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Four bdelloid rotifers new to Korea

Min Ok Song* and Chang-Ho Lee

Department of Biology, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea

**Correspondent: rotisong@gmail.com, minsong@gwnu.ac.kr*

We conducted a taxonomic study of bdelloid rotifers found from terrestrial habitats such as mosses, leaf litter and soil at four different locations in Korea. The study resulted in four new Korean records belonging to two genera in two families, Habrotrochidae and Philodinidae, of bdelloid rotifers: *Habrotrocha soror* Donner, 1950; *H. thienemanni rubella* Donner, 1951; *H. valida* Milne, 1916 and *Philodina nitida nitida* Milne, 1916. These new Korean records are rare and have very limited distributions. *Habrotrocha thienemanni rubella* and *H. valida* were not found again after their original descriptions until the present study. The present study is the third record for both *H. soror* and *P. nitida nitida*. All these four new Korean records are new to Asian fauna as well. Here, diagnostic characteristics and photomicrographs are provided for each new Korean record. In addition, a partial sequence of mitochondrial cytochrome c oxidase subunit 1 gene (mtCOX1) for *P. nitida nitida* is presented.

Keywords: Bdelloidea, Korea, new records, taxonomy, terrestrial habitats

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Introduction

In the genus *Habrotrocha* Bryce, 1910, about 131 taxa are known to the world (Jersabek and Leitner, 2013; Iakovenko *et al*., 2015). In Korea, 41 *Habrotrocha* taxa have been reported (Song, 2015; 2018; Song and Min, 2015). The result of the present study added three species to the Korean record, for a total of 44 taxa in *Habrotrocha*.

In the genus *Philodina* Ehrenberg, 1830, 56 species- and eight subspecies-level taxa have been described and accepted as valid. In Korea, 27 *Philodina* taxa have been reported, including nine new species (Jersabek and Leitner, 2013; Song and Min, 2015; Song and Lee, 2017; 2019; 2020a; 2020b; 2022). The present study brought the Korean record to 27 *Philodina* taxa.

Among the four new Korean records, *H. thienemanni rubella* and *H. valida* were not found again after their original descriptions from Austria and South Africa, respectively, before the present study. The Korean specimens of *H. thienemanni rubella* were found from mosses and soil, and that of *H. valida* from leaf litter and soil. Many bdelloids inhabit the interstitial water of soils, where they play an important role in nutrient cycling (Schöll and Devetter, 2013). The abundance of rotifers in soils are highly variable and affected by environmental factors such as soil moisture, pH, nutrient availability, altitude and soil organic matter. The number of rotifers in soil ranges from 0−2.7 to 4.9−607.9 individuals per 100-g of dry soil (Smykla *et al*., 2010). These rotifers are hydrobionts of semiaquatic habitats and are able to undergo anhydrobiosis to withstand drought (Ricci, 2001; Devetter, 2007).

Here diagnostic characteristics and photomicrographs are presented for each new Korean record. Partial sequence of mitochondrial cytochrome c oxidase subunit 1 gene (mtCOX1) for *P. nitida nitida* is presented as well.

Materials and Methods

The samples were collected from mosses, leaf litter and soil at four different locations in Korea, from June 3 to Oct. 4, 2021. The identified species/subspecies-level taxa as well as detailed habitat information and sampling dates for each locality are listed in Table 1.

The bdelloids were extracted from samples according to a previously described method (Song, 2014). The living rotifers were examined and identified under a light microscope with a magnification of ×400 to ×600. The photography and motion records of living specimens were performed using an Infinity 2 digital camera (Lumenera Corporation, ON, Canada). Measurements were made using GIMP 2.8 (the GNU Image Manipulation Program). The specimens were killed with head, foot, and toes extended, by using the boiling water fixation method (Edmondson,

Table 1. List of species and sampling localities.

