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Polymorphism among sugar beet cyst nematode *Heterodera schachtii* populations as inferred from AFLP and ITS rDNA gene analyses

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Summary. Amplified Fragment Length Polymorphism (AFLP) fingerprinting patterns and sequence analysis of the ITS region were used for a comparative study of *Heterodera schachtii* populations from Western Europe, Australia and Africa, a *H. betae* population from Germany and a *H. glycines* population from the USA. AFLP results revealed a high level of genetic diversity within *H. schachtii*. In Western Europe no grouping that accorded with the geographical origin of the samples was recorded, suggesting that cysts are actively dispersed throughout this region. However, on a larger scale AFLP patterns of *H. schachtii* populations from Europe, Australia and Africa revealed some level of genetic differentiation. Although AFLP data provided enough resolution to distinguish *H. schachtii* populations on a large geographical scale, sequencing of ITS rDNA gene variants did not provide any grouping according to geographical origin or taxonomy. A close relationship between *H. schachtii* and *H. betae* was observed with both techniques used in this study.

Key words: Genetic diversity, intraspecific ITS variation, phytoparasitic nematode.

The sugar beet cyst nematode, *Heterodera schachtii*, is considered a major pest in sugar beet production. This nematode parasitizes over 200 plant species and it is widespread in most sugar beet growing areas in European countries, North and South America, Australia and Africa (Baldwin & Mundo-Ocampo, 1991). Nematode management involves combinations of rotation, host plant resistance, cropping practices, chemical and biological control, all of which may have specific genotype-level interactions with the nematodes (Castagnone-Sereno, 2002; Blok, 2005). Application of pesticides has been reduced in the European Union because of environmental concern, whilst rotation and trap crops have adverse economic consequences for farmers. In a search for ecologically and economically suitable management strategies, identification of genetic relationships at the species level and intra- and interspecies variability is an essential factor (Kaplan *et al.*, 1999; Castagnone-Sereno, 2002). Molecular information, mainly based on PCR of specific parts of the genome, has been shown to provide significant insights into the degree of diversity existing among individuals and populations of plant-parasitic nematodes (Grenier *et al.*, 2001; Rivoal *et al.*, 2003; Plantard & Porte, 2004; Powers *et al.*, 1997; Madani *et al.*, 2004).

Several molecular techniques have been applied to study genetic diversity. The AFLP-technique

has found a wide application in analyses of genetic variation at subspecies level, particularly in investigations of population structure and differentiation, because of the high number of molecular markers provided and because there is no need for prior sequence knowledge. Its use has been demonstrated for cyst nematode populations of *Globodera* spp. (Folkertsma *et al.*, 1996; Grenier *et al.*, 2001; Marche *et al.*, 2001), *H. trifolii* (Wang *et al.*, 2001), *H. schachtii* (Kaplan *et al.*, 1999), root-knot nematode *Meloidogyne* spp. (Semblat *et al.*, 1998; Van der Beek *et al.*, 1998) and stem nematode *Ditylenchus* spp. (Esquibet *et al.*, 2003).

The internal transcribed spacer region (ITS) of the ribosomal DNA is a well-conserved region controlled by concerted evolution (Baldwin *et al.*, 1995). It has been demonstrated to be highly informative as a taxonomic marker at species level within nematodes (Powers *et al.*, 1997; Hugall *et al.*, 1999; Subbotin *et al.*, 2001). Phylogenetic relationships of cyst-forming nematodes were recently analysed using the ITS rDNA gene sequences (Subbotin *et al.*, 2000b, 2001; Tanha Maafi *et al.*, 2003). Sequencing and RFLP of the ITS-rDNA revealed heterogeneity, or presence of many copies with different sequences for rDNA gene clusters, in many species of cyst forming nematodes of the genera *Heterodera* and *Globodera* (Szalanski *et al.*, 1996, 1997; Thiery & Mugniéry, 1996; Blok *et al.*, 1998; Subbotin *et al.*, 2000b; Wouts *et al.*, 2001; Amiri *et al.*, 2002).

The present study is an attempt to evaluate the genetic variability at different levels within *H. schachtii,* using the AFLP technique and ITS sequence analysis. On a large geographic scale, populations from three different continents, i.e. Europe, Australia and Africa, were analysed. On a smaller scale the genetic diversity was studied by comparing several populations from different locations in Western Europe.

MATERIAL AND METHODS

Nematode populations. The sample set used in the present study (Table 1) includes *Heterodera* populations from nine different locations throughout Western Europe: Belgium (four populations), Germany (two populations), The Netherlands (two populations), and France (one population). For one of the Belgian locations (Momalle) five samples were collected in the same field. One African population was collected in Morocco. Three samples from one population collected from a farm growing brassicas in Western Australia were kindly provided by Dr. I. Riley. A population of *H. betae* from Germany (Ge6) and one soybean cyst nematode, *H. glycines*, population were also included. The taxonomical identity of the collected populations was verified by PCR-RFLP analyses using eight restriction enzymes as described by Subbotin *et al.* (2000a) and PCR with species-specific primers (Amiri *et al.*, 2002).

DNA isolation. Genomic DNA of each sample was extracted. Five to seven cysts were soaked in water for 1 h and then crushed under the dissecting microscope; parts of cyst wall and debris were removed. To have a clean suspension, 10 ml suspension of eggs and juveniles were mixed with 40 ml of a 20% sucrose solution in a Falcon tube. To the top of this, 5 ml water was gently added and the tubes were centrifuged for 5 min at 7000 *g*. Eggs and juveniles formed a white layer between the sucrose solution and the water. They were collected and pelleted by a second centrifugation. Total genomic DNA was extracted using the DNeasy animal mini kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions with some modifications: nematodes were crushed in worm lysis buffer (Subbotin *et al.*, 2000a) using a microhomogenator, followed by incubation for 2 h instead of incubation overnight with no crushing step. DNA was stored at -20°C until use.

