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

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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 TCCGTAGGTGAACCTGC-3') and the reverse AB28

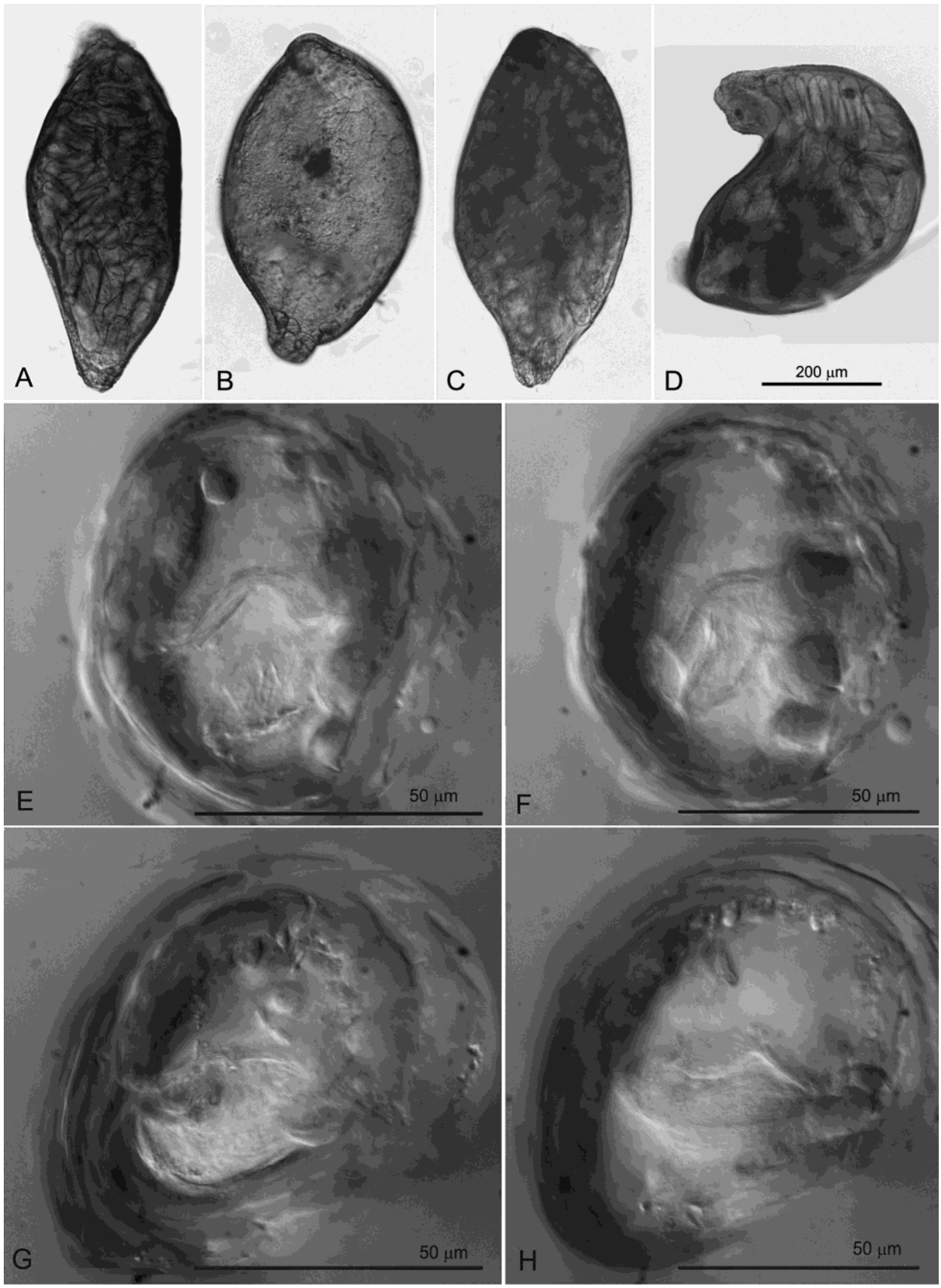

Fig. 1. *Heterodera mothi*. A-D: cyst shape; E, F and G, H: vulval cone at different optic levels.

