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Comparative morphometrics and RAPD studies of *Heterodera schachtii* and *H. betae* populations

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Summary. The morphometrics of 25 populations of *H. schachtii* and two populations of *H. betae* from different origins were compared. Cyst length and width of *H. betae* were greater than those of *H. schachtii*. The fenestral length and semifenestral width did not support distinguishing the *H. betae* populations from the *H. schachtii* populations examined and, therefore, cannot be used to separate these species. In second stage juveniles body length, the hyaline part of the tail, and the tail length were significantly greater for *H. betae* than for *H. schachtii* and enable these species to be differentiated. The RAPD analysis using seventeen decamer primers and carried out on 20 *H. schachtii* populations, two *H. betae* populations and one of *H. glycines* yielded 471 DNA fragments. Some bands generated by primers were specific for *H. schachtii*. The dendrogram constructed on the basis of RAPD data did not arrange *H. schachtii* populations according to their origin but separated the two species.
Key words: beet cyst nematodes, interspecific differences, intraspecific variation.

The beet cyst nematode, *Heterodera schachtii* Schmidt, is an economically important pest of sugar beet (*Beta vulgaris* L.) production worldwide. The species is widespread in most European countries, the USA, Canada, the Middle East, Africa, Australia, and South America (Baldwin and Mundo-Ocampo, 1991; Evans and Rowe, 1998). It causes serious stand yield reductions and decreases sugar content of sugar beet wherever the crop is grown.

A race of *H. trifolii* Goffart attacking sugar beet was first described in The Netherlands by Maas & Heijbroek in 1981 and later was named *H. betae* (Wouts *et al.*, 2001). *Heterodera schachtii* and *H. betae* have similar morphology and share a common host range. However, Maas *et al.* (1982) demonstrated that, unlike *H. schachtii*, *H. betae* passes through a yellow phase during cyst formation and has no males. Maas & Heijbroek (1981), Steele & Whitehand (1984) and Wouts *et al.* (2001) found that *H. betae* could be distinguished morphologically from *H. schachtii* by the larger fenestrae of the cyst vulval cone and the greater length of the body, the stylet, and the hyaline part of the tail of second stage juveniles. *Heterodera betae* has been detected in several European countries and was recently found in Morocco (Amiri *et al.*, 2002).

It has been demonstrated that the DNA technique based on ITS-PCR-RFLP is useful for separating *H. schachtii* from *H. betae* (Wouts *et al.*, 2001; Amiri *et al.*, 2002). Several reports demonstrated the utility of RAPD (Random Amplified Polymorphism DNA) to identify nematode species and to assess the genetic variability within species (Caswell-Chen *et al.*, 1992; Pinochet *et al.*, 1994; Fallas *et al.*, 1996; Ibrahim *et al.*, 1997).

The objective of this work were (i) to study the variation in morphometrics of cysts and second stage juveniles between several populations of *H. schachtii* and two populations of *H. betae* and (ii) to study the genetic diversity of populations of these two species based on RAPD analysis.

MATERIALS AND METHODS

Nematode populations and sample preparation for morphometrics. The morphometrics of 25 po

Table 1. Origin of nematode populations used.

Species	Code	Origin	Morphometrics	RAPD
Heterodera schachtii	Be1	Molembaix, Belgium	+	+
H. schachtii	Be2	Hermé, Belgium	+	-
H. schachtii	Be4	Gingelon, Belgium	+	+
H. schachtii	Be5	Hérines, Belgium	+	+
H. schachtii	Be6	Quivrain, Belgium	+	+
H. schachtii	Be8	Meerdonk, Belgium	+	+
H. schachtii	CPRO	Wageningen, The Netherlands	+	-
H. schachtii	Eng2	Suffolk, England	+	-
H. schachtii	Fr1	Aisne, France	+	+
H. schachtii	Fr2	Nord, France	+	+
H. schachtii	Fr3	Marne, France	+	+
H. schachtii	Fr4	Finistère, France	+	+
H. schachtii	As	Rondebult, South Africa	+	-
H. schachtii	Ge1	Göttingen, Germany	+	+
H. schachtii	Ge2	Shladen, Germany	+	+
H. schachtii	Ge4	Kitsingen, Germany	+	+
H. schachtii	Ir4	Kerma, Iran	+	+
H. schachtii	It	Bologna, Italy	+	-
H. schachtii	Mar5	Ouled M'Barek, Tadla, Morocco	+	+
H. schachtii	Mar27	Bouareg, Mouloya, Morocco	-	+
H. schachtii	Nl4	Stellendam, The Netherlands	+	+
H. schachtii	Nl5	Achthuizen, The Netherlands	+	+
H. schachtii	Sue1	Teckomatorp, Sweden	+	-
H. schachtii	Sue2	Slottaquirden, Sweden	+	+
H. schachtii	Sue3	Fide, Sweden	-	+
H. schachtii	Sue4	Kastlösa, Sweden	+	+
H. schachtii	Wob88	Germany	+	-
H. betae	Ge6	Münster, BBA, Germany	+	+
H. betae	Mar1	Berkane, Mouloya, Morocco	+	-
H. betae	Hb	Merelbeke, Belgium	-	+
H. glycines	Hg1	Arkansas, USA	-	+

