Russian Journal of Nematology, 2002, 10 (2), 139 - 148

Description of the Australian cereal cyst nematode *Heterodera australis* sp. n. (Tylenchida: Heteroderidae)

Sergei A. Subbotin*, Dieter Sturhan, Hans Jürgen Rumpenhorst** and Maurice Moens*****

*Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 117071, Russia,
**Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Nematologie und Wirbeltierkunde, Toppheideweg 88, 48161 Münster, Germany,
***Crop Protection Department, Agricultural Research Centre, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium.

Accepted for publication 10 September 2002

Summary. Populations of cereal cyst nematode sampled in Australia are described as a new species, *Heterodera australis* sp. n. This species is morphologically and morphometrically similar to *H. avenae*. It is distinguished from *H. avenae* and other species of the *H. avenae* complex by sequence and RFLP of the ITS region of rDNA and IEF patterns. The phylogenetic relationships among species of the *H. avenae* complex based on analyses of the ITS-rDNA sequences are presented.

Key words: Australia, cereals, *Heterodera avenae,* IEF, ITS-rDNA.

The cereal cyst nematode (CCN), *Heterodera avenae* Wollenweber, 1924, is a major nematode pest in many countries and found in almost all 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). In Australia, a cyst-forming nematode attacking the roots of cereals was first reported by Davidson (1930). Millikan (1938) recorded the occurrence of CCN in Victoria and provided a brief description. According to Meagher (1977) there is no evidence that *H. avenae* is indigenous in Australia; he assumes that it was probably introduced from Europe in the nineteenth century. CCN is considered as the most important pathogen of wheat and other cereals in the southern wheat belt. More than 2 million hectares in Victoria and South Australia are infested, and annual losses in grain yield in wheat alone are conservatively estimated at USD 70 millions (Brown, 1984).

The Australian populations of CCN were considered as a pathotype quite different from European populations (Andersen & Andersen, 1982; Brown, 1982; Rivoal & Cook, 1993). Thorne (1961) observed some morphological differences between specimens from Australia and Europe and suggested the presence of a geographical variant which "eventually may be set up as a distinct species". Meagher (1974) and McLeod & Khair (1977) compared Australian populations with *H. avenae* from Europe and Canada and concluded that they did not represent a separate species. They assumed that differences in measurements leading to confusion about the identity of *H. avenae* in Australia could arise in part to environmental influences, but also to differences in fixation methods and errors in reporting.

Rumpenhorst (1988) mentioned that three Australian populations, which had been morphologically identified as *H. avenae*, differed in their protein pattern compared with populations from Europe and Israel and should probably be considered as a separate species. Ferris *et al.* (1994) showed that the 2-DGE for Australian isolates differed more from a Swedish *H. avenae* isolate and isolates from Oregon and Idaho than the latter three isolates differed from each other. However, Bossis & Rivoal (1996) using the same method revealed that the Australian isolate was similar to the French population studied, finding an argument in supporting the North European origin of the Australian *H. avenae*. According to data by

Table 1. Species and populations used in the present study.

Species	Location, country	Source of material	Studies*
H. australis sp. n.	York Peninsula, South Australia	F. Charman-Green	M, IEF, RFLP
	Beulah, Victoria, Australia	H. J. Rumpenhorst	M, IEF, RFLP
	Victoria, Australia	J. Wilson	M, IEF
	South Australia sample 1, Australia	M. Moens (originated from A. Khan)	M, RFLP, SEQ
	South Australia, sample 2, Australia	H. J. Rumpenhorst (orig. from W. Wouts)	M, RFLP
	South Australia, sample 3, Australia	I. Riley (originated from J. Lewis)	M, IEF
H. avenae	Grafenreuth, Germany	H. J. Rumpenhorst	IEF
	Taaken, Germany	D. Sturhan	RFLP
	Santa Olalla, Spain	M. Romero	IEF
	unknown, Saudi Arabia	H. J. Rumpenhorst	IEF, RFLP
	Bet Dagan, Israel	D. Orion	IEF
	Niz Oz, Israel	D. Orion	RFLP
	Ha-hoola, Israel	M. Mor	IEF
H. pratensis	Putilovo, Leningrad region, Russia	S. A. Subbotin	SEQ
	Missunde near Schleswig, Germany	D. Sturhan	IEF

* M – morphological or/and morphometrical analysis; IEF – isoelectric focusing of proteins; RFLP – restriction fragment length polymorphism of rDNA; SEQ – sequence and phylogenetic analyses of the ITS-rDNA.

Bekal *et al.* (1997) the ITS region of this Australian population has the same RFLP profile generated by *Hin*fI and *Taq*I as those of other *H. avenae* populations. Australian population clustered with Saudi Arabian and Israeli (Nir Oz) populations in cladogram. Incongruence between 2-DGE data by Ferris *et al.* (1994) and Bossis & Rivoal (1996) and between RFLP data by Bekal *et al.* (1997) and those obtained from the present study, may indicate that populations labelled as "Australian" belong to two different species. Rumpenhorst (unpublished) also found that an Australian population of cereal nematode from Sea Lake had an IEF profile and ITS sequence similar to European populations. Thus, if we exclude the possibility of sample mixing during both studies, the data suggest that currently two species appear to be present in Australian cereal fields: one native Australian species and *H. avenae,* propably introduced from Europe or Asia.

In the present paper, based on analyses of IEF, RFLP and sequences of the ITS of rDNA obtained from populations and species of the *Heterodera avenae* complex, the native Australian populations of CCN are described as a new species.

