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Original article

Characteristics of *Heterodera ripae* Subbotin, Sturhan, Rumpenhorst & Moens, 2003 from Poland

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Abstract

Specimens of plant parasitic nematode species *Heterodera ripae* were isolated from the root zone of common nettle (*Urtica dioica*), a perennial growing near agricultural fields in Winna Góra near Poznań (Wielkopolska region, Poland). The species identity was confirmed by analyses of the structure of the vulval cyst cones, morphology and anatomy of J2, measurements of both developmental stages as well as analyses of the rDNA and mtCOI sequences.

Molecular analyses of the Polish *H. ripae* 28S rDNA sequence revealed that it was the most similar to the *H. ripae* sequences from South Korea and clustered with them in a phylogenetic tree near a closely related *H. ripae* from China and *H. humuli*. Similarly, the mtCOI sequence of the Polish *H. ripae* population clustered with most *H. ripae* sequences deriving from Europe and Asia, however it was more distantly related to the sequence from China.

Under controlled conditions, *H. ripae* pathogenicity and potential harmfulness was tested on three plants: common nettle (*U. dioica*) which served as a control, buckwheat (*Fagopyrum esculentum*), and sorrel (*Rumex acetosa*). During the pathogenicity test, *H. ripae* did not develop new cysts, neither on the roots of *F. esculentum* nor *R. acetosa*. At a population of 100–120 nematode eggs and larvae, cysts of the nematode developed on the common nettle's *U. dioica* roots, however plants showed no signs of growth inhibition. These numbers may have been too low to lead to plant starvation.

It was recommended that soils intended for cultivation of *U. dioica* as a horticultural crop should be tested for the presence of *H. ripae* and that plantations of this plant should be monitored for the presence of other herbivorous nematodes, including cyst-forming species.

Keywords: *Humuli* group, mtCOI, *Fagopyrum esculentum, Rumex acetosa*, *Urtica dioica*

Introduction

Heterodera ripae Subbotin, Sturhan, Rumpenhorst & Moens, 2003 is a cyst-forming nematode belonging to the *Humuli* group. This group includes seven *Heterodera* species. They feed on the roots of dicotyledonous plants (Subbotin *et al.* 2022), of which only two: *H. fici* Kirjanova, 1954 and *H. humuli* Filipjev, 1934 are economically important (Subbotin *et al.* 2010). The former parasitizes fig trees (*Ficus* spp.), while the latter parasitizes hop plants (*Humulus lupulus* L.).

H. ripae parasitizes the roots of common nettle (*Urtica dioica* L.) but has also been reported feeding on the roots of *U. laetevirens* Maxim. (Eroshenko *et al.* 2001). *H. ripae* is a widely distributed cyst nematode species. Its presence has been recorded in many European countries (Subbotin *et al.* 1997; Brzeski 1998; Madani *et al.* 2004; Sturhan and Lišková 2004; Anderson and Manduric 2006; Sturhan 2006; Subotin *et al.* 2022) as well as in Asia (the Russian Far East: Eroshenko *et al.* 2001; China: Wang *et al.* 2012; Yang *et al.* 2024; South Korea: GenBank, unpublished). It occurs in the soil surrounding the nettle rhizosphere, in moist environments, often in the riparian zone of water bodies. The cysts of this nematode were found only in one case among buckwheat roots (Yang *et al.* 2024). *H. ripae* was originally described as *H. riparia* (Subbotin *et al.* 1997), however it had to be renamed (Subbotin *et al.* 2003) after the grass-feeding species *Bidera riparia* Kazachenko, 1993 was transferred to the genus *Heterodera* (Subbotin *et al.* 2003). From Poland, this species was recorded by Brzeski (1998) under the name *H. riparia*, from the soil around the roots of nettles growing on the banks of the Bug and Rawka rivers.

The aim of this article was to: i) present morphological and molecular characterizations of the Polish population of *H. ripae*, ii) test its possible development on buckwheat (*Fagopyrum esculentum* Moench) and sorrel (*Rumex acetosa* L.).

Materials and Methods

Nematode collection and morphological identification

A population of *H. ripae* was found in Winna Góra near Poznań (Greater Poland), in the root zone of a common nettle, growing in clusters near agricultural fields. Soil and plant samples were collected from April to June 2025. Both, empty sheaths and cysts filled with live eggs and larvae, were isolated from the soil adjacent to the plant roots, indicating that they developed at the sampling site. Juveniles J2 were extracted from the soil surrounding the roots using the

centrifugal flotation method (Van Bezooijen 2006). Microscopic slides of J2 individuals and vulval cyst cones were prepared (Van Bezooijen 2006). The species was identified with the keys to the species of the *Humuli* group and other groups of the genus *Heterodera* (Brzeski 1998; Subbotin *et al.* 1997; Subbotin *et al.* 2010; Ni *et al.* 2025; Nemaplex).

