Philodina wonkimi n. sp. and Five New Records of Bdelloids from Korea

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ABSTRACT

A taxonomic study on bdelloid rotifers collected from mosses and/or leaf litter at four different locations in Korea resulted in five new Korean records and a new species, *Philodina wonkimi* n. sp. *Philodina wonkimi* n. sp. is easily distinguished from its congeners by the very long antenna which is much longer than the height of the pseudosegment carrying a dorsal antenna in creeping. Among the five new Korean records, two species- and two subspecies-level taxa are new to Asia as well. *Adineta rhomboidea* Bērzinš, 1950 has been reported from only three European countries including the type locality, and is recorded outside Europe for the first time. Present study is the fourth record for *Philodina eurystephana* Schulte, 1954. In addition, a partial sequence of mitochondrial cytochrome *c* oxidase subunit I gene (mtCOX1) for *P. wonkimi* n. sp. is provided here.

Keywords: Bdelloidea, new species, Adineta, Philodina, Rotifera, taxonomy

INTRODUCTION

In the genus Philodina Ehrenberg, 1830, 53 species- and eight subspecies-level taxa have been described and accepted as valid species in the world to date. In Korea, 19 species- and two subspecies-level taxa have previously been reported including six descriptions of new species (Jersabek and Leitner, 2013; Song and Min, 2015; Song and Lee, 2017, 2019, 2020). Philodina rotifers are oviparous, and have well-developed corona with paired trochal discs, mostly short rostrum, and four toes (Donner, 1965). The species of three other genera in philodinid rotifers, Dissotrocha Bryce, 1910, Embata Bryce, 1910, and Pleuretra Bryce, 1910, have well-developed corona and four toes as well; however, species of these three genera have unique diagnostic characteristics not shared by each other as well as distinguishable from those of Philodina species: Dissotrocha rotifers are viviparous and with long rostrum, Embata species have very wide corona, long foot and big spurs, and Pleuretra rotifers have many transverse striae on trunk ventrally.

The present study on Korean bdelloids from terrestrial habitats such as mosses and/or leaf litter yielded a new species, *Philodina wonkimi* n. sp. This new species is the only *Philodina* species with the very long dorsal antenna which is much longer than the height in creeping as well as the width in feeding of the pseudosegment carrying the antenna. There are four *Philodina* species which have a long antenna: *P. americana* Murray, 1913, *P. dobrogensis* Rudescu, 1960, *P. megalotrocha* Ehrenberg, 1832, and *P. squamosa* Murray, 1906. However, the length of the antenna of these four *Philodina* species is slightly shorter than or as long as the width of the pseudosegment carrying the antenna in feeding. Assuredly, there are significant differences in characteristics between *P. wonkimi* n. sp. and these four congeners, which are discussed in detail at the differential diagnosis for this new species in Results section.

Among the five new Korean records, two species- and two subspecies-level taxa are new to Asia as well: Adineta rhomboidea, Habrotrocha ligula ligula Bryce, 1913, Philodina eurystephana, and P. rugosa rugosa Bryce, 1903. Adineta rhomboidea and Philodina eurystephana are rather rare, and the present study is the fourth record for both of them. The taxonomy and distribution of these two rare species are discussed briefly here.

MATERIALS AND METHODS

The samples were collected from mosses and/or leaf litter

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Table 1. List of species and sampling localities

Species	Habitat	Locality	Previous world records				
Adineta rhomboidea Bērzinš, 1950 ^a	Mosses	4	Poland (6), Sweden (5), Ukraine (8)				
Habrotrocha ampulla Murray, 1911	Mosses	3	Probably cosmopolitan (7, 10, 14)				
Habrotrocha ligula ligula Bryce, 1913 ^a	Mosses and leaf litter	3	Probably cosmopolitan (7, 10, 11)				
<i>Philodina eurystephana</i> Schulte, 1954 ^a	Mosses	2	Germany (13), USA (12), Ukraine (9)				
Philodina rugosa rugosa Bryce, 1903 ^a	Wet leaf litter	1	Cosmopolitan (7, 10)				
Philodina wonkimi n. sp.	Wet leaf litter	1					