MATERIALS AND METHODS

Nematode populations. Populations of the Australian cereal cyst nematode and other species from *H. avenae* complex used in this study were obtained from different sources (Table 1). Second-stage juveniles and males were killed and fixed in hot TAF and processed to glycerine by a slow method. The juveniles, males and vulval cones mounted on permanent slides were examined and measured. All measurements are presented in micrometers (μm) as the mean, the standard error of the mean and the range in parentheses.

Isoelectric focusing. For protein extraction, about 10 full cysts were crushed and homogenized in 20 μl extraction buffer. Water soluble proteins were separated by isoelectric focusing in polyacrylamide gel as implemented by Gäbler *et al.* (2000). The gel was fixed and stained with silver following a modified procedure of Ohms & Heinicke (1983) an then photographed.

PCR, RFLPs and sequencing 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 (20mg/ml, Qiagen)] 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; 16,000g) 2 μl of the DNA suspension was added to PCR reaction mixture containig 5 μl of 10X PCR buffer, 2 μl of $MgCl_2$ (25mM), 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 (Subbotin *et al.*, 2000). The DNA-amplification profile was programmed for initial denaturation and enzyme activation at 95 °C for 15 min, followed

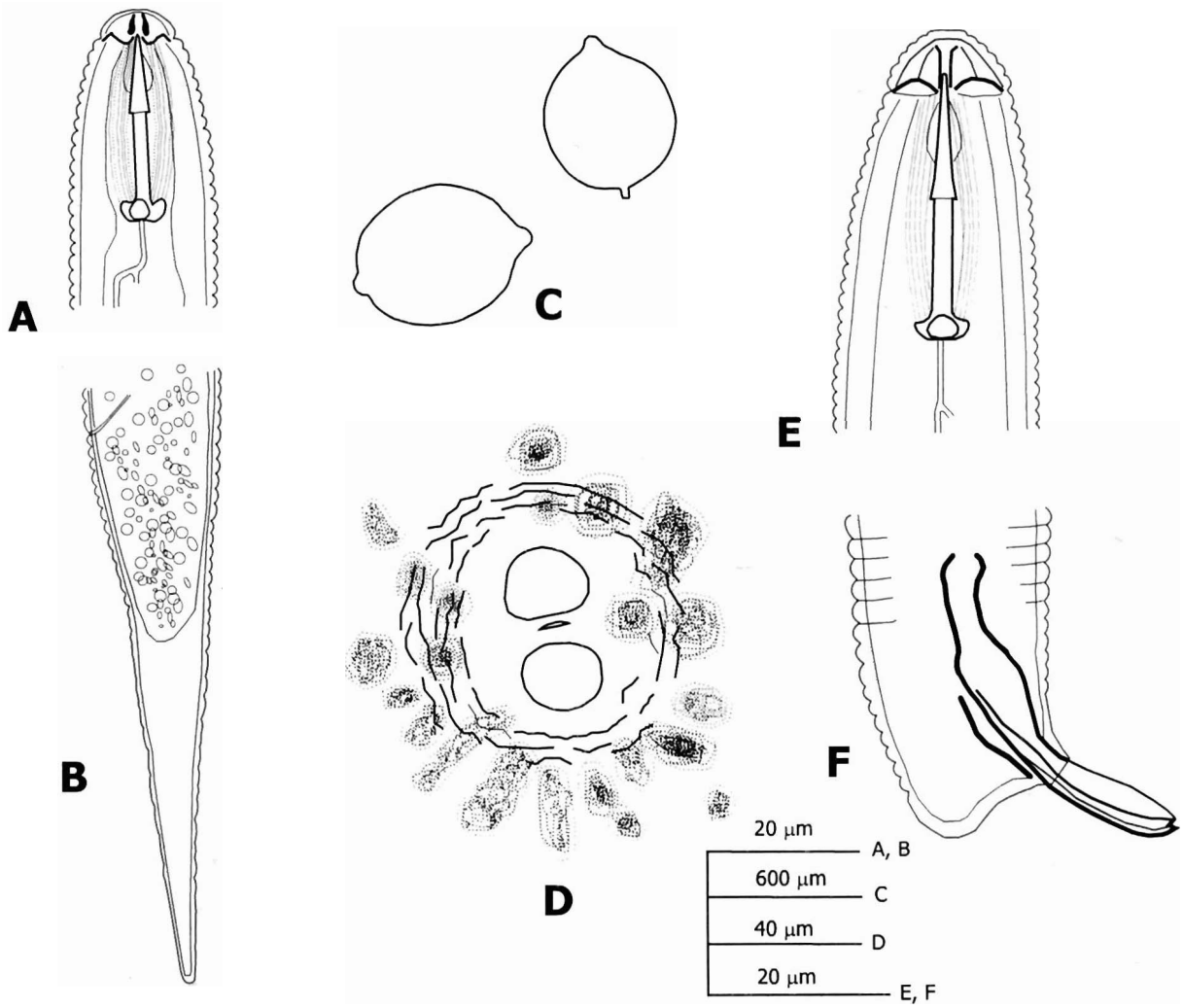

Fig. 1. *Heterodera australis* sp. n. A, B: Second-stage juvenile, head and tail; C: Cysts, D: Vulval cone; E, F. Male, head and tail.

by 35 cycles of 45 sec at 95 °C, 45 sec at 60 °C, 1.5 min at 72 °C, and 10 min at 72 °C. Three to 7 µl of each PCR product were digested with one 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 or MBI Fermentas, Biolabs New England, UK). 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 several times to verify the results.