Molecular identification

Nematode lysis, amplification and sequencing

Six nematode J2 individuals, initially preserved in DESS (Yoder *et al.* 2006), were used for molecular analyses. They were subjected to the washing procedure as in Rybarczyk-Mydłowska *et al.* (2022) and subsequently transferred to separate 0.2 ml polymerase chain reaction (PCR) tubes, each containing 25 µl sterile Milli-Q water. An equal volume of lysis buffer (Holterman *et al.* 2006) was added and a lysis was performed for 3 h at 65°C, followed by 5 min incubation at 100°C. The obtained single nematode lysates were used as DNA templates for PCR reactions.

The 28S rDNA fragment was obtained using the D2A/D3B primer combination (Nunn 1992) while the mtCOI fragments were amplified with the JB3F/JB5R (Derycke *et al.* 2010). PCR reactions included: 12.5 µl of onHybrid PCR Master Mix (2x) (EURx, Gdańsk, Poland), 2.5 µl of Color Load dye (EURx, Gdańsk, Poland), 0.75 µl DMSO (3%), 2.5 µl (5µM) of forward and reverse primer each, 3 µl DNA template and H_2O to 25 µl of a total volume. PCR profile was as follows: an initial denaturation step at 98°C for 10 min, followed by 45 cycles for mtCOI or 40 cycles for 28S rDNA at 98°C for 10 s, 58°C for 30 s and 72°C for 30 s, and a final incubation for 7 min at 72°C. After the initial failure of the PCR reaction for the 28S rDNA, a reamplification was performed, in which as a template 3µl of the post-reaction mixture of the previous PCR was used. After SimplySafe (EURx, Gdańsk, Poland) gel staining and gel electrophoresis, amplicons were visualized under UV illumination. Excess dNTPs and unincorporated primers were removed from the PCR product using the enzymatic mixture of Fast Polar-BAP – Thermosensitive Alkaline Phosphatase (EURx, Gdańsk, Poland) and Exonuclease I – (EURx, Gdańsk, Poland). The obtained rDNA and mitochondrial sequence fragments were subsequently sequenced with an ABI 3500xL genetic analyzer (Applied Biosystems, USA).

Phylogenetic analysis

The newly obtained ribosomal *H. ripae* sequences were analyzed together with publicly available 28S rDNA and mtCOI sequences (GenBank) using the BioEdit program (v. 7.2.5; Hall 1999). Representative sequences of *Mesocriconema* were chosen as an outgroup. The final multiple-sequence alignments comprised of 673 characters in the case of 28S rDNA and 423 characters in the case of mtCOI. Bayesian phylogenies were constructed using the MrBayes v. 3.1 program (Ronquist and Huelsenbeck 2003). In the case of each data set, two independent runs were performed with four Markov chains per run. The program was run for 2,000,000 generations in the case of 28S rDNA and for 4,500,000 in the case of mtCOI and sample frequency was 100 generations in both cases. For the mtCOI data, the partition by codon position was set. The sampled trees from each run were combined in a single 50% majority-rule tree. Stabilization of the likelihood and parameters were checked with the program Tracer (v. 1.6; Rambaut *et al.* 2014).

Host plant test

H. ripae development was tested on the common nettle (*U. dioica*) as a control, as well as buckwheat (*F. esculentum*), and sorrel (*R. acetosa*) plants. Pots (capacity of 1200 ml) filled with substrate (potting soil/gravel ratio 1:1) were planted with nettle, buckwheat or sorrel plants at the 5–6 leaf stage (BBCH 15–16). Five plants of each of the three species (one plant per pot) were inoculated with the nematode. Plants were simultaneously infected with *H. ripae* by introducing 100–120 nematode eggs and larvae into their root zone. The pots were kept under controlled conditions of 20 ± 1°C and day and night length of 16/8 h. After four weeks the plants were removed from the pots. Plant roots were stained with acid fuchsin in lactoglycerol and were examined for the presence of sedentary juvenile stages, females and fresh cysts. The soil was examined for cysts (Van Bezooijen 2006). Sample analysis was performed using an OLYMPUS SZ-6045 stereoscopic microscope with 40x magnification.

Results

Description

Heterodera ripae Subbotin, Sturhan, Rumpenhorst & Moens, 2003

Cysts of *H. ripae* population: lemon shaped with prominent vulval cone, bifenestrate (Fig. 1A), and weak underbridge. Bullae were absent (Fig. 1B). All measurements are shown in Table 1.

