. The morphological classification follows Koste and Voigt (1978), Koste and Shiel (1987, 1989), Shiel and Koste (1992), Segers (2007), and Jersabek and Leitner (2013). All specimens were deposited at the Nakdonggang National Institute of Biological Resources, South Korea.

DNA was extracted from the living rotifers using Extract-N-Amp Blood PCR Kit (Sigma, USA) according to the manufacturer's protocol. Two primer sets, LCO1490-HCO2198 (Folmer et al., 1994) and EukA-EukB (Medlin et al., 1988) were used to obtain the partial cytochrome *c* oxidase subunit 1 (*COI*) sequences and the partial 18S rRNA gene sequences, respectively. PCR amplifications for the LCO1490-HCO2198, *COI* sequences was conducted with following conditions: pre-denaturation 4 min at 94°C, followed by denaturation 30 s at 95°C, annealing 45 s at 42°C and extension 60 s at 72°C for 35 cycles; and a final extension at 72°C for 5 min. For the EukA-EukB, 18S rRNA gene sequences, the conditions were: pre-denaturation 5 min at 94°C, followed by denaturation 30 s at 95°C, annealing 60 s at 55°C and extension 240 s at 72°C for 35 cycles; and a final extension at 72°C for 5 min.

RESULTS AND DISCUSSION

Phylum Rotifera Cuvier, 1817

Class Eurotatoria De Ridder, 1957
Subclass Monogononta Plate, 1889
Order Ploima Hudson & Gosse, 1886
Family Brachionidae Ehrenberg, 1838
Genus *Kellicottia* Ahlstrom, 1938

¹*1. *Kellicottia bostoniensis* (Rousselet, 1908) (Fig. 1)

Material examined. South Korea: Gangwon-do, Gangneung-si, Jukheon-dong, Reservoir, 37°46'55.49"N, 128°51'54.01"E, 22 Jan 2020.

Diagnosis. Oval-shape body with four anterior spines and one caudal spine. Left and right anterior spine of almost same length. Right-middle spine about twice longer than lateral ones. Longest anterior spine occupying 31–42% of total length. Surfaces of all four anterior spines denticulated. Ventral plate slightly narrower than dorsal one. Anterior margin of ventral plate concaved. Trophi malleate type. Fulcrum short and laterally flattened. One small process on middle of manubrium shaft. Serrated teeth on inner margin of rami. Two lateral lamellae on distal end of rami. Eight teeth on each uncus; it goes smaller from the ventral side to the dorsal side.

Measurements. Total length 300–385 µm. Left-lateral spine 17.5–25 µm. Right-lateral spine 15–25 µm. Left-middle spine 105–155 µm. Right-middle spine 35–40 µm. Caudal spine 80–135 µm. Ramus 10.4 µm. Manubrium 14.4 µm. Uncus 6.2 µm. Fulcrum 4.3 µm (n = 10).

Remarks. The genus *Kellicottia* Ahlstrom, 1938 comprises two species, *K. bostoniensis* (Rousselet, 1908) and *K. longispina* (Kellicott, 1879). *Kellicottia longispina* has a cosmopolitan distribution and is larger than *K. bostoniensis*. The most distinguishing feature of the two species is that *K. longispina* has six anterior spines, whereas, *K. bostoniensis* has four. In South Korea, Cho et al. recorded *Kellicottia longispina* in 1978. *Kellicottia bostoniensis* had so far only been recorded in the Nearctic, Neotropic and Western Palearctic regions, except for the Japan record in 1999 (Sudzuki and Kawakita, 1999). To our knowledge, there are no records of *K. bostoniensis* in the Afrotropical, Antarctic, Australasia, and Indomalaya regions. After the record of Japan, this is the second record of *K. bostoniensis* in Eastern Palearctic region.

In this study, we used SEM for trophi observation. The trophi structure of *K. bostoniensis* was first described by De Paggi (2002), using the light microscope. In the Korean population, the structure of fulcrum and ramus is almost identical to that of the Argentina population, but there are some differences in the uncus and manubrium. The uncus of the Korean population has eight teeth, unlike that of the Ar-

Korean name: ¹*보스턴켈리코트윤충(신칭)

