Russian Journal of Nematology, 2004, 12 (2), 161 - 168

Morpometrical and ribosomal DNA sequence analysis of *Globodera rostochiensis* and *Globodera achilleae* from Slovenia

Saša Širca and Gregor Urek

Agricultural Institute of Slovenia, Hacquetova 17, 1001 Ljubljana, Slovenia,
e-mail: sasa.sirca@kis.si

Accepted for publication 17 September 2004

Summary. Differences between two populations of the yarrow cyst nematode *Globodera achilleae* and one population of the potato cyst nematode *G. rostochiensis* from Slovenia were analysed using morphological characters of cysts and second stage juveniles and molecular data of the internal transcribed spacer region ITS 1, 5.8S and ITS 2. *Globodera achilleae* and *G. rostochiensis* were well differentiated using morphometrical method and PCR-RFLP approach. In addition, sequence alignment among *G. rostochiensis* and *G. achilleae* was performed which resulted in 76 nucleotide differences; therefore, 91 % sequence similarity score in the rDNA fragment was determined. Sequences of *G. achilleae* were the most similar to the *G. artemisiae* sequences published in NCBI database. Sequence alignment comparison of *G. achilleae* and *G. artemisiae* resulted in 8 nucleotide difference and a considerable 99 % sequence similarity was estimated.

Key words: identification, internal transcribed spacer, morphology, potato cyst nematode, 5.8S rDNA.

The genus *Globodera* comprises, among others, cyst-forming nematodes that parasitize potato causing severe damage. The most important species are *G. rostochiensis* (Woll.) Behrens, the yellow potato cyst nematode (PCN), and *G. pallida* (Stone), the white PCN. The first report of PCN in Slovenia dates back to 1971 when a single cyst of *G. rostochiensis* was extracted from a soil sample taken from a potato field in the area of Dobrova close to the Slovene-Austrian border (Hržič, 1971). In 1975, a single cyst was found again at the same location. However, no further PCN were encountered in spite of intensive inspections of fields. Only in 1999, a relatively high infestation was discovered in a field located in Libeliče, near the Slovene-Austrian border (Urek & Lapajne, 2001). Spreading of PCN did not occur due to the implementation of appropriate phytosanitary measures and intensive inspections of the fields in Slovenia (Urek & Lapajne, 2001). *Globodera pallida* has not been detected in cultivated soils in Slovenia yet, but it was intercepted frequently in imported consignments. Beside PCN, the yarrow cyst nematode *G. achilleae* has been found repeatedly, mostly in the central part of Slovenia (Klindić & Petrović, 1974; Urek & Hržič, 1993).

The genus *Globodera* comprises more than 10 species, including *G. rostochiensis*, *G. pallida*, *G. artemisiae* (Eroshenko & Kazachenko) Behrens and *G. achilleae* (Golden & Klindić) Behrens, which all are characterized by rounded cysts. *Globodera achilleae* forms cysts on *Achillea millefolium* L. and five other plant species of the Asteraceae but not on potato (Klindić & Petrović, 1974), while *G. rostochiensis* and *G. pallida*, are well known potato pests causing great economic losses worldwide (Trudgill *et al.*, 1975; Turner & Evans, 1998). Therefore, it is necessary to distinguish PCN from other *Globodera* species many of which are considered economically unimportant. Because identification procedures based on morphology can be inaccurate and time consuming, a wide range of molecular tools have been applied for diagnostic purposes (Fleming & Powers, 1998). Several studies have demonstrated that sequences of the internal transcribed spacer regions ITS 1 and ITS 2 of the ribosomal DNA (rDNA) gene cluster are very useful for the

identification of PCN (Ferris *et al.*, 1995; Shields *et al.*, 1996; Subbotin *et al.*, 2000).

In this work the morphometrical and molecular data of the ITS 1, 5.8S and ITS 2 rDNA were used to distinguish *G. rostochiensis* from *G. achilleae* found in Slovenia. PCR was used to amplify the rDNA fragment of ITS 1-5.8S-ITS 2 and fragments of flanking genes. The fragment of rDNA was sequenced and analysed by restriction fragment length polymorphism (RFLP). Neighbour joining tree was constructed using entire ITS region sequences of *G. achilleae* populations from Slovenia (Zadraga and Trbonje), PCN *G. rostochiensis* from Slovenia (Libeliče); and some other European cyst-forming nematodes (sequences from the GenBank). The aspects linked to morphological identification of *Globodera* species are also discussed.