pulations of *H. schachtii* and two populations of *H. betae* were determined (Table 1). Nematodes were maintained in pots kept in the glasshouse or were extracted directly from field soil samples using Seinhorst's method (Seinhorst, 1964). Second stage juveniles were obtained from crushed cysts, and were killed by gentle heat, fixed in TAF and embedded in glycerol on permanent slides following Seinhorst's method (1959). Cyst vulval cones were prepared from brown cysts and mounted in glycerine-gelatine. The specimens were examined and measured under light microscopy. The data were analysed by ANOVA followed by the test of Newmans-Keuls using STAT-ITCF (Anonymous, 1992).

PCR-RAPD. The molecular study comprised 20 populations of *H. schachtii* and two populations of *H. betae*. One population of *H. glycines* was also included and used as outgroup (Table 1). Several cysts of each population were transferred into an Eppendorf tube containing 8 µl distilled water and 10 µl nematode lysis buffer (500 mM KCl, 100 mM Tris-HCl pH 8.0, 15 mM $MgCl_2$, 1.0 mM DTT, 4.5% Tween 20) and crushed with an microhomogenisor Vibro Mixer (Zurich, Switzerland) for 2.5-3 min. Two µl proteinase K (600 µg/ml) (Promega Benelux, Leiden, The Netherlands) were added and the tubes were incubated at 65 °C (1 h) and 95° C (10 min) consecutively and finally centrifuged (1 min; 16 000*g*). Five microliters of the template DNA suspension were added to 20 µl of a PCR mixture containing 2.5 µl 10X Qiagen PCR buffer, 0.5 µl $MgCl_2$, 0.5 µl 10mM dNTP mix, 1.5U *Taq* Polymerase (5U/µl) and 0.5 µl of the single random primers; 15.8 µl double distilled water was added to a final volume. Seventeen decamer random primers were used (Table 2). Amplification was performed in a MJ Research PTC-200 Peltier Thermal Cycler (MJ Research, Landgraaf, The Netherlands). The PCR program consisted of 7 min at 94° C, 44 cycles of 1 min at 94 °C, 1 min at 35° C and 3 min at 72° C; followed by 5 min at 72° C. A negative control containing the PCR mixture without DNA template was also run. Ten microliters of each amplified sample were

Table 2. Decamer primers used in the study of the genetic variation in and between *H. schachtii* and *H. betae* populations.

Sequences of primers used (5'-3')	Sources
AGGTGACCGT	OPERON KIT A code A18
TGCCCAGCTG	OPERON KIT A code A2
GGCACTGAGG	OPERON KIT G code G02
GGGGTGACGA	OPERON KIT D code D13
GAGCGTCGAA	OPERON KIT K code K16
CAGGCCCTTC	OPERON KIT A code A01
AGTCAGCCAC	OPERON KIT A code A03
GGTCCCTGAC	OPERON KIT A code A06
GAAACACCCC	OPERON KIT H code H08
CCTCTAGACC	OPERON KIT F code F19
TGGGGGACTC	OPERON KIT B code B09
CCAGCACTTC	OPERON KIT AE code AE04
TCACGTCCAC	OPERON KIT G code G08
GAACGGACTC	OPERON KIT C code C06
AAGACCCCTC	OPERON KIT E code E06
GTTAGTGCGG	OPERON KIT AC code AC05
CAGCACCCAC	OPERON KIT A code A13

analysed by electrophoresis in a 2.5% agarose gel (100 V, 3 h 30 min), stained with ethidium bromide, visualised and photographed under UV-light.

The DNA fragments were scored for all primers by 1 for the presence or 0 for the absence of the band. Fragments with a length greater than 1500 bp were not considered. The program PAUP 4.0b4a (Swofford, 1998) was used for the fragment analyses. Genetic variation was calculated by means of the pair group method using arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Cyst and vulval cone. Differences in means of cyst length and width were found between the two *H. betae* populations and all *H. schachtii* populations (Table 3). With respect to *H. schachtii*, the Iranian population (Ir4) had the smallest cyst size (565 × 403 µm), whereas the Dutch one (N15) had the largest cysts (856 × 550 µm). The Principal Component Analysis of these data (Amiri, 2002) classified each of the *H. betae* populations (Ge6 and Mar1) in a separate clade and divided the *H. schachtii* populations into four groups.

The vulval slit length, fenestral length, and semifenestral width showed substantial intraspecific variation (Table 3). The fenestral length and semifenestral width did not enable the *H. betae* populations to be distinguished from all the *H. schachtii* populations and, therefore, cannot be used to separate the species. Results of the cluster analysis using the morphometrics of all cyst cone characters (vulval plate length, vulval plate width, vulval slit length, length fenestrate, semifenestral width, semifenestral length, vulval bridge, underbridge, distance vulva-anus) supported this conclusion (Amiri, 2002).