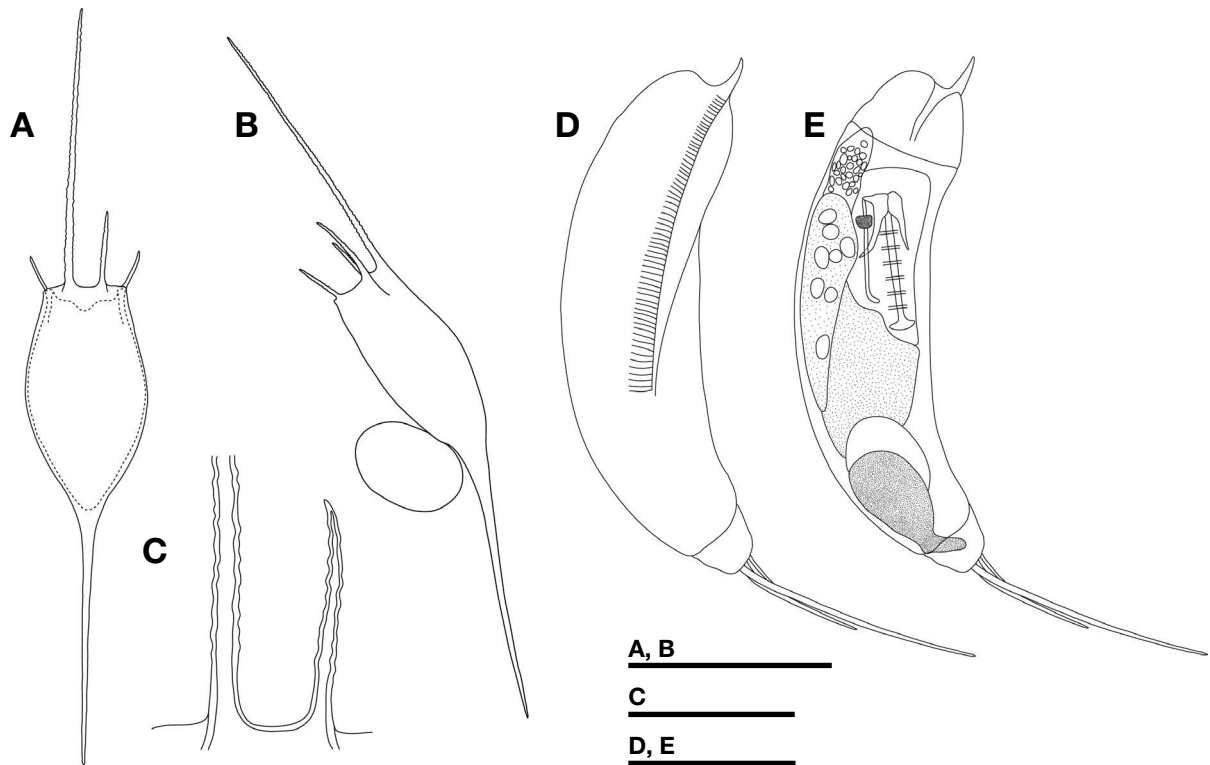


Fig. 1. A-C, *Kellcottia bostoniensis*. A, Dorsal view; B, Lateral view; C, Denticulated middle spines, lower part. D, E, *Trichocerca tenuior*. D, Lateral view; E, Lateral view with inner organs. Scale bars: A, B=100 µm, C=25 µm, D, E=50 µm.

gentina population, which has seven. We also found a minute process on the shaft of the manubrium (Fig. 2A: mp), as was found with *K. longispina* observed with SEM by Segers et al. (1993).

Distribution. Korea, Argentina, Belarus, Brazil, Canada, Finland, France, Germany, Japan, Mexico, Netherlands, Russia, Sweden, United States.

Deposition. NNIBRIV37331.

Family Trichocercidae Haring, 1913

Genus *Trichocerca* Lamarck, 1801

¹***2. *Trichocerca tenuior* (Gosse, 1886) (Fig. 2)**

Material examined. South Korea: Incheon, Michuholgu, 100 Inha-ro, Freshwater pond, 37°27'0.14"N, 126°39'22.50"E, 5 Apr 2019.

Diagnosis. Body curved and elongated cylindrical. Head part divided from body with weak furrow. Head with longitudinal folds. One short spine at the left end of head. Lorica soft and transparent with striated keel. Keel extended from anterior end to 2/3 of lorica length. One eye with red color,

near cerebral organ. Stomach big, orange color, sometimes with several green globules. Vitellarium 8 nuclei. Foot short and stout. Two toes and two to three bristles on the end of foot. Right toe slightly s-shaped, about half length of left toe. Trophi asymmetry, left manubrium (lm) and left uncus (lu) well developed than right manubrium (rm) and right uncus (ru). lm about twice longer than rm and curved end. Unci with a broad main tooth and three to four round teeth. Fulcrum thick, straight, with anchor-shaped extension at the end.

Measurements. Total length 138–158 µm. anterior spine 12.5–22.5 µm. Left toe 67.5–80 µm. Right toe 35–42.5 µm. Trophi 42.5–50 µm (n = 10).