MATERIAL AND METHODS

One population of *G. rostochiensis*, collected from soil of a potato field in Libeliče, and two populations of *G. achilleae*, collected from a corn field in Trbonje and grassland in Zadraga in 2002 were used. Soil samples were airdried for a week and prior to washing, submitted to additional drying at 35°C for 24 hours. Cysts were extracted by sieving using a 250 µm sieve and improved ARS-USDA washing device (Hržič, 1980) and airdried.

Morphometrics. Morphometrical data were generated from ten *G. rostochiensis* and twenty *G. achilleae* mature cysts. A single cyst was cut in drop of water under a dissecting microscope. The posterior part of the cyst, containing juveniles, was used for morphometrical examination of juveniles and cyst. Microscope slide in TAF (trietanolamin-formalin) mounting medium were prepared and examined in order to complete the diagnosis. The anal-vulval region of females and the juvenile morphometrical parameters were analysed using a microscope-computer image analysis system (LUCIA).

DNA extraction and PCR amplification. The anterior parts of the cysts, containing eggs and juveniles, were transferred into 1.5 ml plastic tube. DNA was extracted using the Promega Wizard purification kit according to the manufacturer's instructions. Extracted DNA was diluted in 10 µl of distilled water. A fragment of the partial 18S, ITS1, 5.8S, ITS2 and the partial 28S rDNA was amplified using the primers (forward: 5'-CGT AAC AAG GTA GCT GTA G-3'; reverse: 5'-TCC TCC GCT AAA TGA TAT-3') described by Ferris et al. (1993). PCR reactions contained 1 µl of diluted DNA extract, 10 mM Tris-HCl pH 8.3, 25 mM $MgCl_2$ (Promega), 10 mM of each of the dNTPs (Promega), 1 µM of forward and reverse primers, 1U Taq Polymerase (Promega) and distilled water up to 25 µl. The amplification was carried out in a thermocycler (A&B gene AMP PCR system 2700) using the following program: 94°C for 2.5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 s and polymerisation at 72°C for 1 min, and a final extension cycle at 72°C for 2 min.

ITS-RFLP analysis. A quantity (4 µl) of PCR product was digested with 10 U of the restriction endonucleases. The reactions were carried out with five different restriction enzymes (Fermentas) *Alu*I, *Rsa*I, *Msp*I, *Hinf*I and *Mbo*I at 37°C for 3 h. The restriction fragments were separated on a 2% agarose gel, stained with ethidium bromide and visualised and photographed under UV light.

Sequencing. PCR products were purified using the Quick Yet PCR purification kit (Promega). Sequence reactions contained 2 µl of the BigDye Terminator, 2 µl of the reaction buffer (Sequencing Ready Reaction Kit - Applied Biosystems), 4 µl of the purified PCR and 2 µl of sequence primer. Forward and reverse primers described by Ferris *et al.* (1993) and newly designed reverse primer Glob1 (5'-CTG CGT TCT TCA TCG ATC-3') were used in each separate sequence reaction. The sequence reaction conditions were 25 cycles of 20 s at 96°C, 10 s at 50°C and 3 min at 60°C. The sequence reaction products were purified using 3M sodium acetate and 95% ethanol and analysed using ABI PRISM 310 DNA Sequencer according to manufacturer's instructions. Chromas 2.23 (© 2003 Technelysium Pty Ltd) and BioEdit (©1997-2004 Tom Hall, Ibis Therapeutic Carlsbad, CA) software were used to edit and generate sequence alignments. Sequences of *G. rostochiensis* from Libeliče and *G. achilleae* from Zadraga were deposited in the GenBank (http://www.ncbi.nlm.nih.gov/) (accession numbers AY700060 and AY599498).

Sequence and cluster analysis. For multiple sequence alignment analysis and neighbour joining tree construction the sequences of ITS regions and 5.8 rDNA genes of Slovene *Globodera* species and sequences from the NCBI GenBank of *G. artemisiae* (China), *Punctodera punctata* (The Netherlands), *Cactodera estonica* (Belgium) (Subbotin *et al.*, 2001), *G. artemisiae* (Germany),

G. artemisiae (Sweden) (Manduric & Andersson, 2004), *G. artemisiae* (Estonia) and *G. millefolii* (Estonia) (Ferris *et al.*, 1999) were used. Multiple sequence alignments were made using Clustal X software (Thompson *et al.*, 1997) and neighbour-joining method was applied for tree construction using MEGA3 tool (Kumar *et al.*, unpublished). Bootstrap values based on 1000 re-sampling were determined and the sequence of *Punctodera punctata* (NCBI database accession number AF274416) was used as an out-group taxon.

