Nematology, 2003, Vol. 5(4), 515-538

Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae)

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Received: 30 September 2002; revised: 27 February 2003
Accepted for publication: 5 March 2003

Summary – Species of the *Heterodera avenae* complex, including populations of *H. arenaria*, *H. aucklandica*, *H. australis*, *H. avenae*, *H. filipjevi*, *H. mani*, *H. pratensis* and *H. ustinovi*, obtained from different regions of the world were analysed with PCR-RFLP and sequencing of the ITS-rDNA, RAPD and light microscopy. Phylogenetic relationships between species and populations of the *H. avenae* complex as inferred from analyses of 70 sequences of the ITS region and of 237 RAPD markers revealed that the cereal cyst nematode *H. avenae* is a paraphyletic taxon. The taxonomic status of the Australian cereal cyst nematode *H. australis* based on sequences of the ITS-rDNA and RAPD data is confirmed. Morphometrical and ITS-rDNA sequence analyses revealed that the Chinese cereal cyst nematode is different from other *H. avenae* populations infecting cereals and is related to *H. pratensis*. *Bidera riparia* Kazachenko, 1993 is transferred to the genus *Heterodera* as *H. riparia* (Kazachenko, 1993) comb. n. As a consequence, *H. riparia* Subbotin, Sturhan, Waeyenberge & Moens, 1997 becomes a junior secondary homonym and is renamed as *H. ripae* nom. nov. Morphological, morphometrical characters and RFLP profiles for identification of the nine species presently placed in the *H. avenae* species complex are given.

Keywords – *Bidera riparia*, cereal cyst nematodes, *Heterodera ripae* nom. nov., *Heterodera riparia* comb. n., identification, IEF, ITS-rDNA, PCR-RFLP, phylogeny, RAPD.

The cereal cyst nematode, *Heterodera avenae* Wollenweber, 1924, is a major nematode pest in many countries and found in many cereal growing areas. It has been recorded from many European and Asian countries, northern Africa, Australia, New Zealand, USA and Canada (Meagher, 1977; Baldwin & Mundo-Ocampo, 1991; Rivoal & Cook, 1993). *Heterodera avenae* is the principal nematode species on temperate cereals, and in Europe more than 50% of the fields in major cereal growing areas are infected by this nematode (Rivoal & Cook, 1993). Extensive studies have revealed the presence of several distinct species of *Heterodera* infecting cereals and grasses within studied populations primarily identified as *H. avenae*. Presently, the *H. avenae* complex is considered to contain: *H. avenae*, *H. arenaria* Cooper, 1955, *H. aucklandica* Wouts & Sturhan, 1995, *H. australis* Subbotin, Sturhan, Rumpenhorst & Moens, 2002, *H. filipjevi* (Madzhidov, 1981), *H. mani* Mathews, 1971, *H. pratensis* Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000, and *H. ustinovi* Kirjanova, 1969 (Wouts & Sturhan, 1995; Gäbler *et al.*, 2000; Sturhan & Krall, 2002; Subbotin *et al.*, 2002). An additional species, *Bidera riparia*, belonging to this complex has been described from the Russian Far East by Kazachenko (1993).

The species *H. latipons* Franklin, 1969 and *H. hordecalis* Andersson, 1975 are considered to form a separate species complex within the *H. avenae* group. *Heterodera bifenestra* Cooper, 1955, *H. spinicauda* Wouts, Schoemaker, Sturhan & Burrows, 1995 and *H. turcomanica* Kirjanova & Shagalina, 1965 are clearly not related to species of the *H. avenae*, *H. latipons* and *H. hordecalis* complexes (Wouts & Sturhan, 1995; Gäbler *et al.*, 2000; Subbotin *et al.* 2001; Tanha Maafi *et al.*, 2003), but they are still

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retained in the 'artificial' *H. avenae* group *sensu lato* by Handoo (2002).

The species of the *H. avenae* complex are differentiated from each other by small morphological and morphometrical characters. Recently, Handoo (2002) published keys for morphological identification of some *H. avenae* group species. With an increasing number of species in this group, reliable identification based on morphology is becoming more difficult. Several biochemical and molecular techniques have been used for the separation of species and populations from this complex and showed promising results: enzyme polymorphism analysis (Bergé *et al.*, 1981; Bossis & Rivoal, 1990; Ibrahim & Rowe, 1995; Romero *et al.*, 1996; Andrés *et al.*, 2001; Mokabli *et al.*, 2001), two-dimensional gel electrophoresis (2-DGE) (Ferris *et al.*, 1989, 1994; Bossis & Rivoal, 1996; Romero *et al.*, 1996); isoelectric focusing (IEF) (Rumpenhorst, 1985; Sturhan & Rumpenhorst, 1996; Subbotin *et al.*, 1996), random amplified polymorphic DNA (RAPD) (Lopez-Braña *et al.*, 1996; Sturhan & Rumpenhorst, 1996; Romero *et al.*, 1996), sequences of the ITS-rDNA (Ferris *et al.*, 1994; Subbotin *et al.*, 2001, 2002), restriction fragment length polymorphism (RFLP) of ITS-rDNA (Bekal *et al.*, 1997; Subbotin *et al.*, 1999, 2000, 2002), and PCR-single-strand conformational polymorphism (PCR-SSCP) (Clapp *et al.*, 2000).

This paper reports on our analysis of the ITS-rDNA sequences, morphometric and morphological characters of a wide range of populations and all species of the *H. avenae* complex. Based on molecular phylogenetic relationships, the structure of this complex is discussed. RAPD analysis is used to support the validity of *H. australis.* The most appropriate morphological and morphometrical characters and RFLP-rDNA profiles for identification of the *H. avenae* species complex and populations are given.

Materials and methods

Nematode populations

Sixty seven populations of the *H. avenae* complex were analysed in this paper (see Table 1). The nematode populations were collected during surveys and maintained in pots in the glasshouse. Cysts were isolated from soil by sieving-decanting or centrifugation-flotation methods. Eggs and juveniles were released from squashed cysts. Paratypes of *H. mani* and *Bidera riparia* Kazachenko, 1993 were obtained from Rothamsted Research, Nematode Collection, UK, and Nematode Collection of the Laboratory of Phytonematology, Institute of Biology and Pedology, Vladivostok, Russia, respectively.

PCR and RFLP of the ITS-rDNA

A single cyst was transferred into 20 μl of nematode lysis buffer (2 μl of 10X PCR buffer, 8 μl of double distilled water and 0.06 μl of Proteinase K (20 mg/ml, Qiagen, GmbH, Hilden, Germany)) in an Eppendorf tube and crushed with a microhomogeniser. The tubes were incubated at 60°C (1 h) and 95°C (15 min) consecutively. After centrifugation (1 min; 16 000 *g*) 2 μl of the DNA suspension was added to PCR reaction mixture containing 5 μl of 10X PCR buffer, 2 μl of $MgCl_2$ (25 mM), 200 μM of each dNTP, 1.0 μM of each primer, 0.8U of HotStar *Taq* DNA Polymerase (Qiagen) and double distilled water to a final volume of 45 μl. Primers TW81 and AB28 were used for the PCR reaction as described by Joyce *et al.* (1994) (Table 2). The DNA-amplification profile was programmed for initial denaturation and enzyme activation at 95°C for 15 min, followed by 35 cycles of 45 s at 95°C, 45 s at 60°C, 1.5 min at 72°C, and 10 min at 72°C. A sample 3-7 μl of each PCR product was digested with each of the following restriction enzymes: *Alu*I, *Cfo*I, *Hin*fI, *Ita*I, *Pst*I, *Rsa*I, *Taq*I and *Tru*9I in the buffer stipulated by the manufacturers (Boehringer Mannheim, Germany, MBI Fermentas Inc., New England Biolabs). The digested DNA was loaded on a 1.5% agarose gel, separated by electrophoresis (100V, 1.5 h), stained with ethidium bromide, visualised on UV transilluminator, and photographed with a Polaroid MP4+ Instant Camera System. Procedures for obtaining PCR amplified products and endonuclease digestion were repeated at least twice to verify the results. The length of restriction fragments were calculated by a virtual digestion of sequences using Webcutter 2.0 (http://www.firstmarket.com:cutter/cut2.html).

Cloning and sequencing

PCR products were excised from 1% TBE buffered agarose gels using the QIAquick Gel Extraction Kit (Qiagen), cloned into the pGEM®-T vector and transformed into JM109 High Efficiency Competent Cells (Promega Corporation, USA). The clones of each population were isolated using blue/white selection, submitted to PCR and then cycle sequenced. PCR products of clones from some populations were restricted by *Alu*I, *Rsa*I or *Alw*21I to reveal polymorphic clones. DNA fragments were sequenced in both directions with TW81, AB28, 5.8SM2 or 5.8SM5

Table 1. *Species and populations of the* Heterodera avenae *complex used in this study.*

Species or population group	Location, country	Source of material and/or data	GenBank accession number for the ITS sequence	Studies*
H. arenaria	Lincolnshire, UK	J. Rowe; Subbotin *et al.* (1999, 2001)	AF274396	M, CDA, SEQ
	Unknown, The Netherlands	G. Karssen		M, CDA, RFLP
H. aucklandica	Auckland, New Zealand	W. Wouts; Subbotin *et al.* (1999, 2001)	AF274398	M, CDA, SEQ, RAPD
	Zarren, West Vlaanderen, Belgium	S.A. Subbotin	AY148379	M, CDA, SEQ
	St Albans, UK	S.A. Subbotin	AY148380	M, CDA, SEQ
H. australis	Beulah, Victoria, Australia	H.J. Rumpenhorst; Subbotin *et al.* (2002)	AY148393	M, CDA, RFLP, SEQ, RAPD
	South Australia, Australia (sample 1)	M. Moens (orig. from A. Khan); Subbotin *et al.* (2002)	AY148352	M, RFLP, SEQ
	South Australia, Australia (sample 2)	H.J. Rumpenhorst (orig. from W. Wouts); Subbotin *et al.* (2002)	AY148394	M, CDA, RFLP, SEQ
	Yorke Peninsula, South Australia	F. Charman-Green; Subbotin *et al.* (2002)	AY148395, AY148396	M, CDA, RFLP, SEQ, RAPD
	Victoria, Australia	J. Wilson; Subbotin *et al.* (2002)	AY148392	M, CDA, SEQ, RAPD
	South Australia, Australia (sample 3)	I. Riley (orig. from J. Lewis)		M, RAPD
H. avenae	Taaken, Lower Saxony, Germany	D. Sturhan; Subbotin *et al.* (1999)	AY148353	M, CDA, RFLP, SEQ, RAPD
	Argentan, France	R. Rivoal; Subbotin *et al.* (1999, 2001)	AF274395	M, CDA, SEQ
	St Georges du Bois, France	R. Rivoal; Subbotin *et al.* (1999)		M, CDA
	Knokke, West Vlaanderen, Belgium	Subbotin *et al.* (1999).		M, CDA
	Nuisement sur Coole, France	R. Rivoal; Subbotin *et al.* (1999)	AY148357, AY148359, AY148370, AY148371	M, CDA, SEQ
	Santa Olalla, Spain	M. Romero; Subbotin *et al.* (1999)	AY148354, AY148355, AY148356	M, CDA, RFLP, SEQ, RAPD
	Dedesdorf, Lower Saxony, Germany	D. Sturhan	AY148360	M, RFLP, SEQ
	Grafenreuth, Bavaria, Germany	H.J. Rumpenhorst		M
	Unknown, Morocco	S. Amuri	AY148367, AY148368, AY148369	M, CDA, SEQ, RFLP
	Desert region, India	J. Rowe; Subbotin *et al.* (1999, 2001)	AF274397	M, CDA, SEQ
	Rothamsted Research, UK	J. Rowe	AY148358	SEQ
	Near Delhi, India	H.J. Rumpenhorst	AY148362	M, RFLP, SEQ, RAPD
	Unknown, Saudi Arabia	H.J. Rumpenhorst	AY148361	M, CDA, RFLP, SEQ, RAPD
	Çukurova plain, Turkey	I.H. Elekçioglu	AY148364	M, CDA, RFLP, SEQ, RAPD

