CONCERNING A SWEDISH POPULATION OF *HETERODERA RIPAE* SUBBOTIN, STURHAN, WAEYENBERGE *ET* MOENS, 2003

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Summary. Based on morphological and morphometric characters, a Swedish population of cyst nematodes from *Urtica dioica* was identified as *Heterodera humuli* by Andersson (1979). Subbotin *et al.* (1997) found that cyst nematode populations on *U. dioica* and *Humulus lupulus* could also belong to a species very similar to *H. humuli* and until then not described. A re-examination of our population in 2006, also using molecular biology facilities now available, indicates that the nematode reported in 1979 belongs to that species, *H. ripae* Subbotin, Sturhan, Waeyenberge *et* Moens, 2003. *Heterodera humuli* has not so far been found in Sweden. There are indications that also the species in reports by other authors may have been *H. ripae* rather than *H. humuli*.

Key words: Cyst nematodes, *Heterodera humuli*, Sweden, *Urtica dioica*.

In 1976, on sampling in and around a field of seed potatoes at Flyinge, Skåne, southern Sweden, Andersson (1979) found nematode cysts, the morphology and morphometrics of which were well within the limits of *Heterodera humuli* Filipjev, 1934. The nematode was readily propagated on *Urtica dioica* L., a plant species that grew abundantly in the field border, and mentioned as a host for *H. humuli* (among others by Franklin, 1951; de Grisse and Gillard, 1963). For these reasons and others discussed below, the nematode found was considered to belong to this species. Some twenty years later Subbotin *et al*. (1997) described *Heterodera riparia*, a species closely related to *H. humuli*, and having *U. dioica* as a good host. Later, this nematode was renamed *H. ripae* Subbotin, Sturhan, Waeyenberge *et* Moens (2003). Subbotin *et al.* (1997) also considered *U. dioica* to be a rather poor host of *H. humuli*. Altogether, this new information cast some doubt on the identity of the Swedish population of *H. humuli* reported by Andersson in 1979. Therefore, a renewed investigation was made of this population in 2006, using the appropriate molecular biology tools now available.

MATERIALS AND METHODS

Nematode population. The nematode material originated from the population mentioned above and had been propagated every 1-2 years on *Urtica dioica* in a bucket of the original soil in a glasshouse at Alnarp. The equilibrium density of the population seemed to be about 200-300 eggs/g soil.

Light microscopy observations. Measurements of cysts and eggs had already been made at the beginning of the 1990s, and second-stage juveniles were measured in 2006. Cysts were measured in a dry condition and cyst cones after mounting on slides with glycerine jelly/glycerine as described by Hooper (1985). Eggs were observed in water as temporary mounts on glass microscope slides. All observations and measurements were made using a Leitz Dialux light microscope with interference contrast and *camera lucida* measurement equipment.

Second stage juveniles of the nematode were gently killed by heat in water, and temporary mounts in water were made. The specimens were then observed under a Leitz Dialux 2 microscope and photographed using a Leica DFC320 Digital FireWire Color Camera System, coupled to the microscope, for analysis and documentation. The Leica IM 1000 management application was used for the acquisition, processing, measurement and output of images.

DNA extraction, sequencing and sequence analysis. Forty dry cysts were transferred into an Eppendorf tube and the total genomic DNA was extracted using Extract-N-Amp^{TM} Tissue PCR Kit (Sigma-Aldrich, Inc.) according to the manufacturer's instructions. The sample was homogenised with a sterile, motor-driven pellet pestle (Sigma-Aldrich, Inc.).

A fragment of the 5.8S rDNA and flanking ITS1 and ITS2 regions was amplified by the polymerase chain reaction (PCR) using primers and protocol described by Subbotin *et al.* (2001). The PCR product was treated with the QIAquick PCR Purification Kit.

Single-stranded sequencing was carried out using BigDyeTM, Terminator 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Warrington, UK), and run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.).

Table I. Morphometrics of cysts, eggs and second-stage juveniles of a cyst nematode population described as *Heterodera humuli* by Andersson (1979).