Second stage juveniles. For *H. schachtii*, the mean body length varied between 379 µm (Eng2) and 555 µm (Mar5), the mean hyaline tail length between 22 µm (Eng2) and 35 µm (Fr3) and the mean tail length between 44 µm (Ge2) and 58 µm (Fr3) (Table 4). With respect to the *H. betae* populations, both the body length and the hyaline part of the tail were longer in the German population whereas the tail length was longer in the population from Morocco. The stylet length varied slightly among *H. schachtii* populations but was longer in the two *H. betae* populations. Both the body length and tail length enabled *H. schachtii* to be differentiated from *H. betae*. All these characters were significantly greater in *H. betae* populations than in *H. schachtii* populations. The comparison of other morphometric characters showed that they could not be used for differentiating the two species (Amiri, 2002). The cluster analysis using the combination for all morphometric characters of the J2 clearly separated *H. betae* from *H. schachtii* and divided the 25 *H. schachtii* populations in five groups (Amiri, 2002).

The morphometrical data for cysts of all populations were within the ranges reported by Graney and Miller (1979) Maas and Heijbroek (1981), Ambrogioni *et al.* (1999), and Wouts *et al.* (2001). Compared to the data reported in the latter three papers, we observed a longer fenestral length for *H. betae*. However, this character does not enable

Table 3. Measurement (µm) of cysts, and vulval cones of *Heterodera schachtii* and *H. betae* populations.

Pop (1)	Cyst			Vulval cone			
	n	Length (2)	Width	n	Vulval slit length	Fenestrate length	Semifenestral width
Be1	20	833 cd *(734-917)	519 bcdef (445-603)	27	45 efg (39-51)	38 efgh (35−51)	29 fg (24-35)
Be2	22	825 cde (694-943)	488 defg (406-583)	25	45 efg (39-51)	40 efgh (31−47)	30 defg (24-35)
Be4	20	831 cd (721-917)	509 bcdef (393-668)	24	46 efg (39-51)	41 defgh (31-49)	31 bcdefg (24-39)
Be5	20	803 cdefg (681-904)	485 defg (432-563)	21	44 fgh (39- 47)	39 efgh (35−47)	30 defg (27-35)
Be6	23	800 cdefg (681-904)	498 cdefg (419-590)	24	45 efg (39-55)	40 efgh (35−51)	31 defg (25-39)
Be8	28	819 cde (707-956)	485 defg (367-603)	23	48 cdefg (39-55)	45 cde (37−53)	34.40 bcde (29-43)
CPRO	26	672 j (576-734)	442 gh (367-537)	20	45 efg (39-51)	37 fgh (31−47)	30 defg (24-39)
Eng2	28	755 efgh (642- 865)	479 defg (367-629)	22	45 efg (39-55)	40 efgh (31−47)	34 bcdef (25-41)
Fr1	26	734 ghi (537- 943)	459 fg (328-563)	28	53 abc (39-63)	51 ab (43−59)	43 a (35-59)
Fr2	27	755 efgh (616- 878)	479 defg (354-616)	26	57 ab (51-63)	51 ab (43−59)	41 a (35-49)
Fr3	29	793 cdefgh (601-878)	488 defg (341-563)	27	48 cdefg (39-59)	44 cdefg (35−55)	36 bc (27-43)
Fr4	29	785 cdefgh (629-917)	490 defg (367-576)	27	51 bcde (43-63)	44 cdef (37−55)	36 bcd (29-45)
As	28	646 j (590-799)	412 h (354- 485)	26	44 efgh (31-55)	41 efgh (31−47)	37 b (27-43)
Ge1	25	796 cdefg (648-904)	522 bcde (426-629)	20	49 cdefg (39-55)	42 defg (31−51)	33 bcdef (24-39)
Ge2	25	800 cdefg (675-930)	502 bcdefg (432-557)	22	45 efg (39-51)	41 defgh (35−47)	30 defg (24-35)
Ge4	29	740 fghi (563-904)	468 efg (341-576)	19	45 fgh (39-51)	39 efgh (35−47)	30 efg (25-35)
Ir4	25	565 k (445-655)	403 h (314-537)	20	46 defg (39-55)	48 bc (37−55)	31 bcdef (27-39)
It	25	791 cdefgh (642-983)	523 bcde (380-694)	20	57 a (49-67)	45 cde (37−55)	40 a (31-45)
Mar5	30	686 ij (629-891)	459 fg (354-563)	24	46 efg (39-55)	39 efgh (31−47)	31 cdefg (24-43)
Nl4	26	810 cdef (668- 996)	538 bcd (406-734)	24	45 efg (37-55)	45 cde (37−55)	35 bcde (29-41)
Nl5	24	856 c (642-983)	550 abc (380-668)	27	46 efg (37-55)	44 cdefg (35−53)	34 bcdef (27-45)
Sue1	24	778 defgh (688-891)	487 defg (393-537)	18	46 gh (39-51)	37 gh (31-47)	27 g (24-39)
Sue2	27	782 cdefgh (721-865)	478 defg (393-563)	20	44 fgh (39-51)	40 efgh (35−47)	32 bcdefg (27-37)
Sue4	23	724 hi (642-825)	468 efg (367-550)	22	44 fgh (37-51)	40 efgh (31−51)	29 efg (24-35)
Wob88	22	818 cde (707-943)	517 bcdef (419-668)	18	42 h (39-47)	35 h (31-51)	26 g (24-35)
Ge6**	23	988.72 a (852-1120)	557 ab (419-629)	21	53abcd (47-59)	56 a (47−63)	44 a (39-55)
Mar1**	23	921.59 b (739-1127)	589 a (485-721)	19	52 cdef (43-59)	47 bcd (43−59)	31 defg (27-39)

