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Research Note

First report of *Hemicycliophora conida* from Russia

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Summary

A population of the sheath nematodes, identified as *Hemicycliophora conida*, has been reported from a natural habitat in Moscow, Russia. This paper includes morphological, morphometric and molecular data of the population.

Keywords: *Hemicycliophora conida*; molecular characterization; morphology; sheath nematode; *Rubus idaeus*; Russia

Introduction

Hemicycliophora conida (Thorne, 1955) was originally described from soil samples collected from a sugar-beet field near the village of Ballyculane in Ireland. This species was also reported in the Netherlands (Thorne, 1955, Loof, 1968), Belgium, England, Germany, Switzerland, Italy (Loof, 1968), Poland (Loof, 1968, Brzeski, 1998), Iran (Loof, 1984, Azimi *et al*., 2020), Spain (Castillo *et al*., 1989), the United States (Zeng *et al*., 2012, Subbotin *et al*., 2014), and Iceland (Flis *et al*., 2020).

Recently, *H. conida* was found near the roots of wild red raspberries (*Rubus idaeus* L.) in Serebryany Bor Forest Park in Moscow, Russia. The geographical location of the sampling site is 55°47'10.6"N; 37°24'30.3"E. This is the first record of this species in Russia.

Material and Methods

Nematodes were extracted using a modification of the decanting and sieving method (Flegg, 1967). For morphological studies, the nematodes were killed with hot water, fixed in a 5 % formalin solution, and mounted in glycerin on slides using the Seinhorst technique (Seinhorst, 1959). Molecular studies were performed using the scientific equipment of Core Research Facility of the "Bioengineering" Center (Moscow, Russia). For this work, nematodes frozen in distilled water were used. There were three replicates. Each replicate was a test tube with several specimens of *H. conida*. Their total DNA was extracted using the Wizard Kit (Promega, USA), according to the manufacturer's instructions. The forward Nem_18S_F (5'-CGCGAATRGCTCATTACAACAGC-3') and the reverse Nem_18S_R (5'-GGGCGGTATCTGATCGCC-3') primers (Floyd *et al*., 2005) were used to amplify the fragment of the 18S rRNA gene. The D2-D3 expansion segments of the 28S rRNA gene were amplified using the forward D2A (5'-CAAGTACCGTGAGGGAAAGTTG-3') and the reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (Nunn, 1992). Internal transcribed spacer 1 (ITS1) and a partial sequence of 5.8S ribosomal RNA gene were amplified with the forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer 5.8SM5 (5'-GGCGCAATGTGCATTCGA-3') (Zheng *et al*., 2000). The amplifications were performed in a Tetrad thermal cycler (Bio-Rad, USA). PCR products were purified using the Wizard PCR Preps Kit (Promega, USA). The sequencing of the PCR products was carried out with the same primers using the genetic analyzer

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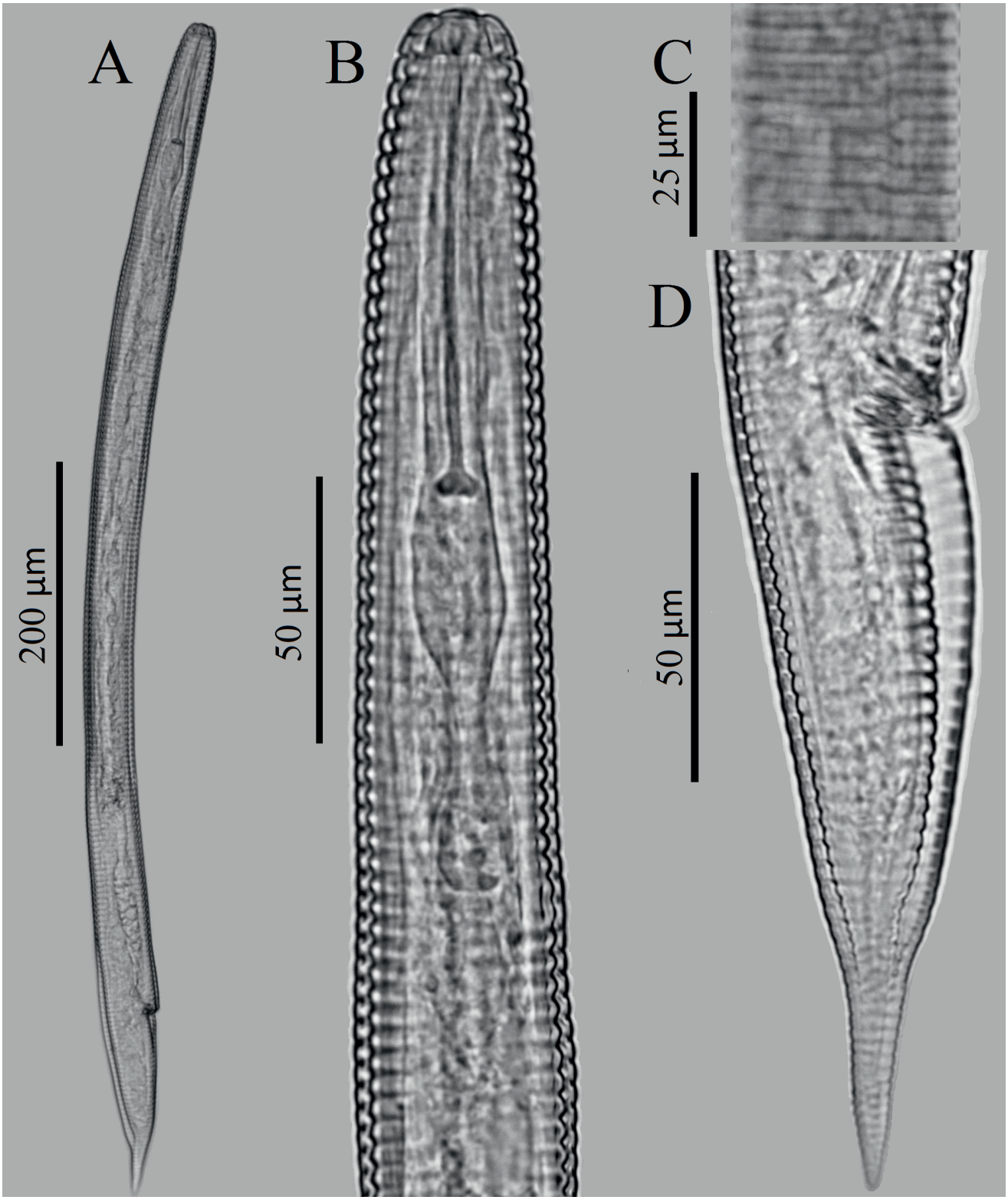