AFLP procedure. The protocol for AFLP fingerprinting was essentially performed as described by Zabeau & Vos (1993) and Vos *et al.* (1995) with some minor modifications. Initially a series of 20 different primer combinations with three additional selective nucleotides were tested on a limited number of samples to screen for scorable patterns. Among them three combinations of *Eco*RI+3 and *Mse*I+3 were chosen: (1) ACA/ACC, (2) ACA/ATC, (3) AGG/AAG. The gels were exposed onto X-ray films (Kodak) for 48 h, which were then used for scoring.

AFLP data analysis. DNA fingerprints were scored by visual inspection for presence (1) or absence (0) of specific AFLP-fragments. Only distinct, major bands were scored. The resulting dataset was analysed using the software package NTSYS-pc version 2.1 (Applied Biostatistic, New York NY, USA, L; Rohlf, 2000) and with TreeCon 1.3b (Version 1.3b; Van de Peer & De Wachter, 1994). Genetic similarities were calculated using Jaccard's coefficient (Jaccard, 1908) with the SIMQUAL module of NTSYS-pc or the DISTANCE ESTIMATION option of Treecon. Similarity matrices were analyzed using the UPGMA (Sokal & Michener, 1958) clustering method in NTSYS-pc (SAHN module).

Calculation of the cophenetic correlation coefficient was done using the COPH option in NTSYS-pc. Reliability of the clusters was tested by bootstrap analysis (Felsenstein, 1985) with 1000 replications using Treecon. Additionally, a PCO-analysis was performed based on the genetic similarity matrix using NTSYS-pc.

ITS-rDNA-PCR and sequencing. The internal transcribed spacer region ITS1 and ITS2 with the 5.8S gene was amplified by PCR with the forward TW81 primer (5′-GTT TCC GTA GGT GAA CCT GC-3′) and the reverse AB28 primer (5′-ATA TGC TTA AGT TCA GCG GGT-3′) as described by Joyce *et al.* (1994). The PCR reactions were carried out in a PTC-100 thermal cycler (MJ, Research, Inc). The PCR reaction consisted of 2.5 µl 10× Qiagen PCR buffer, 0.2 µl of each primer (1 µg/µl) (synthesised by Eurogentec, Merelbeke, Belgium), 1.6 µl 25 mM $MgCl_2$, 0.8 µl 10 mM deoxynucleotides, 0.2 µl Taq DNA polymerase (5U/µl) (Taq PCR Core Kit, Qiagen, Hilden, Germany) and distilled water to a total volume of 25 µl. The following thermal profile was used: 10 min preheating at 95°C followed by 9 cycles of 1 min at 94°C, 1 min at 56°C and 2 min at 72°C, and 24 cycles of 15 s at 94°C, 45 s at 57°C and 1 min at 72°C and 5 min of final extension at 72°C.

Gel purified ITS PCR products were ligated into a pGEM-T vector and transferred to *Escherichia coli* strain JM 109 according to the manufacturer's instructions (Promega, Leiden, The Netherlands). Both DNA-strands were sequenced from all samples by using M13-primers (5′-CAG GAA ACA GCT ATG AC-3′ and 5′-GTT TTC CCA GTC ACG AC-3′), using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits, version 2.0 (AB Applied Biosystems). Sequences were run on a 377 DNASequencer (PE Applied Biosystems, Warrington, UK). The sequences reported here have been deposited in GenBank: EF611100-EF611124.

Phylogenetic analysis of ITS data. A total of 25 newly obtained ITS-rDNA sequences (Table 1) and one sequence from *H. glycines* from GenBank (AF498387) were used for the final analysis. Sequences were aligned with ClustalW using the default settings multiple alignment option (Thompson *et al.*, 1994). Sequence alignment was analysed using Bayesian inference and Maximum Parsimony analysis. Bayesian inference analysis (BI) of the dataset was conducted using MrBayes 3.0 (Huelsenbeck & Ronquist, 2001) with two approaches. A general time-reversible (GTR) model of nucleotide substitution was used; this included a proportion of invariable sites (I) and a gamma distribution (G) of among-site-rate heterogeneity with six rate categories. Analysis was initiated with random starting trees and was run with four chains for 1.0 x 10^6 generations. The Markov chains were sampled at intervals of 100 generations. The log-likelihood values of the sample points stabilized after approximately 10^3 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

An equally weighted maximum parsimony (MP) analysis was conducted with PAUP 4.0b10 (Swofford, 2002) by a heuristic search strategy, with the following options: MULTrees option in effect, tree bisection-reconnection (TBR) branch swapping, 100 replications of random sequence entries, and gaps treated as missing. To assess node support, bootstrap values were estimated from 100 replicates of full heuristic search using 100 random taxon additions. Trees were examined with TreeView (Page, 1996). *Heterodera glycines* was chosen as the outgroup.

RESULTS

AFLP analysis. In an initial test of the AFLP technique 20 primer combinations were evaluated on a subset of samples. Three primer combinations were selected based on the number of amplified markers and the scorability of the pattern. For many combinations, a very dense and ambiguous pattern was obtained, most probably due to the fact that the samples represent a population containing different genotypes. The three primer combinations with the highest resolution and pattern clarity were chosen. An example of a clear and scorable AFLP pattern is shown in Figure 1. A total number of 128 unambiguous fragments were scored, among which 121 were polymorphic (94.5% polymorphism). For the three selected primer combinations, the number of scored bands was 33, 39 and 56, and the polymorphism was 94%, 95% and 94%, respectively. No *H. schachtii*-specific bands were found.

The AFLP data were used to make pairwise comparisons of the genotypes on both shared and unique amplification products to generate a similarity matrix using Jaccard's coefficient. The Jaccard coefficient of band matching is recommended for the analysis of DNA fingerprint data as it only takes into account positive band matching (Weising *et al.*, 1995). The genetic

diversity was calculated as [1 − genetic similarity].

The average genetic diversity of *H. schachtii* was 47.1%. At the small scale, the populations from Western Europe showed a diversity of 40.2%. While the mean diversity between all populations collected in Belgium was only 43.5%, the mean value of genetic diversity between the five samples from the Momalle field in Belgium was 39.7%. The genetic diversity between the two populations collected in The Netherlands was 56.1%, while the three Australian samples showed an average genetic diversity of 35.7%.