DNA fragments of the ITS from *H. australis* sp. n. (sample 2, South Australia) and *H. pratensis* (Putilovo, Russia) were sequenced in both directions with TW81, AB28, or 5.8SM5 primers (Zheng *et al.*, 2000) with BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, UK) according to the manufacturer's instructions. The resulting products were purified using a Centriflex Gel Filtration Cartridge (Edge BioSystems Inc., Gaithersburg, Maryland, USA) and run on a 377 DNA sequencer (PE Applied Biosystems). Sequences of *H. australis* sp. n. (AY148352) and *H. pratensis* (AY148351) are published in the GenBank.

Phylogenetic analysis. Original sequence *H. australis* sp. n. and *H. pratensis* were aligned using ClustalX 1.64 (Thompson *et al.*, 1997) with known sequences of the *H. avenae* complex (Zheng *et al.*, 2000; Subbotin *et al.*, 2001) and sequences of two outgroup taxa *H. hordecalis* Andersson, 1975 and

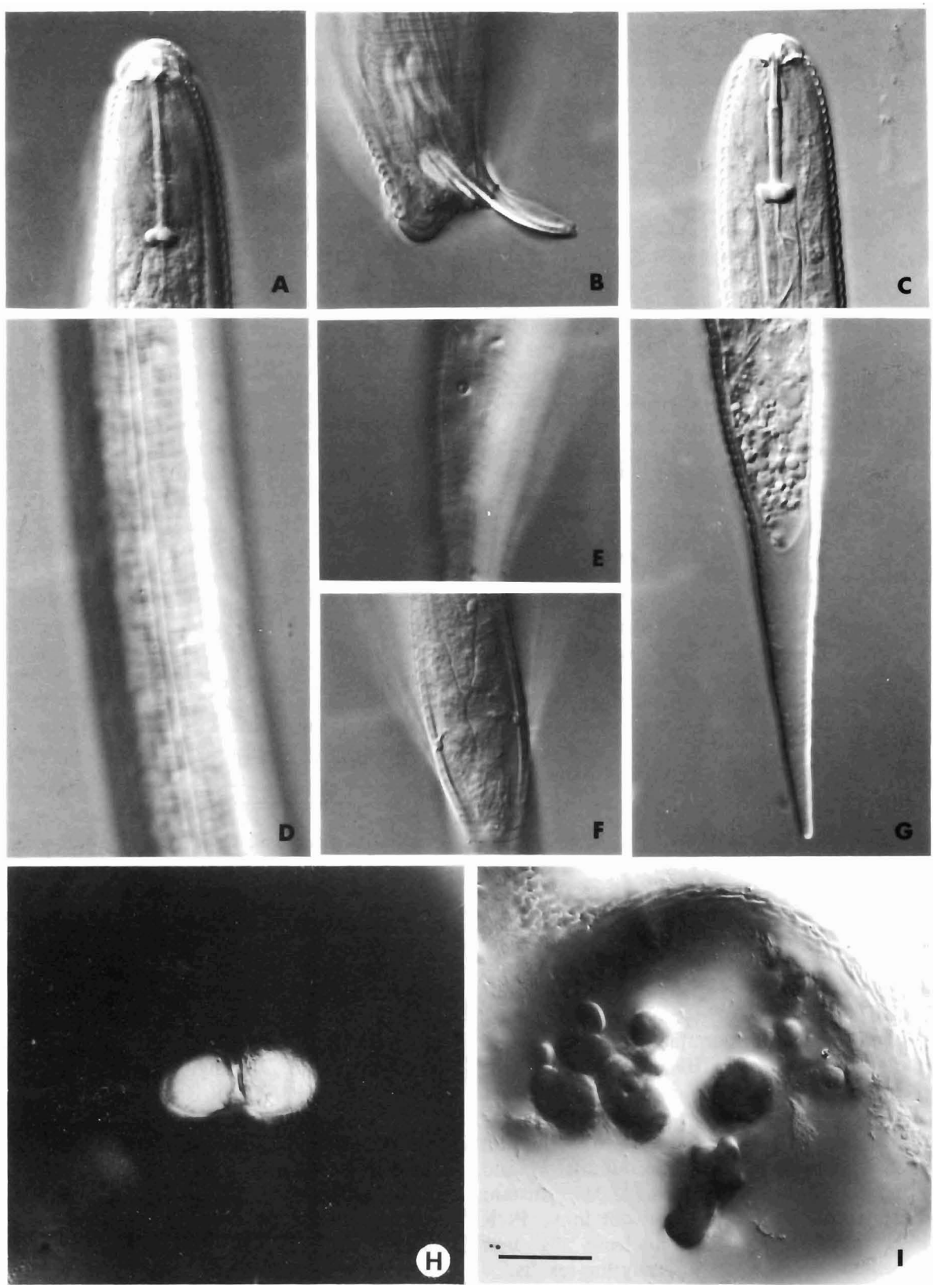

Fig. 2. *Heterodera australis* sp. n. A-B: Male; A: Anterior end; B: Tail and spicules; C-G: Second stage juvenile; C: Anterior end; D: Lateral field at mid-body; E, F: Phasmids in lateral and dorso-ventral view; G: Tail; H, I: Cyst; H: Fenestration in vulval cone; I: Bullae in vulval cone (Scale bar: A-G= 15 µm, H, I = 30 µm).