Table 1. *(Continued).*

Species or population group	Location, country	Source of material and/or data	GenBank accession number for the ITS sequence	Studies*
	Ilam, Mehran-Reza Abad, Iran	Tanha Maafi *et al.* (2003)	AF498378	SEQ
	Nir Oz, Israel	D. Orion; Subbotin *et al.* (2002)	AY148365	M, CDA, SEQ
	Bet Dagan, Israel	D. Orion	AY148363	M, CDA, SEQ
	Ha-hoola, Israel	M. Mor	AY148366	M, CDA, RFLP, SEQ, RAPD
	Unknown, Israel	H.J. Rumpenhorst		M, RAPD
	Villasavary, France	R. Rivoal; Subbotin *et al.* (1999)	AY148372, AY148373, AY148374	M, CDA, SEQ
H. pratensis	Putilovo, Leningrad region, Russia	Subbotin *et al.* (1996; 2002), Gäbler *et al.* (2000)	AY148351	M, CDA, RFLP, SEQ
	Lindhöft, Schleswig-Holstein, Germany	Gäbler *et al.* (2000)	AY148387	M, CDA, RFLP, SEQ
	Near Rotterdam, The Netherlands	B. Schoemaker	AY148388	M, SEQ
	Otterndorf, Lower Saxony, Germany	D. Sturhan	AY148383, AY148384, AY148386, AY148390, AY148391	M, CDA, RFLP, SEQ
	Altenbruch, Lower Saxony, Germany	D. Sturhan	AY148385	M, RFLP, SEQ
	Missunde near Schleswig, Germany	D. Sturhan		M, RFLP, RAPD
	Dangast, Lower Saxony, Germany	D. Sturhan	AY148389	M, RFLP, SEQ
	Lenggries, Bavaria, Germany	D. Sturhan		M, RFLP, RAPD
	Kurilovo, Moscow region, Russia	Subbotin *et al.* (1999), Gäbler *et al.* (2000)		M, CDA
	Östergaard, Schleswig-Holstein, Germany	D. Sturhan		M, RAPD
H. filipjevi	Dushanbe, Tadzhikistan	H.J. Rumpenhorst; Subbotin *et al.* (1996)	AY148402	M, CDA, SEQ
	Saratov, Russia	E. Osipova; Subbotin *et al.* (2001)	AF274409	M, RFLP, SEQ, RAPD
	Vad, Nizhnii Novgorod region, Russia	L. Nasonova	AY148401	M, CDA, SEQ
	Marvast, Iran	H.J. Rumpenhorst (orig. from H. Farivar-Mehin)	AY148404	M, SEQ
	Esfahan, Iran	Tanha Maafi *et al.* (2003)	AF498380	SEQ
	Akenham, UK	H.J. Rumpenhorst (orig. from A.R. Stone)	AY148403	M, CDA, SEQ, RAPD
	Gimbte, near Münster, Germany	H.J. Rumpenhorst	AY148400	M, SEQ
	Torralba de Calatrava, Spain	M. Romero; Subbotin *et al.* (1999)	AY148399	M, CDA, RFLP, SEQ
	Selçuklu, Turkey	H.J. Rumpenhorst (orig. from I.H. Elekçioglu)	AY148397, AY148398	M, CDA, SEQ, RAPD
	Merkez, Turkey	H.J. Rumpenhorst (orig. from I.H. Elekçioglu)		M, CDA, SEQ

Table 1. *(Continued).*

Species or population group	Location, country	Source of material and/or data	GenBank accession number for the ITS sequence	Studies*
H. "avenae" from China	Taigu, Shanxi province, China	Zheng *et al.* (2000)		M, SEQ
	Fonshu county, Beijing sub., China (sample 1)	H.J. Rumpenhorst (originated from D. Peng)	AY148381	M, CDA, RFLP, SEQ
	Tongzhu county, Beijing sub., China	D. Peng	AY148382	M, CDA, RFLP, SEQ
	Pinjgu county, Beijing sub., China	D. Peng		M, CDA, RFLP
	Fonshu county, Beijing sub., China (sample 2)	D. Peng		M, CDA, RFLP
H. mani	Hamminkeln, Germany	D. Sturhan	AY148375, AY148377	M, CDA, RFLP, SEQ
	Bavaria, Germany	H.J. Rumpenhorst	AY148378	M, CDA, RFLP, SEQ, RAPD
	Andernach, Germany	H.J. Rumpenhorst		M, CDA, RFLP, RAPD
	Heinsberg, Germany	H.J. Rumpenhorst	AY148376	M, CDA, RFLP, SEQ, RAPD
	Unknown, Germany	H.J. Rumpenhorst		M, RAPD
H. ustinovi	Forfar, Scotland, UK	Subbotin *et al.* (1999, 2001)	AF274400	M, CDA, SEQ
	Belgium	Subbotin *et al.* (2000)	AY148407	M, CDA, RFLP, SEQ
	Everinghausen, near Bremen, Germany	D. Sturhan	AY148406	M, CDA, RFLP, SEQ
	Zboj, Slovakia	D. Sturhan	AY148405	M, SEQ
Heterodera sp.	Gilan, Bandar Anzali, Iran	Tanha Maafi *et al.* (2003)	AF498379	SEQ
H. riparia comb. n.	Olga, Olga bay, Russian Far East	Kazachenko (1993)		M, CDA

*M – morphological analysis; CDA – canonical discriminant analysis; RFLP – restriction fragment length polymorphism of rDNA; SEQ – sequence and phylogenetic analyses of the ITS-rDNA; RAPD – random amplified polymorphic DNA.

(Zheng *et al.*, 2000) primers (Table 2) with BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. The resulting products were purified using a Centriflex Gel Filtration Cartridge (Edge BioSystems Inc., Gaithersburg, MD, USA) and run on a 377 DNA sequencer (PE Applied Biosystems). Sequences have been deposited in GenBank under accession numbers indicated in Table 1.

Sequence alignment and phylogenetic analysis

Sixty-eight original and known sequences of the *H. avenae* complex (Zheng *et al.*, 2000; Subbotin *et al.*, 2001, 2002; Tanha Maafi *et al.*, 2003) (Table 1) and sequences of the two outgroup taxa *H. hordecalis* (AF274401) and *H. latipons* (AF274402) (Subbotin *et al.*, 2001) were aligned using ClustalX 1.64 with default options (Thompson *et al.*, 1997). Two alignments were created: *i*) alignment of the full data set including the complete ITS sequences of all populations and species, and *ii*) alignment of consensus sequences from all main nematode groups revealed in results of maximum parsimony analyses of the full data set.

The equally weighted maximum parsimony (MP) and maximum likelihood (ML) analyses of the ITS alignment were performed using PAUP* 4b4a (Swofford, 1998). Heuristic search setting was ten random replicates of taxon addition with tree bisection-reconnection (TBR) branch swapping. Gaps were treated as a missing data or coded as a fifth character. Bootstrap support was estimated by a heuristic search from 500 replicates using

Table 2. *Primer sequences used in the present study.*

Name	Position	Sequence 5′-3′
TW81	18S gene	GTT TCC GTA GGT GAA CCT GC
AB28	28S gene	ATA TGC TTA AGT TCA GCG GGT
5.8SM2	5.8S gene	CTT ATC GGT GGA TCA CTC GG
5.8SM5	5.8S gene	GGC GCA ATG TGC ATT CGA
A-16	Random	AGC CAG CGA A
A-18	Random	AGG TGA CCG T
G-05	Random	CTG AGA CGG A
G-10	Random	AGG GCC GTC T
N-05	Random	ACT GAA CGC C
N-06	Random	GAG ACG CAC C
N-12	Random	CAC AGA ACA C
N-14	Random	TCG TGC GGG T
N-16	Random	AAG CGA CCT G

simple addition sequences with TBR swapping. The *g*1 statistic was computed by generating 10 000 random trees using the Randtrees option. Sets of equally parsimonious trees were summarised using strict consensus. For ML analysis, the appropriate substitution model of DNA evolution that best fitted the data set was determined by Akaike Information Criterion with ModelTest 3.04 (Posada & Crandall, 1998). Bootstrap values for ML tree were calculated by a heuristic search from 100 replicates. Alternative phylogenetic hypotheses were tested using Kishino-Hasegawa test as implemented in PAUP*.

RAPD-PCR

Twenty-four populations were used for RAPD study. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen). Samples consisting of 20-25 well filled cysts were homogenised in 20 μl PBS buffer using a microhomogeniser. The homogenate was mixed with 100 μl of buffer ATL of the QIAamp Mini Kit and further processed according to the manufacturer's instructions. The DNA concentration was measured using the TKO 100 mini fluorometer (Hoefer, San Francisco, CA, USA).

Three ng of DNA was used for RAPD-PCR with nine random primers. Sequences of primers supplied from Roth (Karlsruhe, Germany) are given in Table 2. Amplification reactions were performed in 20 μl reaction volumes containing: 2 μm 1X Qiagen PCR buffer, 3 mM $MgCl_2$, 150 μM of the dNTPs, 0.5 μM primer, 0.7 units HotStarTaq DNA polymerase (Qiagen). Amplification was carried out in a Perkin Elmer 2400 thermocycler programmed for an initial enzyme activation step at 95°C for 15 min followed by 40 cycles of 0.5 min at 94°C, 0.5 min at 38°C, and 1.5 min at 72°C. The up ramp from annealing to extension was set to 0.3°C/s. A final extension for 7 min at 72°C ended the run. PCR products were run on 2% agarose gel, stained with ethidium bromide and photographed with a Polaroid camera under UV light.

Similarity analyses were carried out with the RAPDistance version 2.0 (Armstrong *et al.*, 1994) for the RAPD fragment analyses. Data were scored as presence and absence of bands. Smeared and weak bands were excluded. Distance matrixes were calculated using the Dice, Jaccard or Simple Matching coefficients. Diagrams were constructed using the unweighted pair-group method (UPGMA) with the aid of PHYLYP version 3.5 (Felsenstein, 1989).

Light microscopy and statistical analyses of morphometrical characters

Second-stage juveniles (J2) were killed and fixed in hot TAF and processed to glycerine. The J2 and vulval cones mounted in glycerol-gelatine on permanent slides were examined, measured and photographed with Zeiss and Leica light microscopes equipped with Nomarski optics. Morphometrics of several populations were obtained from published articles (Mathews, 1971; Wouts & Weischer, 1977; Madzhidov, 1981; Valdeolivas & Romero, 1990; Robinson *et al.*, 1996; Subbotin *et al.*, 1996, 1999, 2002) and added to our data set for Canonical Discriminant Analysis (CDA). The morphometrical data were analysed with Statistica 5.0. Forward stepwise CDAs of morphometrical characters of the juveniles from 64 populations and the cysts from 35 populations with *a priori* classification were based on sequence and morphological identification. The analysis was conducted as implemented in the Statistica program manual.

Results and discussion

Phylogenetic relationships within the *H. avenae* complex as inferred from sequences of ITS-rDNA

The amplification of the ITS-rDNA of each population yielded one fragment approximately 1050 bp long. No PCR products were obtained in the control lacking the DNA template. Fifty-seven original and 13 known ITS sequences of the *H. avenae* group were aligned. The length of the ITS1+5.8S+ITS2 region sequence alignment for the full data set was 998 characters. The ITS region length

was shorter in the outgroup taxa (961, 962), and varied within ingroup taxa from 964-965 (*H. pratensis*) to 975-976 (*H. filipjevi* and *H. ustinovi*). Nucleotides were distributed on average as A – 18.6%, C – 23.1%, G – 29.6%, T – 28.7%. Sequence divergence for ingroup taxa ranged from 0.0-3.4%, within species without underbridge from 0.0-1.55%, and between ingroup and outgroup from 7.4-10.0%. The 5.8S gene showed minimum variation. The ITS2 region was shorter and showed less sequence divergence than the ITS1.

The alignment of the full sequence data was analysed using MP with gaps coded as fifth character, because application of MP with gaps coded as missing character or ML analyses were problematic because of large numbers of sequence data. Analysis of the alignment of 70 ITS sequences with MP (gaps treated as fifth character) resulted in 20 equally parsimonious trees of 328 length. The strict consensus of these trees is given in Fig. 1. The tree length distribution for 10 000 randomly generated trees was significantly skewed to the left ($-g1 = 0.82904$), suggesting a phylogenetic signal in the data (Hillis & Huelsenbeck, 1992). When *H. latipons* and *H. hordecalis* were used as outgroup taxa, the species having cysts with an underbridge (*H. ustinovi* and *H. filipjevi*) occupied basal positions in the phylogram. Sequences of isolates belonging to the same species clustered together. Cereal cyst nematode populations primarily identified based on morphology as *H. avenae* were distributed within four clades in two main groups. The first main group considered here as *H. avenae sensu stricto* included three clades: *i*) populations from Africa and Asia, *ii*) several populations from France, *iii*) other populations from Europe. Analyses of the Nuisement sur Coole population showed that the single cyst used contained two types of ITS clones belonging to two different groups (Fig. 1). Our unpublished data also revealed that cysts from some Asian populations contained recombinant sequences. Presence of sequences belonging to different groups might be explained as a result of gene flow between these populations. The second main group representing another evolutional branch of the cereal cyst nematodes included *iv*) populations from China designated as *H. 'avenae'*. Our study shows a close relationship of the 'Chinese cereal cyst nematode' with the European *H. pratensis* and the unidentified Iranian *Heterodera* sp. from grassland.

The consensus sequences obtained from each species and main population groups from the complete data set were aligned and analysed by MP (gap treated as missing data) and ML (GTR+G model) analyses. Trees yielded by these analyses are presented in Fig. 2. The Kishino-Hasegawa test rejected constraints when Australian ($-\ln L = 2373.7314$, $P = 0.0119$) or Chinese ($-\ln L = 2381.3186$, $P = 0.0038$) cereal cyst nematodes clustered with European ones. Relationships between *H. avenae*, *H. aucklandica* and *H. mani* are not resolved on these trees.