RESULTS

Morphometric analysis. Morphometric data and other morphological characters of second stage juveniles and cysts indicated that *G. achilleae* can be distinguished from *G. rostochiensis*. Morphometric data are given in Table 1 and 2. The body length and the stylet length of the second stage juveniles distinguished *G. rostochiensis* and *G. achilleae* well. The means of measured *G. rostochiensis* body length and stylet length were 434.4 µm and 20.7 µm, respectively. *G. achilleae* had greater body length than *G. rostochiensis* that ranged from 483.5 µm in the Zadraga population to 516.2 µm in the Trbonje population. Second stage juveniles of both *G. achilleae* populations had longer stylets than *G. rostochiensis*. The mean stylet lengths of *G. achilleae* were 25.1 µm and 24.9 µm for the Trbonje and the Zadraga populations, respectively. Other morphometrical differences between second stage juveniles of both species were less important (Table 1). The rounded stylet knob shape was observed of second stage juveniles of both *G. achilleae* populations and the *G. rostochiensis* population (Fig. 1). Unlike *G. rostochiensis,* the cyst of *G. achilleae* had fewer cuticular ridges (Fig. 2), its distance from anus to vulval basin was smaller and, therefore, the Granek's ratio was also smaller (Table 2).

ITS-RFLP analysis. The PCR amplification yielded a single fragment approximately 1 kbp in length. Restriction site positions of all used enzymes for both Slovenian *Globodera* species are summarized in Table 3. Of the five enzymes used, three resulted in different RFLP profiles of *G. rostochiensis* and *G. achilleae*. With *Alu*I, the *G. rostochiensis* sequence was cut into 4 fragments approximately 380, 325, 150 and 100 bp long. Only one *Alu*I restriction site was detected in the sequence of *G. achilleae* at nucleotide position 14, but the 14 bp long fragment could not be detected in the RFLP analysis. Digestion of sequences with *Rsa*I resulted in three fragments, which were approximately 600, 220 and 170 bp long in *G. rostochiensis* but 600, 280 and 110 bp long in the *G. achilleae* populations. No *Msp*I restriction site was observed in the sequence of *G. rostochiensis*, while one digestion site was observed in *G. achilleae*, which resulted in two fragments, of which one was approximately 510 bp long. Sequence analyses displayed cutting sites for *Hinf*I and *Mbo*I (Figure 3) but no different RFLP product could be observed. All restriction enzymes used in this study separated *G. achilleae* from the white potato cyst nematode *G. pallida* (results not shown).

ITS sequence analysis. We obtained *ca* 900 bp long sequence; 50 bp were not readable at the ends of the fragment. No differences were encountered in the populations of *G. achilleae* from Zadraga and from Trbonje; therefore, both populations shared 100% sequence similarity. *Globodera achilleae* and *G. rostochiensis* differed by 76 nucleotide substitutions, of which 54 were located in the ITS1, 2 in the rDNA 5.8S and 20 in the ITS2. The difference between *G. achilleae* and *G. rostochiensis* resulted in a 91% sequence similarity score.

Sequences of *G. achilleae* and *G. artemisiae* (NCBI database accession number AF274415) were the most similar. They differed by 8 nucleotide substitutions, of which 3 were located in the ITS1 and 5 in the ITS2. Sequences shared a considerable 99% similarity. When align sequences of *G. achilleae* and *G. millefolii* (NCBI database accession number AF161004) were compared there was a sequence similarity of 82%.

Sequences of both *G. achilleae* populations clustered together with sequences of *G. artemisiae* from China, Sweden and Germany. This clade was strongly supported with bootstrap analyses (Fig. 4). Sequences of *G. artemisiae* and *G. millefolii* from Estonia (Ferris *et al.*, 1999) and *C. estonica* from Belgium (Subbotin *et al.*, 2001) formed a separate cluster with high bootstrap values support. Separate clustering with high bootstrap support was also estimated for *G. rostochiensis*.

DISCUSSION

Morphology has been traditionally used for the identification and taxonomic systems of nematodes. In cyst forming nematodes the morphological characters of cysts, juveniles and eggs are important for species identification. The most important features of cysts are found in their terminal region (Golden, 1986). Round cyst-forming species are circumfenestrate, have a vulval