(1): The population codes are cited in table 1;
(2): Length without neck.
*: Means followed by a letter in common are not significantly different (P=0.05); n: number of individuals examined, **: *H. betae* populations.

H. schachtii to be distinguished from *H. betae* as previously found by Maas and Heijbroek (1981).

Heterodera schachtii can be differentiated from *H. betae* by a number of morphometrical characters of second stage juveniles. The length of the body, hyaline part of the tail, and tail of second stage juveniles were considered as taxonomically useful characters for that goal and were used for the morphometrical comparison of several *Heterodera* species (Raski, 1950; Graney & Miller, 1980, 1982; Anderson, 1984; Steel & Whitehand; 1984; Wouts *et al.*, 2001). Variations in certain characters can be explained by the influence of host plants. Several authors argued that this variability is determined in part by the quality of the food source during the development of the females (Trudgill *et al.*, 1970). The level of infestation also is of importance (Von Mende *et al.*, 1998). In order to obtain more consistent observations, it would be interesting to study the first generation obtained after inoculation of equal numbers of juveniles of each population on the same host.

RAPD analysis. The seventeen different random decamer primers used in this study generated a total of 471 DNA fragments for all populations. The number of DNA fragments varied from 2 to 12 with a length between 200 bp and 1500 bp; some were common to all populations (Fig. 1A, B). Primer G02 yielded a multitude of fragments and a major band of different intensity for *H. betae* and *H. glycines*; this band was not generated for the *H. schachtii* populations (Fig. 1A). Amplification with primers AE04, A03, A06 and B09 produced patterns distinguishing *H. schachtii* from the other species by the markers of 300, 520 and

Table 4. Morphometrics (μm) of second stage juveniles of *Heterodera schachtii* and *H. betae* populations.