1, A stream in Bangnae-ri, Hongcheon-gun, Gangwon-do, 37°48'24.5"N, 128°15'42.4"E, 6 May 2019; 2, Bangnae-ri, Nae-myeon, Hongcheon-gun, Gangwon-do, 37°48'21"N, 128°16'02"E, 6 May 2019; 3, Binggye-ri, Chunsan-myeon, Uiseong-gun, Gyeongsangbuk-do, 36°13'51.4"N, 128°45'22.6"E, 7 Jul 2019; 4, Mugeon-ri, Dogye-eup, Samcheok-si, Gangwon-do, 37°15'55.1"N, 129°06'23.2"E, 29 Sep 2019; 5, Berzinš (1950); 6, Bielańska-Grajner et al. (2011); 7, Donner (1965); 8, Yakovenko (2000); 9, Yakovenko (2003); 10, Jersabek and Leitner (2013); 11, Kaya et al. (2010); 12, Örstan (1998); 13, Schulte (1954); 14, Zhuge et al. (1998).

^aNew to Asia.

at four different locations in Korea, from May 6 to Sep 29, 2019. 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). We examined and identified all of the living rotifers 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). We used the photographs for illustrations. Measurements were made by 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, 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) and Segers (2002). Iakovenko et al. (2013) is partially referred to for the scheme of measurements.

DNA was extracted from one specimen of *P. wonkimi* n. sp. using Chelex InstaGene Matrix (Bio-Rad, Hercules, CA, USA) following the protocol of Iakovenko et al. (2013). Partial mitochondrial cytochrome c oxidase subunit I gene (mt-COX1) was amplified following the procedures described in Iakovenko et al. (2013) with some modifications (Song and Lee, 2020). Sequencing was outsourced to Macrogen (Seoul, Korea) using the bdelloids-specific primers. The quality of the sequence was checked by eye using FinchTV1.4.0 (http://www.geospiza.com/finchtv).

RESULTS AND DISCUSSION

As a result of the present study, a new species, Philodina

wonkimi n. sp. and five species/subspecies-level taxa new to Korea were identified (Table 1). This result increased the number of Korean bdelloids records to 137 taxa, of which 104 at species-level and 33 at subspecies-level (Song and Lee, 2020). The five new Korean records included two rather rare species with limited distribution such as Adineta rhomboidea and Philodina eurystephana. Adineta rhomboidea has been reported from Poland (Bielańska-Grajner et al., 2011) and Ukraine (Yakovenko, 2000) after description from Sweden (Bērzinš, 1950), and is recorded outside Europe for the first time by the present study. Philodina eurystephana has been recorded from Ukraine (Yakovenko, 2003) and USA (Örstan, 1998) after description from Germany (Schulte, 1954). The five new Korean records also included three cosmopolitan or probably cosmopolitan species like Habrotrocha ampulla, H. ligula ligula, and Philodina rugosa rugosa. Notably, H. ligula ligula and P. rugosa rugosa are new to Asia as well even though they have cosmopolitan or probably cosmopolitan distributions.

Partial mtCOX1 was amplified using DNA extracted from one specimen of *Philodina wonkimi* n. sp. One sequence (408 bp in length) was obtained and submitted to GenBank under accession number MT414957. The resultant partial mtCOX1 sequence of *P. wonkimi* n. sp. was compared with a database of Rotifera (taxid: 10190) using BLAST (BlastN, http://blast.ncbi.nlm.nih.gov), and top 10 out of 133 sequences producing significant alignments were summarized in Appendix 1. The taxonomy report of this BLAST analysis showed that all of the organisms found in the BLAST hit list were bdelloids including 39 *Philodina* rotifers (Appendix 2). The sequences most similar (89.88%, E value = 6e-148) to that of *P. wonkimi* n. sp. were from *Philodina flaviceps* isolate Ro3c and Ro2h (GenBank accession numbers: DQ890072.1 and DQ890071.1, respectively).