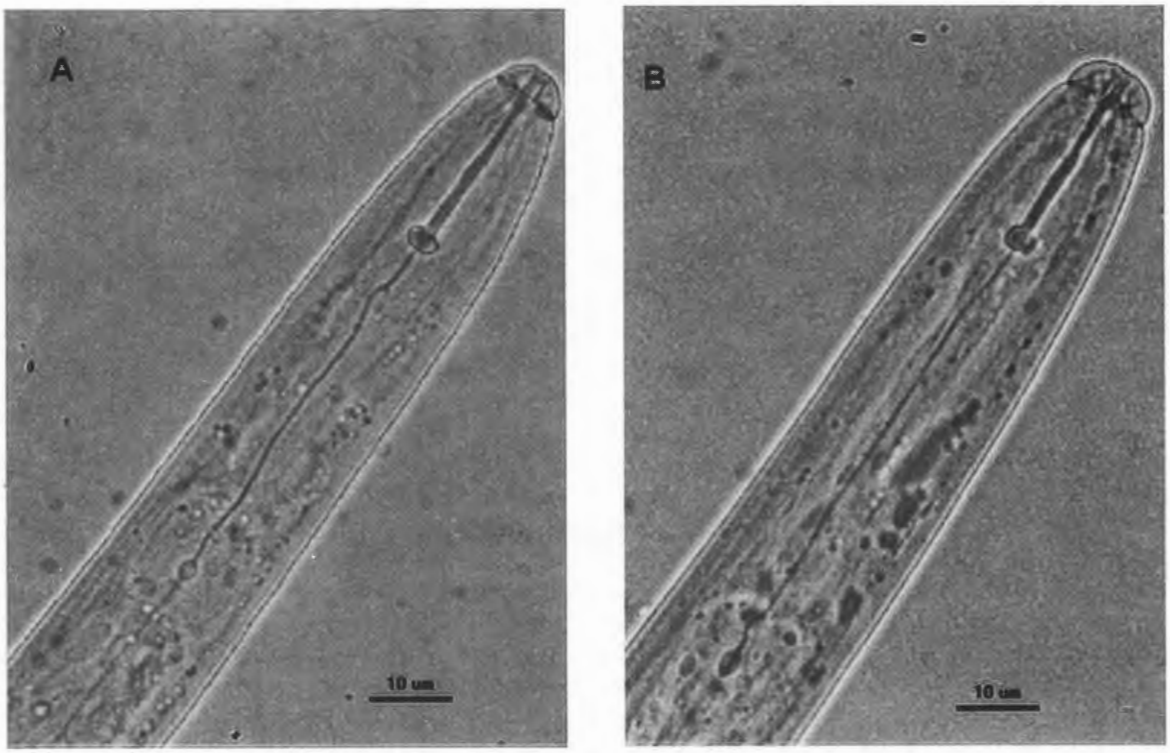

Fig. 1. Light microscope images of the anterior part of the second stage juveniles A: *G. achilleae*; B: *G. rostochiensis*; (Scale bars: 10 µm) found in Slovenia.

Table 1. Morphometrical characteristics of Slovenian *Globodera* second stage juveniles (all measurements in µm).

Character	*G. rostochiensis* Libeliče (n=30)	*G. achilleae* Trbonje (n=40)	*G. achilleae* Zadraga (n=15)
Body length	434.4 ± 16.7 (402.7 - 469.0)	516.2 ± 39.9 (423.8 - 585.0)	483.5 ± 24.7 (455.5 - 535.6)
Body width	20.1 ± 0.6 (18.9 - 22.4)	21.4 ± 1.2 (18.7 - 23.6)	21.6 ± 1.6 (19.3 - 25.0)
Stylet length	20.7 ± 0.6 (19.2 - 21.6)	25.1± 0.8 (23.6 - 26.6)	24.9 ± 0.8 (23.6 - 26.4)
DGO (dorsal gland orifice)	3.8 ± 0.8 (2.4 - 5.0)	4.9 ± 1.1 (2.9 - 7.3)	5.6 ± 1.4 (3.6 - 8.7)
Body width at stylet knobs	16.4 ± 0.5 (15.8 - 17.3)	17.1 ± 0.6 (15.9 - 18.6)	17.7 ± 1.2 (16.3 - 20.4)
Tail length	48.5 ± 2.7 (42.6 - 53.8)	53.2 ± 3.7 (45.0 - 61.7)	49.0 ± 3.5 (45.3 - 57.4)
Tail width	12.7 ± 0.7 (11.3 - 14.4)	13.5 ± 1.0 (10.1 - 15.5)	12.8 ± 0.8 (11.1 - 13.8)
Hyaline part of tail	25.1 ± 1.6 (22.5 - 29.9)	29.8 ± 3.7 (22.4 - 39.1)	25.5 ± 3.2 (21.6 - 32.2)
Distance from centre of median bulb valve to end of head	65.0 ± 6.9 (55.3 - 77.6)	71.0 ± 6.9 (56.1 - 83.1)	68.2 ± 6.4 (58.5 - 80.4)
a	21.4 ± 1.1 (18.9 - 23.0)	24.2 ± 1.5 (20.7 - 27.3)	22.5 ± 1.9 (18.7 - 26.1)
c	9.0 ± 0.6 (7.8 - 11.0)	9.7 ± 0.6 (8.5 - 11.1)	9.9 ± 0.4 (9.3 - 10.5)
c'	3.8 ± 0.3 (3.3 - 4.6)	3.9 ± 0.2 (3.5 - 4.5)	3.8 ± 0.3 (3.5 - 4.5)