Figure 2 shows the UPGMA dendrogram with a cophenetic value of 0.96 (very good fit). Bootstrap values (BT) are indicated on the branches.

On 45% Jaccard's diversity level eight groups could be distinguished. Cluster I (BT = 86%) contained four samples from the Belgian Momalle-field as well as two populations from other locations in Belgium, one Dutch, one German and the French population. Cluster II (BT = 91%) grouped the second Dutch population with the *H. betae* population collected in Germany. Cluster III only contained one population from Belgium (Be2), while cluster IV held one of the Momalle-samples from Belgium. Cluster V (BT = 90%) grouped all samples from Australia. Clusters VI and VII, contained the Moroccan *H. schachtii* population and the *H. glycines* population, respectively.

Genetic relationships among samples were also visualized with Principal Co-ordinate analysis (PCO) (Gower, 1966). The first eigenvector explained 53.2% of the variation, while eigenvector 2 and 3 contained 9.9 and 6.6%, respectively. Figure 3 shows the first two dimensions. This representation of the AFLP data showed all Belgian, the French, and the Dutch populations in the right upper corner. The German *H. betae* population shows a relationship with this group. Australian *H. schachtii* samples were separately grouped at the bottom of the plot, while *H. glycines* and the Moroccan *H. schachtii* were found at the left side.

Table 1. Analysed populations of *Heterodera* spp., their origin and the number of ITS-rDNA sequences obtained.

Species	Population code	Location, country	Numbers of ITS-rDNA sequences
H. schachtii	Be1	Molembaix, Belgium	2
H. schachtii	Be2	Hermé, Belgium	2
H. schachtii	Be3	Bossuit, Belgium	1
H. schachtii	BeM4-1	Momalle, Belgium	–
H. schachtii	BeM4-2	Momalle, Belgium	–
H. schachtii	BeM4-3	Momalle, Belgium	2
H. schachtii	BeM4-4	Momalle, Belgium	2
H. schachtii	BeM4-5	Momalle, Belgium	1
H. schachtii	Ge2	Shladen, Germany	1
H. betae	Ge6	Münster, Germany	2
H. schachtii	Nl1	Rutten, The Netherlands	2
H. schachtii	Nl6	Borsel, The Netherlands	2
H. schachtii	Fr1	Aisne, France	1
H. schachtii	Aus10	Munster, Western Australia	2
H. schachtii	Aus11	Munster, Western Australia	–
H. schachtii	Aus12	Munster, Western Australia	2
H. schachtii	Mar27	Morocco	2
H. glycines	Hg	Arkansas, USA	1

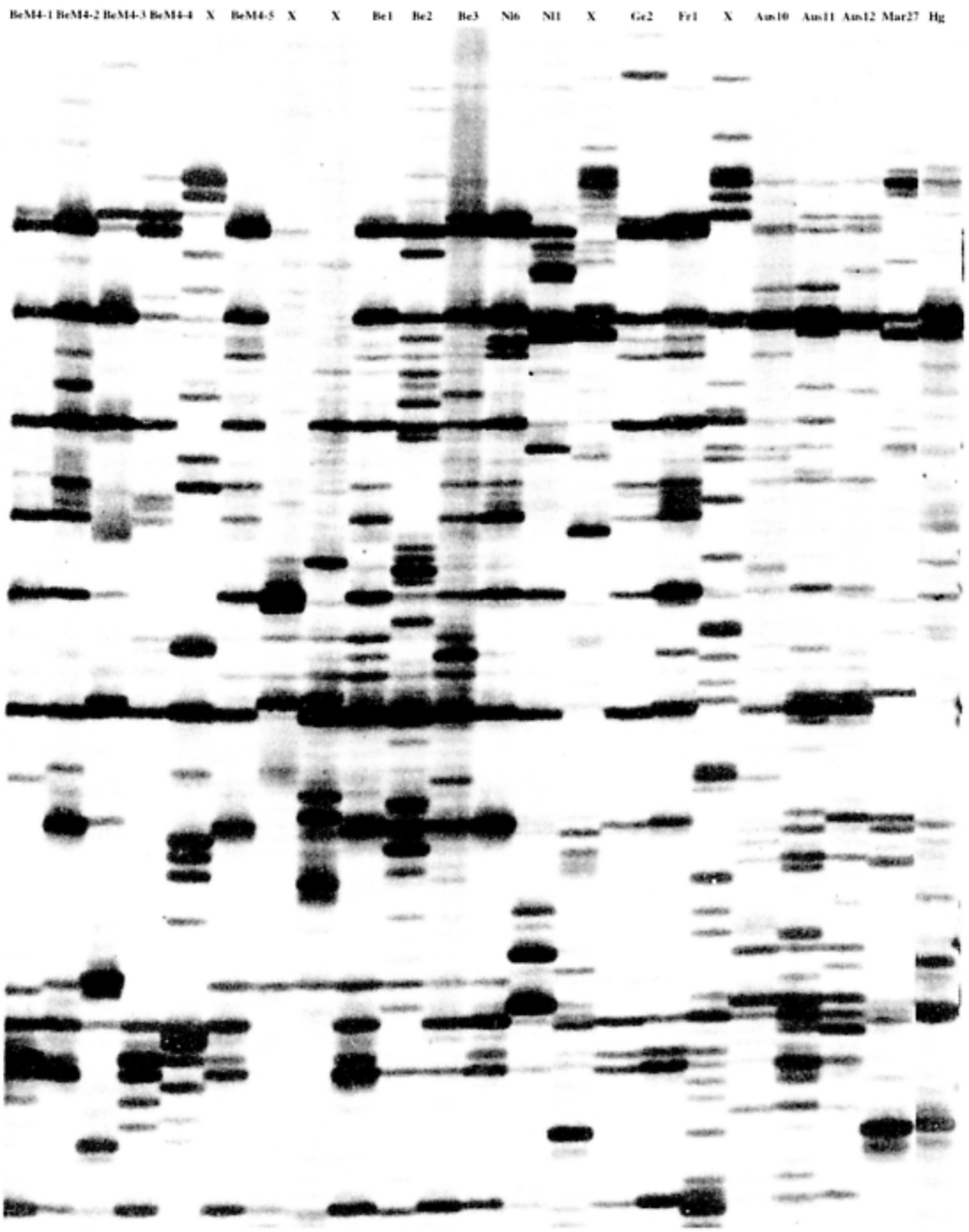

Fig. 1. One of the selected AFLP patterns (*Eco*RI+ACA/*Mse*I+ATC) used in this study. X: sample not used further due to irreproducibility of the pattern.