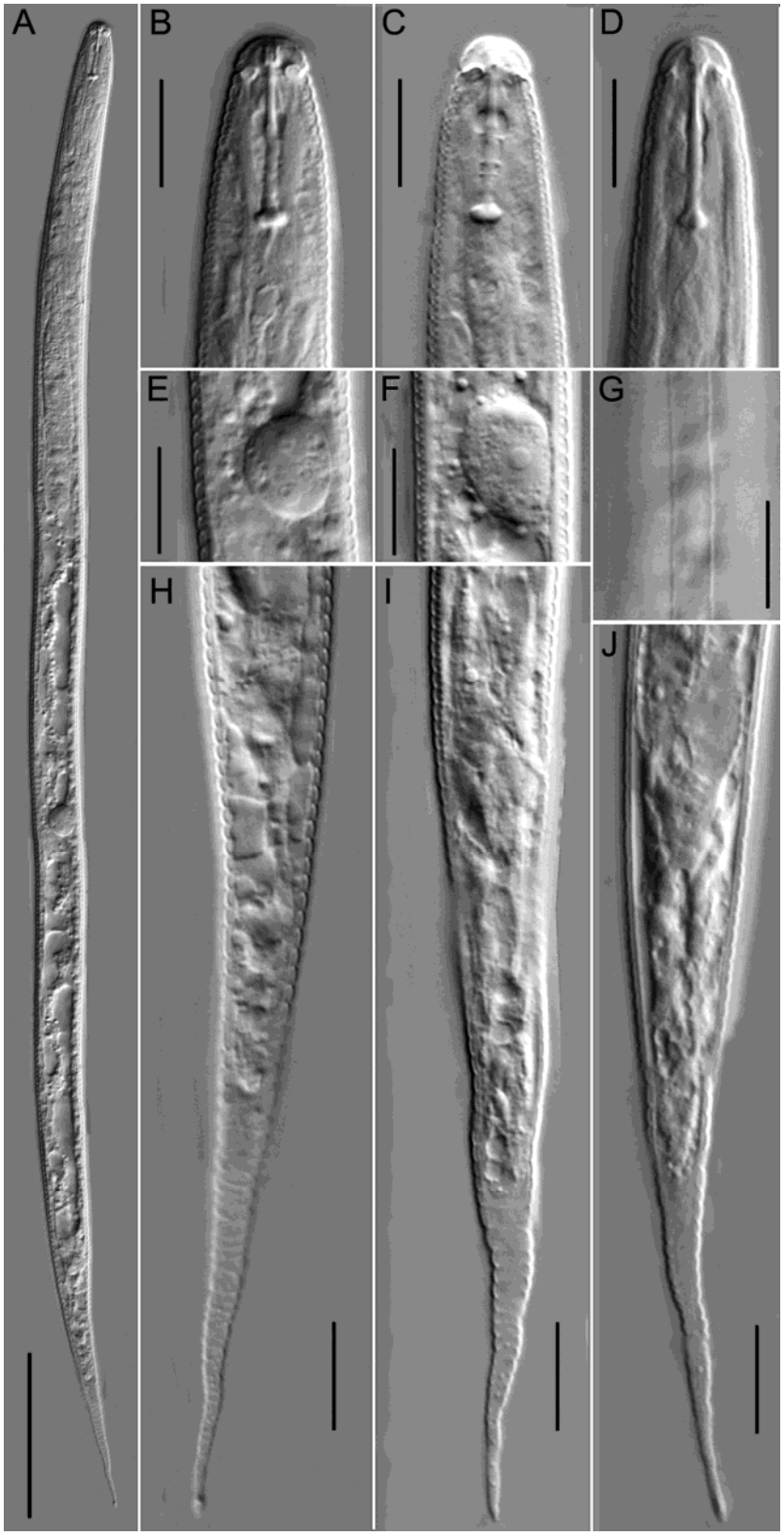

Fig. 2. *Heterodera mothi*. Second-stage juveniles. A: whole body; B, C: head, lateral view; D: head, ventral view; E, F: genital primordium; G: lateral field; H, I: tail, lateral view; J: tail, ventral view. Scale bar = 50 µm for A and 10 µm for B-J.

