Report of *Heterodera mothi* Khan & Husain, 1965 (Tylenchida, Heteroderidae) in Azerbaijan

Alexander Yu. Ryss¹, Gulnara Kazimova² and Sergei A. Subbotin³, ⁴

¹Zoological Institute, Russian Academy of Sciences, Universitetskaya Emb. 1, 199034, Saint Petersburg, Russia
²Institute of Zoology, National Academy of Sciences of Azerbaijan, AZ 1073, Baku, Azerbaijan
³Plant Pest Diagnostic Centre, California Department of Food and Agriculture, 3294 Meadowview Road, 95832-1448, Sacramento, CA, USA
⁴Centre of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii Prospect 33, 117071, Moscow, Russia
e-mail: alryss@gmail.com

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Summary. The description of the *Heterodera mothi* from wheat field of Azerbaijan, Aras River Valley, is given. Morphology of female cysts and the second-stage juveniles, and the phylogenetic relationships of *H. mothi* within the genus *Heterodera* based on the ITS rRNA and COI mtDNA genes, show that species belongs to the *Cyperi* group. This is a first record of the *H. mothi* in Azerbaijan and Caucasus.

Key words: Aras River Valley, Caucasus, molecular phylogeny, taxonomy, wheat field.

The moth cyst nematode, *Heterodera mothi*, was described by Khan and Husain (1965) from roots of *Cyperus rotundus* L. (Cyperaceae, Poales), University campus, Aligarh, UP, India. Later this nematode was reported from several Asian countries including Iraq, Kazakhstan, Nepal and Pakistan (Subbotin et al., 2010a, b). It has been found that *H. mothi* parasitised several weeds. Shahina and Maqbool (1991) reported that it infected roots of *Triticum aestivum*, *Hordeum vulgare* and *Cynodon dactylon* in various localities of Pakistan. Sequence of ITS rRNA gene and PCR-ITS-RFLP profile for this species was provided by Tanha Maafi et al. (2003).

In December 2017 during a nematological survey of wheat fields in Sabirabad district, Aran region of Azerbaijan an unknown cyst nematode was found. Further examination of second-stage juveniles (J2) and cysts showed that it belonged to *H. mothi*. This paper provides a short morphological and molecular characterisation of this species.

MATERIAL AND METHODS

Nematode samples. The soil sample with *H. mothi* was collected in December 2017, in the field of wheat *Triticum aestivum* L. in Sabirabad district, Aran region, Azerbaijan, GIS 39.97265 N, 48.447538 E Aras River Valley. Nematode cysts were extracted from soil using decanting and sieving via 100 µm mesh sieve. To extract J2, a soil sample was poured with water and shaken, with subsequent decanting and sieving via sieves of 100 µm and 38 µm mesh. Debris from the 100 µm mesh sieve was studied under a stereomicroscope and nematode cysts were handpicked. Debris from the 38 µm sieve was placed into a modified Baermann funnel for 16 h to extract J2 (Ryss, 2017a). Vulval plates were prepared by standard procedure (Subbotin et al., 2010a). J2 were fixed and processed to glycerin using the method of Ryss (2017b).

Samples of *H. mothi* and *H. elachista* from Khuzestan, Ahvaz, Iran and Mazandaran, Tonekabon, Soleyman Abad, Iran (Tanha Maafi et al., 2003) were also included in the molecular study.

Light microscopic study. Light microscopic examination and photographs were taken with a Leica microscope equipped with a Nomarski differential interference contrast. Microscopic slides with nematode cysts, vulval plates and juveniles are deposited in the Nematode Collection of the Zoological Institute RAS, St Petersburg, Russia.