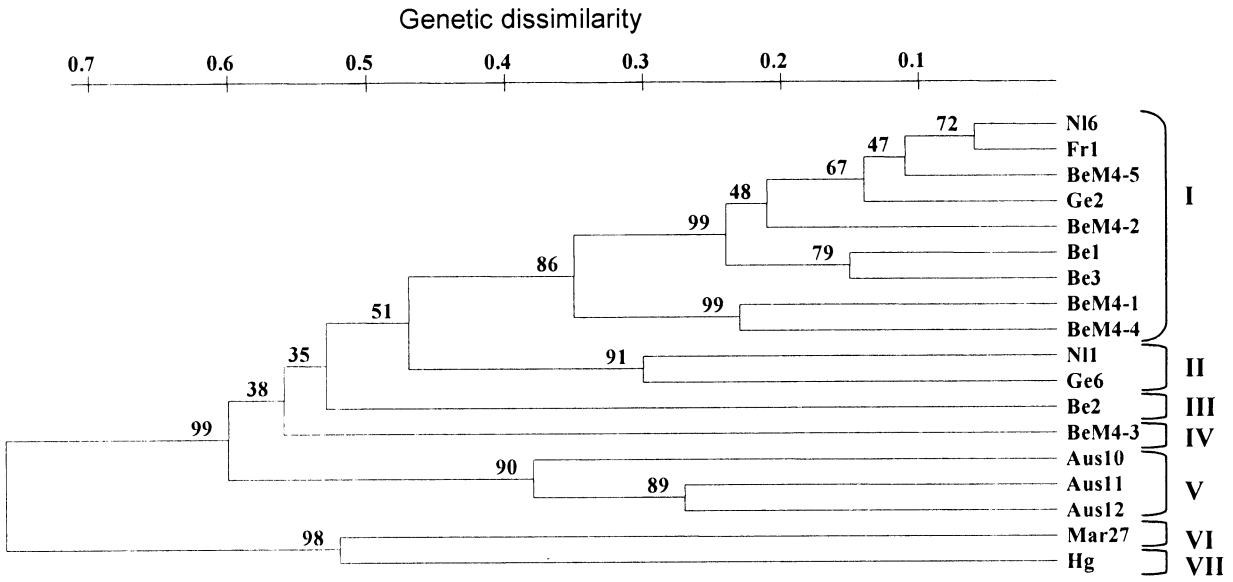

Fig. 2. UPGMA dendrogram based on genetic diversity values calculated using the formula of Jaccard. Bootstrap values are indicated on the branches.

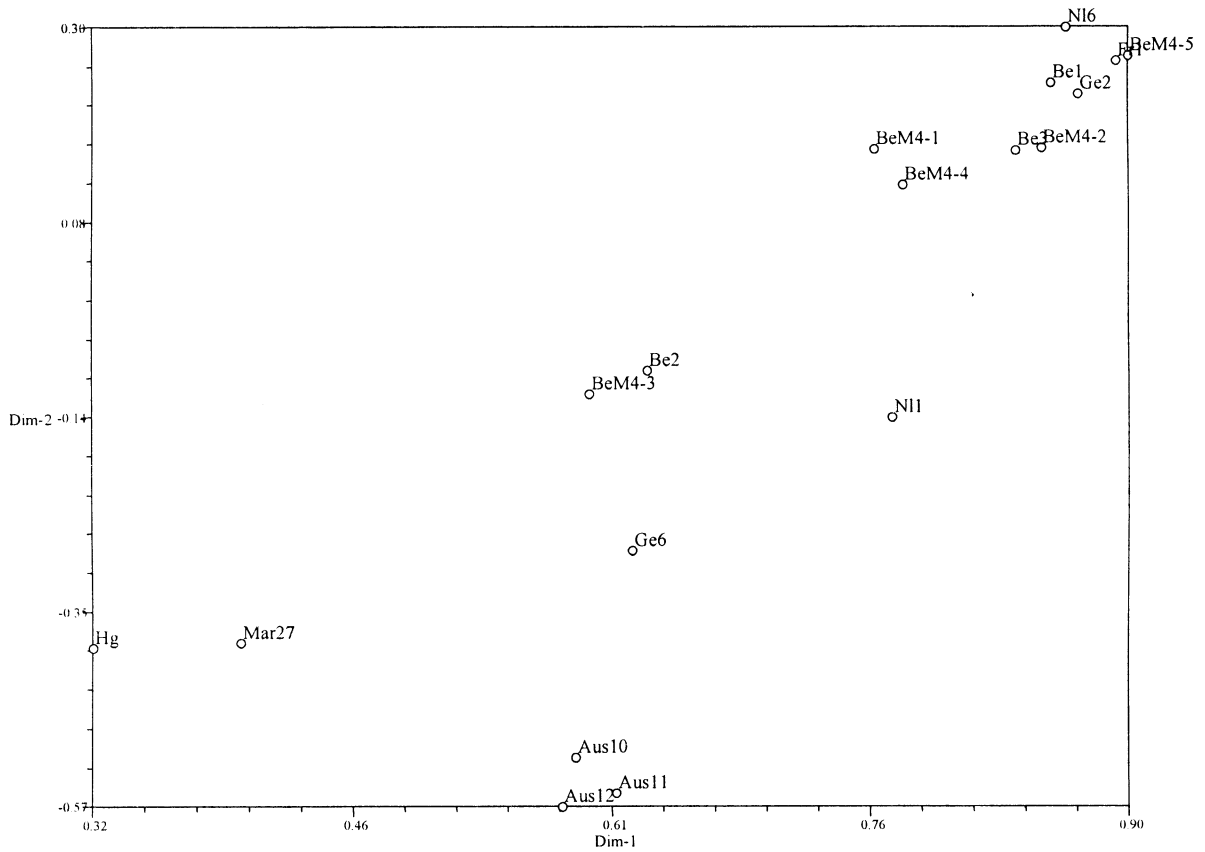

Fig. 3. Principal co-ordinate plot of *Heterodera* populations for the first and second principal co-ordinates estimated with AFLP markers, using the Jaccard similarity matrix.

