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Heterodera pratensis sp. n., a new cyst nematode of the *H. avenae* complex (Nematoda: Heteroderidae)

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Summary. A new cyst-forming nematode belonging to the *Heterodera avenae* complex and specialized on grass hosts is described as *H. pratensis* sp. n. The species is widely distributed in grassy habitats in northern Germany and also has been found in Russia and Estonia. *Heterodera pratensis* sp. n. is well distinguished from the other species in the *H. avenae* complex by protein isoelectric focusing and ribosomal DNA-RFLPs. In its morphological characters it most closely resembles *H. avenae, H. aucklandica* and *H. arenaria* in the absence of an underbridge in the cyst cone. It is distinguished from these species by the shape and colour of the cysts, the arrangement of the bullae, and lengths of juvenile body, tail and hyaline part of tail. Diagnostic rDNA-RFLP profiles for *H. pratensis* sp. n., *H. avenae* and *H. aucklandica* are given.
Key words: Estonia, Germany, *Heterodera aucklandica, Heterodera avenae* group, IEF, ITS-rDNA, Poaceae, Russia.

The existence of undescribed species within the *Heterodera avenae* complex has long been suspected (Cook, 1982; Stone & Hill, 1982; Sturhan, 1976, 1982). In recent years *H. aucklandica* Wouts & Sturhan, 1995 was described from New Zealand, and *H. arenaria* Cooper, 1955 has been redescribed (Robinson *et al.*, 1996). Furthermore, the "British pathotype 3" of *H. avenae*, the "Gotland strain" from Sweden and *H. "mani"* populations from Germany were shown to be representatives of *H. filipjevi* (Madzhidov, 1981) Stelter, 1984 (Sturhan & Rumpenhorst, 1996; Bekal *et al.*, 1997; Ferris *et al.*, 1999).

The *H. avenae* complex with presently six species (*H. avenae* Wollenweber, 1924, *H. arenaria, H. aucklandica, H. filipjevi, H. iri* Mathews, 1971 and *H. mani* Mathews, 1971) appears to be related to the species complex with *H. latipons* Franklin, 1969 and *H. hordecalis* Andersson, 1975 as well as some undescribed species (Gäbler, 1996; Sturhan & Rumpenhorst, 1996; Subbotin *et al.*, 1999). The species *H. bifenestra* Cooper, 1955, *H. turcomanica* Kirjanova & Shagalina, 1965 and *H. spinicauda* Wouts, Schoemaker, Sturhan & Burrows, 1995, which are presently also placed in the "*H. avenae* group", are obviously not related to the *H. avenae* and *H. latipons* species complexes (Sturhan, unpubl.).

Several *Heterodera* populations collected mainly from grasslands from a number of localities in Germany, Russia and Estonia showing only minor morphological and morphometric differences with *H. avenae sensu stricto* and related species, could be positively distinguished from these species by protein isoelectric focusing (IEF) and rDNA-RFLPs (Rumpenhorst, 1994; Sturhan & Rumpenhorst, 1996, Subbotin *et al.*, 1996; 1999). These populations are described here as *H. pratensis* sp. n. and compared with other species of the *H. avenae* group using IEF and rDNA-RFLP methods.

MATERIALS AND METHODS

Nematode populations. Twelve populations of *H. pratensis* sp. n., two populations each of *H. avenae* and *H. filipjevi* and one population of *H.*