Molecular study and phylogenetic analysis. DNA was extracted from several J2 using the proteinase K protocol. DNA extraction, PCR and cloning protocols were as described by Tanha Maafi et al. (2003). The following primer sets were used for PCR: the forward TW81 (5’-GTT TCCGTAAGGTGAACCTGC-3’) and the reverse AB28...
Fig. 1. *Heterodera mothi*. A-D: cyst shape; E, F and G, H: vulval cone at different optic levels.
Fig. 2. *Heterodera mothi*. Second-stage juveniles. A: whole body; B, C: head, lateral view; D: head, ventral view; E, F: genital primordium; G: lateral field; H, I: tail, lateral view; J: tail, ventral view. Scale bar = 50 µm for A and 10 µm for B-J.
**Fig. 3.** Phylogenetic relationships within the *Cyperi* group and *Heterodera sojae*: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to or more than 70% are given for appropriate clades. Original sequence is indicated by bold font.
(5′-ATATGCTTAACTGGTCAAGCTGGT-3′) (Subbotin et al., 2001) primers for amplification of the ITS rRNA gene and the forward primer Het-coxiF (5′-TAGTTGATCGTAAATTTAATGGG-3′) and the reverse primer Het-coxiR (5′-CCTAAAA CATAATGAAAAATGWC-3′) (Subbotin, 2015) primers for amplification of the COI gene. Sequencing was made in Quintara Biosciences (San Francisco, CA, USA). The new sequences were submitted to the GenBank database under accession numbers: GenBank MH144206-MH144209 as indicated in the phylogenetic trees.

The new sequences for the ITS rRNA and COI genes were aligned using Clustal_X 1.83 (Thompson et al., 1997) with default parameters with their corresponding published gene sequences of some Heterodera species (Subbotin et al., 2001; Tanha Maafi et al., 2003; Ding et al., 2012; De Luca et al., 2013; Zhuo et al., 2013, 2014a, b; Kang et al., 2016 and others). Sequence datasets were analysed using Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The best-fitting model of DNA evolution was obtained using jModelTest v. 2 (Darriba et al., 2012) with the Akaike information criterion. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0 × 10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The log-likelihood values of the sample points stabilised after approximately 1000 generations. After discarding burn-in samples, other trees were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

**DESCRIPTION**

*Heterodera mothi* Khan & Husain, 1965 (Figs 1 & 2)

**Cysts** (n = 9) [all measurements are given in µm; values are given as mean ± SD (range)]. Length (L) = 626 ± 81 (542-762) µm; Width (W) = 286 ± 35 (241-331) µm; L/W = 2.2 ± 0.3 (1.9-2.6); fenestral length = 50 ± 3 (47-55) µm; fenestral width = 26 ± 3 (23-31) µm; vulval slit = 38 ± 7 (31-50) µm.

Cysts are ellipsoid, twice as long as wide, brownish. Vulval cone ambifastenate. Buliae prominent, mostly rounded, dark brown. Underbridge present.