Pop	n	L	m.b.w	b.w.a	Stylet length	Length of hyaline part	Tail length
Be1	20	481 cdefghi *	20 abcde	16 ab	32 bc	28 bcd	53 bcdefg
		(441-525)	(18-23)	(14-18)	(29-30)	(24-35)	(43-65)
Be2	21	485 cdefgh	18 de	14 abcd	32 bc	29 bc	54 bcde
		(431-556)	(16-22)	(12-16)	(29-35)	(20-35)	(47-63)
Be4	20	484 cdefgh	19 cde	15 abcd	33 bc	27 bcde	53 bcdefg
		(425-556)	(16-22)	(12-16)	(29-35)	(24-33)	(47-63)
Be5	22	464 defghi	20 abcde	15 abc	33 bc	27 bcde	52 bcdefg
		(419-519)	(16-22)	(13-16)	(29-35)	(24-31)	(43-59)
Be6	20	427 fghij	19 abcde	15 abcd	32 bc	26 bcde	44 fg
		(394-469)	(16-22)	(12-16)	(29-35)	(20-31)	(39-53)
Be8	21	531 cde	19 bcde	14 abcd	32 bc	26 bcde	52 bcdefg
		(463-581)	(16-23)	(12-16)	(31-35)	(22-29)	(41-63)
CPRO	20	428 fghij	21 abcde	15 abcd	31 bc	27 bcde	53 bcdefg
		(400-475)	(19-24)	(13-16)	(27-33)	(24-29)	(49-55)
Eng2	18	379 j	17 e	12 d	28 c	22 e	45 efg
		(356-488)	(17-20)	(12-16)	(27-33)	(22-28)	(41-55)
Fr1	21	530 cde	23 a	15 abcd	32 bc	29 bc	49 bcdefg
		(500-581)	(22-24)	(14-16)	(29-33)	(25-31)	(43-55)
Fr2	22	491 cdefg	22 ab	15 ab	33 bc	28 bcd	54 bcdef
		(425-569)	(19-25)	(12-20)	(29-33)	(23-31)	(47-59)
Fr3	21	495 cdefg	20 abcde	16 a	32 bc	35 a	58 b
		(450-538)	(19-22)	(16-19)	(27-35)	(31-37)	(55-61)
Fr4	18	412 hij	19 bcde	14 abcd	28 c	30 b	52 bcdefg
		(381-519)	(19-24)	(13-18)	(25-33)	(25-37)	(43-63)
As	18	460 efghi	18 e	13 cd	30 c	24 cde	45 defg
		(425-531)	(17- 24)	(12-16)	(27-35)	(22-27)	(43-55)
Ge1	20	444 fghij	19 bcde	14 bcd	32 bc	27 bcde	50 bcdefg
		(394-481)	(18-20)	(12-16)	(29-35)	(24-31)	(41-59)
Ge2	22	418 ghij	19 abcde	14 abcd	32 bc	24 cde	44 g
		(363-463)	(16-22)	(12−16)	(29-35)	(20-28)	(37-55)
Ge4	21	406 ij	20 abcde	16 ab	31 bc	23 de	47 defg
		(356-506)	(18-24)	(13-20)	(27-33)	(20-27)	(37-63)
Ir4	20	521 cde	19 bcde	14 bcd	29c	24 cde	50 bcdefg
		(475-575)	(18-20)	(12−16)	(25-31)	(22-27)	(45-55)
It	21	426 fghij	19 bcde	14 abcd	31 bc	25 cde	49 bcdefg
		(381-462)	(18-20)	(12-16)	(27-33)	(22-27)	(43-53)
Mar5	21	555 c	20 abcde	15 abcd	33 bc	25 bcde	53 bcdefg
		(500-619)	(19-20)	(12-18)	(31-33)	(22-31)	(45-63)
Nl4	21	541 cd	22 abc	15 abc	32 bc	28 bcd	54 bcd
		(500-575)	(20-24)	(14−18)	(29-33)	(24-35)	(47-63)
Nl5	18	479 cdefghi	19 bcde	14 abcd	29 c	25 cde	48 cdefg
		(456-569)	(18-24)	(12-18)	(27-35)	(24-31)	(45-59)
Sue1	18	473 defghi	22 abcd	17 a	32 bc	26 bcde	57 bc
		(419-513)	(20-24)	(15−18)	(27-35)	(22-27)	(49-61)
Sue2	19	523 cde	20 abcde	15 abc	30 bc	24 cde	52 bcdefg
		(506-588)	(20-24)	(14−20)	(27-33)	(22-27)	(49-59)
Sue4	20	472 defghi	20 abcde	14 abcd	31 bc	23 cde	51 bcdefg
		(438-500)	(19-23)	(12-16)	(27-33)	(20-27)	(47-53)
Wob88	19	502 cdef	20 abcde	15 abcd	29 c	26 bcde	47 defg
		(463-575)	(18-24)	(14−18)	(27-33)	(22-37)	(39-71)
Ge6**	24	672 a	20 abcde	15 abcd	38 a	35 a	68 a
		(576-750)	(20-23)	(14-16)	(35-39)	(31-39)	(63-74)
Mar1**	20	6115 b	22 abc	16 ab	35 ab	34 a	71 a
		(581-669)	(20-24)	(16−18)	(31-39)	(31-37)	(66-78)

n: Number of individuals examined; L: Body length; m.b.w: maximum body width; b.w.a: body width at anus. (1): Population codes are given in table 1; *: Means followed by a letter in common are not significantly different (P=0.05); **: *H. betae* populations.