J2 stage body vermiform, slightly ventrally curved after relaxed. Head region slightly offset from the body, 3.9 ± 0.4 µm (3.8–4.6 µm) in height and 8.6 ± 0.4 µm (8.1–9.4 µm) in width (Fig. 1C). Stylet distinct with basal knobs large, slightly concave anteriorly (Fig. 1D). The orifice of the dorsal pharyngeal gland located 4.0–6.8 µm behind the base of the stylet. Median bulb oval, 60–68 µm from anterior. Excretory pore posterior to the hemizonid. Lateral field with four incisures (Fig. 1E). Genital primordium above half the length of the body. The tail conical with finely rounded end (Fig. 1F). Phasmids 30.3–33.1 µm to tail tip. Morphometrics of J2s are summarized in Table 2.

Sequence analyses and phylogenetic relationships

One *H. ripae* 28S rDNA fragment and six identical mtCOI fragments were obtained. They were deposited in GenBank under the following accession numbers: PZ095079 (28S rDNA) and PZ095247 (mtCOI; consensus sequence). BLAST analysis results confirmed the species identity, revealing that the Polish *H. ripae* 28S rDNA sequence was the most similar to the *H. ripae* from South Korea (OQ064081; 99.86% identity, 100% query cover). There was 99.28% sequence similarity between Polish and Chinese sequence (OR468129; 100% query cover) and between Polish and the other South Korean sequence (OQ064081; 100% query cover). The mtCOI sequence of the Polish *H. ripae* population was the most similar to the *H. ripae* MT808350 sequence from Greece (100% identity, 89% query cover) and others like: ON007078 from Russia, MT808349 from Slovakia (both: 99.71% identity, 96% query cover), MT808352 from Belgium, MT808354 from Germany (both: 99.42% identity, 96% query cover). There was 97.49% similarity (query cover 89%) between the Polish and the South Korean sequence (OQ048424) and only 90.96% similarity (query cover 96%) between Polish and the OR468243 sequence from China. These results were in agreement with the obtained phylogenetic analyses (Figs. 2 and 3). Sequences used for these analyses are listed in Table 3. The 28S rDNA Polish *H. ripae* sequence clustered in a well-supported clade with other *H. ripae* from South Korea, which was positioned closely to *H. ripae* from China, *H. humuli* and *H. vallicola.* The mtCOI Polish sequence clustered in a clade including most *H. ripae* sequences, that were most closely related to *H. vallicola.* These relationships were also highly supported.

The Chinese (OR468243) *H. ripae* was positioned in the sister clade, containing representative sequences of *H. amaranthusiae*, *H. humuli* and *H. camelliae* (lower support).

Host plant test

Examination of buckwheat and sorrel roots revealed no signs of infection by *H. ripae*. No sedentary juvenile stages or females were observed. No fresh cysts were isolated from the soil. A few young females of the nematode developed on the common nettle's roots, however no symptoms of wilting were observed in these plants.

Discussion

Based on the investigations of the vulval cyst cone structure, the morphology and anatomy of the J2 juvenile stage, measurements of both of these stages as well as the analyses of the 28S rDNA and mtCOI sequences, the specimens from the Polish population were designated as *H. ripae*. Analysis of the obtained data showed that the dimensions of the Polish specimens differ slightly from the ones previously reported in the literature. Cysts/individuals had slightly longer fenestrae (> 60 μm), a wider vulval bridge (> 18 μm), and a longer vulval slit (> 43 μm). The mean values of these characters in the studied population were also slightly higher than in previously known populations. The mean distance from the vulval slit to the anus was also slightly higher. In several J2 specimens, the measured body lengths were below the previously known range of its variation.

The obtained rDNA and mtCOI sequences revealed high sequence similarity to the *H. ripae* from previous studies (Subbotin *et al*. 2022; Yang *et al.* 2024) and the sequences directly submitted to GenBank (South Korea). The sequences from the Polish populations varied the most from the sequences from China reported for buckwheat (Yang *et al.* 2024), especially in the case of the mitochondrial sequences. Moreover, the results of the pot experiments, conducted under greenhouse conditions, indicated that *H. ripae* individuals did not develop on buckwheat and sorrel roots, which in the first case did not confirm the report of Yang *et al.* (2024). This may be due to the fact that different *H. ripae* haplotypes were obtained in Poland than in the Chinese studies. The mtCOI polymorphism was already observed in other cyst-forming nematode species of the genus *Heterodera* (Subbotin *et al.* 2024).