Because sequences of several populations of the *H. avenae* complex from USA, Sweden and Australia published by Ferris *et al.* (1994) differed in many nucleotide positions, and the sequences of two *H. filipjevi* populations used by Ferris *et al.* (1999) differed in three deletions from the sequences obtained in our study, they were not included in the main phylogenetic analysis. However, analysis of these sequences with our consensus sequence data strongly placed these populations with corresponding population groups or species. American populations (Oregon and Idaho isolates) clustered with European *H. avenae* (data not shown).

RFLP ANALYSIS

The restriction pattern obtained after digestion by eight enzymes for each species or some populations are given in Fig. 3. Table 3 groups the data obtained from all RFLP and shows the enzymes that can be used to separate species. Enzyme *Cfo*I generated five different RFLP profiles within the studied populations and distinguished *H. aucklandica*, *H. mani* and *H. ustinovi* from each other and other species. The restriction profiles obtained with *Pst*I and *Taq*I clearly distinguished *H. filipjevi* from other species. Digestion by *Taq*I showed a unique RFLP profile with heterogeneity in the ITS region for all studied populations of *H. australis*. None of the tested enzymes differentiated European *H. avenae* populations from *H. arenaria*, or *H. pratensis* from Chinese *H. 'avenae'*. Intraspecific polymorphism was revealed within *H. avenae* by *Alu*I and *Rsa*I, between *H. filipjevi* populations by *Cfo*I and between *H. pratensis* populations by *Ita*I. Restriction by *Alu*I and *Rsa*I allowed us to distinguish the following groups within *H. avenae sensu stricto*: *i*) European populations – no restriction by *Alu*I and a fragment more than 1000 bp long after restriction by *Rsa*I, *ii*) Asian populations – PCR product restricted by *Alu*I and *Rsa*I, *iii*) French populations – both previous profiles combined (Subbotin *et al.*, 1999), *iv*) Moroccan population – no restriction by *Alu*I and three fragments by *Rsa*I. *Cfo*I clearly distinguished Spanish and Turkish populations of *H. filipjevi* from others. *Ita*I divided *H. pratensis* into two groups. However, some populations (Dangast, Germany) showed

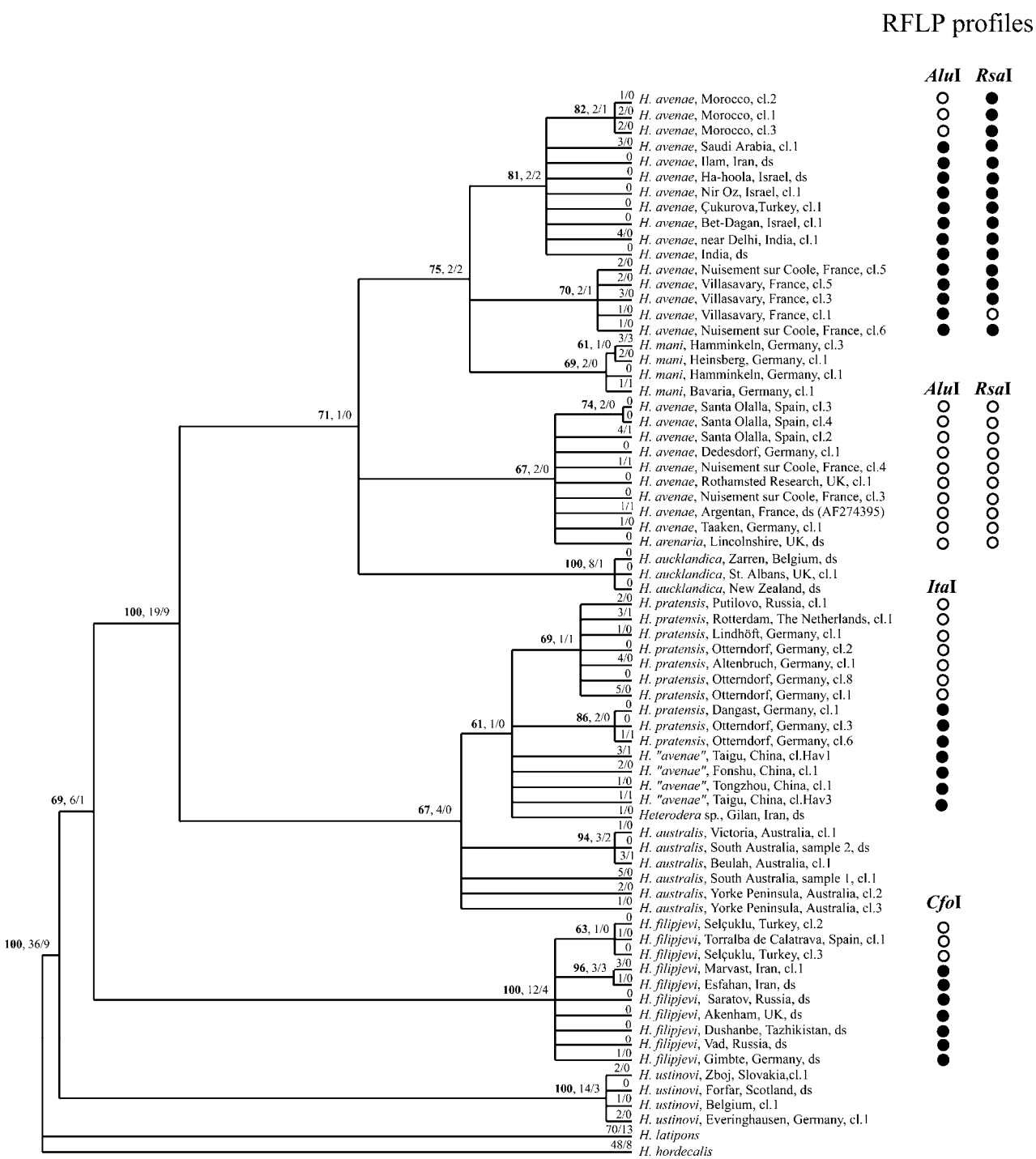

Fig. 1. *Phylogenetic relationships within the* Heterodera avenae *complex inferred from analyses of the full ITS data set. Strict consensus of 20 equally parsimonious trees when gaps were treated as fifth character. (Tree length = 328; number of parsimony-informative characters = 103, CI = 0.8963, HI = 0.1037, RI = 0.9558, RC = 0.8568). Bootstrap in bold and number of nucleotide and gap changes/number of gap changes only are given in appropriate clade. Restriction profiles generated by* AluI *(● – two fragments, ❍ – one fragment);* RsaI *(● – three fragments, ❍ – two fragments);* ItaI *or* CfoI *(● – four fragments, ❍ – five fragments). Abbreviations: cl = sequencing of clone; ds = direct sequencing of PCR product.*

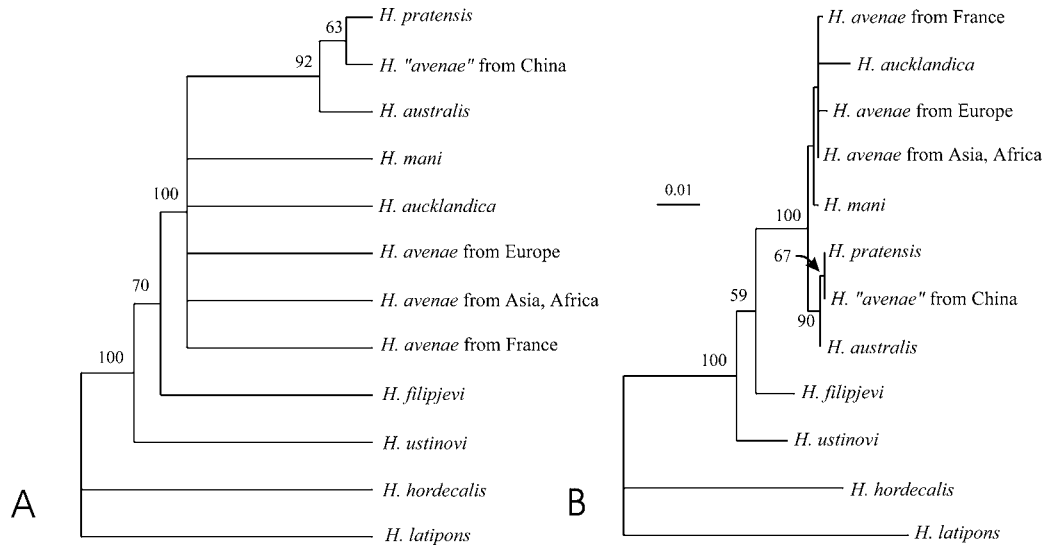

Fig. 2. *Phylogenetic relationships within the* Heterodera avenae *complex inferred from the consensus ITS sequence data. A: Strict consensus of 160 equally maximum parsimonious trees when gaps were treated as missing data. (Tree length = 178, number of parsimony-informative characters = 40, CI = 0.9438, HI = 0.0562, RI = 0.8611, RC = 0.8127); B: Maximum likelihood tree obtained from GTR+G model (Ln = −2342.5267; Lset Base = (0.2295 0.2220 0.2453); Nst = 6 Rmat = (1.8219 2.9769 1.9780 1.3090 2.0227); Rates = gamma Shape = 2.3611; Pinvar = 0). Bootstrap values more than 50% are given in appropriate clade.*

Table 3. *The ITS-rDNA-RFLP profiles yielded by a single enzyme for the* Heterodera avenae *species complex.*

Species Enzyme	*Alu*I	*Cfo*I	*Hin*fI	*Ita*I	*Pst*I	*Rsa*I	*Taq*I	*Tru*9I
H. mani	564, 481	743, 151, 151	497, 318, 189, 41	408, 369, 248, 20	706, 339	706, 318, 21	384, 274, 150, 129, 65, 43	544, 492, 9
H. avenae	566, 482 1044	741, 151, 108, 44	498, 318, 187, 41	408, 370, 246, 20	704, 340	707, 320, 21 1023, 21	384, 275, 148, 129, 65, 43	542, 493, 9
H. aucklandica	562, 481	740, 108, 195	497, 318, 187, 41	407, 370, 246, 20	703, 340	706, 316, 21	531, 275, 129, 65, 43	542, 492, 9
H. ustinovi	567, 484	409, 338, 151, 110, 43	818, 192, 41	408, 371, 252, 20	710, 341	708, 322, 21	533, 276, 134, 65, 43	547, 488, 9, 7
H. filipjevi	571, 482	750, 108, 105, 46, 44 748, 153, 108, 44	820, 192, 41	407, 370, 256, 20	713, 210, 130	706, 326, 21	339, 275, 134, 118, 79, 65, 43	551, 486, 9, 7
H. "avenae" from China	562, 482	741, 151, 108, 44,	816, 187, 41	408, 370, 246, 20	704, 340	707, 316, 21	384, 275, 148, 129, 65, 43	542, 307, 179, 9, 7
H. pratensis	562, 482	741, 151, 108, 44	816, 187, 41	408, 370, 246, 20 371, 344, 246, 63, 20	704, 340	707, 316, 21	384, 275, 148, 129, 65, 43	542, 307, 179, 9, 7
H. australis	562, 484	743, 151, 108, 44	818, 187, 41	410, 370, 246, 20	706, 340	709, 316, 21	532, 386, 275, 148, 129, 65, 43	542, 488, 9, 7

the combined RFLP profile including two types. Distribution of different RFLP types mapped on the phylogram are shown in Fig. 1.

The ITS sequence analyses revealed that populations presently grouped under the name *H. avenae* are polyphyletic. The present analyses including more ITS sequences than a previous study (Subbotin *et al.*, 2002) also supported substantial difference between *H. australis* and *H. avenae*. Our RAPD study permitted an estimate of the genetic divergence of the Australian cereal cyst nematode from the *H. avenae* populations of different geographical origins and other related species.