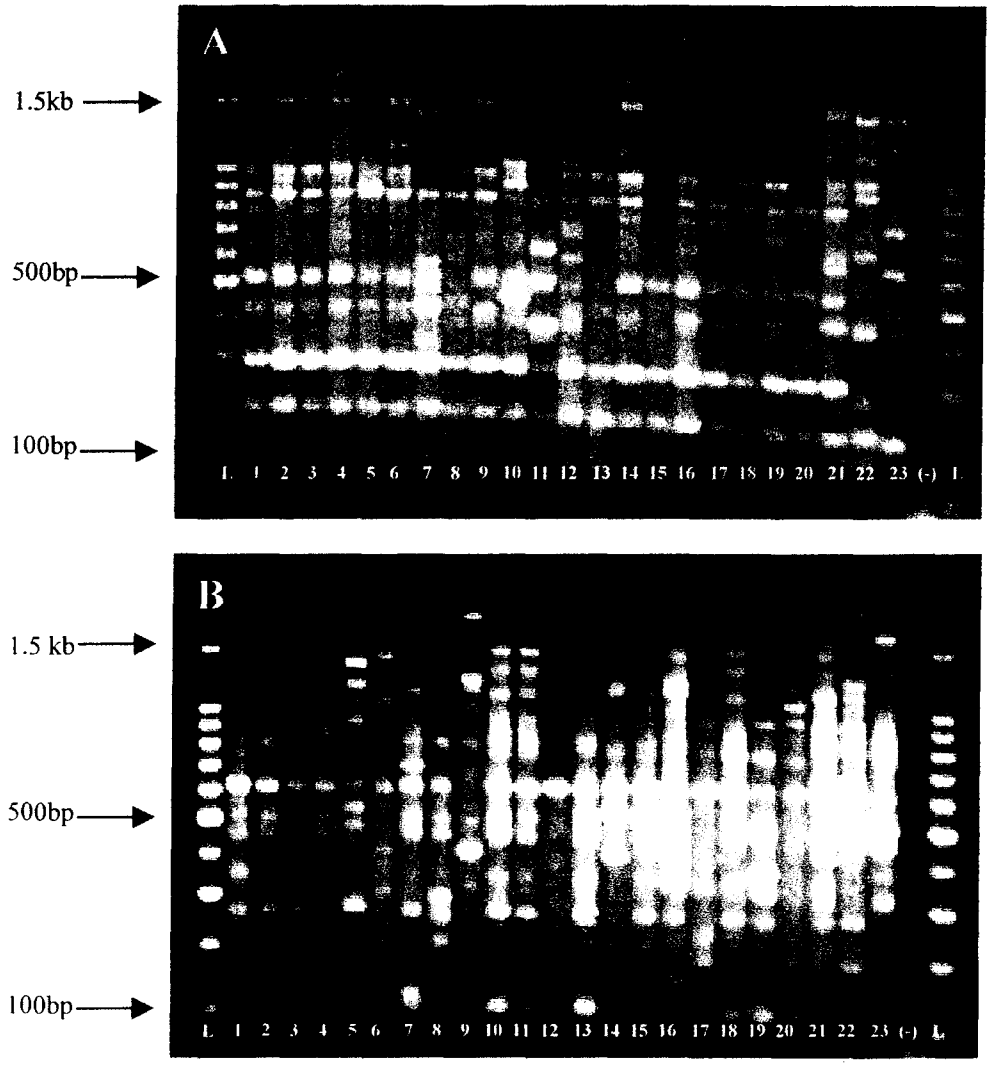

Fig. 1. RAPD profiles of 20 *Heterodera schachtii* populations (1-20), and one population of *H. betae* (21), *H. trifolii* (22) and *H. glycines* (23). Patterns obtained with primers (A) G02 and (B) K16. L: 100 bp ladder; (-): negative control. Populations 1: Be1; 2: Be4; 3: Be6; 4: Ge1; 5: Ge2; 6: Ge4; 7: Fr1; 8: Fr2; 9: Fr3; 10: Mar5; 11: Mar27; 12: Nl4; 13: Nl5; 14: Sue2; 15: Sue3; 16: Sue4; 17: Ir4; 18: Be5; 19: Be8; 20: Fr4; 21: Ge6; 22: Ht2; 23: Hg1. (For population codes, see table 1).

1400 bp, respectively (Amiri, 2002). The UPGMA analysis of the DNA fragments grouped the 20 *H. schachtii* populations together; the *H. betae* populations formed a separate clade (Fig. 2).

The *H. schachtii* populations were not arranged in groups according to their origin. This confirms Caswell-Chen *et al.* (1992) who showed that the similarity found between populations of the Imperial Valley (California) and those collected in the North of California are essentially due to the infestation of fields in the first region, but also by commercial exchanges and the use of the same farm equipment. *H. schachtii* has a large geographical distribution (Baldwin & Mundo-Ocampo, 1991).

Our results also confirm those obtained by Folkertsma *et al.* (1994) who showed that there is no interrelationship between the genetic similarity of populations of *Globodera rostochiensis* and *G. pallida* and their respective origins. Even populations of *G. rostochiensis* and *G. pallida* collected from closely located sites were discriminated in different groups. Similar conclusions were obtained for populations of *Meloidogyne* (Blok *et al.*, 1997) and *Radopholus* (Kaplan, 1994; Fallas *et al.*, 1996; Elbadri *et al.*, 2002).

Although RAPD analysis does not permit grouping of populations of *H. schachtii* according to their geographical origin, it does permit separation of species. Some RAPD markers generated by