In the experiment, the development of a *H. ripae* population was observed on the roots of the nettle, which is a natural host plant for this nematode. However, the nettle plants did not show any wilting symptoms under either natural or controlled conditions. The number of nematodes in the experiment (100–120 eggs and larvae in 100 ml of soil) and the nematode

density in the soil adjacent to the nettle roots (average 350 eggs and larvae in 100 ml of soil) may not have been high enough to lead to starvation and plant death. The combined effect of the nematode and the environment under natural growing conditions was not strong enough to cause the death of the plant. This phenomenon was observed in sugar beet, in soils intensively cultivated with the beet cyst nematode *H. schachtii* Shmidt, 1891 (Smith *et al.* 2004). Nettle plants could also independently control nematodes, due to their natural properties. Recently, aqueous leaf extracts from *U. dioica* have been shown to significantly inhibit egg hatching and increase mortality of *M. incognita* (Kofoid & White, 1919) J2 nematodes (Chandana *et al.* 2025). Nettle has also been shown to have antifungal, antiviral, antibacterial, and insecticidal properties (Martz and Kankaanpää 2025). Nevertheless, it is important to note that although the plant does not suffer from a minor nematode infection, it may be a source of infection for other common nettle plants. Therefore, during cultivation, all possible measures should be taken to prevent the spread of pests.

The most important source of nettle, a perennial plant, is field cultivation. Due to its rich chemical compounds, nettle is used in medicine, cosmetics, and feed production (Grygierzec and Szewczyk 2021; Devkota *et al.* 2022; Martz and Kankaanpää 2025). It is also used as a fiber plant in the spinning industry (Grygierzec and Szewczyk 2021; Viotti *et al.* 2022). Therefore, it is recommended that seedlings of *U. dioica* intended for cultivation as a horticultural plant should be tested for the presence of *H. ripae*, and common nettle plantations should be monitored for the presence of other herbivorous nematodes, including cyst-forming species.

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Table 1. Morphometrics of cysts of *Heterodera ripae*. Data are in a form mean ± SD (range). All abbreviations are used according to Brzeski (1998)

	This study	Brzeski 1998	Subbotin *et al.* 1997	Subbotin *et al.* 1997	Eroshenko *et al.* 2001	Wang *et al.* 2012	Yang *et al.* 2024
Locality			Moscow	Münster	Primorsky Territory	Shenyang	Liupanshui
Host	Poland *Urtica dioica*	Poland *U. dioica*	Russia *U. dioica*	Germany *U. dioica*	Russia *U. laetevirens*	China *Urtica* sp.	China *Fagopyrum esculentum*
n	18		50	25	25	10	10
Body length (excluding the neck) µm	498 ± 59 (350–600)	460 (290–600)	462 ±7.9 (348–580)	415 ± 9.6 (289–497)	505 ± 44 (416–585)	437.6 ± 37.4 (379.4–488.4)	439 ± 31.9 (388–473)
Body width	406 ± 62 (260–490)	340 (210–550)	327 ±9.0 (212–454)	307 ± 9.4 (214–383)	375 ± 52 (299–520)	321.7 ± 40.3 (262.0–378.6)	344 ± 38.9 (284–416)
Length/width	1.2 ± 0.1 (1.1–1.4)	1.3 (1.1–1.8)	1.4 ± 0.3 (1.1–1.8)	1.4 ± 0.02 (1.1–1.7)	1.3 ± 0.02 (1.1–1.6)	1.4 ±0.1 (1.3–1.5)	1.3 ± 0.1 (1.1–1.5)
n	15		35	15	25	10	10
Fenestrate length	54 ± 4.4 (48–65)	(31–60)	46 ± 0.8 (31–58)	46 ± 1.5 (38–56)	48 ± 5.7 (40–55)	47.6 ± 3.6 (42.9–53.7)	44 ± 7.1 (35–53)
Semifenestrae width		26 (17–40)					
Mean Semifenestrae width	25.8 ± 3.2 (18–30)		26.3 ± 0.7 (16.6–38.2)	24.8 ± 0.9 (18–31.7)	27 ± 4.3 (22–34)	25.3 ± 2.4 (22.1–29.5)	23.8 ± 3.9 (20–28)
Underbridge length	79.6 ± 6.9 (70–85)		78 ±2.3 (70–88)	69 ±2.2 (50–90)	71	75.1 ± 3.3 (70.2–79.7)	69.9 ± 13.6 (52–95)
Vulval slit length	39.7 ± 3.4 (33–45)	35 (26–44)	33.8 ± 0.6 (28.2–41.5)	33 ± 0.9 (26–41)	37 ± 4.0 (31–43)	34.2 ± 2.3 (31.2–37.6)	31.9 ± 4.3 (28–39)
Vulval bridge width	17.3 ± 3.7 (10–20)		10.7 ± 0.9 (6.6–18.2)	9 ± 0.5 (6–14)	12 ± 2.2 (9–16)	10.0 ± 1.0 (9.0–11.9)	9.1 ± 1.7 (6.5–12)
Vulva-anal distance	52.1 ± 5.8 (40–58)		47 ±1.4 (36–63)	43 ± 3.9 (24–75)	45 ± 5.3 (37–53)	47.1 ± 5.8 (39.4–55.2)	