Species	Habitat	Locality	Previous world records
**Habrotrocha soror* Donner, 1950	Mosses	2	Austria (Donner, 1950), Brazil (Donner, 1980)
**Habrotrocha thienemanni rubella* Donner, 1951	Mosses and soil	3	Austria (Donner, 1951)
**Habrotrocha valida* Milne, 1916	Leaf litter and soil	1	South Africa (Milne, 1916)
**Philodina nitida nitida* Milne, 1916	Mosses	4	South Africa (Milne, 1916), New Zealand (Haigh, 1965)

* New to Asia.
1 = Gyeongpo Wetland, Unjeong-dong, Gangneung-si, Gangwon-do, 37°47′32.0″N / 128°54′14.2″E, 3 June 2021;
2 = Seorim-ri, Seo-myeon, Yangyang-gun, Gangwon-do, 37°57′35.8″N / 128°31′0.8″E, 27 June 2021;
3 = Cheongsong Eoreumgol Valley, Budong-myeon, Cheongsong-gun, Gyeongsangbuk-do, 36°18′57.3″N / 129°13′23.8″E, 18 Sep. 2021;
4 = Banjeong, Daegwallyeong, Hoenggye-ri, Daegwalnyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′44.7″N / 128°45′49.6″E, 4 Oct. 2021.

1959) instead of narcotization as previously described (Song and Min, 2015). For the preparation of permanent mounts, we used the method of Stemberger (1979). The classification scheme is based on those of Melone and Ricci (1995) as well as Segers (2002). Iakovenko *et al*. (2013) is partially referred to for the scheme of measurements.

DNA was extracted from one specimen of *Philodina nitida nitida* using Extract-N-Amp^{TM} Tissue PCR Kit (Sigma-Aldrich, MO, USA) following the manufacturer's instructions. Partial mitochondrial cytochrome c oxidase subunit I gene (mtCOX1) was amplified following the procedures described in Iakovenko *et al*. (2013) with some modifications (Song and Lee, 2022). Sequencing was outsourced to Macrogen (Seoul, Korea) using universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994). The quality of the sequence was checked by eye using FinchTV1.4.0 (http://www.geospiza.com/finchtv). The sequence alignment was done by ClustalW program implemented in MEGAX (https://www.megasoftware.net/).

Results and Discussion

Phylum Rotifera Cuvier, 1817 윤형동물문
Class Eurotatoria De Ridder, 1957 진윤충강
Subclass Bdelloidea Hudson, 1884 질형아강
Order Philodinida Melone and Ricci, 1995 선윤충목
Family Habrotrochidae Bryce, 1910 협관윤충과
Genus *Habrotrocha* Bryce, 1910 협관윤충속

1. *Habrotrocha soror* Donner, 1950
큰샘협관윤충 (신칭)

Habrotrocha soror Donner, 1950, pp. 326–327, figs. 19a–d; Donner, 1980, p. 132, fig. 2a.

Material examined. One specimen found from mosses at Seorim-ri, Seo-myeon, Yangyang-gun, Gangwon-do, 37°57′35.8″N, 128°31′0.8″E, on 27 June 2021.

Diagnosis. Rostrum long; twice as long as its width. Pharyngeal tube very long and wavy; one and half as long as trophi length. Gastric glands big, very long and filled with coarse granules. Preanal pseudosegment constricted posteriorly. Anal pseudosegment plump anteriorly and tapering gradually to foot. Foot short. Spurs short, conical and with wide interspace; interspace one and half times as wide as spur base.

Measurements. Total length in creeping 211–215 μm. Greatest trunk width in creeping 26–28 μm. Greatest rump width in creeping 24–26 μm. Greatest neck width in creeping 26–28 μm. Spur length 5 μm.

Remarks. One of the significant diagnostic characteristics of *H. soror* is the big and long gastric glands, which are filled with coarse granules. In bdelloids, gastric glands are usually round, and much smaller than mastax. In *H. soror*, the gastric glands are long, narrow sac-shaped, and much longer than trophi length.

This species has been reported from Brazil (Donner, 1980) after the original description from Austria by Donner (1950). The present study is the third report of this species globally, and the first one from Asia.

World distribution. Austria, Brazil and Korea.

Deposition. Deposited in the collection of the Nakdonggang National Institute of Biological Resources, Sangju-si, Gyeongsangbuk-do, Korea (NNIBR2021523IV8274).

