

## Morphological Characteristics and Distribution of *Globodera* Species in Slovenia

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### Abstract

Surveillance on cyst nematodes in Slovenia started already in 1963. We have found *Globodera rostochiensis* for the first time in 1971 and for the second time in 1975 (two cysts). In 1999 cysts of yellow potato cyst nematode were found again. A small hot spot was detected along the Slovene Austrian border. Cysts of *Globodera rostochiensis* were extracted from samples taken in 1.6 hectare field. In 2001 we surveyed 194 fields with the surface of 468.29 ha. We discovered another hot spot of *Globodera rostochiensis* in the Central Slovenia. Cysts of *Globodera achilleae* are seldom discovered in Slovenia. During the period between 1980 and 2000 we extracted altogether 206 cysts. In 2001 we found three fields where we extracted at first seven cysts. With more detailed survey we extracted another 30 cysts. In a few soil samples taken from the imported potatoes from Italy we have intercepted cysts of *Globodera pallida*. For that reason shipments were returned. In a similar way in 2002 shipments from Croatia were returned because of *G. rostochiensis* presence. Specimens of *Globodera rostochiensis* and *G. achilleae* species found in Slovenia were morphometrically handled.

**Keywords:** cyst nematodes; *Globodera*; Slovenia; morphology; geographical distribution

### INTRODUCTION

In the area of former Yugoslavia the cyst-forming nematodes were studied rather intensively. GRUJIČIĆ (1958) reported for the first time on sugar beet cyst nematode (*Heterodera schachtii*). GRUJIČIĆ and KRŃJAIĆ (1966) reported on the spreading of *Heterodera cruciferae* in the area of Serbia and they discovered a rather strongly felt presence of this nematode in the fields in which cabbage was grown intensively (Futog, Bačko Petrovo Selo). KORUNIĆ (1968) reported on the spreading of cyst-forming nematodes in Croatia. He established seven species: *H. punctata*, *H. rostochiensis*, *H. humuli*, *H. avenae*, *H. cruciferae*, *H. trifolii* and *H. schachtii*. Data from 1974 provided by KLINDIĆ and PETROVIĆ (1974) dealt with the spreading of the species *Heterodera* (*Globodera*) *achilleae* in the area of Bosnia (Fojnica), Croatia (Žumberak, Delnice) and Slovenia (Sorško polje). KORUNIĆ and OŠTREC (1981) reported on the spreading of sugar beet cyst nematode (*Heterodera schachtii*) in the area of Croatia, and, in the same year, Krnjaić reported

on the spreading of this nematode in the whole area of Yugoslavia (KRŃJAIĆ *et al.* 1981).

Until now, several articles have been published on the spreading of nematodes in the area of Slovenia (HRŽIČ & UREK 1987, 1988, 1989a,b, 1990a,b, 1992, 1993, 1995, 1996). *Heterodera trifolii* is evidently the most frequent species in Slovenia. Beside *H. trifolii* six other species of the genus *Heterodera*: *H. schachtii*, *H. cruciferae*, *H. goettingiana*, *H. galeopsidis*, *H. humuli* and *H. carotae* could be found in Slovenia. In addition to *Heterodera* species one species with pear formed cysts (*Punctodera punctata*) and two species with round formed cysts, *Globodera rostochiensis* and *G. achilleae* have been extracted from the arable soil in Slovenia. In a few soil samples taken from the imported potatoes from Italy we intercepted *Globodera pallida* cysts.

### MATERIAL AND METHODS

In frame of a systematic surveillance of arable soil we have been taking soil samples from the fields of

seed and ware potato, and beet situated in various Slovene regions (Dolenjska, Gorenjska, Koroška, Prekmurje and Štajerska) for several years and inspecting them to the presence of cyst-forming nematodes of the *Heteroderidae* family. The intensity of sampling (the number of checkpoints) is co-ordinated with the intensity of potato production in a particular region and it is directed towards areas in which seed potato and sugar beet are grown.

Soil samples were taken with special augers from 10 cm depth. Each soil sample (500–1000 cm<sup>3</sup>) consisted of 50–70 cores. The number of samples was depended on the size of the field and on the uniformity of studied land. One sample/0.5 ha was taken from smaller areas (up to 5 hectares) while the number of samples taken from larger areas was smaller. At least one sample was taken from an area measuring up to 4 hectares.