Table 2. Morphometrics of J2 of *Heterodera ripae*. Data are given as form mean ± SD (range). All abbreviations are used according to Brzeski (1998)

	This study	Brzeski 1998	Subbotin *et al.* 1997	Subbotin *et al.* 1997	Eroshenko *et al.* 2001	Wang *et al.* 2012	Yang *et al.* 2024
Locality			Moscow	Münster	Primorsky Territory	Shenyang	Liupanshui
Host	Poland *Urtica dioica*	Poland *U. dioica*	Russia *U. dioica*	Germany *U. dioica*	Russia *U. laetevirens*	China *Urtica* sp.	China *Fagopyrum esculentum*
n	15		52	25	20	12	10
L body length	352 ± 12 (321–371)	370 (330–410)	373 ± 2.1 (342–407)	359 ± 2.3 (336–381)	370 ± 26 (330–432)	400.9 ± 30.1 (352.8–444.9)	369 ± 21.2 (340–415)
Body with at mid-body	17.8 ± 1.4 (15–20)		18.0 ± 0.1 (16.3–20.1)	16.7 ± 0.1 (15.2–18.4)	19.0 ± 1.0 (18–21)	20.7 ± 2.0 (18.1–24.2)	18.3 ± 0.8 (17–19)
Body with at anus	11.4 ± 0.7 (10–13)		11.2 ± 0.1 (9.9–12.8)	10.7 ± 0.1 (10.0–11.6)	11 ± 1.2 (9–12)		12 ± 0.8 (11–13)
a	19.9 ± 1.5 (17.8–22.8)	20 (18–23)	20.7 ± 0.1 (18.7–22.8)	21.6 ± 0.1 (19.7–22.6)	21 ± 1.7 (18–24)	19.5 ± 1.9 (16.7–23.9)	20.2 ± 1.7 (17.9–24.5)
b		3.4 (2.9–4.0)	3.3 ± 0.03 (2.9–4.0)	3.7 ± 0.04 (3.5–4.0)	3.4 ± 0.2 (3.2–3.7)	3.5 ± 0.3 (2.9–3.9)	3.4 ± 0.3 (3–3.8)
b’	2.2 ± 0.1 (2.1–2.6)	2.3–2.6					
c	7.6 ± 0.6 (6.6–8.6)	8.3 (7.1–10)	8.0 ± 0.1 (7.4–10.0)	8.1 ± 0.1 (7.1–8.9)	7.7 ± 1.7 (7.1–10.1)	9.3 ± 1.6 (7.2–12.2)	6.5 ± 0.3 (6–7.1)
c’	4.1 ± 0.3 (3.3–4.9)	3.8 (3.2–5.0)	4.1 ± 0.04 (3.4–4.5)	4.2 ± 0.1 (3.8–5.0)	-	3.6 ± 0.2 (3.2–4.0)	4.8 ± 0.2 (4.4–5.2)
Stylet length	21.8 ± 0.7 (20–22)	22 (20.5–24.5)	21.7 ± 0.1 (20.3–23.5)	22.1 ± 0.1 (20.8–23.8)	24 ± 0.7 (22–25)	21.6 ± 0.7 (20.2–22.4)	20.3 ± 0.5 (20–21)
Tail length	46.7 ± 4.3 (40–59)	44 (36–52)	47 ± 0.4 (36–50)	45 ± 0.6 (41–52)	46 ± 4.7 (38–54)	43.7 ± 6.1 (33.6–52.2)	57 ± 4 (48–61)
Hyaline part of tail (H)	22.8 ± 1.8 (19–25)	21 (16–27)	22.9 ± 0.3 (18.1–27.5)	23 ± 0.3 (18.4–25.6)	23 ± 2.5 (20–32)	21.6 ±0.7 (20.2–22.4)	25 ± 1 (23.0–26.0)

Table 3. Information about GenBank sequences used for phylogenetic analyses of this study