2. *Habrotrocha thienemanni rubella* Donner, 1951
붉은깃고대협관윤충 (신칭)

Habrotrocha thienemanni var. *rubella* Donner, 1951, pp. 616–618, fig. 3.

Material examined. Two specimens from mosses and soil at Budong-myeon, Cheongsong-gun, Gyeongsangbuk-do, 36°18′57.3″N, 129°13′23.8″E, on 18 Sep. 2021.

Diagnosis. Rostral lamella short and round. Rostrum thick and short. Antenna pseudosegment round laterally. Pharyngeal tube slightly longer than trophi length. Corona much narrower than cingulum. Sulcus very narrow. Pedicel high. Upper lip two layered; upper layer lower than trochal discs, thin and triangle-shaped; much narrower than lower

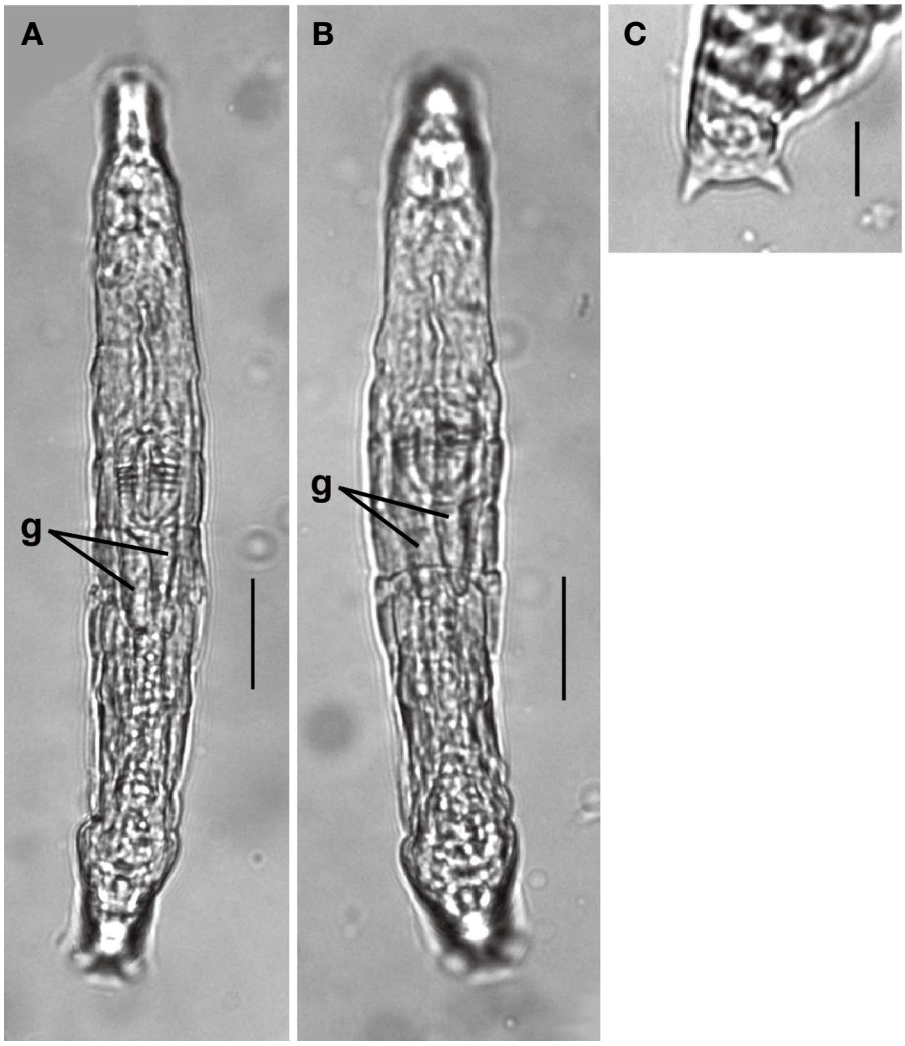

Fig. 1. *Habrotrocha soror* Donner, 1950. A, B. creeping, dorsal view; C. foot and spurs, dorsal view (Scales: A, B = 25 μm; C = 10 μm). g, gastric gland.

layer; lower layer arched, narrow and much lower than upper layer; cingulum rimmed by lower layer. Trunk, rump and foot granulated. Spurs conical and with very narrow interspace.