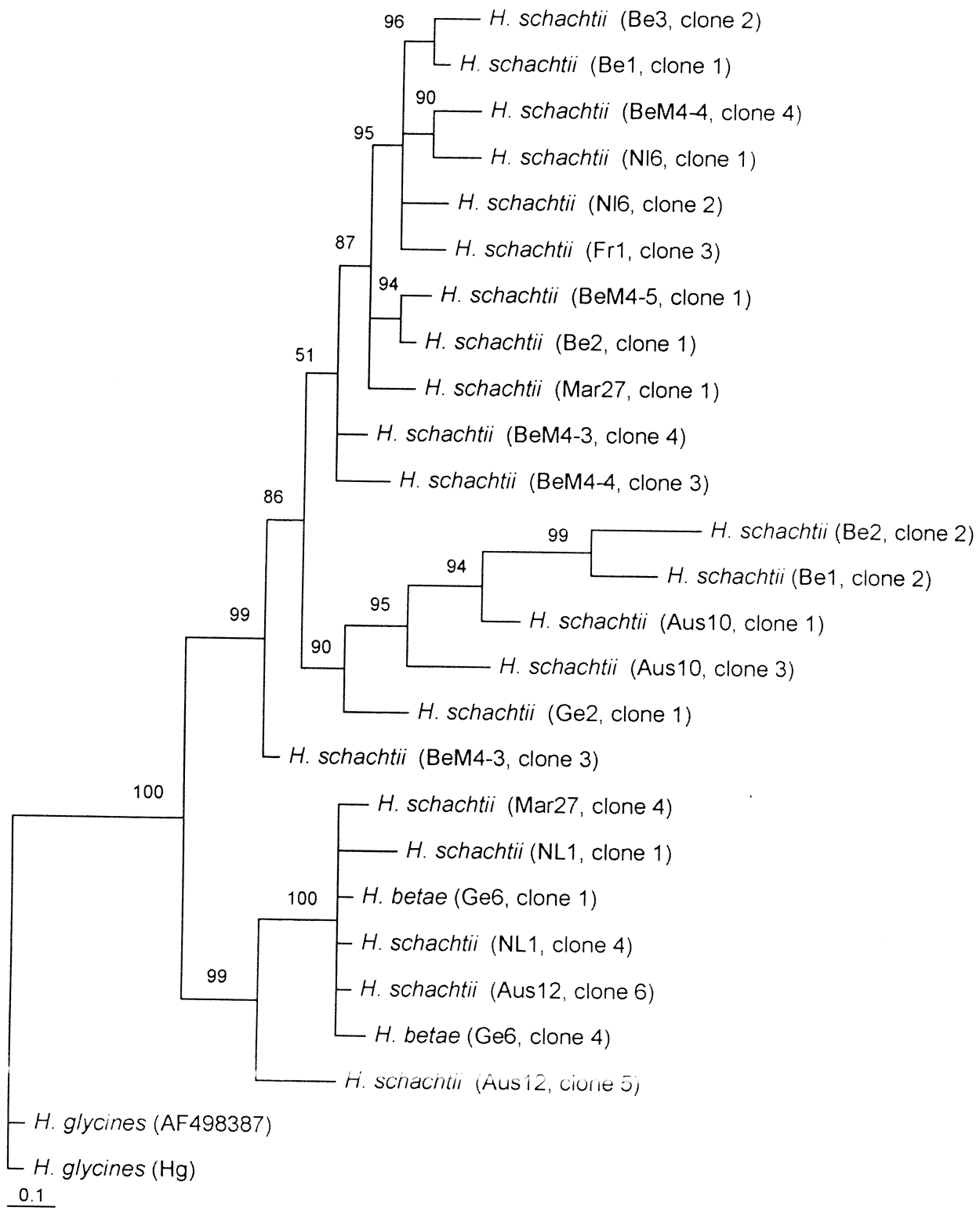

Fig. 4. Phylogenetic relationships with the ITS rDNA clones of *H. schachtii* and *H. betae*. The 50% majority rule consensus tree from Bayesian analysis generated with the GTR+I+G model. Posterior probabilities greater than 50% are given for appropriate clades.

ITS analysis. As previous PCR-RFLP analysis revealed intensive ITS-rDNA heterogeneity in *H. schachtii* (Amiri *et al.*, 2003; Tahna Maafi *et al.*, 2003) and *H. betae* (Wouts *et al.*, 2001), the PCR-ITS products were cloned before sequencing. For ten samples, two different clone variants of ITS were examined. The ITS-rDNA sequence lengths ranged from 1024 to 1030 bp. The ITS alignment included 1030 positions, of which 44 characters were parsimony-informative. The % G+C content of the ITS1 and ITS2 was 55.0% and 50.3%, respectively. The range of nucleotide differences in the ITS region of *H. schachtii* within populations varied from 0 to 26 nucleotides or from 0% to 2.54%.

Unweighted Maximum Parsimony (MP) analysis of the ITS-rDNA gene alignment, with gaps coded as missing data, generated 24 equally most parsimonious trees. Two distinct clades were evident in all trees (data not shown). The Majority Rule consensus BI tree given in Figure 4 is congruent with the MP tree and also contains two main clades, confirmed by very high posterior probabilities. The first clade (PP = 99%) as well as the second clade (PP = 99%) contained *H. schachtii* ITS clone variants from Western Europe, Australia and Africa. Within these clades no grouping according to geographical origin was observed. For most populations where different ITS clone variants were investigated, these variants did not cluster together in the tree. The second clade also contained the two ITS clone variants detected in *H. betae*.