Species	28S rDNA		mtCOI	
	GenBank number	Country	GenBank number	Country
Heterodera amaranthusiae	-	-	PX529407	China
Heterodera arenaria	-	-	MG522942	United Kingdom
Heterodera aucklandica	DQ328688	New Zealand	MG523089	United Kingdom
Heterodera australis	OR701399	Australia	PQ877274	Australia
Heterodera avenae	GU595444	China	MW767617	China
	LT159823	Algeria	PQ517160	NI*
Heterodera betae	LC208670	Netherlands	MW345314	Germany
Heterodera bifenestra	-	-	MW374275	The Netherlands
Heterodera cajani	DQ328693	India	MW345412	India
	KY660057	NI*	-	-
Heterodera camelliae	PP923572	China	PP919169	China
Heterodera cardiolata	-	-	MW374266	Pakistan
	-	-	MW374265	Pakistan
Heterodera carotae	KX463288	Italy	MN820659	South Africa
	KX463291	Canada	MW363076	Italy
Heterodera ciceri	-	-	MW345320	Syria
Heterodera circeae	-	-	MW363065	Germany
	-	-	MW363066	Germany
Heterodera cruciferae	KP114546	NI*	MW363074	USA
	LC884693	Japan	MW363078	USA
	JX402414	Italy	-	-
Heterodera cyperi	-	-	MW374286	Spain
Heterodera daverti	KT163230	Italy	ON926517	Australia
Heterodera dunensis	-	-	MT511095	Spain
Heterodera elachista	KC618464	Italy	KC618473	Italy
	KM017066	NI*	MH144207	Iran
Heterodera fengi	JX566451	China	-	-
Heterodera fici	LT996914	NI*	MT808373	Iran
	-	-	MT911895	Greece
Heterodera filipjevi	HM147945	China	PQ096969	USA
	-	-	PQ096972	Greece
Heterodera galeopsidis	-	-	MW345310	Iran
Heterodera glycines	JQ067684	NI*	MT644154	Korea
	LC208673	Japan	PQ285514	China
Heterodera goettingiana	DQ328697	Germany	MW363085	Germany
	-	-	MW363087	France
	-	-	MW363086	Iran
Heterodera goldeni	-	-	MW374280	Iran
	-	-	MW374278	Iran

Heterodera guangdongensis	KM224882	China	-	-
	MF425686	Vietnam	MF425735	Vietnam
Heterodera hainanensis	JX081323	China	-	-
Heterodera hordecalis	LT159828	Algeria	PQ096964	Greece
	LT159829	Algeria	PQ096965	Greece
Heterodera humuli	MG598808	Iran	MT808372	Kyrgyzstan
	-	-	OQ558837	USA
Heterodera koreana	LC202114	Japan	MW642454	Korea
	LC202132	Japan	OL813219	NI*
Heterodera latipons	HM560854	Jordan	MG523129	Russia
Heterodera litoralis	DQ328691	New Zealand	MT808378	New Zealand
Heterodera mani	PQ621790	Portugal	PQ096967	Greece
Heterodera medicaginis	-	-	MW345342	Russia
	-	-	PX519311	China
Heterodera mediterranea	LT990599	NI*	MW345393	Spain
	LT990600	NI*	MW345409	Spain
	-	-	MW345407	Spain
Heterodera microulae	-	-	MT576084	China
Heterodera orientalis	EU284033	USA	-	-
Heterodera oryzae	-	-	MT823012	South Korea
Heterodera oryzicola	DQ328694	India	-	-
Heterodera persica	-	-	MW363067	Iran
	-	-	MW363068	Iran
Heterodera pratensis	MN741142	South Korea	KC172916	Belgium
	-	-	KU147200	Russia
Heterodera ripae	-	-	MT808349	Slovakia
	-	-	MT808350	Greece
	-	-	MT808352	Belgium
	-	-	MT808354	Germany
	-	-	MT808356	Russia
	OQ064080	South Korea	ON007078	Russia
	OQ064081	South Korea	OQ048424	South Korea
	OR468129	China	OR468243	China
	PZ095079	Poland	PZ095247	Poland
Heterodera sacchari	-	-	MW374284	Ghana
Heterodera salixophila	-	-	MT808384	Germany
	DQ328690	Belgium	MT808391	Belgium
	-	-	MT911890	Russia
Heterodera schachtii	JQ040526	USA	MW345384	Spain
	-	-	MW856645	China
	-	-	PQ724975	South Korea
Heterodera sojae	KU160509	Korea	MT644153	Korea
	KU160511	Korea	PX560107	China
	-	-	PX559963	China
	-	-	PX559861	China
Heterodera sonchophila	-	-	MW345341	Estonia

Heterodera sorghi	DQ328689	India	-	-
Heterodera sturhani	PX647854	China	KU147199	China
Heterodera trifolii	LC208669	Japan	MZ882164	NI*
Heterodera tropica	PV962729	Mexico	PV962830	Mexico
Heterodera turcomanica	-	-	MT813456	Iran
Heterodera urticae	DQ328696	Belgium	-	-
Heterodera ustinovi	-	-	MG523094	Belgium
Heterodera vallicola	- OR399076	- Japan	MT808357 OR486007	Vietnam Japan
Heterodera zeae	GU145612	Greece	PX506304	China
Mesocriconema curvatum	-	-	KJ787851	USA
Mesocriconema xenoplax	MG680454	Portugal	-	-