Table 2. Morphometrics of cysts and second-stage juveniles of *Heterodera australis* sp. n. (measurements in µm).

Population	York Peninsula, Australia (Paratypes)	Beulah, Australia	Victoria, Australia	South Australia, (sample 2)
Cysts				
n	16	6	25	9
Length excl. neck	734±26 (576-960)	780±28 (696-888)	748±18 (624-912)	763±16 (696-840)
Width	528±17 (432-672)	564±14 (528-624)	540±12 (432-648)	557±10 (504-600)
Length/width	1.4±0.03 (1.2-1.9)	1.4±0.1 (1.3-1.5)	1.4±0.03 (1.1-1.8)	1.4±0.04 (1.2-1.6)
Vulval areas				
n	15	7	18	5
Fenestral length	52±1.1 (43-58)	49±1.2 (43-52)	51±1.2 (43-62)	47±2.3 (39-52)
Mean semifenestral width	25±0.7 (19-30)	23±1.0 (19-27)	24±0.5 (19-27)	23±1.6 (19-27)
Vulval bridge width	11±0.4 (7.8-14)	11±0.7 (7.8-14)	9.9±0.4 (7.8-12)	10±0.7 (7.8-12)
Vulval slit length	8.3±0.3 (5.8-10.5)	8.0±0.3 (7.0-9.7)	7.7±0.2 (5.8-9.7)	7.7±0.2 (7.0-8.1)
Vulva-anus distance	57±2.2 (50-70)	50±3.1 (39-58)	53±1.3 (47-62)	53±3.7 (43-58)
Juveniles				
n	20	30	16	26
Body length (L)	562±5.1 (519-608)	583±2.7 (558-617)	569±4.9 (533-593)	576±4.1 (544-619)
a	28±0.3 (25-31)	28±0.1 (27-30)	28±0.3 (26-30)	29±0.2 (27-32)
b	4.5±0.1 (4.1-5.0)	4.7±0.03 (4.3-5.0)	4.7±0.1 (4.5-5.1)	4.6±0.03 (4.3-4.9)
c	8.3±0.2 (7.4-9.5)	8.3±0.1 (7.8-9.2)	8.4±0.1 (8.0-9.2)	8.2±0.1 (7.8-8.9)
Stylet length	26±0.1 (25-27)	26±0.1 (25-27)	25±0.1 (25-27)	26±0.1 (25-27)
Lip region height	**3.9±0.03 (3.6-3.9)**	**3.8±0.03 (3.6-4.1)**	**4.0±0.1 (3.6-4.4)**	3.8±0.04 (3.4-4.1)
Lip region width	**9.4±0.1 (8.8-9.8)**	**9.2±0.1 (8.8-9.8)**	**9.2±0.1 (8.8-9.8)**	9.1±0.1 (8.8-9.8)
DGO	5.5±0.2 (4.9-6.4)	6.2±0.1 (4.9-6.9)	5.6±0.1 (4.9-6.4)	6.2±0.1 (4.9-7.4)
Anterior end to valve of median bulb (MB)	70±1.7 (61-76)	77±0.4 (74-80)	69±1.0 (64-78)	78±1.0 (60-85)
Anterior end to excretory pore	108± 0.8 (99-115)	115±0.6 (107-120)	112±1.3 (100-118)	112±0.9 (104-121)
Oesophagus length (cardia)	125±1.8 (115-135)	125±0.5 (118-130)	120±1.5 (113-127)	126±0.8 (118-132)
Body width at mid-body	20±0.2 (20-21)	21±0.1 (20-21)	20±0.1 (20-21)	20±0.1 (20-21)
Body width at level of anus (BWA)	15±0.1 (15-16)	15±0.1 (15-16)	15±0.1 (14-16)	15±0.1 (15-16)
Hyaline part of tail length (H)	43±1.0 (33-50)	45±0.6 (39-50)	45±0.6 (38-48)	43±0.7 (37-49)
Tail length	68±0.9 (59-74)	70±0.6 (61-76)	68±0.7 (59-71)	71±0.5 (67-76)
Tail length/BWA	3.4±0.1 (3.0-3.7)	3.4±0.02 (3.1-3.6)	3.3±0.04 (2.9-3.5)	3.5±0.03 (3.2-3.8)
H/Stylet length	1.7±0.04 (1.3-2.0)	1.8±0.02 (1.5-2.0)	1.8±0.02 (1.6-1.9)	1.7±0.02 (1.4-1.9)
L/MB	8.1±0.2 (7.1-9.5)	7.6±0.04 (7.1-8.1)	8.3±0.1 (7.3-9.2)	7.4±0.1 (6.8-9.2)

H. latipons Franklin, 1969 (Subbotin *et al.*, 2001). The equally weighted maximum parsimony analysis of the ITS alignment was performed using PAUP* 4b4a (Swofford, 1998). Heuristic search setting were 10 random replicates of taxon addition with tree bisection - reconnection (TBR) branch swapping. Gaps were treated as a fifth character. Bootstrap support was estimated by a heuristic search from 500 replicates using simple addition sequences with TBR swapping.

DESCRIPTION
Heterodera australis sp. n.
(Figs. 1 & 2)

Holotype cyst: L (excluding neck) = 780 µm; width = 560 µm; length/width ratio = 1.4; neck length = 105 µm.

Paratype cysts: See Table 2.