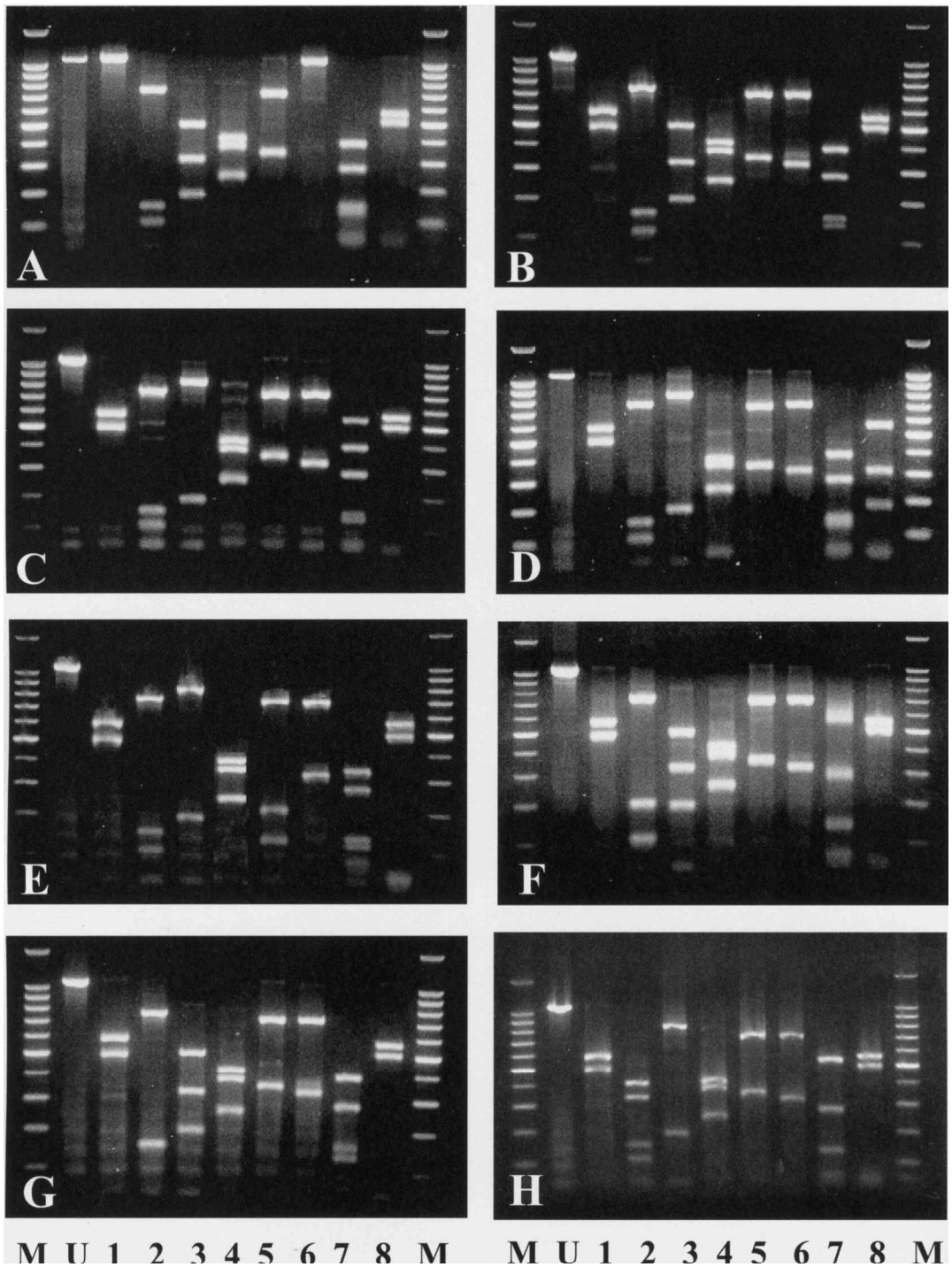

Fig. 3. *RFLP-ITS-rDNA profiles of species from the* Heterodera avenae *complex. A:* H. avenae *(Taaken, Germany); B:* H. avenae *(Nir-Oz, Israel); C:* H. australis *(South Australia, sample 1); D:* H. pratensis *(Lindhöft, Germany); E:* H. filipjevi *(Vad, Russia); F:* H. aucklandica *(Auckland, New Zealand); G:* H. mani *(Hamminkeln, Germany); H:* H. ustinovi *(Forfar, Scotland, UK). Code: M = 100 bp DNA marker (Promega); U = unrestricted PCR product; 1 =* Alu*I; 2 =* Cfo*I; 3 =* Hin*fI; 4 =* Ita*I; 5 =* Pst*I; 6 =* Rsa*I; 7 =* Taq*I; 8 =* Tru*9I.*

Table 4. *Morphometrics of cysts and second-stage juveniles of species and populations of the* Heterodera avenae *complex. Measurements are given in the form: mean ± standard deviation (range) and are in μm.*

Species Population	*H. avenae* Nir Oz, Israel	*H. avenae* Ha-hoola, Israel	*H. avenae* Saudi Arabia	*H. avenae* Çukurova, Turkey	*H. mani* Hamminkeln, Germany	*H. mani* Andernach, Germany	*H. mani* Heinsberg, Germany
Cysts (n)	10	15	10	25	10	11	15
Length excl. neck	809 ± 33 (648-1008)	835 ± 34 (552-1056)	701 ± 17 (600-768)	845 ± 22 (672-1056)	580 ± 14 (504-624)	598 ± 16 (504-672)	624 ± 14 (528-696)
Width	583 ± 17 (504-672)	589 ± 23 (408-720)	483 ± 14 (432-552)	601 ± 16 (432-744)	468 ± 20 (336-552)	478 ± 29 (360-648)	507 ± 16 (408-600)
Length/width	1.4 ± 0.03 (12-1.5)	1.4 ± 0.03 (1.1-1.6)	1.5 ± 0.04 (1.3-1.7)	1.4 ± 0.03 (1.2-1.7)	1.3 ± 0.04 (1.1-0.5)	1.3 ± 0.1 (1.0-1.5)	1.2 ± 0.03 (1.0-1.4)
Vulval areas (n)	6	12	4	5	4	12	7
Fenestral length	50 ± 1.7 (43-54)	53 ± 1.1 (47-58)	45 ± 2.5 (39-50)	55 ± 1.7 (51-59)	52 ± 1.9 (50-58)	52 ± 1.0 (47-58)	55 ± 1.8 (47-58)
Mean semifenestral width	22 ± 0.4 (21-23)	25 ± 0.7 (19-28)	24 ± 1.9 (19-27)	21 ± 1.0 (20-23)	25 ± 0.9 (23-27)	27 ± 0.4 (23-29)	27 ± 0.9 (23-31)
Vulval bridge width	10 ± 1.1 (6.6-14)	12 ± 0.7 (7.8-16)	12 ± 0.8 (9.7-14)	12 ± 1.1 (7.8-14)	8.2 ± 0.5 (7.8-9.7)	12 ± 0.5 (9.7-16)	9.7 ± 0.6 (7.8-12)
Vulval slit length	8.7 ± 0.5 (7.8-11)	8.5 ± 0.2 (7.8-9.7)	8.1 ± 0.6 (6.6-9.7)	10 ± 1.0 (7.8-12)	9.1 ± 0.9 (7.8-12)	8.6 ± 0.4 (7.8-12)	7.8 ± 0.4 (6.6-9.7)
Underbridge length	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Vulva-anus distance	57 ± 1.9 (54-62)	49 ± 2.5 (43-62)	56 ± 3.4 (50-62)	55,70	48 ± 5.6 (39-58)	51 ± 1.7 (39-58)	50 ± 4.9 (43-70)
Juveniles (n)	15	8	15	8	20	12	17
L	535 ± 3.4 (517-568)	557 ± 8.5 (504-583)	552 ± 3.9 (529-572)	572 ± 7.2 (541-603)	526 ± 3.0 (501-556)	559 ± 4.5 (531-588)	526 ± 3.2 (504-549)
a	26 ± 0.3 (24-28)	27 ± 0.5 (25-29)	26 ± 0.2 (25-28)	27 ± 0.3 (26-28)	25 ± 0.2 (24-28)	27 ± 0.3 (26-30)	26 ± 0.3 (23-28)
b	4.6 ± 0.1 (4.2-4.9)	4.6 ± 0.1 (4.4-4.9)	4.5 ± 0.1 (4.2-4.8)	4.9 ± 0.1 (4.6-5.1)	4.3 ± 0.04 (4.0-4.7)	4.5 ± 0.07 (4.0-4.9)	4.2 ± 0.04 (3.9-4.6)
c	8.6 ± 0.1 (8.2-8.9)	8.5 ± 0.1 (8.1-8.9)	8.3 ± 0.1 (7.9-8.9)	8.2 ± 0.1 (7.6-8.5)	9.0 ± 0.1 (8.1-11)	8.2 ± 0.1 (7.6-9.0)	8.7 ± 0.15 (7.9-11)
Stylet length	26 ± 0.1 (26-27)	26 ± 0.3 (25-27)	27 ± 0.2 (26-27)	27 ± 0.2 (26-27)	26 ± 0.2 (24-27)	26 ± 0.2 (25-27)	26 ± 0.1 (25-27)
Lip region height	4.0 ± 0.1 (3.6-4.9)	3.7 ± 0.1 (3.4-4.1)	3.8 ± 0.04 (3.4-3.9)	3.9 ± 0.04 (3.7-4.1)	3.9 ± 0.04 (3.7-4.4)	3.8 ± 0.03 (3.6-4.1)	3.9 ± 0.05 (3.4-4.4)
Lip region width	9.5 ± 0.1 (8.8-9.8)	9.4 ± 0.1 (8.8-9.8)	9.5 ± 0.1 (8.8-9.8)	9.7 ± 0.1 (9.3-9.8)	9.8 ± 0.04 (9.3-10.3)	9.8 ± 0.03 (9.5-9.8)	9.8 ± 0.04 (9.3-10)
DGO	5.6 ± 0.2 (4.9-6.4)	5.4 ± 0.2 (4.9-5.9)	5.8 ± 0.1 (4.9-5.9)	5.4 ± 0.2 (4.9-5.9)	6.1 ± 0.2 (4.9-6.9)	6.4 ± 0.2 (4.9-7.4)	6.5 ± 0.1 (5.9-7.4)
Anterior end to valve of median bulb (MB)	69 ± 1.0 (64-76)	74 ± 1.5 (69-78)	74 ± 0.9 (67-79)	74 ± 1.0 (71-76)	73 ± 1.1 (64-83)	77 ± 1.3 (72-86)	74 ± 1.3 (66-89)
Anterior end to excretory pore	108±0.9 (102-115)	106 ± 3.2 (88-117)	110 ± 1.3 (103-119)	111 ± 1.7 (102-118)	107 ± 0.9 (100-114)	110 ± 1.1 (103-116)	103 ± 0.6 (100-109)
Pharynx length (cardia)	118 ± 1.3 (108-123)	123 ± 1.9 (113-127)	123 ± 1.4 (113-132)	117 ± 1.7 (108-123)	121 ± 0.9 (113-127)	125 ± 1.3 (120-132)	125 ± 1.4 (118-137)
Body diam. at mid-body	21±0.2 (20-22)	21 ± 0.2 (20-22)	21 ± 0.1 (21-22)	21 ± 0.1 (21-22)	21 ± 0.2 (19-22)	21 ± 0.1 (20-21)	20 ± 0.1 (20-22)
Body diam. at level of anus (BWA)	16±0.1 (16-17)	15 ± 0.1 (15-16)	16 ± 0.1 (15-16.2)	16 ± 0.2 (15-17)	16 ± 0.1 (15-16)	15 ± 0.2 (15-16)	15 ± 0.06 (14-15)
Tail length	63 ± 0.6 (60-67)	66 ± 1.1 (59-69)	66 ± 0.8 (61-71)	70 ± 1.1 (67-76)	59 ± 0.9 (49-67)	68 ± 0.9 (64-74)	61 ± 1.1 (49-67)
Hyaline part of tail length (H)	38 ± 0.7 (34-43)	41 ± 0.9 (37-45)	41 ± 1.0 (34-49)	47 ± 0.6 (45-50)	37 ± 0.8 (28-44)	42 ± 1.2 (38-51)	40 ± 0.6 (34-43)
Tail length/BWA	3.0 ± 0.1 (2.8-3.4)	3.2 ± 0.1 (2.9-3.4)	3.1 ± 0.03 (2.8-3.3)	3.3 ± 0.04 (3.1-3.5)	2.8 ± 0.04 (2.3-3.2)	3.2 ± 0.04 (3.1-3.7)	3.0 ± 0.06 (2.4-3.3)
H/Stylet length	1.5 ± 0.03 (1.3-1.7)	1.6 ± 0.03 (1.5-1.7)	1.5 ± 0.04 (1.4-1.8)	1.8 ± 0.03 (1.7-1.9)	1.4 ± 0.03 (1.1-1.7)	1.7 ± 0.05 (1.5-2.0)	1.5 ± 0.03 (1.3-1.8)
L/MB	7.7 ± 0.1 (6.9-8.5)	7.7 ± 0.1 (7.2-8.1)	7.5 ± 01 (7.1-8.0)	7.8 ± 0.1 (7.3-8.2)	7.2 ± 0.1 (6.3-8.1)	7.3 ± 0.2 (6.2-8.2)	7.1 ± 0.1 (5.8-7.8)