* NI = No information

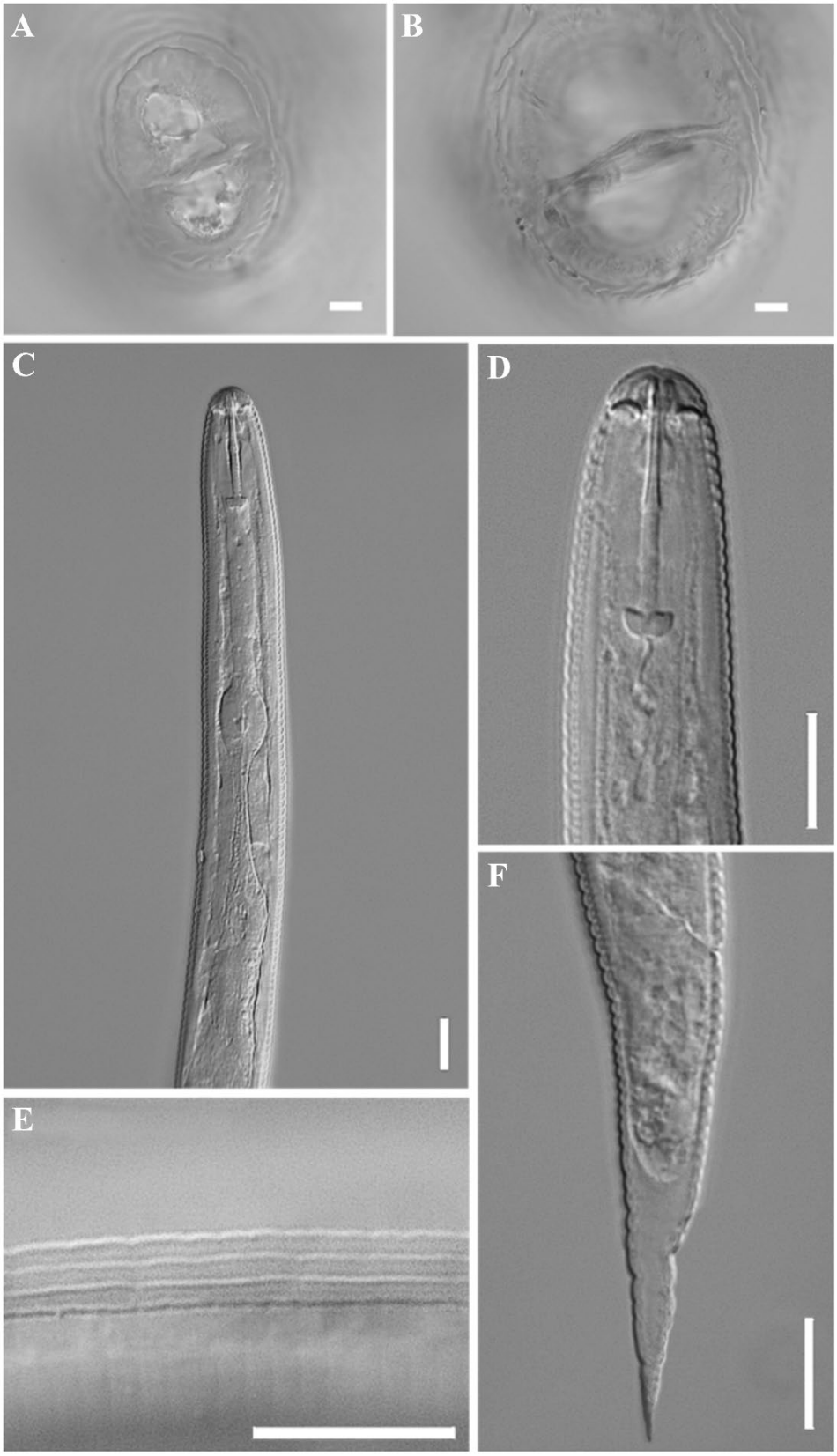

Fig. 1. *Heterodera ripae*. Vulval cone. (A) anterior view, (B) underbridge, (C) anterior end, (D) stylet, (E) lateral field, (F) tail. Scale = 10 µm