Measurements. Total length in creeping 179–190 μm. Total length in feeding 146–157 μm. Greatest trunk width in creeping 24–28 μm. Greatest trunk width in feeding 42 μm. Greatest rump width in creeping 20–22 μm. Corona width 13–14 μm. Cingulum width 21 μm. Cingulum pad width 20 μm.

Remarks. *Habrotrocha thienemanni rubella* is distinguished from *H. t. thienemanni* Hauer, 1924 by the following characteristics: (1) corona is slightly wider than cingulum in *H. t. thienemanni*, while it is much narrower than cingulum in *H. t. rubella* and (2) the pedicel of *H. t. rubella* is higher than that of *H. t. thienemanni*. Donner (1951) described that the body color of *H. t. rubella* was always reddish brown. However, the body color of the Korean specimens was pale instead of reddish brown.

This subspecies-level taxon was not found again after the original description from Austria by Donner (1951). Therefore, the present study is the second report of this subspecies. New to Asia as well.

World distribution. Austria and Korea.

Deposition. Deposited in the collection of the Nakdonggang National Institute of Biological Resources, Sangju-si, Gyeongsangbuk-do, Korea (NNIBR2021523IV8275).

3. *Habrotrocha valida* Milne, 1916 건장협관윤충 (신칭)

Habrotrocha valida Milne, 1916, pp. 160–161, pl. 13, figs. 16–16a.

Material examined. One specimen found from leaf litter and soil at Gyeongpo Wetland, Unjeong-dong, Gangneung-