Table 4. *(Continued).*

Species Population	*H. aucklandica* Zarren, Belgium	*H. aucklandica* St. Albans, UK	*H. pratensis* Otterndorf, Germany	*H. ustinovi* Everinghausen, Germany	*H. riparia* comb. n. Russia (Kazachenko, 1993)	*H. arenaria* The Netherlands
Cysts (n)	4	–	5	9	10	7
Length excl. (* incl.) neck	480 ± 27 (440-560)	–	672 ± 67 (408-768)	610 ± 27 (480-768)	582 (480-652)*	925 ± 27 (840-1056)
Width	346 ± 23 (320-416)	–	523 ± 62 (288-624)	459 ± 22 (360-600)	428 (320-480)	706 ± 30 (576-792)
Length/width	1.4 ± 0.02 (1.3-1.5)	–	1.3 ± 0.04 (1.2-1.4)	1.3 ± 0.1 (1.1-1.6)	1.4 (1.2-1.6)	1.3 ± 0.03 (12-1.5)
Vulval areas (n)	9	5	5	6	10	6
Fenestral length	49 ± 1.8 (38-55)	47 ± 3.8 (39-58)	45 ± 0.9 (43-47)	51 ± 1.6 (47-58)	40 (33-49)	51 ± 1.8 (47-56)
Mean semifenestral width	24 ± 1.1 (20-28)	28 ± 1.6 (23-31)	22 ± 0.9 (19-23)	26 ± 1.7 (19-31)	18 (17-23)	26 ± 1.7 (19-31)
Vulval bridge width	9.4 ± 0.3 (7.5-10)	8.9 ± 0.8 (7.8-12)	8.5 ± 0.4 (7.8-9.7)	9.6 ± 0.5 (7.8-12)	4.8 (3.9-6.5)	11 ± 1.2 (6.6-16)
Vulval slit length	6.4 ± 0.4 (5.9-7.5)	7.4 ± 0.4 (5.8-8.1)	7.6 ± 0.7 (5.8-9.7)	9.1 ± 0.6 (7.8-12)	10.6 (9.1-13)	10 ± 0.4 (8.9-12)
Underbridge length	Absent	Absent	Absent	72 ± 3.4 (66-85)	71.5	Absent
Vulva-anus distance	–	56 ± 2.9 (50-66)	49 ± 3.4 (43-54)	56 ± 1.9 (54-58)	–	61 ± 2.6 (50-70)
Juveniles (n)	20	16	13	5	25	16
L	494 ± 5.8 (433-544)	494 ± 3.4 (470-521)	516 ± 5.4 (480-548)	612 ± 8.5 (588-632)	452 (432-487)	654 ± 5.1 (625-684)
a	25 ± 0.3 (22-28)	25 ± 0.2 (23-26)	26 ± 0.3 (24-27)	29 ± 0.7 (27-31)	23.9 (21.0-26.8)	29 ± 0.4 (27-32)
b	4.3 ± 0.1 (3.7-4.9)	4.1 ± 0.1 (3.8-4.6)	4.1 ± 0.1 (3.8-4.4)	4.6 ± 0.1 (4.5-4.8)	2.8 (2.3-3.1)	4.7 ± 0.1 (4.4-5.1)
c	7.2 ± 0.1 (6.6-8.0)	6.5 ± 0.1 (6.1-6.7)	8.0 ± 0.1 (7.5-8.5)	7.3 ± 0.1 (7.1-7.7)	8.4 (7.8-9.1)	8.1 ± 0.1 (7.8-8.7)
Stylet length	25 ± 0.1 (24-27)	24 ± 0.1 (24-25)	25 ± 0.2 (25-26)	27 ± 0.2 (26-27)	22	29 ± 0.2 (27-31)
Lip region height	4.1 ± 0.03 (3.8-4.4)	3.9 ± 0.1 (3.4-4.4)	3.9 ± 0.1 (3.4-4.4)	4.0 ± 0.1 (3.9-4.4)	3.9	4.4 ± 0.1 (3.9-5.1)
Lip region width	9.2 ± 0.03 (8.9-9.7)	9.0 ± 0.1 (8.8-9.8)	9.2 ± 0.1 (8.8-9.8)	9.8 ± 0.02 (9.8-9.9)	7.8	10 ± 0.1 (10-11)
DGO	6.5 ± 0.2 (5.1-8.2)	6.1 ± 0.2 (4.9-6.9)	4.9 ± 0.1 (3.9-5.4)	6.7 ± 0.1 (6.4-7.0)	5.2-6.6	7.7 ± 0.2 (5.9-8.8)
Anterior end to valve of median bulb (MB)	76 ± 0.4 (71-79)	75 ± 0.9 (69-80)	76 ± 1.2 (67-83)	77 ± 1.1 (75-80)	63 (55-70)	88 ± 0.7 (83-92)
Anterior end to excretory pore	101 ± 1.2 (92-113)	98 ± 0.6 (93-101)	103 ± 1.1 (96-110)	112 ± 1.9 (107-117)	90 (85-95)	126 ± 1.5 (108-137)
Pharynx length (cardia)	114 ± 1.1 (105-122)	122 ± 1.3 (111-128)	124 ± 1.7 (113-132)	132 ± 1.5 (130-137)	–	139 ± 1.9 (123-152)
Body diam. at mid-body	20 ± 0.2 (18-21)	20 ± 0.1 (19-21)	20 ± 0.1 (20-21)	21 ± 0.2 (21-22)	–	22 ± 0.1 (22-24)
Body diam. at level of anus (BWA)	14 ± 0.1 (13-15)	15 ± 0.1 (15-16)	15 ± 0.1 (14-16)	16 ± 0.2 (15-16)	13 (12-14)	18 ± 0.1 (17-18)
Tail length	69 ± 1.2 (62-78)	76 ± 0.5 (74-79)	65 ± 0.6 (61-87)	83 ± 1.1 (80-86)	57 (48-64)	81 ± 0.6 (76-86)
Hyaline part of tail length (H)	46 ± 1.1 (38-52)	48 ± 0.8 (39-52)	42 ± 0.9 (37-49)	55 ± 1.4 (52-60)	37 (34-42)	55 ± 1.2 (47-62)
Tail length/BWA	3.5 ± 0.1 (3.1-4.1)	3.9 ± 0.03 (3.6-4.1)	3.2 ± 0.03 (3.0-3.4)	3.9 ± 0.1 (3.7-4.1)	–	3.6 ± 0.03 (3.3-3.9)
H/Stylet length	1.8 ± 0.04 (1.5-2.1)	1.9 ± 0.03 (1.6-2.1)	1.7 ± 0.04 (1.4-1.9)	2.1 (2.0-2.3)	1.6 (1.5-1.8)	1.9 ± 0.04 (1.6-2.1)
L/MB	6.5 ± 0.1 (5.8-7.1)	6.6 ± 0.1 (6.2-7.0)	6.8 ± 0.1 (6.2-7.3)	8.0 ± 0.1 (7.8-8.3)	–	7.5 ± 0.1 (6.9-7.9)

Table 4. *(Continued).*

Species Population	*H. filipjevi*, Akenham, UK	*H. filipjevi*, Selçuklu, Turkey	*H. filipjevi* Merkez, Turkey	*H. "avenae"* Fonshu county, (sample 1), China	*H. "avenae"* Fonshu county, (sample 2), China	*H. "avenae"* Tongzhou county, China	*H. "avenae"* Pinjgu county, China
Cysts (n)	25	15	10	9	9	9	20
Length excl. neck	796 ± 12 (696-936)	786 ± 17 (672-888)	816 ± 16 (744-888)	597 ± 25 (504-696)	632 ± 24 (552-792)	612 ± 36 (456-768)	638 ± 12 (504-696)
Width	592 ± 9.4 (528-672)	562 ± 10.2 (480-600)	616 ± 12 (552-672)	437 ± 13 (384-504)	480 ± 17 (432-576)	432 ± 28 (312-576)	461 ± 9 (384-528)
Length/width	1.3 ± 0.01 (1.3-1.4)	1.4 ± 0.02 (1.2-1.5)	1.3 ± 0.02 (1.1-1.5)	1.4 ± 0.05 (1.2-1.5)	1.3 ± 0.05 (1.1-1.5)	1.5 ± 0.8 (1.2-1.9)	1.4 ± 0.02 (1.2-1.5)
Vulval areas (n)	7	5	–	10	8	6	8
Fenestral length	54 ± 2.0 (47-62)	59 ± 1.9 (54-66)	–	46 ± 0.8 (43-50)	46 ± 1.4 (39-50)	45 ± 1.9 (39-50)	40 ± 0.9 (35-43)
Mean semifenestral width	29 ± 1.1 (23-31)	28 ± 0.8 (27-31)	–	23 ± 1.3 (19-31)	23 ± 0.8 (19-27)	24 ± 1.2 (19-27)	21 ± 0.6 (19-23)
Vulval bridge width	13 ± 0.6 (12-16)	12 ± 0.4 (12-14)	–	10 ± 0.9 (6.6-16)	8.3 ± 0.5 (6.6-12)	8.5 ± 0.8 (6.6-12)	8.0 ± 0.6 (5.8-10)
Vulval slit length	9.3 ± 0.6 (7.8-12)	9.5 ± 0.9 (7.8-12)	–	8.6 ± 0.4 (6.6-12)	7.9 ± 0.1 (7.8-8.1)	7.7 ± 0.3 (6.6-8.9)	8.0 ± 0.7 (5.8-12)
Underbridge length	74 ± 3.9 (70-78)	76 ± 8.5 (66-93)	–	–	–	–	–
Vulva-anus distance	52 ± 5.6 (43-62)	52 ± 1.3 (50-54)	–	57 ± 1.5 (50-62)	52 ± 1.6 (47-58)	53 ± 1.6 (50-58)	49 ± 2.2 (43-58)
Juveniles (n)	16	15	12	20	12	15	11
L	522 ± 5.6 (488-558)	543 ± 7.5 (494-592)	545 ± 7.5 (494-588)	513 ± 3.5 (480-537)	537 ± 6.0 (495-570)	496 ± 4.0 (468-523)	488 ± 5.6 (456-523)
a	25 ± 0.3 (23-27)	26 ± 0.3 (23-28)	25 ± 0.4 (23-26)	25 ± 0.2 (23-27)	27 ± 0.3 (25-29)	25 ± 0.3 (24-27)	25 ± 0.3 (23-26)
b	4.3 ± 0.1 (4.0-4.7)	4.1 (3.8-4.4)	4.3 ± 0.1 (4.1-5.0)	4.5 ± 0.1 (4.0-4.8)	4.6 ± 0.1 (4.2-4.9)	4.5 ± 0.1 (4.0-4.9)	4.4 (4.0-4.9)
c	8.8 ± 0.1 (8.2-9.4)	8.8 (7.7-9.5)	8.4 ± 0.2 (7.3-9.2)	8.1 ± 0.1 (7.5-9.1)	8.1 ± 0.1 (7.4-8.8)	7.9 ± 0.1 (7.3-8.6)	7.9 ± 0.1 (7.2-8.5)
Stylet length	24 ± 0.2 (24-26)	25 ± 0.2 (25-27)	25 ± 0.2 (23-26)	24 ± 0.1 (24-25)	25 ± 0.2 (25-26)	24 ± 0.1 (24-25)	24 ± 0.3 (23-27)
Lip region height	3.9 ± 0.1 (3.2-4.4)	4.3 ± 0.1 (3.7-4.9)	4.2 ± 0.1 (3.9-4.9)	4.1 ± 0.06 (3.4-4.4)	4.1 ± 0.1 (3.4-4.9)	3.4 ± 0.1 (2.9-3.9)	4.0 ± 0.1 (3.6-4.4)
Lip region width	9.7 ± 0.1 (9.3-10)	9.9 ± 0.1 (9.6-10.8)	10 ± 0.1 (9.8-10.8)	9.3 ± 0.09 (8.8-9.9)	9.2 ± 0.1 (8.8-9.8)	9.2 ± 0.1 (8.8-9.8)	9.0 ± 0.1 (8.8-9.8)
DGO	5.7 ± 0.2 (3.9-6.9)	6.1 ± 0.3 (4.9-7.8)	6.4 ± 0.2 (5.9-7.4)	6.4 ± 0.1 (5.4-6.9)	6.0 ± 0.2 (4.9-6.4)	5.8 ± 0.1 (4.9-6.3)	6.2 ± 0.2 (4.9-6.9)
Anterior end to valve of median bulb (MB)	74 ± 1.4 (67-86)	80 ± 1.2 (73-89)	75 ± 1.3 (69-81)	72 ± 1.0 (67-87)	71 ± 1.1 (64-76)	69 ± 2.1 (59-83)	65 ± 1.1 (59-70)
Anterior end to excretory pore	105 ± 1.6 (88-113)	112 ± 1.5 (100-123)	111 ± 1.4 (101-117)	103 ± 1.0 (98-112)	109 ± 1.5 (98-116)	96 ± 1.6 (85-104)	95 ± 1.6 (85-103)
Pharynx length (cardia)	123 ± 2.3 (109-134)	131 ± 1.9 (118-138)	128 ± 1.6 (118-132)	116 ± 1.2 (107-126)	118 ± 2.3 (103-127)	111 ± 2.4 (98-123)	111 ± 2.0 (98-123)
Body diam. at mid-body	21 ± 0.2 (20-22)	21 ± 0.1 (21-22)	22 ± 0.2 (21-23)	21 ± 0.1 (20-22)	20 ± 0.1 (20-21)	20 ± 0.2 (18-21)	20 ± 0.2 (9-21)
Body diam. at level of anus (BWA)	16 ± 0.2 (15-17)	16 ± 0.2 (15-17)	16 ± 0.2 (15-17)	15 ± 0.1 (15-16)	15 ± 0.1 (15-16)	14 ± 0.1 (14-15)	14 ± 0.1 (14-15)
Tail length	59 ± 0.7 (53-64)	62 ± 1.0 (54-67)	65 ± 0.8 (61-71)	63 ± 0.9 (55-72)	66 ± 0.8 (61-70)	63 ± 0.7 (57-68)	62 ± 1.1 (57-68)
Hyaline part of tail length (H)	35 ± 0.7 (29-39)	37 ± 0.8 (32-45)	41 ± 1.0 (35-48)	40 ± 0.6 (34-45)	42 ± 0.6 (39-47)	39 ± 0.7 (34-44)	38 ± 0.8 (34-42)
Tail length/BWA	2.8 ± 0.03 (2.6-3.1)	2.9 ± 0.04 (2.6-3.2)	3.0 ± 0.1 (2.8-3.3)	3.1 ± 0.04 (2.7-3.5)	3.3 ± 0.04 (3.1-3.5)	3.1 ± 0.1 (2.9-3.5)	3.2 ± 0.1 (2.9-3.5)
H/Stylet length	1.5 ± 0.03 (1.3-1.7)	1.5 ± 0.03 (1.3-1.8)	1.7 ± 0.04 (1.4-1.9)	1.6 ± 0.02 (1.4-1.8)	1.7 ± 0.02 (1.5-1.8)	1.6 ± 0.03 (1.4-1.8)	1.7 ± 0.03 (1.5-1.8)
L/MB	7.1 ± 0.1 (6.2-7.6)	6.8 ± 0.1 (1.5-1.8)	7.4 ± 0.1 (6.4-8.1)	7.1 ± 0.09 (5.8-7.7)	7.7 ± 0.2 (6.6-8.4)	7.3 ± 0.2 (5.7-8.3)	7.5 ± 0.1 (6.6-8.1)