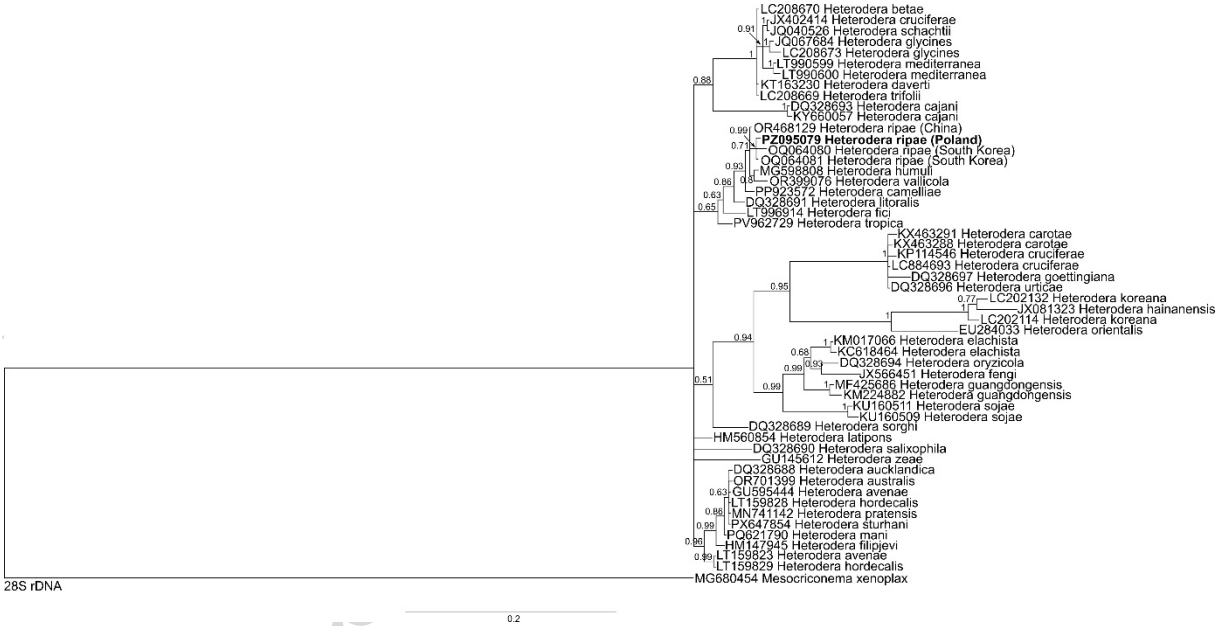

Fig. 2. 28S rDNA-based Bayesian phylogeny of the genus *Heterodera*. The new sequence of the Polish *H. ripae* is indicated in bold. Numbers near nodes indicate posterior probabilities

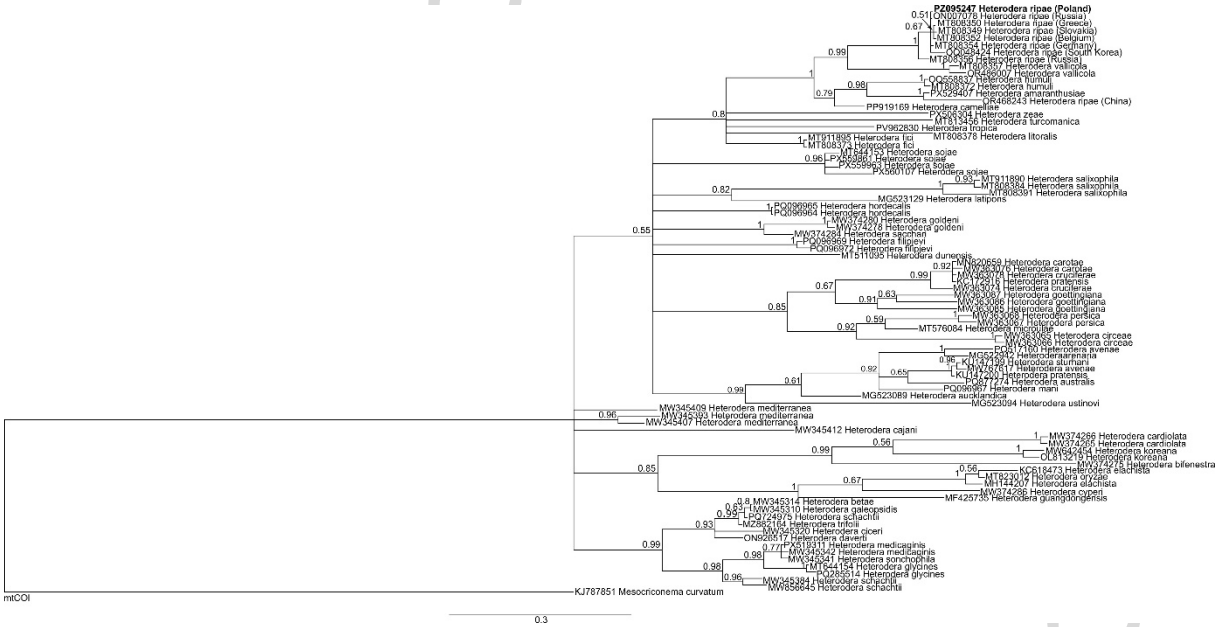

Fig. 3. mtCOI -based Bayesian phylogeny of the genus *Heterodera*. The new sequence of the Polish *H. ripae* is indicated in bold. Numbers near nodes indicate posterior probabilities