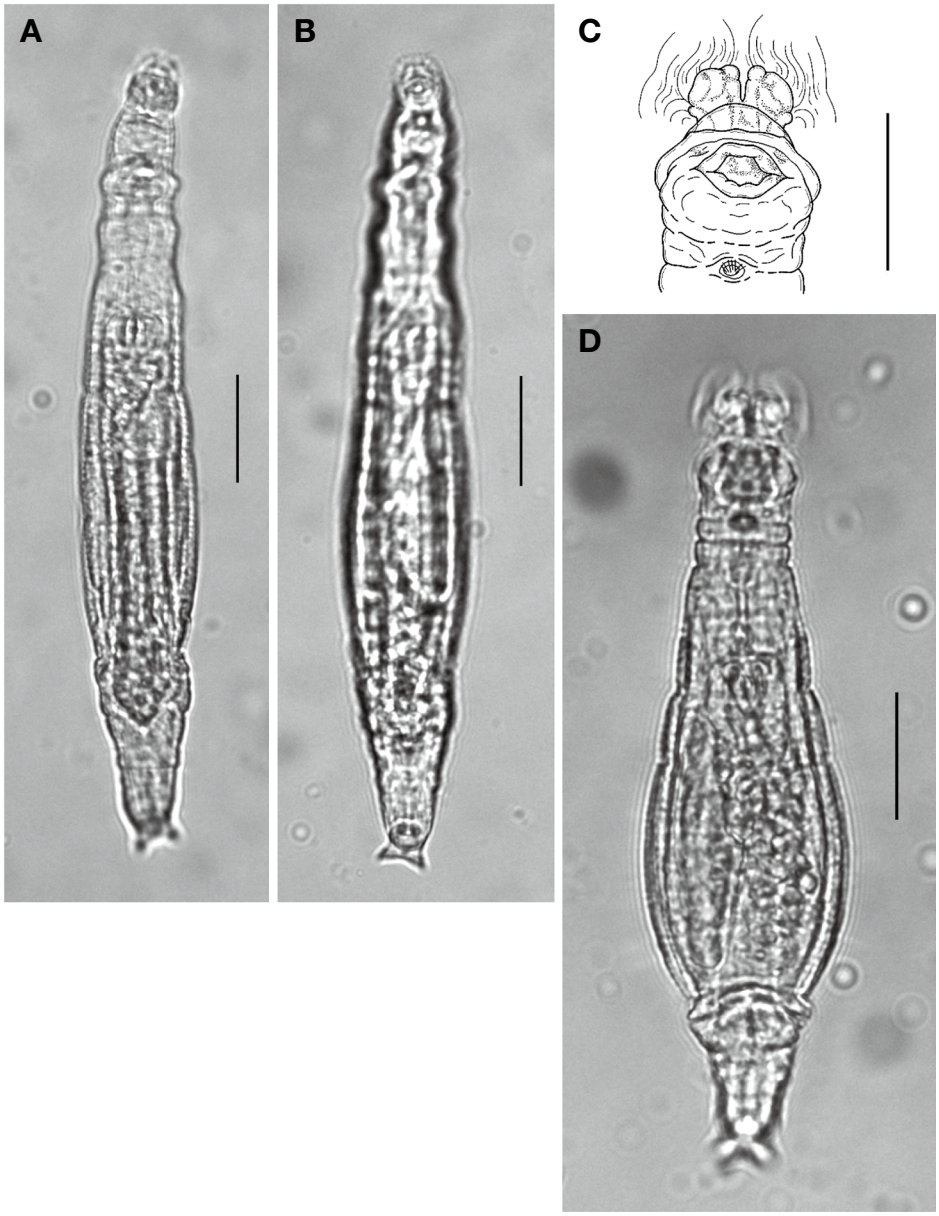

Fig. 2. *Habrotrocha thienemanni rubella* Donner, 1951. A, B. creeping, dorsal view; C. feeding head and neck, dorsal view; D. feeding, dorsal view (Scales: A, B, D = 25 μm; C = 20 μm).

si, Gangwon-do, 37°47′32.0″N, 128°54′14.2″E, on 3 June 2021.

Diagnosis. Rostrum short. Rostral lamella very short and without median notch. Corona slightly narrower than cingulum. Sulcus very narrow; trochal discs and pedicels almost attached. Pedicel very short. Upper lip arched; median lobe truncated and slightly wavy; lower than trochal discs. Antenna short; about 1/4 of neck width. Teeth 6/6. Foot with 4 pseudosegments; much narrower than rump. Spurs conical and with narrow interspace; interspace much narrower than spur base width.

Measurements. Total length in creeping 264 μm. Total length in feeding 185 μm. Corona width 28 μm. Cingulum width 25 μm. Cingulum pad width 31 μm. Greatest trunk width in feeding 71 μm. Spur length 7.5 μm.

Remarks. The characteristics of the Korean specimen agree well with the original description except the length of the spurs. The spurs were as long as the spur base width in the original description, while that of the Korean specimen was slightly longer than the spur base width.

Milne (1916) mentioned that there was a band or ridge which was parallel to the anterior margin of the upper lip. The same structure was observed in the Korean specimen, and it turned out to be the disc retractor, even though Milne