Fig. 1. Light photomicrographs of *Hemicycliophora conida*.
A) Entire female, B) Anterior end, C) Lateral field at mid-body, D) Posterior end

Table 1. Measurements of 15 females of *Hemicycliophora conida* from Moscow, Russia.
If not otherwise stated, measurements are in µm and in the form: mean ± standard deviation (range).

Characters	Measurements
L	823 ± 68 (710–930)
Body width	41.5 ± 4.3 (35–50)
Pharynx length	153 ± 12.5 (132.5–165)
Tail length	80 ± 9.8 (66–95)
a	19.8 ± 2 (17.4–24.2)
b	5.3 ± 0.1 (5.1–5.5)
c	10.5 ± 1.2 (8.9–12.5)
c'	2.8 ± 0.3 (2.3–3.3)
V, %	85.4 ± 0.8 (84.3–87.1)
VL/VB	3.2 ± 0.3 (2.7–3.9)
Lip width	19 ± 1.5 (16–22.5)
Lip height	6.6 ± 0.6 (5.6–7.8)
Stylet	85.6 ± 6 (75–92.5)
DGO	9.7 ± 1.2 (8–12)
R	236.9 ± 14.8 (206–257)
Rst	23.9 ± 3 (18–27)
Roes	42.6 ± 4.8 (36–49)
Rex	45.4 ± 4.5 (39–52)
RV	45.1 ± 2.7 (41–51)
RVan	13.2 ± 2.8 (10–18)
Ran	32.7 ± 3.1 (28–38)

ABI 3730 (Applied Biosystems, USA). Low-quality segments of sequences at the 5' and 3' ends were removed. Then, the newly obtained sequences were submitted to the GenBank database under accession numbers OL765280 (18S rRNA gene), OL765287 (28S rRNA gene), and OL913796 (ITS1).

Ethical Approval and/or Informed Consent

For this study formal consent is not required.

Results and Discussion

Hemicycliophora conida (Thorne, 1955)
(Fig. 1, Table 1)

Female. Body straight to slightly ventrally arcuate. Cuticular sheath fitting closely or loosely to body, more loosely on posterior end. Lateral field with occasional breaks of transverse striae and anastomoses bordered by two lines for a short distance; sometimes it looks like two longitudinal rows of blocks. Each annulus with two rows of scratches. Labial region broad, truncated, with two distinct annuli. Stylet with posteriorly sloping knobs sized about 7 µm across. Excretory pore 3–4 annuli posterior to pharynx base. Hemizonid distinct or indistinct, two annuli long, located anterior to excretory pore. Reproductive system mono-prodelphic, outstretched, oocytes in single row except for anterior end. In studied specimens, spermatheca of only one female was filled with sperm. Eggs (n=3) 78–82 x 24–26 µm in size, one per uterus. Vulval lips modified, elongated. Tail elongate conoid, tip is usually straight; in some specimens, it is curved slightly dorsally, while on others, it is curved slightly ventrally. Males were not found.

Remarks. Morphology and morphometric data of the Moscow population of *H. conida* resemble the original description and other descriptions of the species (Chitambar & Subbotin, 2014). According to Loof (1968), *H. conida* can be divided into two morphological forms (I and II), differing in the number of annuli (227–284 vs 180–220), the excretory pore position from the anterior end (42–53 annuli vs 36–43), and the stylet length (78–96 vs 69–86 µm). He considered these forms to be "conspecific." The population does not exactly correspond to Form I and overlaps with Form II. Some specimens from the studied population had a slightly smaller number of annuli, as compared to those in Form I, while other specimens had a slightly smaller stylet length, as compared to those in Form I.

Molecular characterization. The sequences of the 18S rRNA gene, the D2-D3 expansion segments of the 28S rRNA gene, and the ITS1 obtained from different individuals in this study were identical

within each marker. The sequences of the 18S rRNA gene of the studied specimens were identical to the sequence AJ966471.
Three other sequences of *H. conida* deposited in GenBank EU669914, KJ934172, KJ934173 share only 98.23 %, 98.12 %, and 97.56 % similarity, respectively. However, according to Van den Berg *et al*. (2018), the sequences under the accession numbers: KJ934172–KJ934174 identified in GenBank as *H. conida* actually belong to *H. parvana*.
The sequences of the D2–D3 expansion segments of the 28S rRNA gene were identical to the sequences of *H. conida* KF430448, KF430447, FN433875 and share 99.69 % similarity with *H. conida* sequence MN628433.
The sequences of Internal transcribed spacer 1 (ITS1) were identical to the sequence of *H. conida* from Spain (KF430580), share 99.77 % similarity with the sequence of *H. conida* from Iran (MT901584), whereas similarity to the sequence of this species from the United States (KF430579) was only 97.92 %.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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