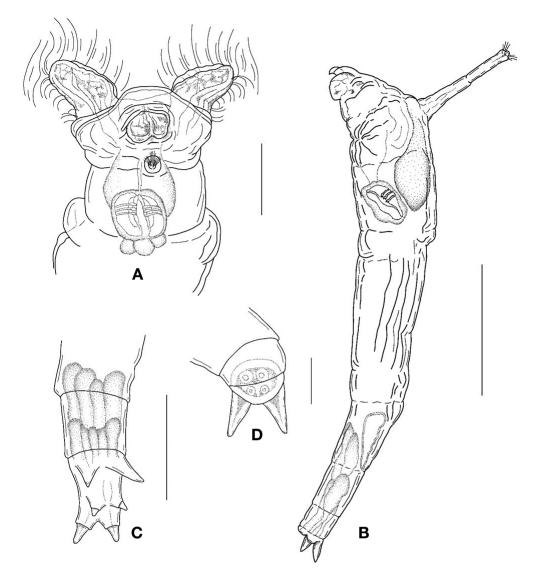


Fig. 1. *Philodina wonkimi* n. sp. A, Feeding head and neck, dorsal view; B, Creeping, ventrolateral view; C, Foot, spurs and toes, dorsal view; D, Foot and spurs, dorsal view. Scale bars: A, $C=25 \mu m$, $B=50 \mu m$, $D=10 \mu m$.

Phylum Rotifera Cuvier, 1817 Class Eurotatoria De Ridder, 1957 Subclass Bdelloidea Hudson, 1884 Order Philodinida Melone and Ricci, 1995 Family Philodinidae Ehrenberg, 1838 Genus *Philodina* Ehrenberg, 1830

^{1*}*Philodina wonkimi* new species (Figs. 1, 2) urn:lsid:zoobank.org:act:3DC9400E-3699-4680-91B7-54EF7B144F40 Holotype and paratypes. In permanent slides, deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (holotype: NIBRIV0000862815; two paratypes: NIBRIV0000862816, and NIBRIV0000862817). Type locality. A stream in Bangnae-ri, Hongcheon-gun, Gangwon-do, Korea. GPS coordinates based on WGS84 datum 37°48'24.5"N, 128°15'42.4"E.

Material examined. Four specimens sorted out from wet leaf litter around a stream in Bangnae-ri, collected by Min Ok Song and Chang-Ho Lee on 6 May 2019.

Differential diagnosis. The most prominent characteristic

Korean name: 1*김원선윤충

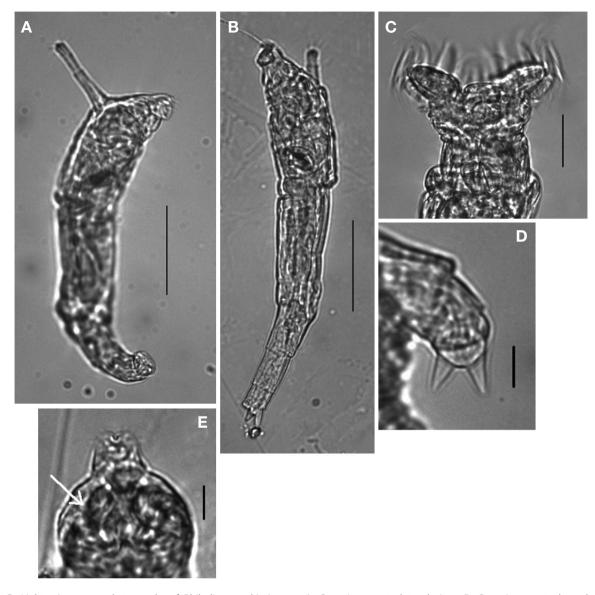


Fig. 2. Light microscopy photographs of *Philodina wonkimi* n. sp. A, Creeping, ventrolateral view; B, Creeping, ventrolateral view; C, Feeding head and neck, dorsal view; D, Foot and spurs, dorsal view; E, Rostrum and head, ventral view (an arrow pointing at the unfolding corona). Scale bars: A, $B=50 \mu m$, $C=25 \mu m$, D, $E=10 \mu m$.

of *Philodina wonkimi* n. sp. is the very long dorsal antenna which is much longer than the height in creeping as well as the width in feeding of the pseudosegment carrying the antenna. Among the *Philodina* species with smooth integument under a light microscope and without eye spots, this new species seems to be the only *Philodina* species with such a long antenna.