Paratype males (n=5): L = 1556±48 (1405-1695) µm; width = 31±0.4 (29-31) µm; height of lip region = 6.4±0.2 (5.9-6.9) µm; width of lip region = 12±0.2 (12-13) µm; stylet length = 29±0.3 (28-30) µm; distance of opening of dorsal oesophageal gland from stylet base = 6.5±0.4 (5.4-7.4) µm; distance from anterior end to median bulb valve = 106±3 (102-119) µm; distance from anterior end to excretory pore = 181±19 (147-210) µm; oesophagus length = 168±17 (137-196) µm; length of genital tract = 770±53 (637-896) µm; a = 51±1.8 (45-56): b = 9.8±0.8 (8.6-11.4); length of spicules = 43 µm; length of gubernaculum = 12 µm.

Paratype juveniles: See Table 2.

Paratype eggs (n=20): L = 135±1.6 (122-148) µm; width = 46±0.6 (43-54) µm; ratio of length to

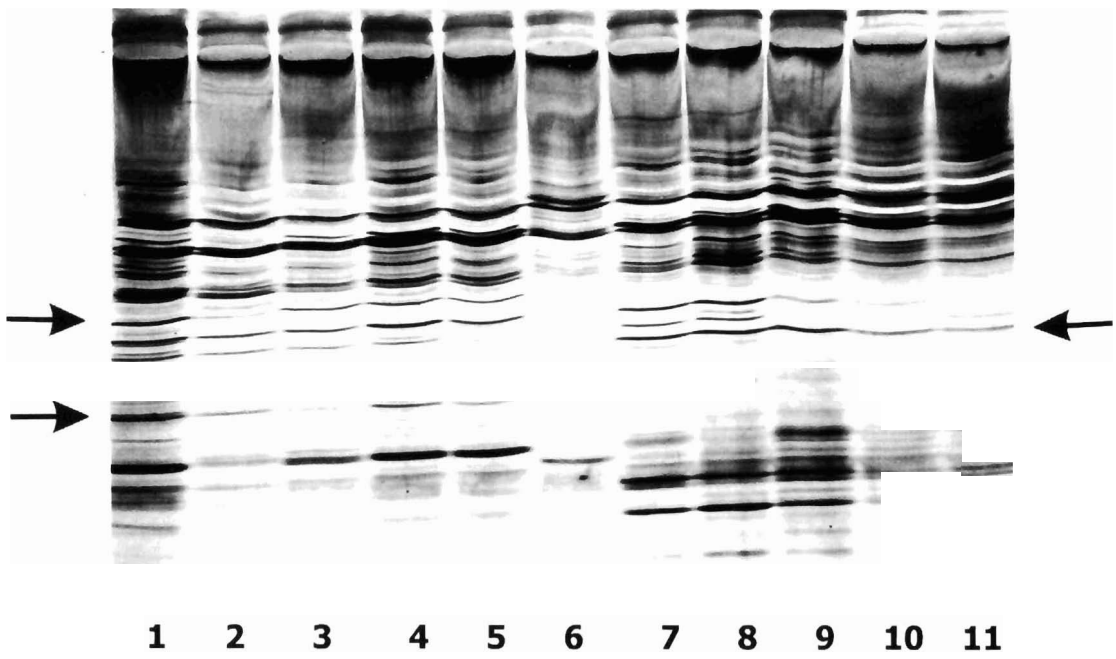

Fig. 3. IEF protein patterns of species from the *H. avenae* complex. 1: *H. avenae* (Santa Olalla, Spain); 2: *H. avenae* (Grafenreuth, Germany); 3: *H. avenae* (Bet Dagan, Israel); 4: *H. avenae* (Ha-hoola, Israel); 5: *H. avenae* (Saudi Arabia); 6: *H. pratensis* (Missunde, Germany); 7: *H. australis* sp. n. (South Australia, sample 2); 8: *H. australis* sp. n. (Beulah, Australia); 9: *H. australis* sp. n. (South Australia, sample 3); 10: *H. australis* sp. n. (York Peninsula, Australia); 11: *H. australis* sp. n. (Victoria, Australia). Arrows indicate specific bands.

width = 2.9±0.1 (2.3-3.4).

Cysts. Lemon-shaped, generally elongate with distinct vulval cone. Cyst colour varies from yellow brown to dark brown. Subcrystalline layer distinct; egg sac not observed. Vulval cone bifenestrate, fenestrae oval to almost circular, almost horseshoe shaped in older cysts. Vulval slit short. Bullae numerous, at different levels in vulval cone, distinct, deeper brown than cyst cuticle, irregular in form and variable in size. Underbridge absent.

Males. General morphology as typical for the genus. Lip region dome-shaped, distinctly set off, with 4-5 annules and a labial disc measuring 8 x 4.5 µm. Stylet strong, knobs rather small, slightly indented or almost flat anteriorly. Median bulb oval, occupying about half of the body diameter, with 4 µm long valve. Lateral field with four incisures and irregular areolation. Cuticle annules 2 µm wide at mid-body. Tail short and rounded, smooth at the end. Phasmids absent. Spicules with broad, bidentate tip.

Juveniles. Body slightly curved ventral. Lip region set off, rounded, more than two times wider than high, with two indistinct annules and wide labial disc. Cephalic framework well developed. Cuticle only slightly thickened posterior to lip region. Stylet strong, stylet knobs slightly to moderately concave; stylet base 5.5–6 µm wide and 2.5–3 µm high. Median bulb oval, with 3.5 µm long valve. Oesophageal glands well developed. Lateral field with four incisures, but mostly only the inner two incisures distinct; outer bands generally areolated. Cuticle annules 1.8–2.0 µm wide at mid-body. Tail gradually tapering to a rounded terminus, with hyaline portion mostly occupying almost two thirds of its total length. Phasmids prominent, wide and lens-like, 2–5 body annules posterior to anus.