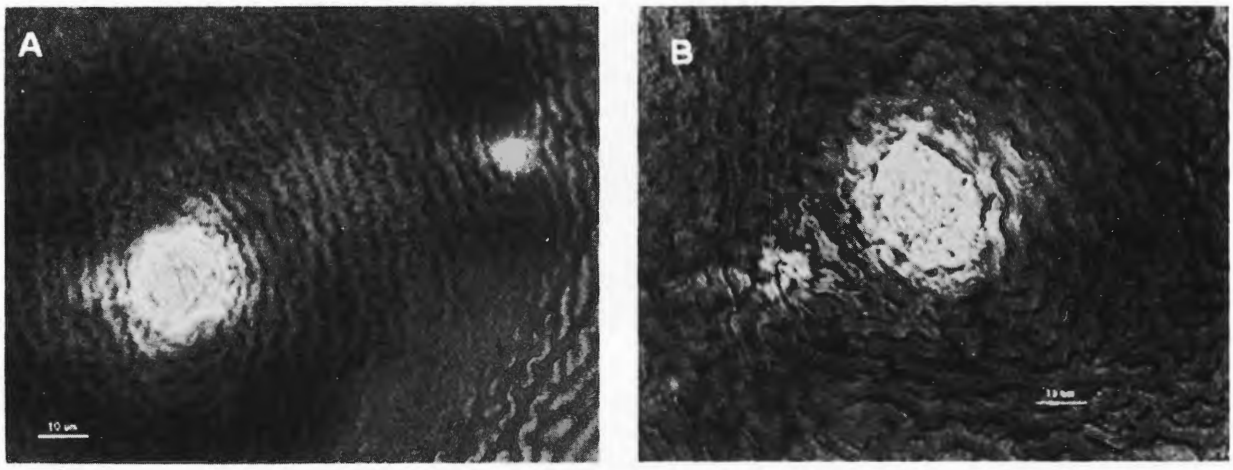

Fig. 2. Light microscope images of the posterior part of the cysts A: *G. rostochiensis*; B: *G. achilleae*; (Scale bars: 10 μm) found in Slovenia.

Table 2. Morphometrical data of Slovenian *Globodera* cysts characters (all meqasurements in μm).

Character	*G. rostochiensis* Libeliče (n=10)	*G. achilleae* Trbonje (n=10)	*G. achilleae* Zadraga (n=10)
Vulval basin diameter [μm]	17.9 ± 1.7 (14.6 - 19.4)	22.3 ± 3.8 (18.7 - 32.3)	20.1 ± 1.6 (18.1 - 22.1)
Distance vulval basin to anus [μm]	64.8 ± 4.7 (58.1 - 73.3)	29.0 ± 5.7 (18.1 - 34.9)	28.6 ± 3.7 (23.8 - 34.1)
No. of cuticular ridges	18.3 ± 1.6 (16 - 22)	5.7 ± 1.2 (4 - 7)	5.6 ± 0.8 (5 - 7)
Granek's ratio	3.6 ± 0.4 (3.0 - 4.3)	1.3 ± 0.3 (0.9 - 1.7)	1.4 ± 0.1 (1.3 - 1.5)

Table 3. RFLP of *G. rostochiensis* and *G. achilleae* rDNA-ITS sequences, generated using BioEdit software (©1997-2004 Tom Hall, Ibis Therapeutic Carlsbad, CA) (compare with Fig. 3).

Restriction enzyme	Sequence recognition site	Restriction fragments length (bp)	
		G. rostochiensis Libeliče	*G. achilleae* Zadraga
*Alu*I	AG/CT	381, 326, 148, 100, 33, 14	998, 14
*Rsa*I	GT/AC	603, 221, 169, 9	608, 283, 112, 5, 4
*Msp*I	C/CGG	1002	511, 501
*Hinf*I	G/ANTC	921, 81	1012
*Mbo*I	/GATC	537, 226, 165, 35, 16, 14	560, 236, 165, 35, 16