Remarks. *Trichocerca tenuior* is cosmopolitan and has been collected from various habitat such as detritus, algal mats, periphyton, psammon, still waters, and moor pools (Shiel and Koste, 1992). The Korean population was collected from an artificial pond using plankton net. In the annual sampling of the pond, *T. tenuior* was collected only in winter, at a water temperature of 8–10°C. The morphology of *T. tenuior* is similar to that of *T. insulana*, *T. tigris*, and *T. intermedia* (Shiel and Koste, 1992). Unlike *T. tenuior*, the body of the three

Korean name: ¹*날씬쥐윤충(신칭)

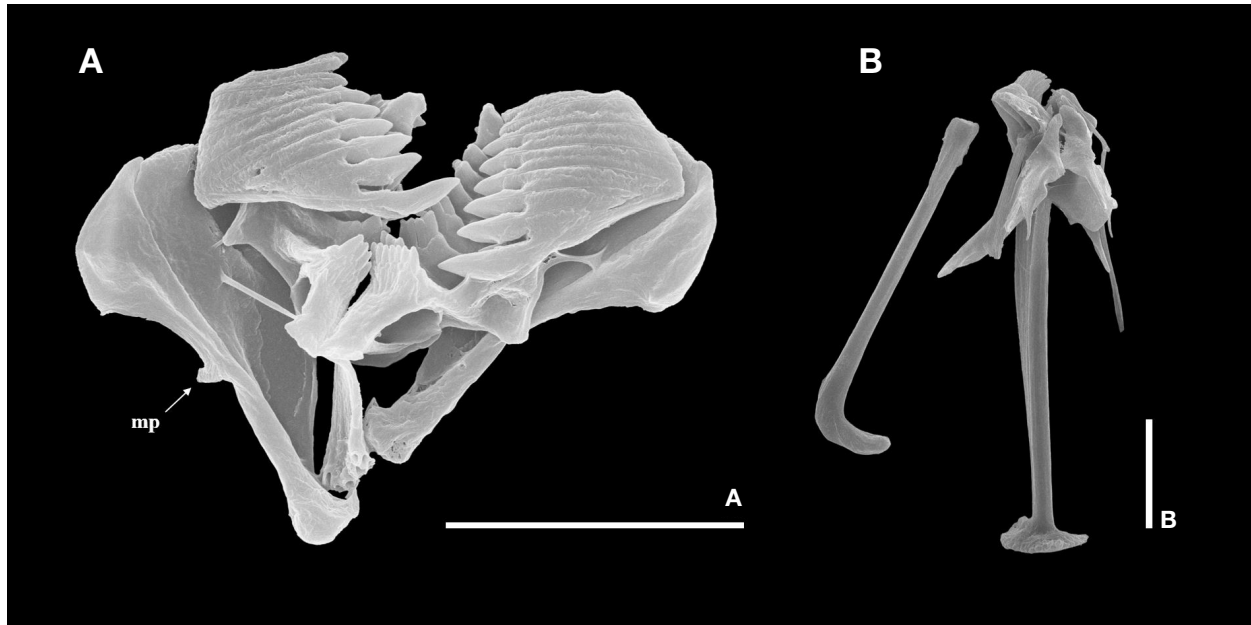


Fig. 2. A, *Kellicottia bostoniensis* (trophi); B, *Trichocerca tenuior* (trophi). Scale bars: A, B = 10 μ m.

other species is noticeably narrow in the low third. The trophi structure of *T. intermedia* and *T. tigris* is somewhat different in that the left manubrium has a double crutch at the terminal end. The terminal end of the left manubrium of *T. tenuior* and *T. insulana* is only slightly bent. *Trichocerca insulana* has a trophi structure similar to *T. tenuior* but is distinguished from *T. tenuior* by having two toes of almost the same length (Koste and Voigt, 1978).

Distribution. Cosmopolitan.

Deposition. NNIBRIV35123.

Molecular data. Partial *COI* sequences (660 bp) and partial 18S rRNA gene sequences (1,691 bp) were obtained from three specimens (GenBank accession Nos., *COI*: MT551601, MT551602, MT551603; 18S rDNA: MT549299, MT549300, MT549301). We conducted pairwise alignment with Geneious 8.1.9 (Biomatters, New Zealand) using the *COI* and 18S rRNA gene sequences of *T. tenuior* on the NCBI (GenBank accession Nos., *COI*: DQ297795; 18S rDNA: DQ297723). The sequence length of the alignment was 660 bp for *COI*, and 1,608 bp for 18S rDNA. The identity between the Korean population and NCBI data was 76.2% for *COI*, and 99.8% for 18S rDNA.