Type locality. Cysts with eggs and juveniles defined as type specimens were collected early 1999 by Franky Charman-Green (South Australian Research and Development Institute) from a cereal field in York Peninsula, South Australia (precise locality unknown). Other populations were collected from different localities in South Australia and Victoria.

Type material. Holotype cyst, paratype cysts and cone tops, males, second-stage juveniles and

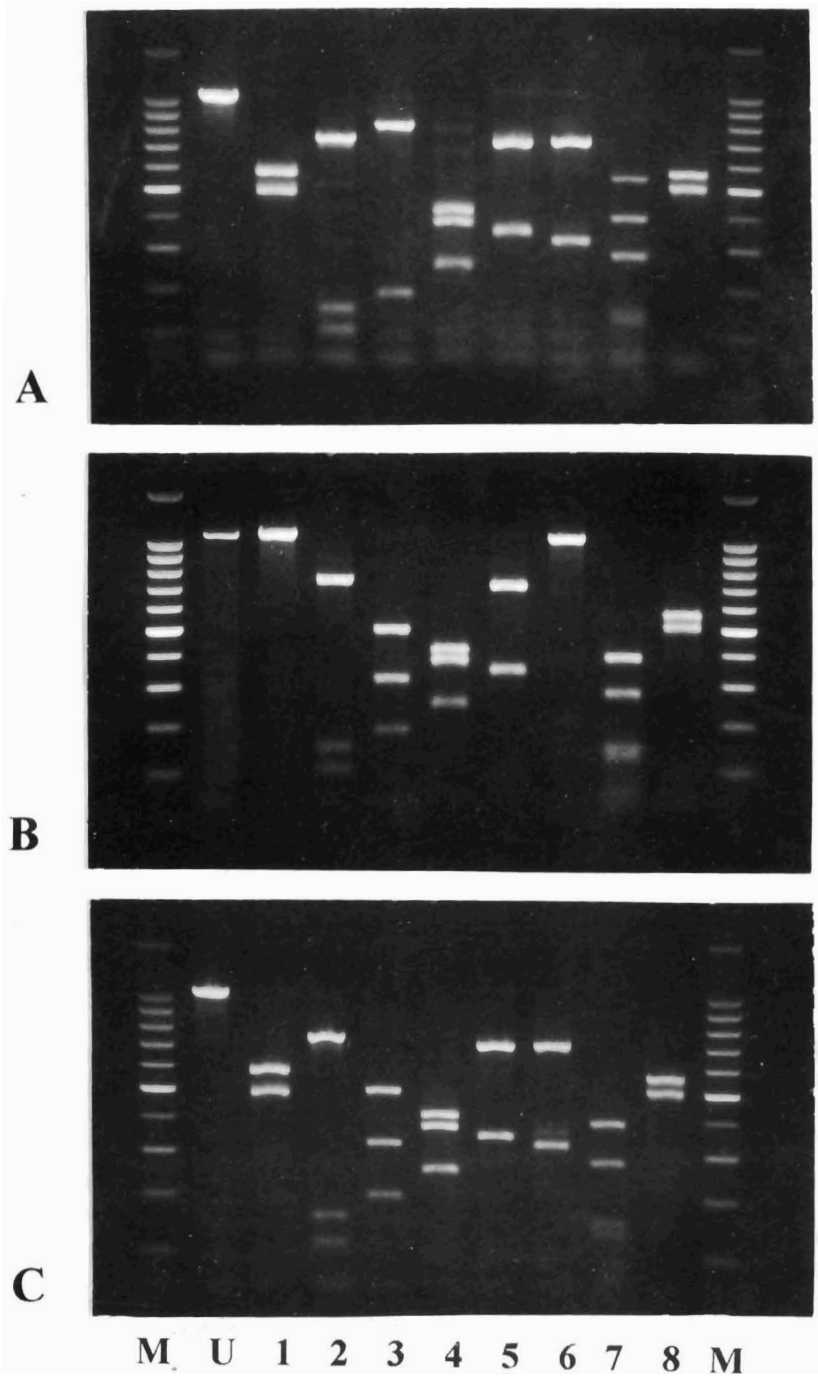

Fig. 4. RFLPs-ITS-rDNA profiles of species from the *H. avenae* complex. A: *H. australis* sp. n. (South Australia, sample 1), B: *H. avenae* (Taaken, Germany), C: *H. avenae* (Nir Oz, Israel). Code: M – 100 bp DNA marker (Promega), U – unrestricted PCR product, 1 – *Alu*I; 2 – *Cfo*I; 3 – *Hin*fI; 4 – *Ita*I; 5 – *Pst*I; 6 – *Rsa*I; 7 – *Taq*I; 8 – *Tru*9I.