DISCUSSION

AFLP results. On the small geographic scale, AFLP data of the *H. schachtii* populations from Western Europe reveal high levels of genetic diversity and a lack of differentiation according to geographical origin. These observations are indicative of a high level of gene flow among the *H. schachtii* populations in this region. The data of five samples from the same field in Belgium (Momalle) revealed a very high level of genetic variability within populations at field scale. Some of these *H. schachtii* samples were more closely related to populations from other fields than to samples collected in the same field. Also, populations from the same country (Belgium) did not form a distinctive cluster, but are intermingled with populations from The Netherlands and France. In conclusion, *H. schachtii* being native to Western Europe, AFLP analysis reveals that within this region, there is no correlation between the genetic similarity of (sub)populations of this species and their geographical proximity. These data agree with previous conclusions based on RAPD analysis (Amiri *et al.*, 2003) and the study of Plantard & Porte (2004), who used micro-satellite loci to study population diversity of *H. schachtii* in the north of France and found high levels of gene flow on the small scale. All data confirm a significant level of dispersal of *H. schachtii* within its native region, which is most probably caused by passive transport of cysts within and among fields by human activities, water or wind (White, 1953; Norton & Niblack, 1991; Gavassoni *et al.*, 2001).

On the large scale, the two representations (UPGMA dendrogram and PCO) of the AFLP results reveal a clear divergence between the *H. schachtii* populations from Western Europe, the African *H. schachtii* population, and the *H. schachtii* populations collected in Australia. This kind of large-scale genetic differentiation, in contrast to an extensive gene flow at the small-scale, was previously also observed for *Globodera* populations from Peru (Picard *et al.*, 2004).

Compared to previous RAPD-data (Amiri *et al.*, 2003) that presented both species-specific markers and distinctive clustering for *H. schachtii* and *H. betae* our AFLP data did not provide a clear separation of both species. The German *H. betae* population shows a genetic relationship to the *H. schachtii* populations from Western Europe.

ITS results. Our sequence analysis of ITS rDNA clone variants revealed very high levels of intraspecific variation of the ITS-rDNA sequence (up to 2.54%), while in previous studies this value did not exceed 1.6-1.8% (Subbotin *et al.*; 2000b; Tanha Maafi *et al.*, 2003; Madani *et al.*, 2004). These observations confirm again the complexity and heterogeneity of ITS for *H. schachtii* (Amiri *et al.*, 2002). Although ITS repeat units are usually considered to be rapidly homogenized by concerted evolution, the occurrence of intra-specific and intra-individual ITS polymorphisms has also been reported for *Caenorhabditis* (Butlter *et al.*, 1981), *Meloidogyne* (Zijlstra *et al.*, 1995; van der Beek *et al.*, 1998), *Globodera* and *Heterodera* spp. (Szalanski *et al.*, 1997; Blok *et al.*, 1998; Subbotin *et al.*, 2000b).

Moreover, when two ITS clone variants were sequenced from the same population these sequences did not cluster together in BI and MP trees in most cases, demonstrating a very high level of ITS variation at intraspecific and intrapopulation level. Apparently, concerted evolution has not homogenized all rDNA variants within these populations. It is possible that the analysed populations of *H. schachtii* are constituted

of a mixture of individuals with very divergent ITS regions. However, if this kind of divergent individuals would be present in a population, the outcrossing nature of *H. schachtii* would lead to hybridizations between them, and subsequently result in homogenization of the variants by concerted evolution. Therefore, the fact that high intrapopulation variation was observed in most of the analysed populations makes the hypothesis of mixed populations very unlikely.

A more likely explanation for the observed ITS clone variants might be the presence of several major rDNA gene loci in the genome of *H. schachtii*. Since it has been demonstrated that intrachromosomal homogenization is faster than interchromosomal recombination (Liao, 1999; Parkin & Butlin, 2004), the process of concerted evolution might not have had enough time to homogenize rDNA sequences across different loci. Additional studies are needed to find out the arrangement of ITS diversity and the evolutionary forces acting on it in the *Heterodera* genome.

ITS clone variants from populations from Western Europe, Africa and Australia are scattered throughout the cladogram. Apparently, our ITS-rDNA dataset does not group isolates according to their geographical origin or taxonomic grouping.

Comparison between AFLP and ITS data and general conclusions. A high level of variability in ITS sequence at the intraspecific and intra-population level has been observed in this and previous studies. From the results of this study it is clear that the AFLP fingerprinting method, which covers multiple loci across the genome, provides a higher resolution of clustering between the analysed populations of *H. schachtii* at large scale, in comparison with the ITS data.

Based on the AFLP data we can conclude that *H. schachtii* shows some degree of differentiation at large scale (Europe *vs* Australia and Africa). However, at local scale a very high level of variability and gene flow within and between populations was observed.

Based on the close relationship between *H. schachtii* and *H. betae* demonstrated by both genetic analyses used in this study, two hypotheses can be postulated regarding the origin of *H. betae*. The first one is the possibility that *H. betae* represents a polyploid parthenogenetic form that has evolved from autopolyploidization of the diploid, amphimictic *H. schachtii* or another very closely related species. This hypothesis was proposed before by Triantaphyllou & Hirschmann (1964) to explain the origin of the clover cyst nematode *H. trifolii* and other polyploid species from the *Schachtii* group. The second hypothesis is that the polyploid *H. betae* originated from interspecific hybridization of two diploid, amphimictic species, *i.e.* allopolyploidization (Subbotin *et al.*, unpublished). In this case *H. schachtii* is suggested as one of the parent species. These two hypotheses suggesting that *H. betae* were formed by a polyploidization and/or an ancient hybridization event should be investigated by more detailed analyses of the ITS-rDNA, including more ITS variant copies from other species and populations of cyst forming nematodes of the *Schachtii* group. In addition, the analysis of genetic markers with a codominant nature is highly recommended to elucidate the origin of *H. betae*.