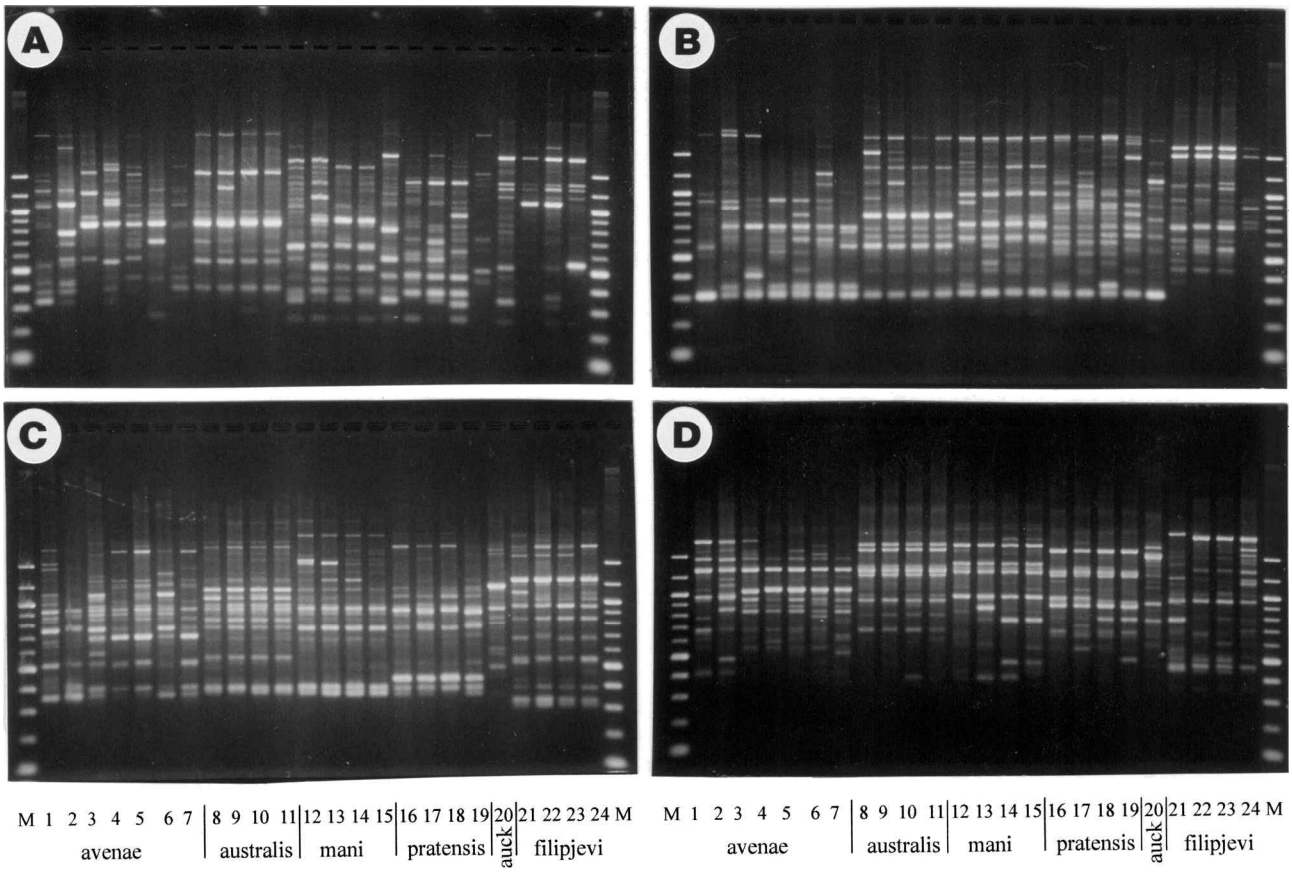

Fig. 4. *RAPD patterns of 26 populations of the* Heterodera avenae *complex. Primers. A: A-16; B: A-18; C: N-05; D: G-10. Populations. 1:* H. avenae *(Taaken, Germany); 2:* H. avenae *(Santa Olalla, Spain); 3:* H. avenae *(Çukurova plain, Turkey); 4:* H. avenae *(Saudi Arabia); 5:* H. avenae *(Ha-hoola, Israel); 6:* H. avenae *(Israel); 7:* H. avenae *(near Delhi); 8:* H. australis *(South Australia, sample 3); 9:* H. australis *(Beulah, Australia); 10:* H. australis *(Victoria, Australia); 11:* H. australis *(Yorke Peninsula, Australia); 12:* H. mani *(Bavaria, Germany); 13:* H. mani *(Heinsberg, Germany); 14:* H. mani *(Andernach, Germany); 15:* H. mani *(Germany); 16:* H. pratensis *(Missunde, Germany); 17:* H. pratensis *(Östergaard, Germany); 18:* H. pratensis *(Lindhöft, Germany); 19:* H. pratensis *(Lenggries, Germany); 20:* H. aucklandica *(Auckland, New Zealand); 21:* H. filipjevi *(Saratov, Russia); 22:* H. filipjevi *(Akenham, UK); 23:* H. filipjevi *(Torralba de Calatrava, Spain); 24:* H. filipjevi *(Selçuklu, Turkey). M = 100 bp DNA ladder (Biolab).*

RAPD analysis

A total of 237 polymorphic bands, ranging from 200-2500 bp in size, were produced using nine primers (Fig. 4). The number of bands produced per primer ranged from 21-31. Five bands were found to be specific for the Australian cereal cyst nematode populations. To analyse the similarities between populations, we calculated the distance matrixes using the Dice, Jaccard or Simple Matching coefficients. The UPGMA trees obtained with these types of matrixes showed some differences in topologies between calculations based on Dice or Jaccard and Simple Matching. All trees revealed seven clades: *i*) populations of *H. avenae sensu stricto* from Asia, *ii*) populations of *H. avenae*, *sensu stricto* from Europe, *iii*) *H. aucklandica*, *iv*) *H. australis*, *v*) *H. pratensis*, *vi*) *H. mani* and *vii*) *H. filipjevi* (Fig. 5). In the diagram the Australian cereal cyst nematode clustered with *H. pratensis.*

Morphometric and canonical discriminant analyses

The morphometrics of cysts and J2 for 23 populations are presented in Table 4. All morphometric charac-

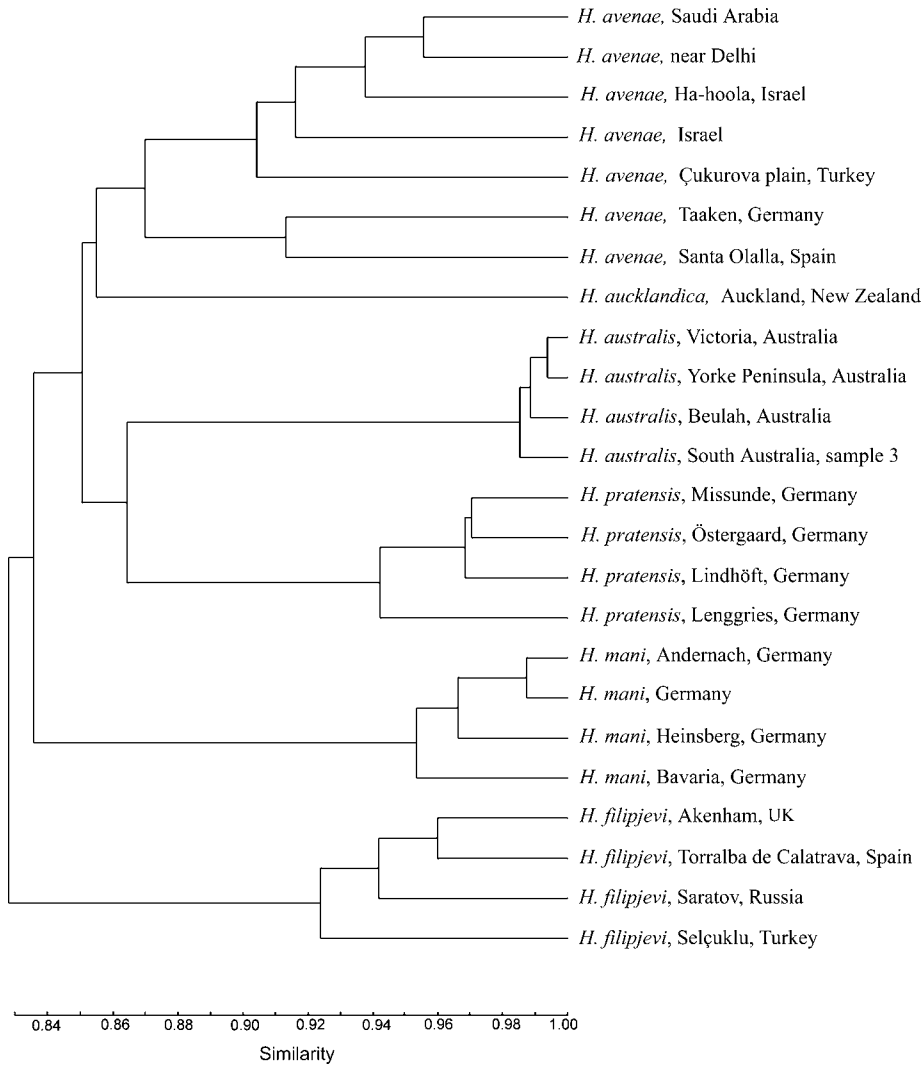

Fig. 5. *UPGMA cluster diagram based on Simple Matching similarity measure estimated from 237 RAPD markers for 24 populations of the* Heterodera avenae *complex.*

ters showed extensive variation and overlapping between species. Principal co-ordinate and stepwise discriminant analyses of morphometrical characters of cysts and J2 were successfully used to differentiate several species of the *H. avenae* complex (Stone & Hill, 1982; Valdeolivas & Romero, 1990; Subbotin *et al.*, 1999, 2002). Our present study, which includes significantly more populations and species, supports the previous conclusions that juvenile characters (lengths of body, stylet, tail, hyaline part of tail) and cyst characters (cyst length and width, fenestral length and width) can be used for identification of some species within the *H. avenae* complex.

The CDA of the means of four juvenile characters from 64 populations calculated four canonical variables. Standardised coefficients for four canonical variables are given in Table 5. The first discriminant function was more strongly influenced by stylet and tail lengths whereas the second function was marked mostly by tail length and body length. The first three functions accounted for over 98% of the explained variance. Several groups were distinguished based on the scatter plot of population means of the first two canonical axes (Fig. 6A). *Heterodera arenaria*, *H. ustinovi*, *H. filipjevi* and *H. aucklandica* formed well separated groups. However within the two groups:

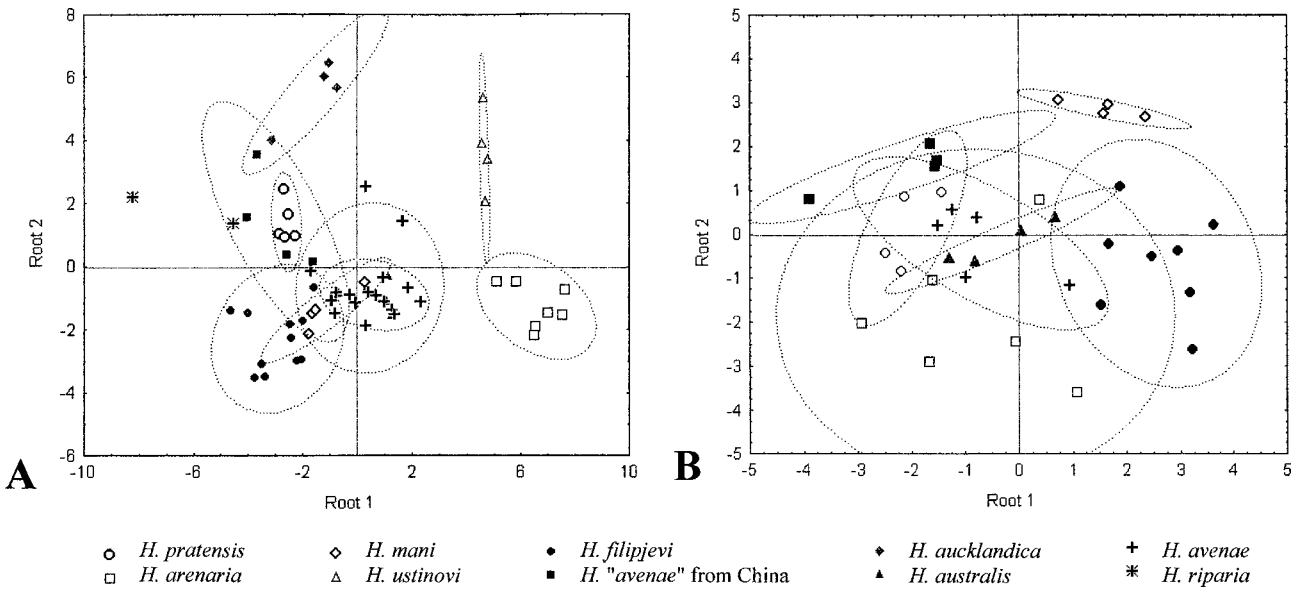

Fig. 6. *Canonical discriminant analysis of the* Heterodera avenae *complex. Scatterplots of population means on the first and second axes. A: Juveniles; B: Cysts.*