Among the *Philodina* species with eye spots, *P. megalotrocha* Ehrenberg might be most similar to *P. wonkimi* n. sp. in the general morphology including the long antenna. However, besides the absence of the eye spots, *P. wonkimi* n. sp. can be distinguished from *P. megalotrocha* by the following characteristics: (1) the feeding trunk of *P. wonkimi* n. sp. is rather slim and not as round as that of *P. megalotrocha*, (2) the rump of *P. megalotrocha* is much narrower than trunk, while the trunk is gradually tapering to rump in *P. wonkimi* n. sp., (3) the neck is much narrower than cingulum in *P. wonkimi* n. sp., while it is almost as wide as the cingulum in *P. megalotrocha*, and (4) the upper lip of *P. wonkimi* n. sp. is much lower than trochal discs, while that of *P. megalotrocha* is slightly higher or as high as trochal discs. Another *Philodina* species with eye spots, *P. squamosa* also has a

long antenna which is as long as the width of a pseudosegment carrying an antenna; however, the trunk of *P. squamosa* is covered with open book-shaped thick and viscous cuticular plates, while that of *P. wonkimi* n. sp. is smooth.

Both Philodina americana and P. dobrogensis are without eve spots and have a long antenna; however, their antennae are not as long as that of P. wonkimi n. sp. In addition to the antenna which is just as long as the width of the pseudosegment carrying the dorsal antenna, P. dobrogensis is different from *P. wonkimi* n. sp. as follows: (1) the upper lip of *P. dobrogensis* is rather flat and truncated medially, while that of *P. wonkimi* n. sp. is arched, (2) the dental formula of P. dobrogensis is 5/5, while that of P. wonkimi n. sp. is 3/3, (3) the spurs of *P. dobrogensis* are without interspace, while those of P. wonkimi n. sp. have interspace which is a half as wide as spur base width, and (4) the toes of P. dobrogensis have equal lengths, while the ventral toes are much longer and thicker than the dorsal ones in P. wonkimi n. sp. Regarding P. americana, the presence of long antenna is the only diagnostic characteristic of P. americana shared with P. wonkimi n. sp., and even the antenna itself is shorter than the height of the pseudosegment carrying the dorsal antenna, while the antenna of P. wonkimi n. sp. is much longer than that. The general morphology of P. americana is quite different from that of P. wonkimi n. sp. For example, P. americana has a big hump on either side of antenna, which is absent in *P. wonkimi* n. sp.

Description. No eye spots. Body with smooth integument when observed under the light microscope. Rostral lamella bilobed; each lobe round and without interspace. Rostrum as long as it is wide. Corona much wider than cingulum: the ratio of corona width to cingulum width about 1.42-1.43:1. Sulcus slightly wider than pedicel width; the ratio of sulcus width to pedicel width about 1.28:1. Pedicels short. Upper lip arched; much lower than trochal discs and higher than sulcus base. Cingulum pad slightly narrower than cingulum. Antenna very long and with two segments; much longer than height of pseudosegment carrying antenna in creeping; wider than width of pseudosegment carrying antenna in feeding. Pharyngeal tube shorter than trophi length. Dental formula 3/3. Trunk tumbler-shaped and gradually tapering to foot; first pseudosegment of trunk widest; middle part of trunk cylindrical; fourth pseudosegment narrower than rump when fully stretched in dorsal view; preanal pseudosegment rather plump anteriorly and abruptly tapering to anal pseudosegment; anal pseudosegment gradually tapering to foot. Foot with four pseudosegments, cylindrical and gradually tapering to spur pseudosegment; foot length about 1/5 of total body length in creeping. Spurs rather big, conical, and with blunt tips; interspace about a half of spur base width;

the ratio of spur length to spur pseudosegment width about 1:1.35; the ratio of spur length to spur base width about 1.63:1. Four toes with two pseudosegments; ventral toes much longer and thicker than dorsal ones.