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

Species	Origin	Source	Methods of study[1]
H. pratensis sp. n.	Lindhöft, Schleswig-Holstein, Germany	D. Sturhan, Germany	MS, IEF, RFLP
H. pratensis sp. n.	Östergaard, Schleswig-Holstein, Germany	D. Sturhan, Germany	MS, IEF, RFLP
H. pratensis sp. n.	Missunde near Schleswig, Germany	D. Sturhan, Germany	MS, IEF, RFLP
H. pratensis sp. n.	Benstaben near Bad Oldesloe, Germany	D. Sturhan, Germany	MS
H. pratensis sp. n.	Otterndorf near Cuxhaven, Germany	D. Sturhan, Germany	MS
H. pratensis sp. n.	Freiburg/Elbe, Germany	D. Sturhan, Germany	MS, IEF, RFLP
H. pratensis sp. n.	Stade, Germany	D. Sturhan, Germany	MS
H. pratensis sp. n.	Cranz near Hamburg, Germany	D. Sturhan, Germany	MS
H. pratensis sp. n.	Gandesbergen/Weser, Germany	D. Sturhan, Germany	IEF
H. pratensis sp. n.	Putilovo, Leningrad region, Russia	S. Subbotin, Russia	MS, IEF, RFLP
H. pratensis sp. n.	Kurilovo, Moscow region, Russia	S. Subbotin, Russia	MS
H. pratensis sp. n.	Assamalla, Estonia	E. Krall, Estonia	MS, IEF
H. arenaria	The Netherlands	D. Sturhan, Germany	IEF
H. aucklandica	One Tree Hill, Auckland, New Zealand	W.M. Wouts, New Zealand	IEF, RFLP
H. avenae	Taaken, Germany	H.J. Rumpenhorst, Germany	IEF, RFLP
H. avenae	Grafenreuth, Germany	H.J. Rumpenhorst, Germany	IEF
H. filipjevi	Baimak, Russia	V. Balakhnina, Russia	IEF
H. filipjevi	Pushkin, Russia	S. Subbotin, Russia	IEF
H. iri	Gandesbergen/Weser, Germany	D. Sturhan, Germany	IEF
H. mani	Hamminkeln, Germany	D. Sturhan, Germany	IEF

[1]MS – morphological and/or morphometric study; IEF – isoelectric focusing of proteins; RFLP – restriction fragment length polymorphism of rDNA.

aucklandica, H. arenaria, H. iri and *H. mani* were used for this study (Table 1). Prior to the study, the nematodes were in general maintained in pots, planted with grasses, in the greenhouse. The nematodes were isolated from soil by the sieving-decanting or the centrifugation-flotation methods. Eggs and second-stage juveniles were released from squashed cysts. Some air-dried cysts were stored at room temperature in plastic specimen tubes for biochemical and molecular studies.

Light microscopy. The nematodes were killed and fixed in hot TAF and processed to glycerine by a modified slow method. The specimens mounted on permanent slides were examined, measured and photographed with Zeiss and Leica light microscopes equipped with Nomarski optics. All measurements are presented in micrometers (µm) as the mean, the standard error of the mean and the range in parentheses.

Isoelectric focusing (IEF). For protein extraction 4-5 cysts containing eggs and second-stage juveniles were crushed and homogenized in 15 µl extraction buffer (0.2 mM acetic acid with 1% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulphonate, CHAPS) in a micro-homogenizer. Water soluble proteins from the homogenates were separated by isoelectric focusing in thin layer (300 µm) polyacrylamide gels (5% polymer, 3% crosslinker and 5% Servalyt; SERVA, Heidelberg) with a pH-range from 3-10. 10 µl homogenate were placed in each slot of the applicator strip. The Desatronic 2000/300 power supply (Desaga) was set to 2040 V_{max}, 6 mA_{max}, 8 W_{max} and 3000 Vh at 5 °C. After about 3 hours the gel was fixed and stained with silver following a modified procedure of Ohms and Heinicke (1983).

rDNA-RFLP. Methods of DNA extraction from one or two cysts as described by Subbotin *et al.* (1999) were used for this molecular study. After centrifugation (1 min; 16 000*g*) 4 µl of the DNA suspension was added to the PCR reaction mixture containing 5 µl 10X *Taq* incubation buffer, 10 µl 5X Q-solution, 200 µM of each of the dNTPs (*Taq* PCR Core Kit, Qiagen, Germany), 1.5 µM of each primer (synthesised by Life Technologies, Merelbeke, Belgium), 0.8U *Taq* Polymerase (*Taq* PCR Core Kit, Qiagen, Germany) and double distilled water to a final volume of 50 µl. Forward primer TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and reverse primer AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) were used in the PCR reaction. The DNA-amplification profile carried out in a GeneE (New Brunswick Scientific,

Wezembeek-Oppem, Belgium) DNA thermal cycler consisted of 4 minutes 94 °C; 35 cycles of 1 min 94 °C, 1.5 min 55 °C, and 2 min 72 °C; and 10 min 72 °C. After DNA amplification, 5 µl product was run on a 1% agarose gel. The remainder was stored at -20 °C. Seven microliters of each PCR product were digested with one of the following restriction enzymes: *Alu*I, *Cfo*I, *Hin*fI, *Pst*I, *Rsa*I, *Taq*I, *Tru*9I (Promega Corporation, USA) and *Ita*I (Boehringer Mannheim, Belgium) in the buffer stipulated by the manufacturers. The digested DNA was loaded on a 1.5% agarose gel, separated by electrophoresis, stained with ethidium bromide, visualised on a 2011 Macrovue UV transilluminator, and photographed with a Polaroid MP4+ Instant Camera System. Procedures for obtaining PCR amplified products and endonuclease digestion of these products were repeated several times to verify the results.