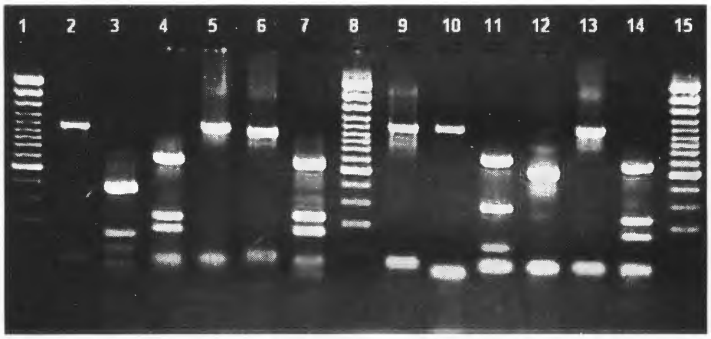

Fig. 3. Restriction fragments of amplified ITS rDNA regions of *G. rostochiensis* from Libeliče (lanes: 2 – 7) and *G. achilleae* from Zadraga (lanes: 9 – 14) with different restriction enzymes: *Alu*I (lanes: 3 & 10), *Rsa*I (lanes: 4 & 11), *Msp*I (lanes: 5 & 12), *Hinf*I (lanes: 6 & 13), *Mbo*I (lanes: 7 & 14), undigested PCR (lanes: 2 & 9) and Marker 100 bp (Fermentas) (lanes: 1, 8 & 15).

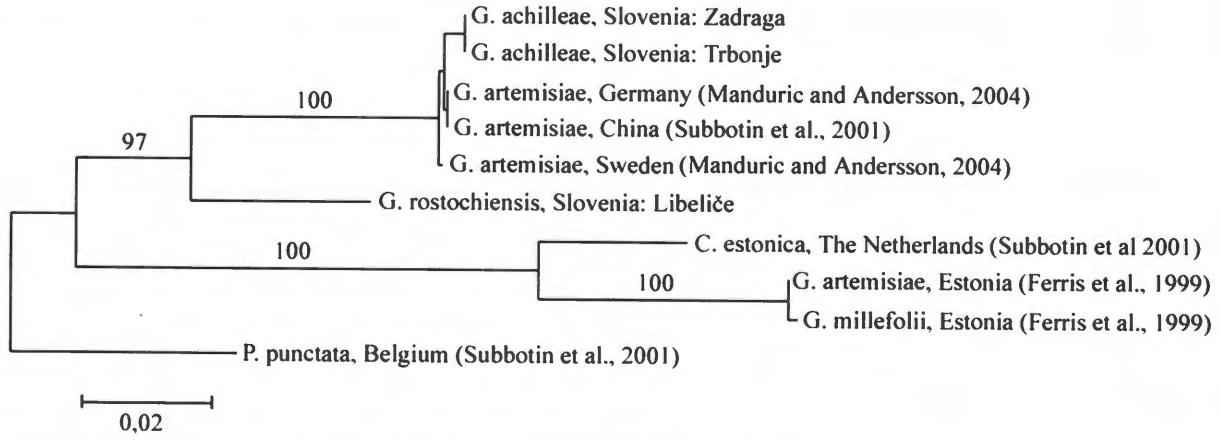

Fig. 4. ITS1, 5.8S and ITS2 rDNA region cluster analyses of the sequences of *G. achilleae* populations from Slovenia (Zadraga and Trbonje); PCN *G. rostochiensis* from Slovenia (Libeliče); and some other European cyst-forming nematodes (sequences from GenBank). Neighbour joining tree was constructed with MEGA3 software. Bootstrap values below 60 % are not given.

fenestra and small anal pore, except for *Punctodera* species, which have an anal fenestra about the same size as the vulval fenestra (Golden, 1986). Among all measured characters of cysts and juveniles in this study the distance from vulval basin to the anus, number of cuticular ridges, Granek's ratio, juvenile body length, juvenile stylet length and distance from centre of median bulb to the end of head differ between *G. achilleae* and *G. rostochiensis* found in Slovenia. The morphological results obtained in this study supported conclusions made earlier (Mulvey & Golden, 1983) that all mentioned characters are significant for the identification of *G. achilleae* and *G. rostochiensis* nematodes.

Morphometrics are also useful for differentiating *G. achilleae* and *G. artemisiae*. The data presented here and elsewhere (Eroshenko & Kazachenko, 1972; Golden & Klindić, 1973; Brzeski, 1998; Fleming & Powers, 1998) show that *G. artemisiae* and *G. achilleae* are morphologically similar. Both species form similar mature cysts with regard to the number of cuticular ridges, vulval basin diameter, Granek's ratio etc. The size ranges of mature cyst characters of both species seem to overlap. However, they can be distinguished from each other by the size ranges of characters of second stage juveniles. The mean value of stylet lenght of *G. artemisiae* second stage juvenile is 21 µm and the distance from head end to the centre of valve in median bulb is 64 µm (Brzeski, 1998). Both characters were greater in *G. achilleae*. The means of the stylet length were 25.1 and 24.9 µm and the distance from head end to the centre of valve were 71 and 69 µm for Trbonje and Zadraga populations, respectively. These dimensions are

similar to measurements of *G. achilleae* made by others (Golden & Klindić, 1973; Brzeski, 1998; Fleming & Powers, 1998). Regarding the shape of the stylet knobs of second stage juveniles, there is some inconsistency in the literature. The shape of the stylet knobs of Slovenian *G. achilleae* populations was rounded and this is also mentioned in other work (Golden & Klindić, 1973; Fleming & Powers, 1998). By contrast, Brzeski (1998) described the shape of stylet knobs *G. achilleae* as anchored. However, these morphological characters of the juveniles in particular are useful for separating *G. achilleae* from *G. artemisiae*.