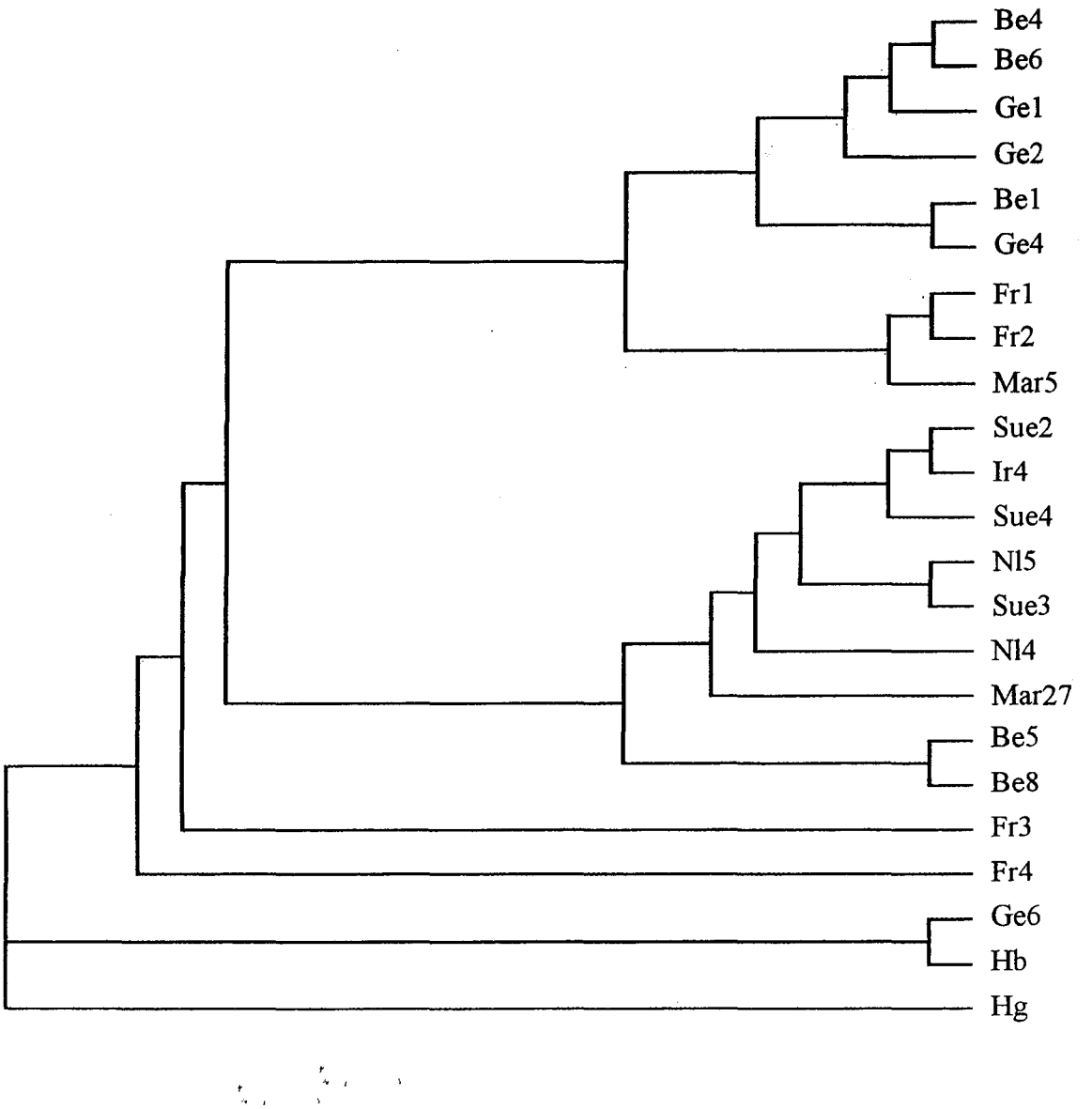

Fig. 2. Genetic relationships between populations of *Heterodera schachtii* and *H. betae* constructed on the basis of RAPD marker analysis.

primers are specific for *H. schachtii* and can be exploited for sequencing work and the development of specific primers for this species.

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REFERENCES

Amiri, S. 2002. *Diversité et identification des nematodes a kyste de la betterave, Heterodera schachtii et H. betae.* PhD thesis, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Belgium. 168 pp.

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.

Ambrogioni, L., Cotroneo, A., Moretti, F., Tacconi, R. & Irdani, T. 1999. First record of the yellow beet cyst nematode (*Heterodera trifolii*) in Italy. *Nematologia Mediterranea.* 27: 151-158.

Anderson, S. 1984. First record of yellow beet cyst nematode (*Heterodera trifolii*) in Sweden. *Växtskyddsnotiser* 48: 11.

Anonymous. 1992. *Manuel d'utilisation de STAT-ITCF.* Versailles, France, 63 pp

Baldwin, J.G & Mundo-Ocampo, M. 1991. Heteroderinae, cyst and non cyst forming nematodes. In: *Manual of Agricultural Nematology.* (W.R Nickel,. Ed). pp. 275-362, New York, Marcel Dekker Inc.

Blok, V.C., Phillips, M.C., McNicol, J.W. & Fargette, M. 1997. Genetic variation in tropical *Meloidogyne* spp. as shown by RAPD. *Fundamental and Applied Nematology* 20: 127-133.

Caswell-Chen, E.P., Willamson, V.M. & Wu, F.F. 1992. Random amplified polymorphic DNA analysis of *Heterodera cruciferae* and *H. schachtii* populations. *Journal of Nematology* 24: 343-351.

Elbadri, G.A.A., De Ley, P, Waeyenberge, L., Vierstraete, A., Moens, M. & Vanfleteren, J. 2002. Intraspecific variation in *Radopholus similis* isolates assessed with restriction fragment length polymorphism and DNA sequencing of the internal transcribed spacer region of the ribosomal RNA cistron. *International Journal for Parasitology* 32: 199-205.