Soil samples were air dried. Organic soil particles including cysts from soil samples were extracted on 250 µm sieve using improved ARS-USDA washing device. The content remaining on the sieve was cleaned from the remaining inorganic particles using funnel system and transferred to the filter paper. A suitable

form obtained for microscopic inspection. Cysts were determined on the basis of morphological characteristics along with the use of determination keys and computer program for the image analysis called Lucia.

## RESULTS AND DISCUSSION

Until now, two species with round formed cysts belonging to genus *Globodera*, *G. rostochiensis* and *G. achilleae* were found in Slovenia. The first actual finding of PCN in Slovenia dates back to 1971 when one cyst of *G. rostochiensis* was extracted from the soil sample taken from ware potato field in the area of Dobrova near Dravograd near the Austrian-Slovene border. In 1975, a potato nematode cyst was found again in this area. In spite of a more thorough survey of the field in which the mentioned cysts had been found, no additional potato nematode cysts were extracted from the soil at that time. In Slovenia, potato nematode was not encountered after the year 1975 until 1999 in spite of intensive inspection of fields. In 1999, *G. rostochiensis* was found again in soil samples taken from the field located near the Slovene-Austrian border (UREK & LAPAJNE 2001). In the year



Figure 1. Distribution map of *Globodera* species in Slovenia

Table 1. Morphologic characteristics and measurements of cysts and J2 larvae – Slovenian population of *Globodera* sp.

Characteristics		<i>Globodera rostochiensis</i> (Woll., 1923) Behrens 1975	<i>Globodera achilleae</i> GOLDEN & KLINDIČ (1973)
Cysts	L – cyst length ( $\mu\text{m}$ )	355–820	370–581
	W – cyst width ( $\mu\text{m}$ )	292–792	271–545
	Fenestra diameter ( $\mu\text{m}$ )	10.3–21.7	13.6–16.5
	Distance fenestra to anus ( $\mu\text{m}$ )	42.5–81.2	21.5–37.7
	Distance fenestra to anus/fenestra diameter	1.9–4.7	0.9–1.4
	No. of cuticular ridges between vulva and anus	12–22	58
J2 larvae	L – body length ( $\mu\text{m}$ )	434–469	472–519
	Stylet length ( $\mu\text{m}$ )	19.8–21.5	23.7–27
	Tail length ( $\mu\text{m}$ )	38.6–51.5	48.6–53
	Body width at anus level ( $\mu\text{m}$ )	10.1–12.8	13–14.7
	Hyaline part of tail ( $\mu\text{m}$ )	20.6–29.1	21–26.4
	Stylet knob shape	anterior surface rounded	anterior surface rounded

2001, *G. rostochiensis* was found for the first time also in the central part of Slovenia, i.e. near Šenčur in Gorenjska (Figure 1).

The first report concerning yarrow nematode *G. achilleae* dates back to 1973, when the new species belonging to the cyst-forming nematodes with round cysts was described by GOLDEN and KLINDIČ (1973). Cysts were extracted from the soil samples taken from the fields in the surroundings of Fojnica situated in Bosnia and Herzegovina. Host tests showed that yarrow, *Achillea millefolium* L., was heavily attacked whereas the potato was not (GOLDEN & KLINDIČ 1973). The spreading of yarrow nematode *Heterodera (Globodera) achilleae* in Bosnia and Herzegovina (Fojnica), Croatia (Žumberak, Delnice), and Slovenia (Sorško polje) was reported by KLINDIČ and PETROVIČ in 1974. The cysts of yarrow nematode are relatively uncommon and were extracted mainly from the soil samples taken in the central part and sometimes in the north-eastern part of Slovenia (UREK & HRŽIČ 1993). Until present, eight hot spots have been registered in the area of Gorenjska and one in each area of Koroška, Dolenjska and Štajerska (Figure 1).

In nematology, morphology has traditionally been the basis used for identification and systematics. In cyst nematodes, several stages are important and useful both in morphology and in identification. The cysts,

juveniles and eggs, are of the greatest value and the most widely used for that purpose (GOLDEN 1986). Based on the microscopic examination of morphologic characteristics and measurements of J2 larvae and cysts, we established following characteristics of Slovenian populations of *Globodera* species presented in Table 1.

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