Stage	Character	Measurements (µm) and quotients		
		Mean	Range	s^1
Cyst body (n = 122)	Length (excluding neck)	430.4	273-687	74.2
	Width	333.5	172-545	66.7
	Length/width	1.31	1.03-1.65	0.14
Cyst vulval area	Fenestra length (n = 38)	51.5	33.5-61.0	6.5
	Fenestra width (n = 38)	20.6	12.0-25.5	3.0
	Vulval slit length (n = 37)	36.3	25.5-45.0	5.6
	Vulval bridge width (n = 38)	13.3	7.5-22.5	3.3
	Underbridge length (n = 18)	78.9	61-101	11.1
	Underbridge width (n = 18)	7.1	3.0-15.5	3.4
	Vulva-anus distance (n = 38)	49.1	35-70	8.3
	Fenestra length/width (n = 38)	2.52	2.02-3.16	0.25
Egg (n = 100)	Length	92	76-107	4.9
	Width	39	35-45	2.0
	Length/width	2.37	1.79-2.87	0.18
Juvenile (n = 30)	Body length	394	333-429	26.5
	Body width (mid-body)	19.4	17.8-22.4	1.00
	Stylet length	20.6	19.1-23.0	0.94
	Tail length	44.9	37.6-52.3	3.8
	Hyaline tail length	22.7	19.0-29.1	2.0
	a	20.3	17.5-22.1	1.4

[1] s = standard deviation.

The sequences were reviewed and edited using BioEdit 7.0.0. Assembly into a complete sequence was performed in Contig Express in Vector NTI (InforMax, Inc. Frederick, MD, U.S.A.). A sequence similarity search was made by comparing our sequence with all sequences available in the public nucleotide database at NCBI (National Center for Biotechnology Information, Bethesda, MD, U.S.A.) using nucleotide-nucleotide BLASTn software.

RESULTS

The measurements of cysts, eggs and second-stage juveniles are summarized in Table I.

The gene sequence considered was 996-bp long and contained a part of the internal transcribed spacer 1 (595 bp), the complete 5.8S rRNA gene (158 bp) and a part of the internal transcribed spacer 2 (243 bp). Sequence data is available online at: http://www.ncbi.nlm.nih.gov, accession number DQ846902. A comparison revealed a 100% sequence homology with AF274407, *H. ripae* from Germany, whereas a distinctly lower homology was found with *H. humuli*.

DISCUSSION

Most cyst and juvenile characters of our population are more similar to the *H. riparia* (= *H. ripae*) populations than to the *H. humuli* populations given by Subbotin *et al.* (1997: Tables 1 and 2). An exception is the juvenile length, which is more similar to that of the *H. humuli* populations. However, the gene sequencing conclusively shows that our population belongs to *H. ripae*.

As mentioned above, several authors have reported *Urtica dioica* to be a host of *Heterodera humuli*. Filipjev, referred to by Franklin (1951), assumed that cysts found on *U. dioica* belonged to *H. humuli*. In the population mentioned in 1979 by the first author (Andersson, 1979), the population now re-described, the size of the cysts was on average somewhat smaller than cysts from most *H. humuli* populations mentioned in the literature. However, de Grisse and Gillard (1963) found that *H. humuli* cysts from *U. dioica* were smaller (actually of about the same size as our cysts) than from hops. It is not clearly stated in their paper if the population with the small cysts was originally derived from hops, but it seems likely from the context; however some uncertainty may remain.

Still another reason in 1979 to believe that our population belonged to *H. humuli* was a paper by Webley

(1974), who found many cysts in England of what he considered to be *H. humuli* in organic debris in the bottom of a filled-in well from the 9th century. As the most common weed seeds found in the debris together with the cysts were from *U. dioica*, and as hops were not introduced into England until the 16th century, he concluded that *U. dioica* had been the host of the *H. humuli* cysts. In the light of the present knowledge, it does not seem unlikely that the cysts might have been from *H. ripae*. If possible, a DNA examination of Webley´s material should be carried out. A general conclusion of the splitting of *H. humuli* into two species may be that older reports of *H. humuli* on *U. dioica* should be treated with caution unless numerous cysts of the same population have been produced on hops.

Heterodera humuli has so far not been found in Sweden, and there appears to be no reliable record of the hop cyst-nematode for other Scandinavian countries. *Heterodera ripae* has, among other records, been reported from the Baltic states Estonia and Latvia (Subbotin *et al.*, 1997).

ACKNOWLEDGEMENTS

The authors are indebted to Mrs. Christina Harström, Mrs. Greta Mårtensson and Mr. Maimoun Hassoun for technical assistance, and to Dr. Dieter Sturhan for valuable criticism of the typescript.

Accepted for publication on 15 November 2006.

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