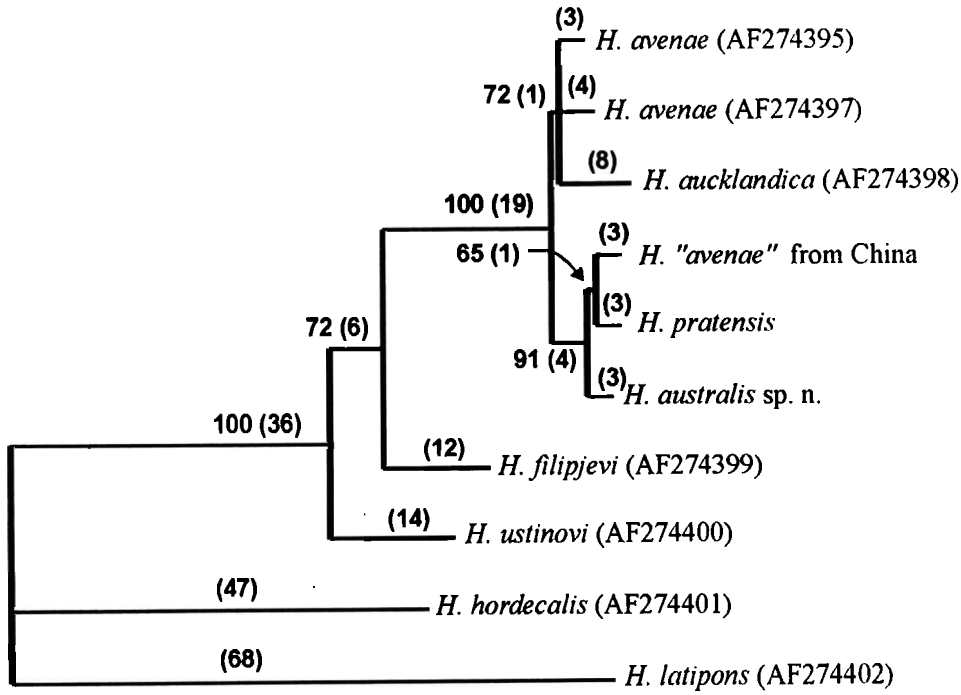

Fig. 5. Phylogenetic relationships within the *H. avenae* complex inferred from analyses of the ITS sequences. A single parsimonious tree, when gaps were treated as fifth character (tree length = 232; consistency index = 0.9483; homoplasy index = 0.0517; retention index = 0.8846; rescaled consistency index = 0.8389). Bootstrap volumes are given in bold and number of changes in brackets.

eggs are deposited in the German Nematode Collection, Biologische Bundesanstalt, Münster, Germany. Additional paratypes are deposited at Institute of Parasitology of RAS, Moscow, Russia, Rothamsted Experimental Station, Harpenden, England, and in the USDA Nematode Collection, Beltsville, Maryland, USA. *Heterodera australis* sp. n. specimens from other Australian populations are also deposited in all four nematode collections.

Remark. Meagher (1974) gave a detailed description, including drawings and photomicrographs of a population collected from a field close to Sea Lake, Victoria. McLeod and Khair (1977) provided measurements and morphological details of seven populations from Victoria, South Australia, Western Australia and New South Wales. We consider the populations studied as most probably conspecific with *H. australis* sp. n.

IEF. Samples from *H. avenae* populations and *H. australis* sp. n. produced consistent banding patterns on IEF gels (pH range 3-10); several distinct bands allow to distinguish both species (Fig. 3). The results of our present IEF study of populations of *H. avenae, H. pratensis* and *H. australis* sp. n. are in accordance with published data, which showed the IEF technique to be sensitive and robust and able to differentiate the *H. avenae* complex species (Rumpenhorst, 1985; Sturhan & Rumpenhorst, 1996; Rumpenhorst *et al.*, 1996; Subbotin *et al.*, 1996; Gäbler *et al.*, 2000).

RFLP analysis. The restriction patterns obtained after digestion by eight enzymes of populations from the *H. avenae* complex are given in Fig. 4. Digestion by *Taq*I shows a unique RFLP profile with heterogeneity in the ITS region for all studied populations of *H. australis* sp. n. The restriction profiles obtained with *Hin*fI distinguish *H. australis* sp. n. from *H. avenae.*

Phylogenetic relationships of *H. australis* sp. n. with other species of the *H. avenae* complex. Analysis of the alignment with 10 ITS sequences using maximum parsimony (gaps treated as fifth character) resulted a single parsimonious tree with two main groups for the *H. avenae* complex species. *Heterodera australis* sp. n. clustered with high bootstrap support with *H. pratensis* and *H. "avenae"* from China (Fig. 5). Detailed phylogenetic analyses of more than 60 sequences of the *Avenae* group species using maximum parsimony and maximum likelihood revealed that the Australian

cereal cyst nematode is clearly separated from other species (Subbotin *et al.*, unpublished).

Differential diagnosis. Comparison of the morphology and morphometrics of *H. australis* sp. n. with morphological characteristics of other species of the *H. avenae* complex (Williams & Siddiqi, 1972; Subbotin *et al.*, 1999) revealed that this species is morphologically indistinguishable from *H. avenae*. The measurements of all stages of both species overlap. *Heterodera australis* sp. n. differs from *H. filipjevi* (Madzhidov, 1981) and *H. ustinovi* Kirjanova, 1969 by absence of an underbridge in the cysts, from *H. arenaria* Cooper, 1955 by longer stylet, tail and hyaline part of tail in the second-stage juveniles, from *H. pratensis* Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000 and *H. aucklandica* Wouts & Sturhan, 1995 by shorter body in the juveniles, from *H. mani* Mathews, 1971 by shorter tail and hyaline part of tail in the juveniles.

The new species is easily differentiated from *H. avenae* and other species of the *H. avenae* complex by RFLPs and sequences of the ITS region of rDNA and IEF patterns. Also, several RAPD markers distinguish *H. australis* sp. n. from the other species (Subbotin *et al.*, unpublished).