Table 5. *Standardised coefficients of canonical variables for juveniles.*

Variable	Root 1	Root 2	Root 3	Root 4
Tail length	0.52948	1.25416	0.90675	1.28261
Body length	0.40866	−1.04444	−0.59408	0.37444
Stylet length	0.70317	−0.15008	0.74199	−0.18019
Hyaline part of tail	−0.07946	−0.00674	−1.02745	−1.56568
Eigenval	12.50830	5.42982	0.56694	0.19421
Cum. Prop	0.66892	0.95929	0.98961	1.00000

i) *H. pratensis*, *H.* '*avenae*' from China and *H. riparia* comb. n., *ii*) *H. avenae*, *H. mani* and *H. australis*, the species were poorly separated with these variables. Results of the CDA for cysts (length and width of cyst, fenestral length, mean semifenestral width) from 35 populations are presented in Table 6 and shown in Fig. 6B. *Heterodera filipjevi* and *H. mani* can be separated from other species based on cyst characters.

Morphological characterisation

Photomicrographs of anterior and posterior ends of the J2 are presented in Figs 7 and 8. A strong stylet is typical for *H. arenaria* and *H. ustinovi*; a comparatively slender and short one for *H. aucklandica*. Stylet knobs vary from almost flat to slightly anteriorly projecting in *H. arenaria*, *H. aucklandica*, *H. pratensis*, *H. riparia* comb. n. and *H. 'avenae'* from China, flat to moderately anteriorly projecting in *H. avenae* and *H. australis*, moderately projecting in *H. filipjevi* and moderately to strongly projecting in *H. mani*. The tail terminus varies from narrowly rounded in *H. aucklandica* to rather broadly rounded in *H. filipjevi* and *H. mani*. However, shapes of stylet knobs and of tail terminus often exhibit a considerable intraspecific variation. Interspecific differences in tail length and 'slenderness' are mostly more evident. The mean hyaline tail portion among all species varies between 59 and 68% of the total tail length.

Size, shape and colour of cysts are also variable between species. More rounded cysts are typical for *H. mani* and *H. pratensis*, whereas *H. avenae* and *H. australis* have elongated lemon-shaped cysts. The colour of the cyst cuticle is generally brown to dark brown in *H. avenae*, *H. australis* and *H. mani*, whereas other species have mostly lighter coloured cysts. The vulval cone exhibits similar structures in all studied species; only the fenestral length of *H. filipjevi* was on average longer than that in other species, and the bullae are situated below the fenestrae, whereas in other species they were at different levels in the vulval cone.

A distinct underbridge with bifurcated arms is present in vulval cones of *H. ustinovi* and *H. filipjevi*. No underbridge was found in the vulval cones of all *H.*

mani populations in our study. Rarely, an underbridge-like structure, as described by McLeod and Khair (1997), was visible in cysts of *H. avenae*. We failed to discriminate *H. australis* from *H. avenae* based on morphometric and morphological characters of both J2 and cysts.

Based on analyses of published and original data we present a table with morphological and morphometric characters useful for identification of species of the *H. avenae* complex (Table 7). The ranges given for the morphometric characters are calculated based on the means.

In conclusion, the molecular and biochemical study revealed the presence of additional species within the *H. avenae* complex, which were not derived from the morphological study. The present phylogenetic analysis of the complete and reduced ITS sequence data showed that the currently recognised *H. avenae* is a paraphyletic species whose taxonomic status needs to be re-evaluated. Evidence obtained from ITS sequences, RFLP, RAPD and IEF (Subbotin *et al.*, 2002) confirmed that the Australian cereal cyst nematode is genetically isolated and we conclude that it represents an independent evolutionary unit which should be recognised as a distinct species.

Species in the *Heterodera avenae* complex

Presently, we recognise within the *H. avenae* complex the nine species listed below plus a group of populations whose taxonomic status needs further study. The *H. avenae* complex is generally considered, together with the *H. latipons* and *H. hordecalis* complex, to form the *Avenae* group within the genus *Heterodera*.

Heterodera avenae *Wollenweber, 1924*

The cereal cyst nematode was first found as a parasite of cereals in 1874 in Germany. It is widespread throughout Europe and is present in the Mediterranean region of Africa, in Asia and North America. It includes several pathotypes and ecotypes. Detailed morphological and morphometrical descriptions of several populations have been given by several authors (Franklin, 1951; Williams & Siddiqi, 1972; Wouts & Weischer, 1977). *Heterodera avenae* is distinguished from other species by IEF (Rumpenhorst, 1985; Sturhan & Rumpenhorst, 1996; Gäbler *et al.*, 2000), RFLP and sequence of the ITS-rDNA, except from *H. arenaria* (Subbotin *et al.*, 1999, 2001; present study) and, except from *H. australis* (Subbotin *et al.*, 2002), by morphology and morphometrics.

Heterodera arenaria *Cooper, 1955*

A cyst nematode from marram grass, *Ammophila arenaria* (L.) Link., was first recorded by Triffit (1929). Cooper (1955) included a marram root eelworm, *H. major* var. *arenaria*, in his key to British species of *Heterodera*. *Heterodera arenaria* was subsequently raised to species level by Kirjanova and Krall (1971) and redescribed by Robinson *et al.* (1996). The species is distributed along the coast of the North Sea in England, Scotland (Robinson *et al.*, 1996), Germany (D. Sturhan, unpubl.) and the Netherlands (G. Karssen, pers. comm.). Reports of Kirjanova and Krall (1971) about the finding of cyst nematodes identified as *H. arenaria* in many Russian regions, Belorussia, Estonia and Lithuania, seem to refer rather to *H. ustinovi*. *Heterodera arenaria* differs from other species in morphometrics (Robinson *et al.*, 1996) and by IEF (Gäbler *et al.*, 2000).

Heterodera mani *Mathews, 1971*

This species was described from a grass field in Northern Ireland. Host-range tests showed that *H. mani* reproduced readily on *Lolium* spp., *Dactylis glomerata*, *Festuca* and several other grasses, but not on *Agrostis* spp., wheat, oats and barley (Matthews, 1971; Mowat, 1974). This species is known from Northern Ireland (Mathews, 1971), Germany (Sturhan, 1976, 1982) and the Netherlands (Maas & Brinkman, 1977). Several reports of *H. mani* or *H. mani*-like cyst nematodes from cereals or grasslands by several authors (Romero *et al.*, 1973; Maqbool, 1981; Cook, 1982; Rumpenhorst, 1985; Bekal *et al.*, 1997) were subsequently identified as *H. filipjevi*, although they may belong to other species. *Heterodera mani* is distinguished by morphological and morphometric characters (Mathews, 1971; present study), IEF (Sturhan & Rumpenhorst, 1996; Gäbler *et al.*, 2000), RFLP and sequence of the ITS-rDNA (present study).

Heterodera ustinovi *Kirjanova, 1969*

Kirjanova (1969) described this cyst-forming nematode from the roots of *Agrostis tenuis* from the Beskids, eastern part of Eastern Carpathians, Ukraine. Because of the inadequate description, the taxonomic status of *H. ustinovi* has long been controversial. Recently, Sturhan and Krall (2002) re-examined paratypes and studied original material collected from places close to the type locality. They concluded that *H. iri* Mathews, 1971 is a junior synonym of *H. ustinovi*. The species has been found in Northern Ireland (Mathews, 1971), Scotland (Subbotin *et al.*, 1999), Poland (Brzeski, 1998), Germany (Sturhan, 1976,

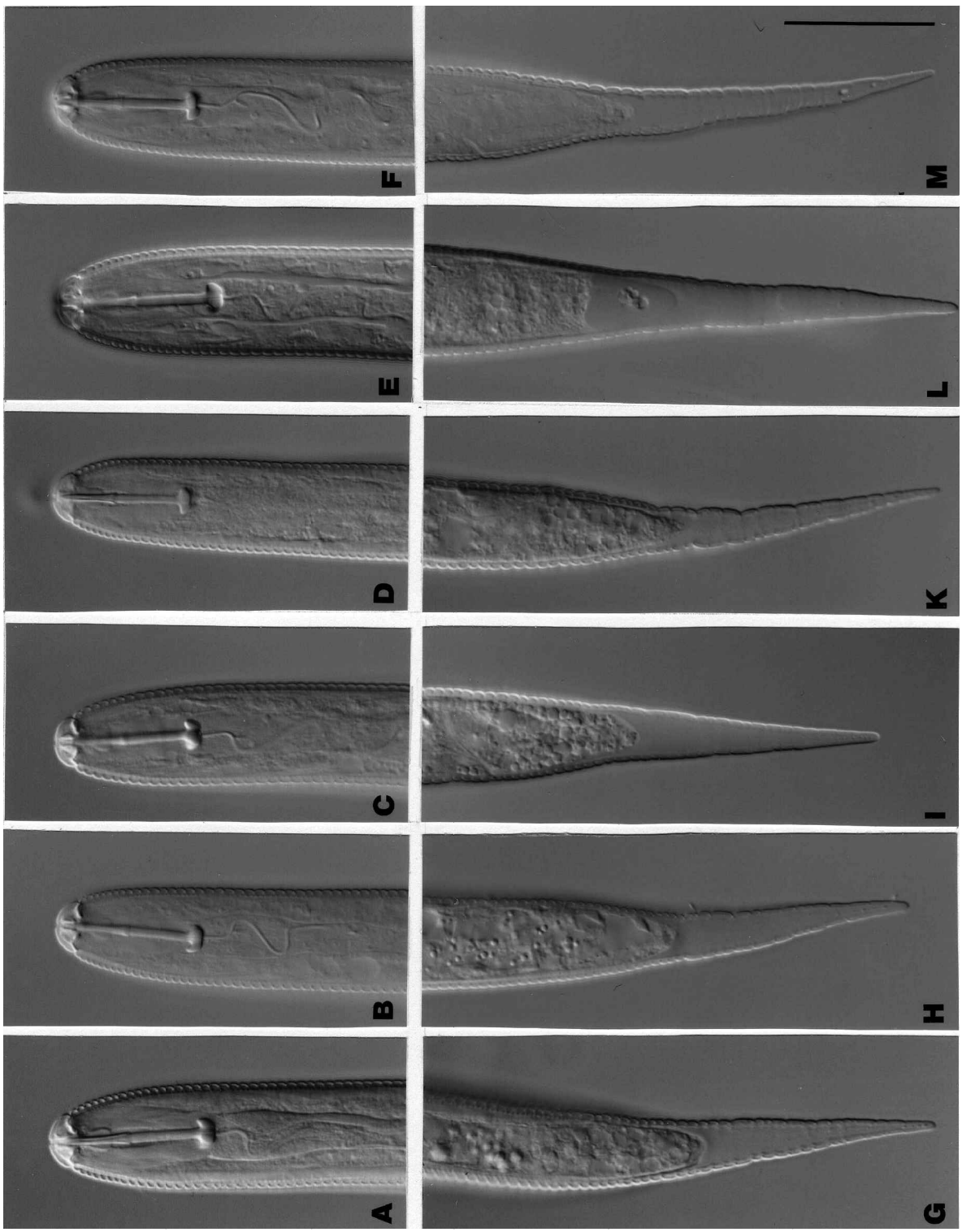

Fig. 7. *Anterior and posterior ends of the second-stage juveniles of species belonging to the* Heterodera avenae *complex. A, G:* H. avenae *(Knokke, Belgium); B, H:* H. avenae *(Ha-hoola, Israel); C, I:* H. australis *(Beulah, Australia); D, K:* H. aucklandica *(St. Albans, UK); E, L:* H. arenaria *(The Netherlands); F, M:* H. ustinovi *(Everinghausen, Germany). (Scale bar = 30 μm.)*