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REFERENCES

AMIRI, S., SUBBOTIN, S.A. & MOENS, M. 2003. Comparative morphometrics and RAPD studies of *Heterodera schachtii* and *H. betae* populations. *Russian Journal of Nematology* 11: 91-99.

AMIRI, S., SUBBOTIN, S.A. & MOENS, M. 2002. Identification of the beet cyst nematode *Heterodera schachtii* by PCR. *European Journal of Plant Pathology* 108: 497-506.

BALDWIN, J.G. & MUNDO-OCAMPO, M. 1991. Heteroderinae, cyst and non-cyst-forming nematodes. In: *Manual of Agriculture Nematology* (W.R. Nickle Ed.). pp. 275-362. New York: Marcel Dekker Inc.

BALDWIN, B.G., CAMPBELL, C.S., PORTER, J.M., SANDERSON, M.J., WOJCIECHOWSKI, M.F. & DONOGHUE, M.J. 1995. Utility of nuclear ribosomal DNA internal transcribed spacer sequences in phylogenetic analysis of angiosperms. *Annals of the Missouri Botanical Garden* 82: 247-277.

BLOK, V.C. 2005. Achievements in and future prospects for molecular diagnostics of plant-parasitic nematodes. *Canadian Journal of Plant Pathology* 27: 176-185.

BLOK, V.C., MALLOUCH, G., HARROWER, B., PHILIPS, M.S. & VRAIN, T.C. 1998. Intraspecific variation in ribosomal DNA in population of potato cyst nematode *Globodera pallida*. *Journal of Nematology* 30: 262-274.

BUTLER, M.H., WALL, S.M., LUEHRSEN, K.R., FOX, G.E. & HECHT, R.M. 1981. Molecular relationships

between closely related strains and species of nematodes. *Journal of Molecular Evolution* 18: 18-23.

CASTAGNONE-SERENO, P. 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes. *Euphytica* 124: 193-199.

ESQUIBET, M., GRENIER, E., PLANTARD, O., ANDALOUSSI, A. & CAUBEL, G. 2003. DNA polymorphism in the stem nematode *Ditylenchus dipsaci*: development of diagnostic markers for normal and giant races. *Genome* 46: 1077-1083.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

FOLKERTSMA, R.T., VAN DER VOORT, R., DE GROOT, K.E.D., VAN ZANDVOORT, P.M., SCHOTS, A., GOMMERS, F.J., HELDER, J. & BAKKER, J. 1996. Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. *Molecular Plant-Microbe Interactions* 9: 47-57.

GAVASSONI, W.L., TYLKA, G.L. & MUNKVOLD, G.P. 2001. Relationships between tillage and spatial patterns of *Heterodera glycines*. *Phytopathology* 91: 534-545.

GOWER, J.C. 1966. Some distance properties of latent root and vector method used in multivariance analysis. *Biometrika* 5: 325-338.

GRENIER, E., BOSSIS, M., FOUVILLE, D., RENAULT, L. & MUGNIÉRY, D. 2001. Molecular approaches to the taxonomic position of Peruvian potato cyst nematodes and gene pool similarities in indigenous and imported populations of *Globodera*. *Heredity* 86: 277-290.

HUELSENBECK, J.P. & RONQUIST, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

HUGALL, A., STANTON, J. & MORITZ, C. 1999. Reticulate evolution and the origins of ribosomal internal transcribed spacer diversity in apomictic *Meloidogyne*. *Molecular Biology and Evolution* 16: 157-164.

JACCARD, P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin de la Societe Vaudoise de Sciences Naturelles* 44: 223-270.

JOYCE, S.A., RIED, A., DRIVER, F. & CURRAN, J. 1994. Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: *COSR 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes* (A.M. Burnell, R.U. Ehlers, J.P. Masson Eds.) pp 178-187. Proceedings of Symposium & Workshop. St. Patrick's College, Maynooth, Co, Kildare, Ireland, Luxembourg, European Commission, DG XII.

KAPLAN, M., CASWELL-CHEN, E.P. & WILLIAMSON, V.M. 1999. Assessment of host induced selection on three geographic isolates of *Heterodera schachtii* using RAPD and AFLP markers. *Phytopathology* 89: 68-73.

LIAO, D. 1999. Concerted Evolution: Molecular Mechanism and Biological Implications. *American Journal of Human Genetics* 64: 24-30.

MADANI, M., VOVLAS, N., CASTILLO, P., SUBBOTIN, S.A. & MOENS, M. 2004. Molecular characterization of cyst nematode species (*Heterodera* spp.) from the Mediterranean basin using RFLPs and sequences of ITS-rDNA. *Journal of Phytopathology* 152: 229-234.

MARCHE, L., VALETTE, S., GRENIER, E. & MUGNIÉRY, D. 2001. Intraspecies DNA polymorphism in the tobacco cyst-nematode complex. (*Globodera tabacum*) using AFLP. *Genome* 44: 941–946.

NORTON, D.D. & NIBLACK, T.L. 1991. Biology and ecology of nematodes. In: *Manual of Agricultural Nematology* (W.R. Nickle, Ed.), pp. 47-72. Marcel Dekker, New-York.

PAGE, R.D.M. 1996. TreeView: An application to display phylogenetic trees on personal computers. *Computational Applications in Biosciences* 12: 357-358.

PARKIN, E.J. & BUTLIN, R.K. 2004. Within- and between-individual sequence variation among ITS1 copies in the meadow grasshopper *Chorthippus parallelus* indicates frequent intrachromosomal gene conversion. *Molecular Biology and Evolution* 21: 1595-1601.

PICARD, D., PLANTARD, O., SCURRAH, M. & MUGNIÉRY, D. 2004. Inbreeding and population structure of the potato cyst nematode (*Globodera pallida*) in its native area (Peru). *Molecular Ecology* 13: 2899-2908.