ACKNOWLEDGEMENTS

The study was in part financed by a grant of the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. The authors thank F. Charman-Green, A. Khan, J. Lewis, M. Mor, D. Orion, I. Riley, M. Romero, J. Wilson, and W. Wouts for supplying nematode materials.

REFERENCES

Andersen, S. & Andersen, K. 1982. Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. *EPPO Bulletin* 12: 379-386.

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

Bekal, S., Gauthier, J.P. & Rivoal, R. 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. *Genome* 40: 479-486.

Bossis, M. & Rivoal, R. 1996. Protein variability in cereal cyst nematodes from different geographic regions assessed by two-dimensional gel electrophoresis. *Fundamental and Applied Nematology* 19: 25-34.

Brown, R.H. 1982. Studies on the Australian pathotype of *Heterodera avenae*. *EPPO Bulletin* 12: 413-421.

Brown, R.H. 1984. Cereal cyst nematode and its chemical control in Australia. *Plant Disease* 68: 922-928.

Davidson, J. 1930. Eelworms (*Heterodera schachtii* Schm.) affecting cereals in South Australia. *Journal of the Department of Agriculture of South Australia* 34: 378-385.

Ferris, V.R., Ferris, J.M., Faghihi, J. & Ireholm, A. 1994. Comparisons of isolates of *Heterodera avenae* using 2-D PAGE protein patterns and ribosomal DNA. *Journal of Nematology* 26: 144-151.

Gäbler, C., Sturhan, D., Subbotin, S.A. & Rumpenhorst, H.J. 2000. *Heterodera pratensis* sp. n., a new cyst nematode of the *H. avenae* complex (Nematoda: Heteroderidae). *Russian Journal of Nematology* 8: 115-126.

McLeod, R.W. & Khair, G.T. 1977. Observations on the morphology of *Heterodera avenae* in Australia. *Nematologica* 23: 382-389.

Meagher, J.W. 1974. The morphology of the cereal cyst nematode (*Heterodera avenae*) in Australia. *Nematologica* 20: 1-8.

Meagher, J.W. 1977. World dissemination of the cereal-cyst nematode (*Heterodera avenae*) and its potential as a pathogen of wheat. *Journal of Nematology* 9: 9-15.

Millikan, C.R. 1938. Eelworm (*Heterodera schachtii* Schmidt) disease of cereals. *Journal of Agriculture Victoria* 36: 452-468.

Ohms, J.P. & Heinicke, D.H.K. 1983. Pathotypen des Kartoffelnematoden I. Schnellbestimmung der Artzugehörigkeit mittels Isoelektrofokussierung. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 90: 258-264.

Rivoal, R. & Cook, R. 1993. Nematode pests of cereals. In: *Plant Parasitic Nematodes in Temperate Agriculture*. (K. Evans, D.L. Trudgill, & J.M. Webster, Eds.). pp. 259-303. CAB International, Wallingford.

Rumpenhorst, H.J. 1985. Vergleichende elektrophoretische Untersuchungen von Proteinen einiger Zystennematoden von Getreide und Gräsern. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 226: 64-74.

Rumpenhorst, H.J. 1988. Protein taxonomic studies on *Heterodera avenae* and related species. *Biologische Bundesanstalt für Land- und Forstwirtschaft in Berlin und Braunschweig, Jahresbericht 1987* (publ. 1988), p. 67.

Rumpenhorst, H.J., Elekçioglu, I.H., Sturhan, D., Öztürk, G. & Enneli, S. 1996. The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. *Nematologia mediterranea* 24: 135-138.

Sturhan, D. & Rumpenhorst, H.J. 1996. Untersuchun-

gen über den *Heterodera avenae*-Komplex. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 317: 75-91.

Subbotin, S.A., Rumpenhorst, H.J. & Sturhan, D. 1996. Morphological and electrophoretic studies on populations of the *Heterodera avenae* complex from the former USSR. *Russian Journal of Nematology* 4: 29-38.

Subbotin, S.A., Waeyenberge, L., Molokanova, I.A. & Moens, M. 1999. Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLPs. *Nematology* 1: 195-207.

Subbotin, S.A., Waeyenberge, L. & Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. *Nematology* 2: 153-164.

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

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

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Thorne, G. 1961. *Principles of Nematology.* New York, McGraw-Hill Book Company, 553 pp.

Williams, T.D. & Siddiqi, M.R. 1972. *Heterodera avenae. C.I.H. Descriptions of plant-parasitic nematodes.* Set. 1, N. 2. 4 pp.

Zheng, J., Subbotin, S.A., Waeyenberge, L. & Moens, M. 2000. Molecular characterisation of Chinese *Heterodera glycines* and *H. avenae* populations based on RFLPs and sequences of rDNA-ITS regions. *Russian Journal of Nematology* 8: 109-113.

Subbotin S.A., Sturhan D., Rumpenhorst H. J., Moens M. Описание Австралийской овсяной нематоды *Heterodera australis* sp. n. (Tylenchida: Heteroderidae).

Резюме. Популяции овсяной цистообразующей нематоды из Австралии описываются как новый вид *Heterodera australis* sp. n.. Морфологически и мофометрически новый вид близок к *H. avenae*, но отличается от этого и других видов этого комплекса по нуклеотидным последовательностям и профилям RFLP ITS участка рибосомальной ДНК, а также спектрам изоэлектрофокусировки белков. Представлена схема филогенетических отношений между видами комплекса *H. avenae*, основанная на анализе последовательностей рибосомальной ДНК.