RESULTS

Heterodera pratensis sp. n. (Figs. 1-3, Tables 2 & 3)

Holotype cyst: L (excluding neck) = 660 µm; width = 510 µm; length/width ratio = 1.3; L (including neck) = 760 µm; vulval slit length = 10 µm; vulval bridge width = 5.9 µm; semifenestral widths = 26, 27 µm; semifenestral lengths = 20, 23 µm.

Cysts. See Table 2.

Paratype females (n=14): L (including neck) = 557±12 (450-695) µm; width = 343±15 (180-470) µm; length/width ratio = 1.7±0.1 (1.4-2.5); length of neck = 127±2.2 (105-145) µm; length of stylet = 29±0.6 (25.0-30.5) µm; distance of opening of dorsal oesophageal gland from stylet base (n=7) = 4.3±0.2 (4.0-5.2) µm; distance of median bulb from anterior end (n=8) = 97±2.4 (88-103) µm; length of median bulb = 31±0.6 (27-34) µm; width of median bulb = 32±0.6 (27-35) µm; distance of excretory pore from anterior end (n=8) = 133±5 (110-150) µm.

Paratype males (n=19): L = 1170±18 (1050-1330) µm; width = 29±0.2 (28-30) µm; height of lip region = 6.5±0.1 (5.7-7.2) µm; width of lip region = 11±0.1 (10-12) µm; length of stylet = 29±0.2 (26-31) µm; height of stylet knobs = 2.7±0.03 (2.4-2.8) µm; width of stylet base = 5.4±0.1 (4.8-5.6) µm; distance of opening of dorsal oesophageal gland from stylet base = 4.6±0.1 (4.0-5.2) µm; distance of median bulb from anterior end = 99±1.3 (90-110) µm; distance of hemizonid from anterior end = 142±2.3 (125-162) µm; distance of excretory pore from anterior end = 150±2.5 (126-176) µm; distance of posterior end of oesophageal glands from anterior end = 457±9 (370-510) µm; length of genital tract = 595±27 (390-740) µm; T = 51±2 (31-63); length of spicules = 40±0.4 (38-44) µm; length of gubernaculum = 13±0.2 (12-14) µm; a = 40±0.6 (37-47); b' (n=15) = 2.6±0.1 (2.1-3.2).

Second-stage juveniles. See Table 3.

Paratype eggs (n=40): L = 120±1 (110-130) µm; width = 44±0.2 (43-47) µm; length/width ratio = 2.7±0.02 (2.5-2.9).

Cysts. Lemon-shaped with low vulval cone, generally elongate but occasionally almost spherical, filled with about 300 eggs, subcrystalline layer distinct; egg sac not observed. Cuticle with irregular zig-zag-pattern; colour varying from pale to medium brown, becoming increasingly darker with age but mostly remaining more or less transparent (eggs generally visible through cyst wall). Vulval cone bifenestrate, fenestrae oval to almost circular, in older cysts generally flattened on side of vulval bridge and becoming horse-shoe shaped; longest axis of fenestrae parallel to vulval slit. Vulval bridge narrow, often broken in older cysts. Vulval slit short, about half as long as width of fenestrae. Bullae numerous, distinct, mostly globular but variable in shape, darker than cyst cuticle, situated mostly in a circle at different levels in vulva cone (up to 130 µm below vulva), but in general not immediately below fenestrae, often more concentrated under the distal ends of both fenestrae; underbridge absent. Anus 22-38 µm away from ventral fenestra, with no peculiar cuticular structures around it.

Females. Body ovoid with low vulval cone. Cuticle colourless or yellow to pale brown; thick white subcrystalline layer present (removed in specimens studied). Remnants of the J_4 cuticle usually still attached to the anterior end. Cuticle with irregular rugose line pattern except on the anterior part of the neck. Cephalic region with anterior lip annule 5-7 µm wide and labial disc 3-4 µm wide. Stylet knobs rounded, sloping, base of stylet 4.1-4.5 µm in diameter. Median bulb globular or slightly wider than long, valve plates 8.5-11 µm long. No egg sac observed.