PLANTARD, O. & PORTE, C. 2004. Population genetic structure of the sugar beet cyst nematode *Heterodera schachtii*: a gonochoristic and amphimitic species with highly inbred but weakly differentiated populations. *Molecular Ecology* 13: 33-41.

POWERS, T.O., TODD, T.C., BURNELL, A.M., MURRAY, P.C.B., FLEMING, C.C., SZALANSKI, A.L., ADAMS, B.A. & HARRIS, T.S. 1997. The rDNA internal transcript spacer region as a taxonomic marker for nematodes. *Journal of Nematology* 29: 441-450.

RIVOAL, R., VALETTE, S., BEKAL, S., GAUTHIER, J.P. & YAHYAOUI, A. 2003. Genetic and phenotypic diversity in the gramineous cyst nematode complex, inferred from PCR-RFLP of ribosomal DNA and morphometric analysis. *European Journal of Plant Pathology* 109: 227-241.

ROHLF, F.J. 2000. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, Setauket, New York, USA.

SEMBLAT, J.P., WAJNBERG, E., DALMASSO, A., ABAD, P. & CASTAGNONE-SERENO, P. 1998. High resolution of DNA fingerprinting of parthenogenetic root knot nematodes using AFLP analysis. *Molecular Ecology* 7: 119-125.

SOKAL, R.R. & MICHENER, C.D. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 38: 1409-1438.

SUBBOTIN, S.A., WAEYENBERGE, M. & MOENS, M. 2000A. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. *Nematology* 2: 153-164.

SUBBOTIN, S.A., HALFORD, P., WARRY, A. & PERRY, R.N. 2000B. Variation in ribosomal DNA sequences and phylogeny of *Globodera* parisitising solanaceous plants. *Nematology* 2: 591-604.

SUBBOTIN, S.A., VIERSTRAETE, A., DE LEY, P., ROWE, J., WAEYENBERGE, L., MOENS M. & VANFLETEREN, J.R. 2001. Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS region of ribosomal DNA. *Molecular Phylogenetics and Evolution* 21: 1-16.

SWOFFORD, D.L. 2002. PAUP. Phylogenetic analysis using parsimony and other methods. Version 4. Sunderland. Massachusetts. Sinauer Associates. 128 pp.

SZALANSKI, A.L., SUI, D.D., HARRIS, T.S. & POWERS, T.O. 1997. Identification of cyst nematodes of agronomic and regulatory concern by PCR-RFLP of ITS1. *Journal of Nematology* 29: 255-267.

TANHA MAAFI, Z., SUBBOTIN, S.A. & MOENS, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on the ITS sequences of rDNA. *Nematology* 5: 99-111.

THIERY, M. & MUGNIÉRY, D. 1996. Intraspecific rDNA restriction fragment length polymorphism in *Globodera* species, parasites of solanaceaous plants. *Fundamental and Applied Nematology* 19: 471-479.

THOMPSON, J.D., GIBSON, T.J., PLEWINIAK, F. & HIGGINS, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876-4882.

TRIANTAPHYLLOU, A.C. & HIRSCHMANN, H. 1964. Reproduction in plant and soil nematodes. *Annual Reviews in Phytopathology* 2: 57-80.

VAN DE PEER, Y. & DE WACHTER, R. 1994. TREECON for windows: A software package for the construction and drawing of the evolutionary trees for the Microsoft windows environment. *Computational Applications in Bioscience* 10: 569-570.

VAN DER BEEK, J.G., FOLKERTSMA, R., ZIJLSTRA, C., VAN KOERT, P.H.G., POLEIJ, L.M. & BAKKER J. 1998. Genetic variation among parthenogenetic *Meloidogyne* species revealed by AFLPs and 2D-protein electrophoresis contrasted to morphology. *Fundamental and Applied Nematology* 21: 401-411.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DER LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., KUIPER, M. & ZABEAU, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407-4414.

WANG, S., RIGGS, R.D. & YANG, Y. 2001. Grouping of populations of *Heterodera trifolii* by host preference and AFLP pattern. *Nematology* 3: 667-674.

WEISING, K., NYBOM, H., WOLFF, K. & MEYER, W. 1995. *DNA Fingerprinting in Plants and Fungi.* CRC Press, Boca Raton, USA.

WHITE, J. 1953. Wind-borne dispersal of potato-root eelworm. *Nature* 172: 686-687.

WOUTS, W.M., RUMPENHORST, H.J. & STURHAN, D. 2001. *Heterodera betae* sp. n., the yellow beet cyst nematode (Nematoda: Heteroderidae). *Russian Journal of Nematology* 9: 33-42.

ZABEAU, M. & VOS, P. 1993. *Selective restriction fragment amplification: A general method for DNA fingerprinting.* Publication 0534858A1. European patent Application EP 534858A1. Munich, Germany.

Madani M., Kyndt T., Colpaert N., Subbotin S.A., Gheysen G., Moens M. Полиморфизм популяций свекловичной цистообразующей нематоды *Heterodera schachtii* по результатам AFLP и генетического анализа последовательностей ITS-rDNA.

Резюме. Метод AFLP (выявление полиморфизма в длинах амплифицированных фрагментов) и анализ последовательностей ITS-участка были использованы для сравнительного изучения популяций *Heterodera schachtii* из Западной Европы, Австралии и Африки, а также популяций *H. betae* из Германии и *H. glycines* из США. Результаты AFLP показали высокий уровень генетического разнообразия в пределах вида *H. schachtii*. В Западной Европе не было отмечено группировок популяций, соответствующих их географическому происхождению, что можно рассматривается как свидетельство активного распространения этой нематоды в данном регионе. В то же время, результаты AFLP показывают, что определенные различия существуют между популяциями *H. schachtii* из Европы, Австралии и Африки. Хотя данные AFLP анализа показали некоторое разрешение в разграничении популяций *H. schachtii* из различных географических регионов, результаты генетического анализа последовательностей ITS rDNA не выявляют каких-либо географических или таксономических группировок. Очевидная филогенетическая близость *H. schachtii* и *H. betae* обнаружена при использовании каждого из использованных методов.