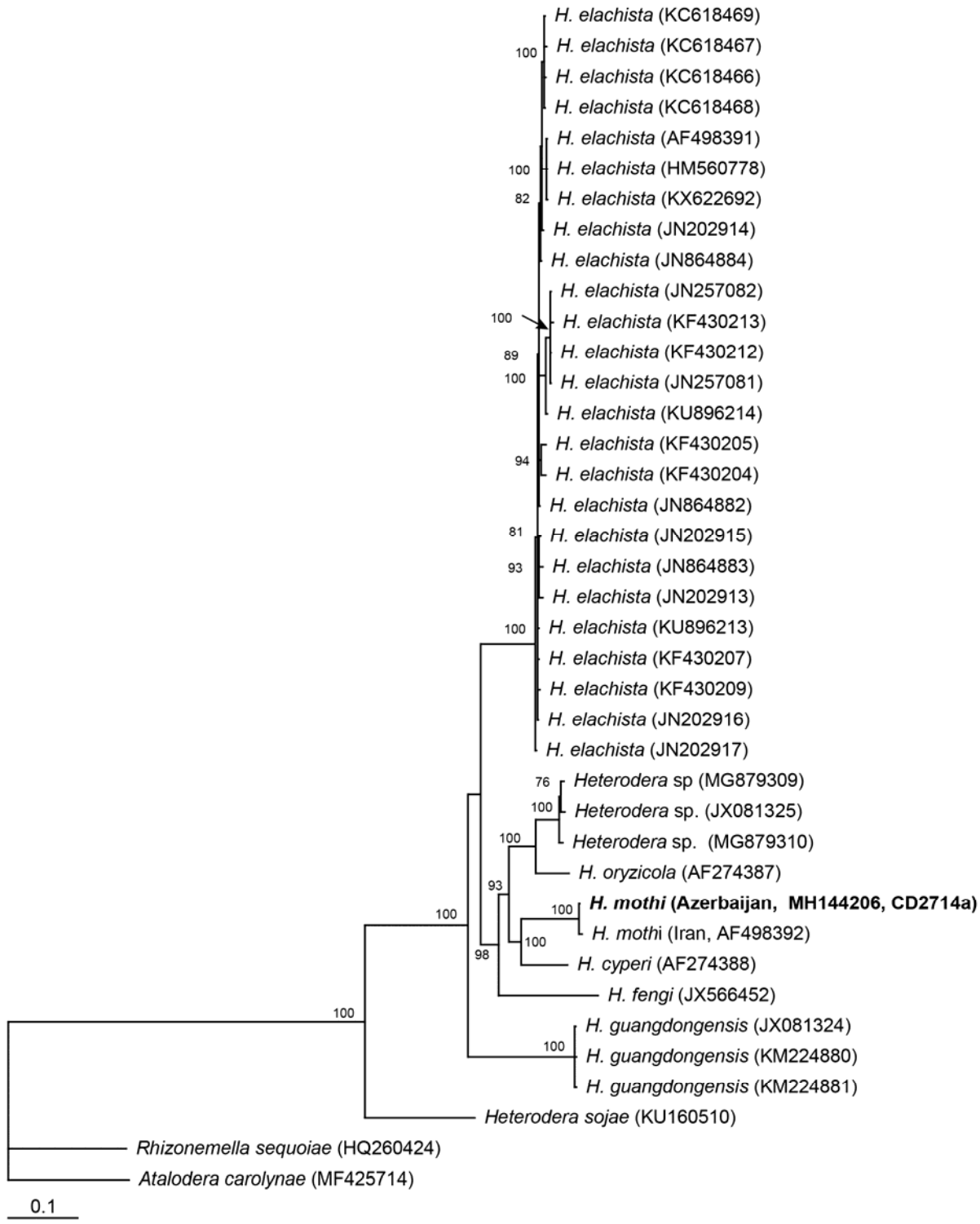

Fig. 3. Phylogenetic relationships within the *Cyperi* group and *Heterodera sojae*: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA *g*ene sequence alignment under the GTR + I + G model. Posterior probabilities equal to or more than, 70% are given for appropriate clades. Original sequence is indicated by bold font.

(5'-ATATGCTTAAGTTCAGCGGGT-3') (Subbotin *et al.*, 2001) primers for amplification of the ITS rRNA gene and the forward primer Het-coxiF (5'-TAGTTGATCGTAATTTTAATGG-3') and the reverse primer Het-coxiR (5'-CCTAAAA CATAATGAAAATGWGC-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 ambifenestrate. Bullae 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 = 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 width = 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. cyperi* formed a clade with PP = 100% (Fig. 3).