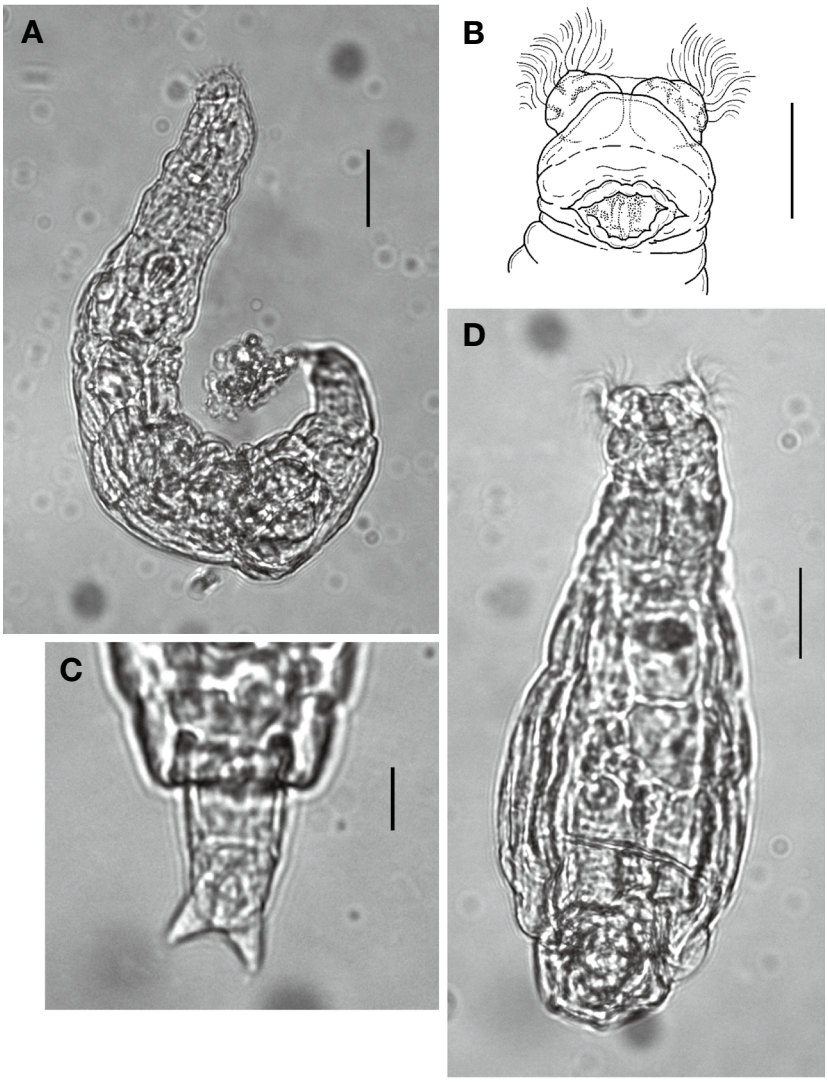

Fig. 3. *Habrotrocha valida* Milne, 1916. A. creeping, dorsal view; B. feeding head and neck, dorsal view; C. rump, foot and spurs, dorsal view; D. feeding, dorsal view (Scales: A, D = 25 μm; B = 20 μm; C = 10 μm).

(1916) thought it was a part of upper lip.

This species was not found again after the original description from South Africa in 1916. The present study is the second report of this species.

World distribution. South Africa and Korea.

Deposition. Deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (VSJAIV 0000000398).

Family Philodinidae Ehrenberg, 1838 선윤충과
Genus *Philodina* Ehrenberg, 1830 선윤충속

4. *Philodina nitida nitida* Milne, 1916 광채선윤충 (신칭)
Philodina nitida Milne, 1916, pp. 67–68, pl. 5, figs. 9–9a.

Material examined. Three specimens from mosses at Banjeong, Daegwallyeong, Hoenggye-ri, Daegwalnyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′44.7″N, 128°45′49.6″E, on 4 Oct. 2021.

Diagnosis. Rostrum much narrower than head in creeping. Upper lip arched, bilobed medially, and lower than trochal discs. Corona much wider than cingulum. Sulcus as wide as pedicel width. Cingulum pad slightly expanded anterolaterally. Two small eye spots. Pharyngeal tube slightly shorter than trophi length. Teeth 3 + 1/1 + 3. Last trunk pseudosegment round squarish posteriorly. Anal pseudosegment much narrower than a half of the greatest trunk width in creeping. Foot with four pseudosegments. Spurs long, thin and divergent; interspace slightly wider than spur base width.

Measurements. Total length in creeping 202 μm. Total length in feeding 167 μm. Greatest trunk width in creep-