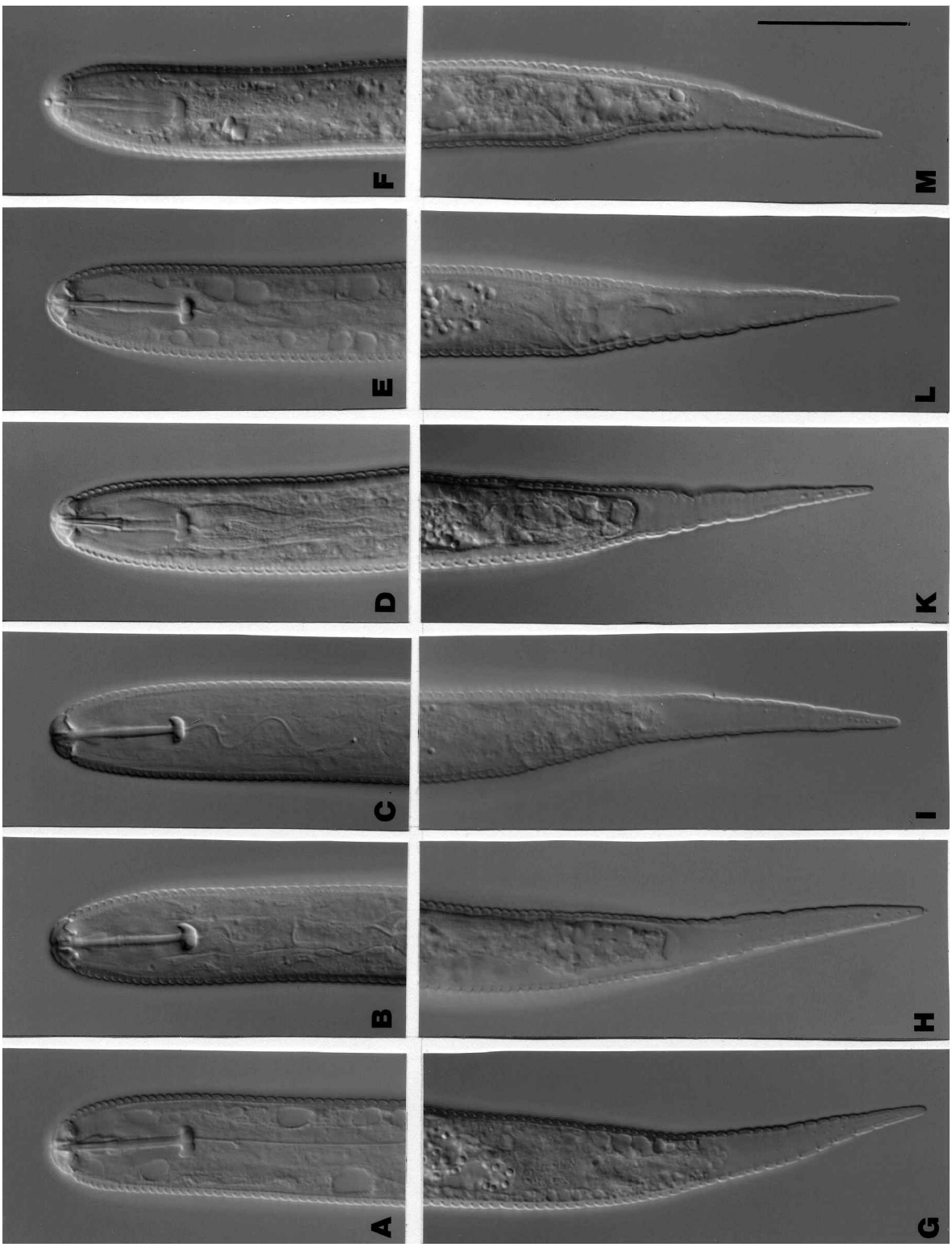

Fig. 8. *Anterior and posterior ends of the second-stage juveniles of species belonging to the* Heterodera avenae *complex. A, G:* H. filipjevi *(Selçuklu, Turkey); B, H:* H. mani *(Andernach, Germany); C, I:* H. 'avenae' *from China (Fonshu county, sample 1); D, K:* H. pratensis *(Lindhöft, Germany); E, L:* H. pratensis *(Otterndorf, Germany); F, M:* H. riparia *comb. n. (paratype). (Scale bar = 30 µm.)*

Table 6. *Standardised coefficients of canonical variables for cysts.*

Variable	Root 1	Root 2	Root 3	Root 4
Mean semifenestral width	0.592555	−0.00849	−0.913691	0.329079
Cyst length	−0.113336	−1.16282	−0.329080	−0.356684
Fenestral length	0.566883	0.23455	0.880617	−0.507318
Cyst width	0.325365	0.27693	0.611818	0.981792
Eigenval	3.766098	2.37738	0.746677	0.078792
Cum. Prop	0.540411	0.88155	0.988694	1.000000

Table 7. *Morphological and morphometrical characters useful for identification of the* Heterodera avenae *species complex (for measurements the ranges of means are given in μm).*

Species	Cyst			Juvenile				
	Cyst length	Under-bridge	Fenestral length	Body length	Stylet length	Stylet knob shape	Tail length	Hyaline part of tail
H. avenae	696-845	absent	45-55	505-580	26-28	flat to moderately concave	60-70	38-48
H. arenaria	693-925	absent	45-51	587-654	28-29	strongly developed, flat or concave	74-81	48-55
H. filipjevi	690-928	present	50-59	506-552	24-26	slightly to moderately concave	54-65	31-41
H. pratensis	672-760	absent	43-45	505-535	24-25	flat or slightly concave	65-69	42-45
H. aucklandica	480-690	absent	46-49	494-508	24-25	flat	69-76	46-48
H. ustinovi	610-675	present	42-51	593-638	25-27	strongly developed, concave	83-94	55-62
H. mani	580-624	absent	52-55	526-559	24-26	strongly developed and deeply concave	59-68	37-42
H. australis	734-780	absent	47-52	562-583	25-26	slightly concave	68-71	43-45
H. riparia comb. n.	582	absent	40	452-486	22-24	flat or slightly concave	57-61	36-37

1982), Belgium (Subbotin *et al.*, 2000), Slovakia (Sturhan & Krall, 2002), Russia, Estonia, Belorussia, Ukraine (Kirjanova & Krall, 1971) and USA (LaMondia, 1992). Several reports of this species from Asia (Iran, Kazakhstan, India) based solely on identification of cysts should be re-validated. *Heterodera ustinovi* is distinguished from other species by morphology and morphometrics (Mathews, 1971), IEF (Gäbler *et al.*, 2000), RFLP and sequence of the ITS-rDNA (Subbotin *et al.*, 1999, 2001; present study).

Heterodera filipjevi *(Madzhidov, 1981) Stelter, 1984*

In 1964 a cyst-forming nematode identified as *H. avenae* was found in the vicinity of Dushanbe, Tadzhikistan (Kirjanova & Krall, 1971) and was subsequently described as a new species by Madzhidov (1981) on the presence of a characteristic vulval underbridge in the cysts and differences in morphometrics of juveniles and cysts from other species. Subbotin *et al.* (1996) showed that *H. filipjevi* is in fact widely distributed throughout the territory of the former USSR, the populations studied not being *H. avenae* as previously presumed. *Heterodera filipjevi* infects wheat, rye, corn, false wheat and other grasses. It has been recorded from Tadzhikistan (Madzhidov, 1981), Russia (Subbotin *et al.*, 1996), Uzbekistan (Narbaev, pers. comm.), Ukraine (Subbotin *et al.*, 1996), Turkey (Rumpenhorst *et al.*, 1996), Iran (Sturhan, 1996), Bulgaria, Germany (Sturhan, 1982), Sweden (Andersson, 1973), England (Cook, 1975), Poland (Brzeski, 1998), Estonia (Krall *et al.*, 1999) and Spain (Romero, 1977; Valdeolivas & Romero, 1990). *Heterodera filipjevi* differs from other species by morphology and morphomet-

rics (Madzhidov, 1981; Subbotin *et al.*, 1999; present study), IEF (Sturhan & Rumpenhorst, 1996; Subbotin *et al.*, 1996), RFLP and sequence of the ITS-rDNA (Bekal *et al.*, 1997; Subbotin *et al.*, 1999, 2001; present study).

Heterodera aucklandica *Wouts & Sturhan, 1995*

This species was first isolated in 1975 from turf samples from Auckland, New Zealand. It occurred on the native grass *Microlaena stipoides* and was finally described as a distinct species by Wouts and Sturhan (1995). In Europe, *H. aucklandica* was found in natural grasslands on a stream bank at Zarren, Belgium (Subbotin *et al.*, 1999) and on a river bank at St. Albans, UK (present study). It differs from other species by morphology and morphometrics (Wouts & Sturhan, 1995; present study), IEF (Sturhan & Rumpenhorst, 1996; Gäbler *et al.*, 2000), RFLP and sequence of the ITS-rDNA (Subbotin *et al.*, 1999, 2001; present study).

Heterodera pratensis *Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000*

A morphological and morphometrical description of this species was given by Gäbler *et al.* (2000). This species infects *Elytrigia repens* and other grasses. Attempts to rear the type population on barley and wheat failed. However, a population identified as *H. pratensis* was collected from a barley field in Estonia. *Heterodera pratensis* is known from Germany, Russia, Estonia (Gäbler *et al.*, 2000) and the Netherlands (present study). It differs from other species of the *H. avenae* complex by morphometrics, IEF (Gäbler *et al.*, 2000) and sequence of the ITS-rDNA (Subbotin *et al.*, 1999; present study).

Heterodera australis *Subbotin, Sturhan, Rumpenhorst & Moens, 2002*

The Australian cereal cyst nematode is morphologically and morphometrically very similar to *H. avenae* and so far found only in Australia. It differs from all species in the *H. avenae* complex by IEF, RFLP and sequence of the ITS-rDNA (Subbotin *et al.*, 2002; present study).

Heterodera riparia *(Kazachenko, 1993) comb. n.* (= Bidera riparia *Kazachenko, 1993, syn. n.)*

This species was described from roots of false wheat (*Elytrigia repens*) and *Phragmites australis* along the coast of the Olga Bay, Okhot Sea, Kamchatka. Morphological description and measurements of cysts and juveniles of this species were given by Kazachenko (1993) and are presented in part in Table 2. Several paratype slides of juveniles and cone tops were kindly put at our disposal by Dr I.P. Kazachenko. Results of our re-measurements of juveniles (n = 9) are as follows: L = 486 ± 3.8 (470-500) μm; stylet length = 24 ± 0.1 (23-25) μm; length of hyaline part of tail = 36 ± 0.7 (32-39) μm and tail length = 61 ± 0.6 (58-64) μm. *Heterodera riparia* comb. n. is similar to *H. pratensis* and *H. 'avenae'* from China in morphometrics and other morphological characteristics, although its relationships with these species are unclear. Biochemical and molecular studies are still lacking.

Because *Bidera* is considered to be a junior synonym of *Heterodera*, *H. riparia* Subbotin, Sturhan, Waeyenberge & Moens, 1997, a species parasitising nettle (*Urtica* spp.) now becomes a junior secondary homonym of *H. riparia* (Kazachenko, 1993) comb. nov. The name *H. ripae* nom. nov. is herein proposed as a replacement name for *H. riparia* Subbotin, Sturhan, Waeyenberge & Moens, 1997.

Heterodera 'avenae' *from China*

In 1989, a cyst nematode morphologically similar to, and identified as, *H. avenae* was found on wheat in China (Chen *et al.*, 1989; Wang *et al.*, 1991). A subsequent survey revealed that this nematode was widely distributed and it has been found in 26 regions and eight provinces or cities, including Hubei, Beijing, Hebei, Henan, Inner Mongolia Autonomous Region, Qinghai, Shanxi and Anhui (Chen *et al.*, 1989; Wang *et al.*, 1991; Peng *et al.*, 1995, 1996; Zheng *et al.*, 1996). Peng and Cook (1996) concluded that pathotypes of Chinese populations of cereal cyst nematode may differ considerably from those of other regions, but they did not allocate numbers for pathotypes using the scheme of Andersen and Andersen (1982) because some host reactions had still to be validated. In an independent study, Zheng *et al.* (1997) showed the Taigu and Guthen populations to be distinctive new pathotypes. Sturhan and Rumpenhorst (1996) showed that Chinese cereal cyst nematode populations have an IEF profile different from other species. Morphological and morphometrical descriptions of several stages were given by Zheng *et al.* (1996). Our present studies have shown that populations from China appear to form a distinct group within the *H. avenae* complex. Morphologically and genetically these populations are close to *H. pratensis.* Until detailed comparative morphological studies and a molecular analysis of the Chinese cereal cyst nematode have been conducted (with inclusion of *H. riparia* comb. n. and the Asian *Heterodera* sp. from grassland), the taxonomic status of the Chinese populations, presently designated by us as *'avenae'*, remains uncertain.

Acknowledgements

The study was in part financed by a grant of the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. The authors thank S. Amiri, F. Charman-Green, I.H. Elekçioglu, A. Eroshenko, A. Ireholm, G. Karssen, I.P. Kazachenko, M. Mor, L. Nasonova, D. Orion, E. Osipova, D. Peng, I. Riley, R. Rivoal, D. Romero, J.A. Rowe, B. Schoemaker, J. Wilson, W.M. Wouts and J. Zheng for supplying nematode materials.

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