Measurements. Total length in creeping 223–234 μ m. Corona width 70–75 μ m. Cingulum width 49–53 μ m. Cingulum pad width 46–50 μ m. Antenna length 42–44 μ m. Antenna pseudosegment width in feeding 38–40 μ m. Antenna pseudosegment height in creeping 29–33 μ m. Head length in feeding 35 μ m. Greatest trunk width in feeding 56–57 μ m. Greatest trunk width in creeping 41–56 μ m. Greatest foot width in creeping 16–17 μ m. Trophi length 19 μ m. Spur length 7.3–8.6 μ m.

Distribution. The new species is known only from its type locality.

Etymology. The new species is named after Dr. Won Kim, Professor Emeritus of the School of Biological Sciences at Seoul National University, in recognition of his excellent mentoring during the author's PhD study (M. O. Song). **Barcode.** GenBank accession number MT414957.

Philodina eurystephana Schulte, **1954** (Fig. 3) *Philodina eurystephana* Schulte, 1954: 610, figs. 38a-e.

Material examined. One specimen sorted out from mosses at the bottom of a mountain in Bangnae-ri, collected by Min Ok Song and Chang-Ho Lee on 6 May 2019.

Diagnosis. No eye spots. Corona slightly wider than cingulum and much wider than cingulum pad. Sulcus as wide as pedicel width. Upper lip arched and with median depression; median depression narrower than a half of pedicel width and slightly wavy medially. Upper lip slightly higher than sulcus base and much lower than trochal discs. Feeding head area between upper lip and antenna pseudosegment short and football-shaped. Pharyngeal tube shorter than trophi length. Spurs narrow, conical and with wide interspace; spur slightly longer than double of spur base width; interspace slightly wider than spur base width. Egg with rough surface.

Measurements. Corona width 47–48 μ m. Cingulum width 45 μ m. Cingulum pad width 42 μ m. Greatest trunk width in feeding 64–79 μ m. Trophi length 16 μ m. Spur length 6.6 μ m.

Remarks. *Philodina eurystephana* is rather rare and has been reported as parts of species lists from only two countries, Ukraine (Yakovenko, 2003) and USA (Örstan, 1998), after the description from Germany by Schulte (1954).

The general morphology of the Korean specimen agrees well with that in the original description except the following characteristics: (1) the median depression of upper lip of

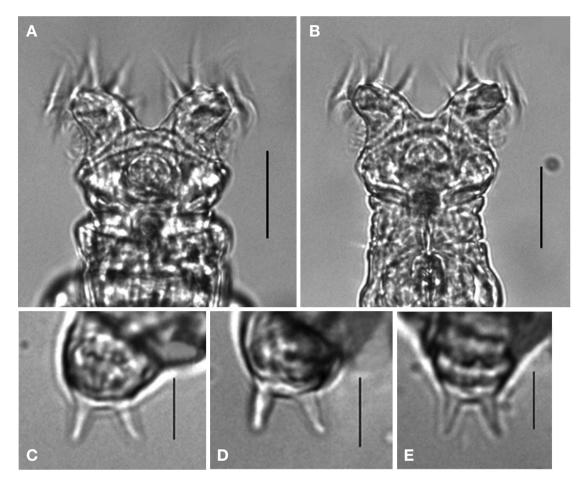


Fig. 3. Light microscopy photographs of *Philodina eurystephana* Schulte, 1954. A, B, Feeding head and neck, dorsal view; C–E, Foot and spurs, dorsal view. Scale bars: A, B=25 µm, C–E=10 µm.

the original illustration is slightly wider than that of the Korean specimen, and (2) the egg is pear-shaped in the original description, while it is oval in the Korean specimen. The interspace between spurs is slightly wider than or twice as wide as spur base width in the original illustrations (fig. 38b and fig. 38d, respectively, of p. 610). In the Korean specimen, the interspace between spurs is slightly wider than spur base width, as illustrated in fig. 38b of Schulte (1954).

Order Adinetida Melone and Ricci, 1995 Family Adinetidae Hudson and Gosse, 1886 Genus *Adineta* Hudson and Gosse, 1886

Adineta rhomboidea Bērzinš, 1950 (Fig. 4)

- Adineta vaga rhomboidea Bērzinš, 1950: 5, fig. 15 (cited from Donner, 1965).
- Adineta rhomboidea: Yakovenko, 2000: 17-18, fig. 8; Bielańska-Grajner et al., 2011: 105, fig. 2.