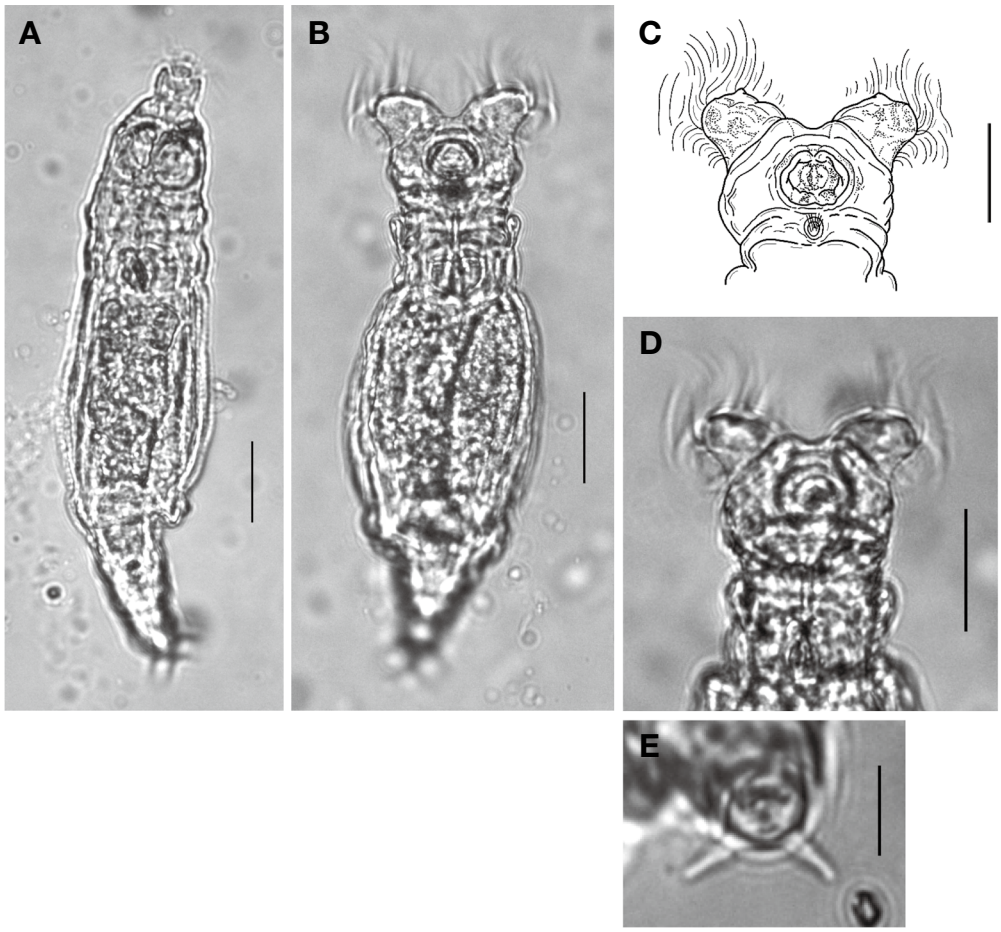

Fig. 4. *Philodina nitida nitida* Milne, 1916. A. creeping, dorsal view; B. feeding, dorsal view; C. feeding head, dorsal view; D. feeding head and neck, dorsal view; E. foot and spurs, ventral view (Scales: A, B, D = 25 μm; C = 20 μm; E = 10 μm).

ing 52 μm. Corona width 43–44 μm. Cingulum width 35 μm. Cingulum pad width 36–38 μm. Spur length 8 μm.

Remarks. Milne described this subspecies-level taxon, *Philodina nitida nitida*, with a variety, *P. n.* var. *decens* (Milne, 1916). The major difference between *P. n. nitida* and *P. n. decens* is in the morphology of feeding head. The ratio of corona width to cingulum width is 1.25 : 1 in *P. n. nitida*, while it is 1.5 : 1 in *P. n. decens*. In addition, the median notch on upper lip is narrow in *P. n. nitida*, while it is wide in *P. n. decens*. Haigh (1965) reported *P. nitida* from New Zealand. In the illustration of the feeding head, the median notch was very wide, which implied that the New Zealand specimens were *P. n. decens*. Therefore, the present study rediscovered *P. n. nitida* 105 years after the original description from South Africa.

Partial mtCOX1 was amplified using DNA extracted from one specimen of *P. nitida nitida*. One sequence (672 bp in length) was obtained and submitted to GenBank under accession number OP644282.

World distribution. South Africa, Korea.

Deposition. Deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (DSEVIV 0000003943).

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