Males. General morphology as typical for the genus. Body of heat-killed specimens C-shaped, twisted about longitudinal axis. Lip region hemi-

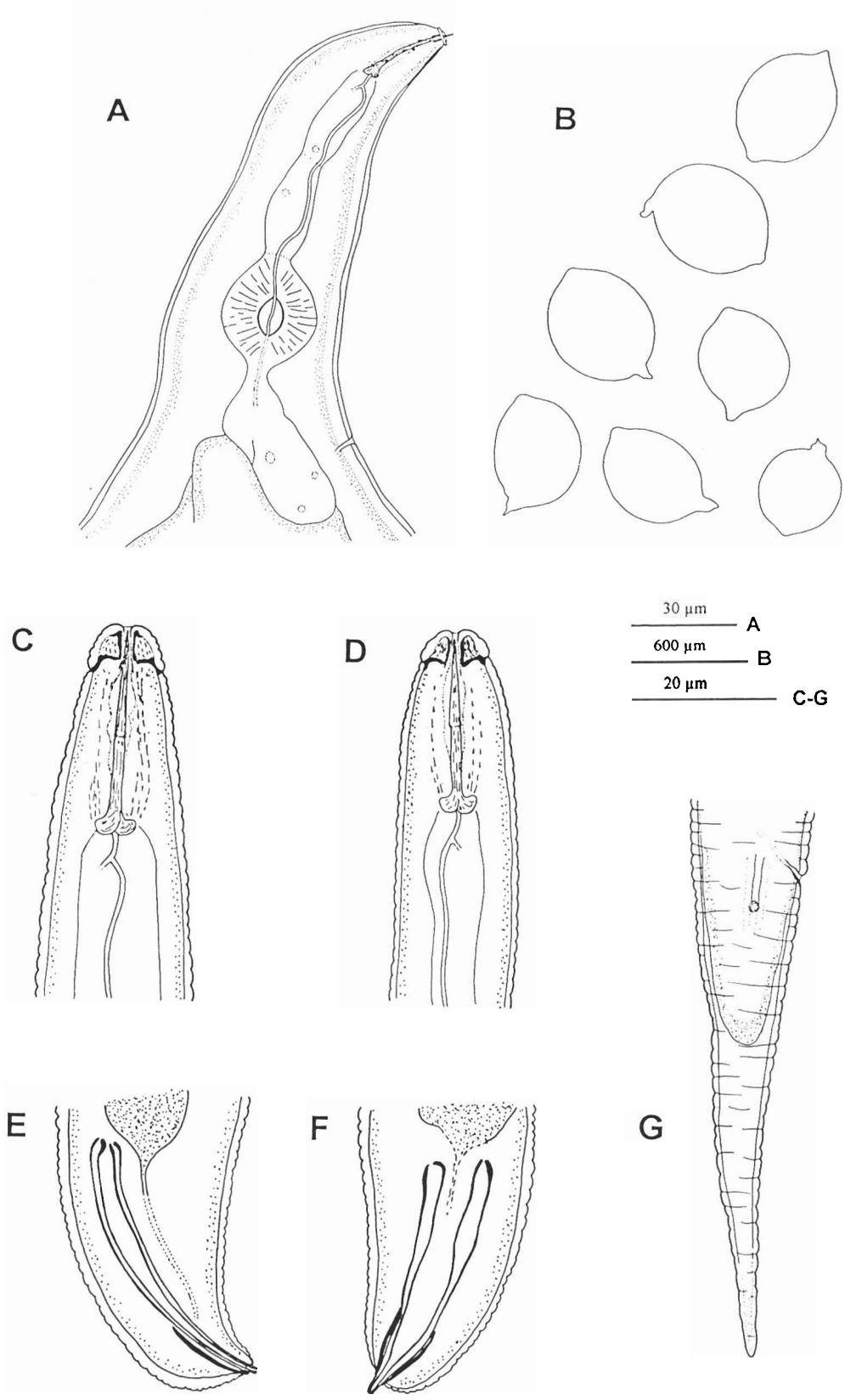

Fig. 1. *Heterodera pratensis* sp. n. A: Female, anterior end; B: Cysts; C: Male, head; E, F: Male, posterior end, lateral and dorsoventral view; D: Second-stage juvenile, head; G: Second-stage juvenile, tail.