Material examined. One specimen sorted out from mosses at the bottom of a mountain near Moss Valley in Mugeon-ri, collected by Min Ok Song and Chang-Ho Lee on 29 Sep 2019.

Diagnosis. Rostrum wide and narrower than head width; the ratio of head width to rostrum width about 1.27:1. Rostral lamella bilobed and each lobe rhomboidal leaf- or eagle beak-shaped in ventral view; without interspace between lobes anteriorly; interspace between rostral lamella arched and slightly wider than width of rostral lamella when rostrum rolled forward. Neck thick and slightly wider than head width. Flat rhomboidal elevation on rump dorsally. Spurs narrow and slightly longer than spur pseudosegment.

Measurements. Rostrum width 22 μm. Head width 28 μm. Trunk width 32–44 μm. Rump width 23–26 μm. Spur length 7.7 μm.

Remarks. This species was described as a subspecies of *Adineta vaga* (Davis, 1873), *A. vaga rhomboidea*, by Bērzinš (1950). Yakovenko (2000) considered it as a distinct spe-

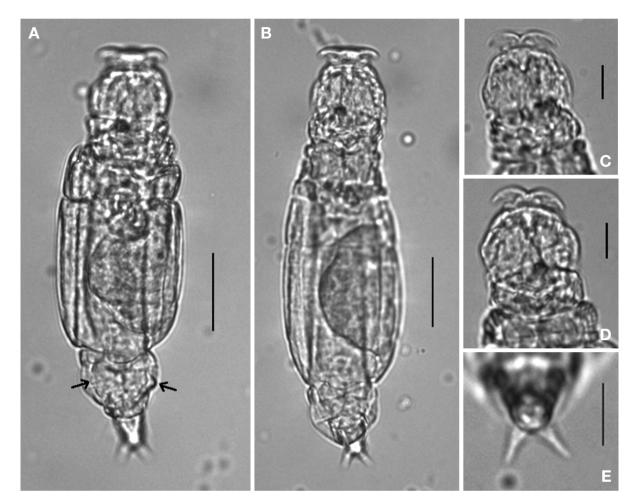


Fig. 4. Light microscopy photographs of *Adineta rhomboidea* Bērzinš, 1950. A, Creeping, dorsal view (two arrows pointing at the lateral corners of the rhomboidal elevation on rump); B, Creeping, dorsal view; C, D, Head and rostrum, ventral view; E, Foot and spurs, ventral view. Scale bars: A, $B=25 \mu m$, $C-E=10 \mu m$.

cies since it has some characteristics such as thick neck and rhomboidal elevation on rump which are sufficiently different from the characteristics of *A. vaga* (Jersabek and Leitner, 2013).

The morphology of the Korean specimen agrees well with that of the original description including the rhomboidal protuberance on dorsal area of rump. However, the triangular light-refracting body on either side of antenna was not observed in the Korean specimen. In her report on the Ukraine bdelloids, Yakovenko (2000) mentioned that there were a pair of small rotating bodies on each side of the dorsal antenna; however, it was not clear whether those small rotating bodies are the same as the triangular light-refracting bodies in the original description. Plewka (2014) commented that, contrary to the description of Bērzinš (1950), the light refracting bodies in the neck segment were not visible in the Italian specimens he observed. In the original illustration (fig. 15) of Bērzinš (1950), rostrum was much wider than a half of the head width, elevated, and trilobed anteriorly. In the Korean specimen, the width of rostrum was about 2/3 of the head width, and the rostral lamella was bilobed and without interspace between them anteriorly (Fig. 4C, D). The rostral lamella tended to roll forward and seldom stretched fully. When the rostral lamella was rolled forward, the anterior margin of rostrum appeared to be trilobed (Fig. 4A, B) in ventral/dorsal view, which is similar to the morphology of the rostrum in the original illustration (Bērzinš, 1950, fig. 15).

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Appendix 1. BLAST results.

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Appendix 2. Taxonomy report of BLAST analysis.