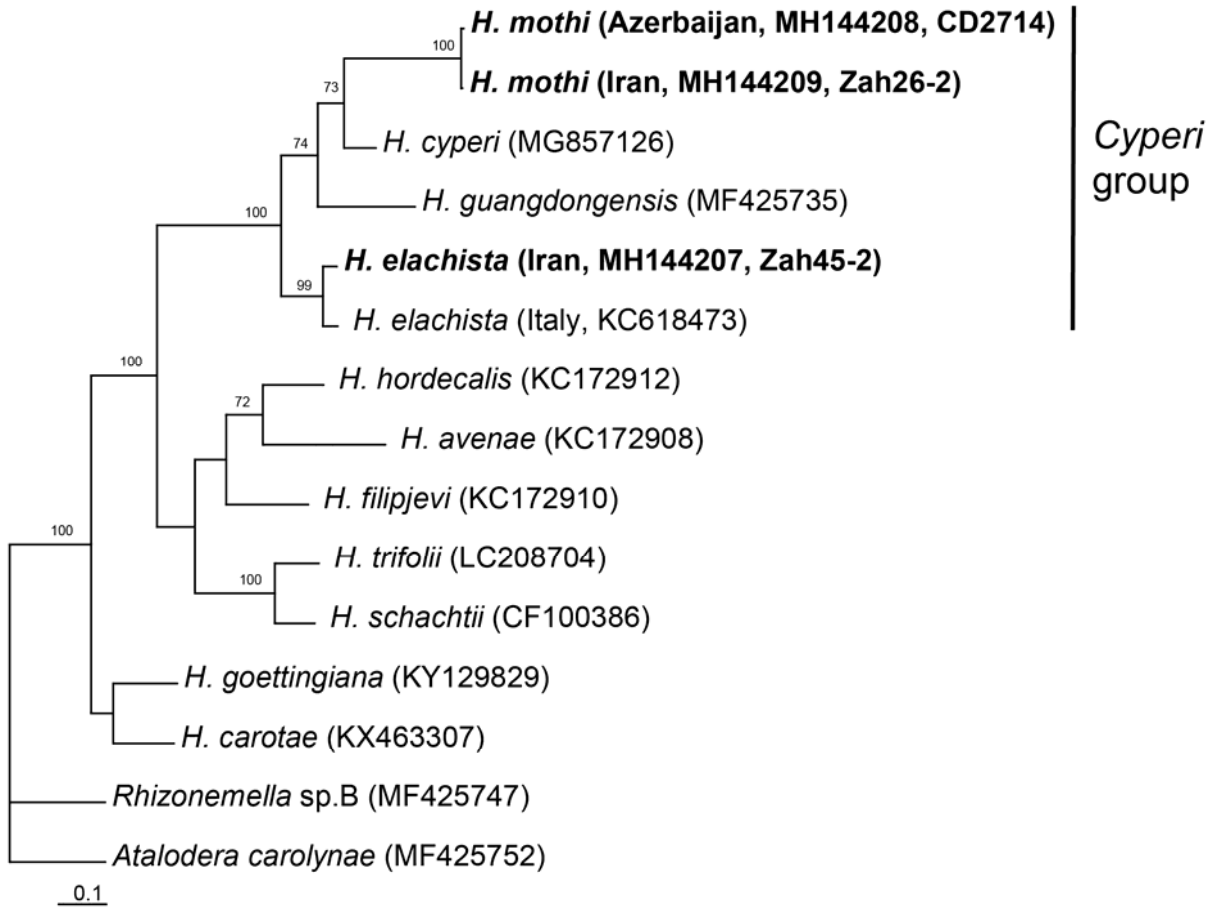

Fig. 4. Phylogenetic relationships within the genus *Heterodera*: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the *COI* mtDNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to or more than, 70% are given for appropriate clades. 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 *Herodera 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 рибосомальной РНК и *COI* митохондриальной ДНК, показывают, что данный вид принадлежит к группе видов *Cyperi*. Данная находка *Herodera mothi* – первая для Азербайджана и Кавказа в целом.