Family Lepadellidae Haring, 1913

Genus *Lepadella* Bory de St. Vincent, 1826

¹*3. *Lepadella triptera* (Ehrenberg, 1830) (Fig. 3)

Korean name: ¹*혹등안갑윤충 (신칭)

Material examined. South Korea: Chungcheongbuk-do, Okcheon-gun, Gunbuk-meon, Jio-ri, Freshwater pond, 36°19'49.79"N, 127°34'3.16"E, 31 May 2019.

Diagnosis. Outline of lorica oval to circular. Anterior margin of lorica slightly concaved. Head opening wide, semi-elliptical shape. Two lateral eyes on the head. Body transparent and finely textured surface. Stomach and mastax large. Other inner organs difficult to recognize because of opaque color. One large keel in the middle of the dorsal plate. Keel extended from anterior end to posterior end. In cross section, height of dorsal plate gradually increases from outside to middle half, and rapidly increases from middle half to middle. Two lateral antennae located 2/3 of dorsal plate. Ventral plate almost flat, slightly convex in middle. Foot opening elliptical shape and smoothly tapered to end. Three pseudo-segmented foot, distal one is the longest. Two toes short and pointed.

Measurements. Lorica length 57.5–67.5 μ m. Lorica width 45.0–50 μ m. Toe length 12.5–20 μ m (n = 3).

Remarks. *Lepadella triptera*, a small, transparent species, has a cosmopolitan distribution and lives in various habitat, ranging from fresh to brackish water (Koste and Shiel, 1989). The large keel in the middle of the dorsal plate is a notably unique feature of *L. triptera* and therefore, easily distinguished from other *Lepadella* species. *Lepadella rhomboides* also has a high arch on the dorsal plate, but the shape of its lorica and the head opening are different from those of *L.*

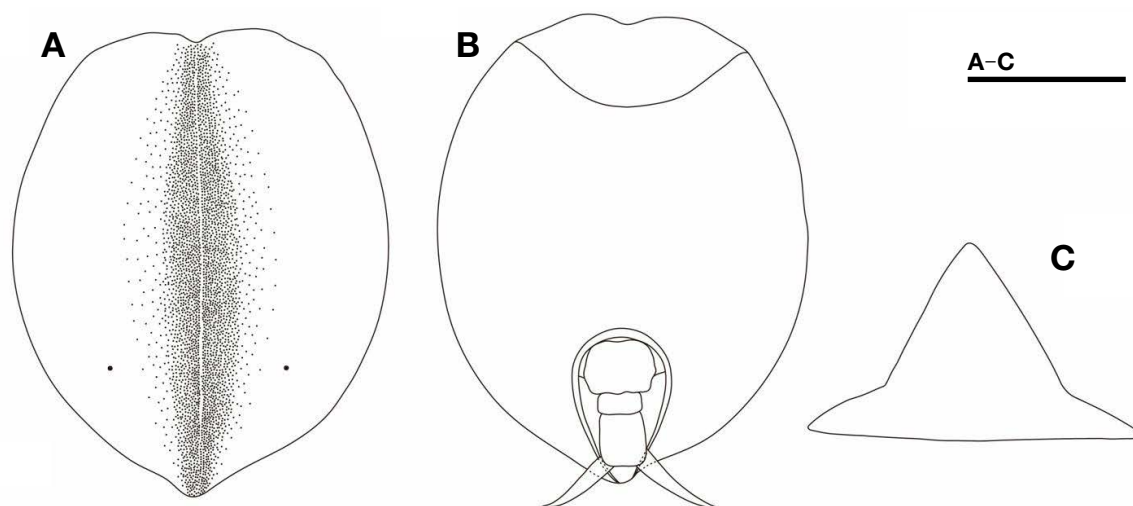


Fig. 3. *Lepadella triptera*. A, Dorsal view; B, Ventral view; C, Cross section (transverse plane). Scale bar: A-C=20 μ m.

triptera. The outline of the lorica is variable, rhombus to circular (Koste and Voigt, 1978). The Korea population collected in this study had only oval to circular shapes.

Distribution. Cosmopolitan.

Deposition. NNIBRIV35125.

Molecular data. Partial *COI* sequence (660 bp) and partial 18S rRNA gene sequence (1,689 bp) were obtained from one specimen (GenBank accession number, *COI*: MT551600, 18S rDNA: MT549298). To our knowledge, this is the first genomic data of *Lepadella triptera*.

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CONFLICTS OF INTEREST

Gi-Sik Min, a contributing editor of the Animal Systematics, Evolution and Diversity, was not involved in the editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

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