Evans, K. & Rowe, J.A. 1998. Distribution and economic importance. In: *The Cyst Nematodes.* (S.B. Sharma, Ed). London, UK, Kluwer Academic Publishers. pp. 1-30.

Fallas, G.A., Hahn, M.L. Fargette, M., Burrows, P.R. & Sarah, J.-L. 1996. Molecular and biochemical diversity among isolates of *Radopholus* spp. from different areas of the world. *Journal of Nematology* 28: 422-430.

Folkertsma, R.T., Rouppe van der Voort, J.N.A.M., van Gent-Pelzer, M.P.E., de Groot K.E, van den Bos. W.J., Schots, A., Bakker, J. & Gommers, F.J. 1994. Inter- and intraspecific variation between populations of *Globodera rostochiensis* and *G. pallida* revealed by random amplified polymorphic DNA. *Phytopathology* 84: 807-811.

Graney, L.S. & Miller, L.I. 1979. Morphology of second-stage larvae of four isolates of *Heterodera schachtii. Journal of Nematology* 11: 299.

Graney, L.S. & Miller, L.I. 1980. Differentiation of five isolates of *Heterodera schachtii* as races of the sugar beet cyst nematode. *Journal of Nematology* 12: 223.

Graney, L.S. & Miller, L.I. 1982. Comparative morphological studies of *Heterodera schachtii* and *H. glycines.* Nematology in the Southern Region of United States. *Southern Cooperative Series Bulletin* 276: 96-107.

Ibrahim, S.K., Baldwin, J.G., Robert, P.A. & Hyman, B.C. 1997. Genetic variations in *Nacobbus aberrans*: An approach towards taxonomic resolution. *Journal of Nematology* 29: 241-249.

Kaplan, D.T. 1994. Molecular characterisation of the burrowing nematodes sibling species, *R. citrophilus* and *R. similis.* In: *Advances in Molecular Plant Nematology.* (F. Lamberti, C. De Giorgi, & D.M. Bird, Eds). pp. 77-83. Plenum Press, New York.

Maas, P.W.Th. & Heijbroek, W. 1981. Het geel bieteysteaaltje, een biotype van *Heterodera trifolii. Gewasbescherming* 12: 205-218.

Maas, P.W.Th., Du Bois, E. & Dede, J. 1982. Morphological and host range variation in the *Heterodera trifolii* complex. *Nematologica* 28: 263-270.

Pinochet, J., Cenis, J.L., Fernadez, C., Doucet, M. & Marull, J. 1994. Reproductive fitness and Random Amplified Polymorphic DNA variation among isolates of *Pratylenchus vulnus. Journal of Nematology* 18: 391-392.

Raski, D.J. 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. *Phytopathology* 40: 135-151.

Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* 4: 67-69.

Seinhorst, J.W. 1964. Methods for the extraction of *Heterodera* cysts from not previously dried soil samples. *Nematologica* 10: 87-94.

Steele, A.E. & Whitehand, L. 1984. Comparative morphometrics of eggs and second-stage juveniles of *Heterodera schachtii* and race of *H. trifolii* parasitic on sugar beet in the Netherlands. *Journal of Nematology* 16: 171-177.

Swofford, D., L. 1998. *PAUP*. Phylogenetic analysis using parsimony and other methods.* Version 4. Sinauer, Sunderland, MA. 128 pp.

Trudgill, D. L., Parrott, D. M. & Stone, A. R. 1970. Morphometrics of males and larvae of ten *Heterodera rostochiensis* population and the influence of resistant hosts. *Nematologica* 16: 410-416.

Von Mende, N., Gravato Nobre, M.J. & Perry, R.N. 1998. Host finding, invasion and feeding. In: *The Cyst Nematodes.* (S.B. Sharma Ed.). pp. 1-30. London, UK, Kluwer Academic Publishers.

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.

Amiri S., Subbotin S.A., Moens M. Сравнительная морфометрия и RAPD анализ популяций *Heterodera schachtii* и *H. betae* .

Резюме. Проведено сравнение морфометрических данных личинок и цист из 25 популяций *H. schachtii* и двух популяций *H. betae*. Длина и ширина цист *H. betae* превышают таковые у *H. schachtii*. Длина фенестр и ширина полуфенестр не отличают изученные популяции *H. betae* от популяций *H. schachtii,* и потому эти характеристики не могут использоваться для разграничения этих видов. Длины тела личинок 2 стадии, их хвостового конца и гиалиновой части были значительно больше у *H. betae,* чем у *H. schachtii,* что позволяет диагностировать эти виды. RAPD анализ с использованием 17 декамерных праймеров для 20 популяций *H. schachtii,* двух популяций *H. betae* и одной популяции *H. glycines* выявил 471 фрагмент ДНК. Некоторые из генерированных этими праймерами полос спектра были специфичны для *H. schachtii*. Созданная на основе данных RAPD дендрограмма не распределяет изученные популяции *H. schachtii* в соответствии с их происхождением, но выделяет виды нематод.