**Second-stage juveniles** (n = 5) [all measurements are given in µm; values are given as mean ± SD (range)]. L = 433 ± 24 (416-461) µm; a = 28.3 ± 1.7 (27.2-30.3); b = 5.4 ± 0.5 (4.7-5.7); b’ = 2.9 ± 0.4 (2.4-3.2); c = 6.0 ± 0.3 (5.7-6.3); c’ = 6.4 ± 0.7 (5.6-6.9); ratio: distance from anterior body end to genital primordium, divided to body length = 55 ± 2 (54-57)%; stylet length = 16.5 ± 0.1 (16-17) µm; stylet cone = 8 ± 0.2 (8-9) µm; stylet knob width = 2.7 ± 0.3 (2.4-2.9) µm; stylet knob height = 1.8 ± 0.1 (1.6-1.8) µm; dorsal pharyngeal gland (DGO) = 5.4 ± 0.7 (5-6) µm; body width = 15.3 ± 0.2 (15-16) µm; lateral field width = 4.5 ± 0.2 (4-5) µm; pharynx measured up to a pharyngeal-intestinal valve = 81.2 ± 6.2 (75-88) µm; pharynx measured up to pharyngeal gland lobe end = 152 ± 9 (132-170) µm; pharyngeal gland globe = 70 ± 13 (57-82) µm; lip region length = 7.0 ± 0.2 (6.9-7.2) µm; lip region height = 3.5 ± 0.1 (3.3-3.5); anterior end to median bulb valve = 59 ± 9 (53-69) µm; median bulb width = 8 ± 0.1 (7-9) µm; median bulb length = 13 ± 0.2 (12-14) µm; anterior end to excretory pore = 86.2 ± 8.5 (77-94) µm; hemizonid length = 3 µm; genital primordium from anterior = 239 ± 9 (230-248) µm; genital primordium length = 11.4 ± 1.2 (10-13) µm; genital primordium width = 8.3 ± 0.3 (8-9) µm; tail length = 72.4 ± 7.8 (66-81) µm; anal body width = 11.4 ± 0.6 (11-12) µm; hyaline part of tail length = 34.6 ± 4.8 (31-40) µm; body width at hyaline part of tail = 5.3 ± 0.8 (4-6) µm. Body straight or slightly, ventrally curved. Lateral field 25-30% of body width, with three lines, a central incisure is weakly recognisable. Head hemispherical, set off, its width twice more than height, bearing 3 annuli and lip disc surrounded by 6 sectors. Cephalic framework strongly sclerotised. Stylet cone slightly less than half of its length. Stylet knobs moderate, nearly rounded or slightly sloping posteriorly. Median bulb oval with a central moderate valve. Hemizonid 3 µm wide, hemispherical in lateral view. Excretory pore just posterior to hemizonid. Gland lobe ventral, 57-82 µm, large nucleus of dorsal pharyngeal gland anterior to closely distanced small subventral glands nuclei. The pharynx to the gland lobe end occupies one third of body length. Genital primordium almost rounded, situated in the centre of the body attaching ventrally to body wall, consisting of two large germinal cells with large nuclei, and two small apical cells. Tail almost straight, 6-7 times longer than its width, with long annulated hyaline zone, gradually tapering to digital tip.

Plant host for the Azerbaijan population of *H. mothi* remained unknown.

**Molecular characterisation and phylogenetic relationships.** The ITS rRNA gene sequences of *H. mothi* of Azerbaijan and Iran differed in 2 bp, or 0.2%. In the ITS rRNA phylogenetic tree *H. mothi* and *H. cypéri* formed a clade with PP = 100% (Fig. 3).
Fig. 4. Phylogenetic relationships within the genus *Heterodera*: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the COI mtDNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to or more than, 70% are given for appropriate clades. Original sequences are indicated by bold font.

The COI gene sequence fragments of *H. mothi* from Azerbaijan and Iran used for the analysis was 454 bp. Sequences differed from each other in 1 nucleotide or 2.2%. The COI gene sequences of *H. elachista* from Iran and Italy were different in 12 bp or 2.9%. *Heterodera mothi* was in a sister relationship with *H. cyperi* (PP = 73%). The Cyperi group containing four species was highly supported in the COI phylogenetic tree (Fig. 4).

Descriptions and measurements of the Azerbaijan population of *H. mothi* are generally similar with those given for several Asian populations (Subbotin et al., 2010b). There is also a single report of *H. mothi* from Georgia, USA by Minton et al. (1973); however, because of this article does not contain any morphological and morphometric information, we consider it as doubtful and likely belonging to close relative species and morphologically similar species *H. cyperi* Golden, Rau & Cobb, 1962. *Heterodera mothi* is distributed in Western, South and Central Asia and the South Caucasus region of Eurasia, but *H. cyperi* are reported in several states of the USA and Mexico and in Spain. Both species parasitise plants from the genus *Cyperus* (Cyperaceae, Poales).

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А.Ю. Рысс, Г. Казимова и С.А. Субботин. Находка *Heterodera mothi* Khan & Husain, 1965 (Tylenchida, Heteroderidae) в Азербайджане. Резюме. Дано описание находки цистообразующей нематоды *Heterodera mothi* в Азербайджане в долине реки Аракс. Морфология цист самок и личинок второй стадии, а также филогенетические взаимоотношения *H. mothi* с другими видами рода *Heterodera*, проанализированные на основании генов ITS и рибосомальной РНК и COI рибосомной ДНК, показывают, что данный вид принадлежит к группе видов *Cyperi*. Данная находка *Heterodera mothi* – первая для Азербайджана и Кавказа в целом.