Biochemical and molecular methods enabled more precise and routine identification of plant-parasitic nematodes (Ferris *et al.*, 1995; Shields *et al.*, 1996; Subbotin *et al.*, 2000) as well as conformation of morphological identifications. The PCR-RFLP approach assured rapid *Globodera* species identification and differentiation between PCN and the yarrow cyst nematode and, thus, a more reliable PCN monitoring in Slovenia. Molecular methods represent also a powerful tool for studying intra- and interspecific nematode phylogenetic relationship. Genetic divergence within *Globodera* genus reflects the species groupings based on geographical origin and host plants (Subbotin *et al.*, 2001). Our examination supported this by comparing sequences of *G. rostochiensis, G. achilleae* and *G. artemisiae*. *Globodera rostochiensis* originates from Andean regions of South America and parasitizing Solanaceae plants including potato (Turner & Evans, 1998). *Globodera achilleae* and *G. artemisiae* are known to be of European origin but have different host range, with the exception of *Achillea millefolium* (Klindić & Petrović, 1974; Brzeski, 1998). *Globodera achilleae* and *G. rostochiensis* shared 91% sequence similarity in 890 bp long rDNA fragment. By contrast, the closely related species *G. artemisiae* and *G. achilleae* showed 99% sequence similarity.

Globodera achilleae and *G. millefolii* are supposed to be very closely related species. Furthermore, Brzeski (1998) reported that *G. achilleae* may be conspecific with *G. millefolii* and, therefore, additional research is needed to prove its junior synonym. According to our results, which showed relative low (82%) sequence similarity score between *G. achilleae* and *G. millefolii,* it is difficult to conclude that they are the same species. In addition, it can be concluded that *G. achilleae* and *G. millefolii* are not closely related and that they are two distinct species.

On the other hand the sequences of *G. millefolii* and *G. artemisiae* from Estonia (Ferris *et al.*, 1999) showed high similarity (99%) and formed a separate cluster together with *C. estonica* from Belgium (Subbotin *et al.*, 2001) with high bootstrap support. However, sequences of *G. artemisiae* (from China, Germany and Sweden) and *G. achilleae* (from Slovenia) formed separate cluster. Therefore, it is difficult to make a final conclusion, since sequences of the same species i.e. *G. artemisiae* from Estonia (Ferris *et al.*, 1999) and *G. artemisiae* from China, Germany and Sweden (Subbotin *et al.*, 2001; Manduric & Andersson, 2004) displayed low (83%) alignment similarity score. Additional phylogenetic and taxonomical studies are needed in the future to solve this problem.

ACKNOWLEDGEMENT

We thank Dr. Hans J. Schroers for critical review of the manuscript.

REFERENCES

Brzeski, M.W. 1998. *Nematodes of Tylenchina in Poland and Temperate Europe.* Muzeum I Inst. Zool. Polska Akad. Nauk, Warsaw, 396 pp.

Eroshenko, A. & Kazachenko, I.P. 1972. [*Heterodera artemisiae* sp. n. (Nematoda: Hetroderidae) – the new species of cyst-forming nematode from the Primorsk area] *Parazitologiia*, 6: 166-170.

Ferris, V. R., Ferris, J. M. & Faghihi J. 1993. Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. *Fundamental and Applied Nematology* 16: 177-184.

Ferris, V. R., Ferris, J. M., Miller, L. I. & Faghihi J. 1995. Ribosomal DNA Comparisons of *Globodera* from two continents. *Journal of Nematology* 27: 273-283.

Ferris, V. R., Krall, E., Faghihi, F. & Ferris, J. M. 1999. Phylogenetic relationship of *Globodera millefolii, G. artemisiae,* and *Cactodera salina* based on ITS region of ribosomal DNA. *Journal of Nematology,* 31: 498-507.

Fleming, C. C. & Powers, T. O. 1998. Potato cyst nematode diagnostics: morphology, differential hosts and biochemical techniques. In: *Potato Cyst nematodes: Biology, Distribution and Control* (Marks, R. J. & Brodie, B. B. Eds). pp. 91-114. Wallingford, UK. CAB International.

Golden, A. M. 1986. Morphology and identification of cyst nematodes. In: *Cyst nematodes* (Lamberti, F. and Taylor, C. E. Eds). pp. 75-99. New York, Plenum Press.

Golden, A. M. & Klindić, O. 1973. *Heterodera achilleae* n. sp. (Nematoda: Heteroderidae) from yarrow in Yugoslavia. *Journal of Nematology 5*: 196-201.

Hržič, A. 1971. [*Investigations of nematodes as direct plant pests and vectors of plant diseases*]. Agricultural Institute of Slovenia, Ljubljana, Technical report, 15 p.

Hržič, A. 1980. [*Research on correlation of cyst distal region anatomy and morphology in cyst forming nematodes Heterodera*]. PhD thesis, University of Ljubljana, 129pp.

Klindić, O. & Petrović, D. 1974. [Appendix on the new nematode species *Heterodera achilleae* (Golden & Klindić)] . *Zashtita bilja*, 25(128-129), 141-162.

Manduric, S. & Andersson, S. 2004. The identity of a Swedish *Globodera* (Nematoda: Hetroderidae) population, following comparisons with known populations of *G. artemisiae* (Eroshenko and Kazachenko, 1972) Behrens, 1975. *Russian Journal of Nematology* 12: 39-44.

Mulvey, R. H. & Golden, A. M. 1983. An illustrated key to the cyst-forming genera and species of Heteroderidae in the Western Hemisphere with species morphometrics and distribution. *Journal of Nematology* 15: 1-59.

Shields, R., Fleming, C.C. & Stradford, R. 1996. Identification of potato cyst nematodes using the polymerase chain reaction. *Fundamental and Applied Nematology* 19: 167-173.

Subbotin, S.A., Halford, P.D., Warry, A. & Perry, R.N. 2000. Variants in ribosomal DNA sequences and phylogeny of *Globodera* parasitizing solanaceous plant. *Nematology* 2: 591-604.

Subbotin, A.S., Vierstraete, A., De Ley, P., Rowe, J., Waeyenderge, L., Moens, M. & Vanfleteren, J.R. 2001. Phylogenetic relationship within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. *Molecular Phylogenetics and Evolution* 21: 1-16.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research* 24: 4876-4882.

Trudgill, D.L., Evans, K. & Parrott, D.M. 1975. Effect of potato cyst nematodes on potato plants. II. Effect on haulm size, concentration of nutrients in haulm tissue and tuber yield of nematode resistant and nematode susceptible potato variety. *Nematologica* 21: 183-191.

Turner, S.J. & Evans, H. 1998. Origins, Distribution and Biology of PCN. In: *Potato Cyst nematodes: Biology, Distribution and Control* (Marks, R. J. & Brodie, B. B. Eds). pp. 91-114. Wallingford, UK. CAB International.

Urek, G. & Hržič, A. 1993. [Importance of cyst-forming nematodes and their geographical dispersal in Slovenia]. In: Lectures and papers presented at the 1st Slovenian conference on plant protection in Radenci, February 24-25, 1993 (Maček, J. Ed). pp. 81-93. Ljubljana, Plant Protection Society of Slovenia.

Urek, G. & Lapajne, S. 2001. The incidence of potato nematode, *Globodera rostochiensis* (Woll., 1923) Behrens, 1975, in Slovenia. *Research Reports. Biotechnical Faculty of University of Ljubljana* 77: 49-58.

Širca S., Urek G. Анализ морфометрии и последовательностей рибосомальной ДНК *Globodera rostochiensis* и *Globodera achilleae* из Словении.

Резюме. Различия между двумя популяциями тысячелистниковой цистообразующей нематоды *Globodera achilleae* и одной популяцией картофельной цистообразующей нематоды *G. rostochiensis* из Словении были проанализированы по морфологическим особенностям цист и личинок второго возраста и по молекулярным данным, полученным для рибомсомальных последовательностей участков ITS 1, 5.8S и ITS 2. *Globodera achilleae* и *G. rostochiensis* хорошо дифференцируются по данным морфометрии и при использовании PCR-RFLP. Также было получено выравнивание нуклеотидных последовательностей *G. rostochiensis* и *G. achilleae*, которое выявило 76 различий на уровне нуклеотидов. Сравниваемые популяции были идентичны на 91%. Полученные последовательности *G. achilleae* были близки к депонированным в базе данных NCBI последовательностям *G. artemisiae*. Анализ выравнивания, содержащего последовательности *G. achilleae* и *G. artemisiae*, выявил лишь 8 нуклеотидных отличий, и высокий уровень сходства (99%).