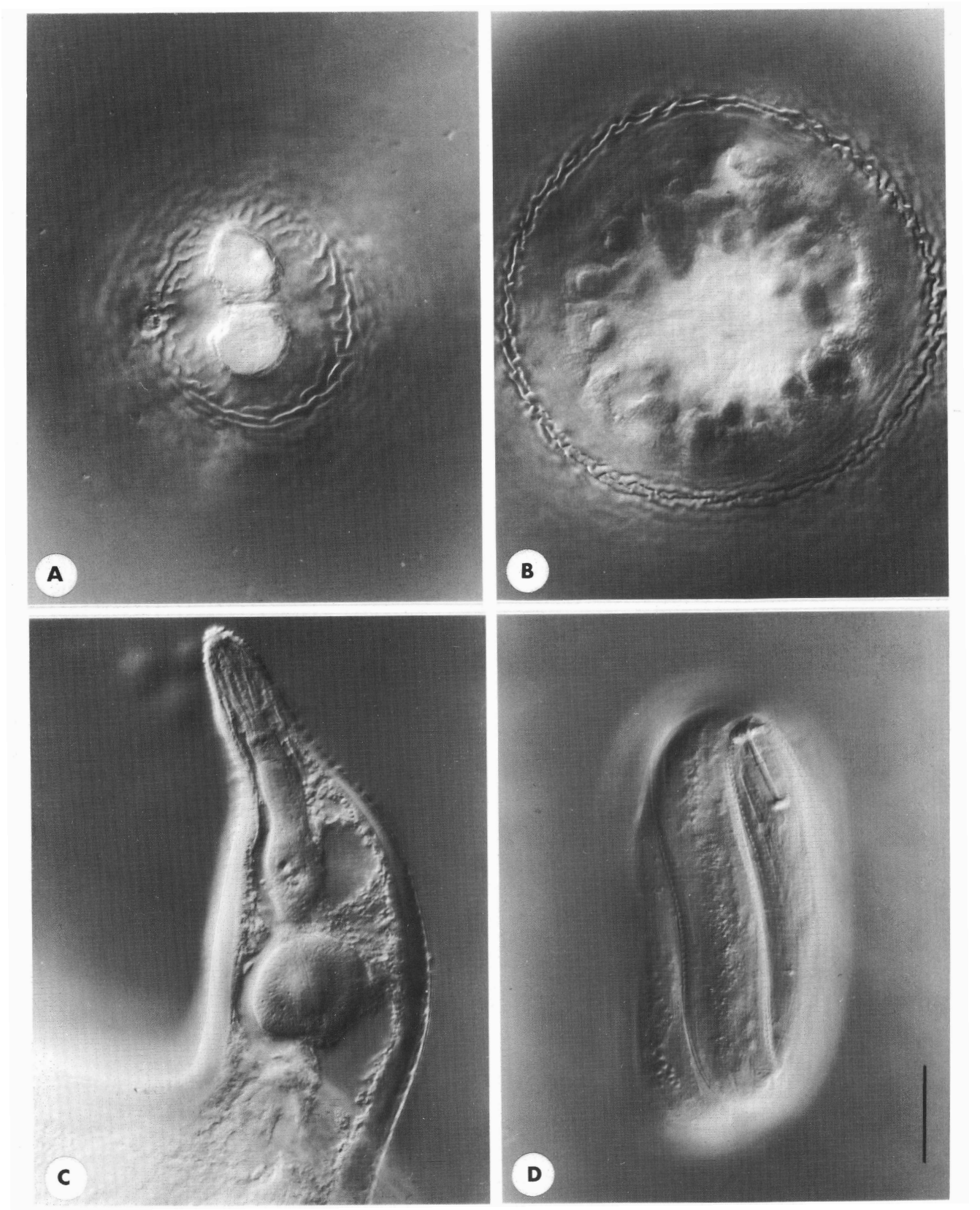

Fig. 2. *Heterodera pratensis* sp. n. A: Fenestration in vulval cone; B: Bullae in vulval cone; C: Female, anterior end; D: Second-stage juvenile, folded within egg (Scale bar: A, B - 35 µm; C, D - 25 µm).

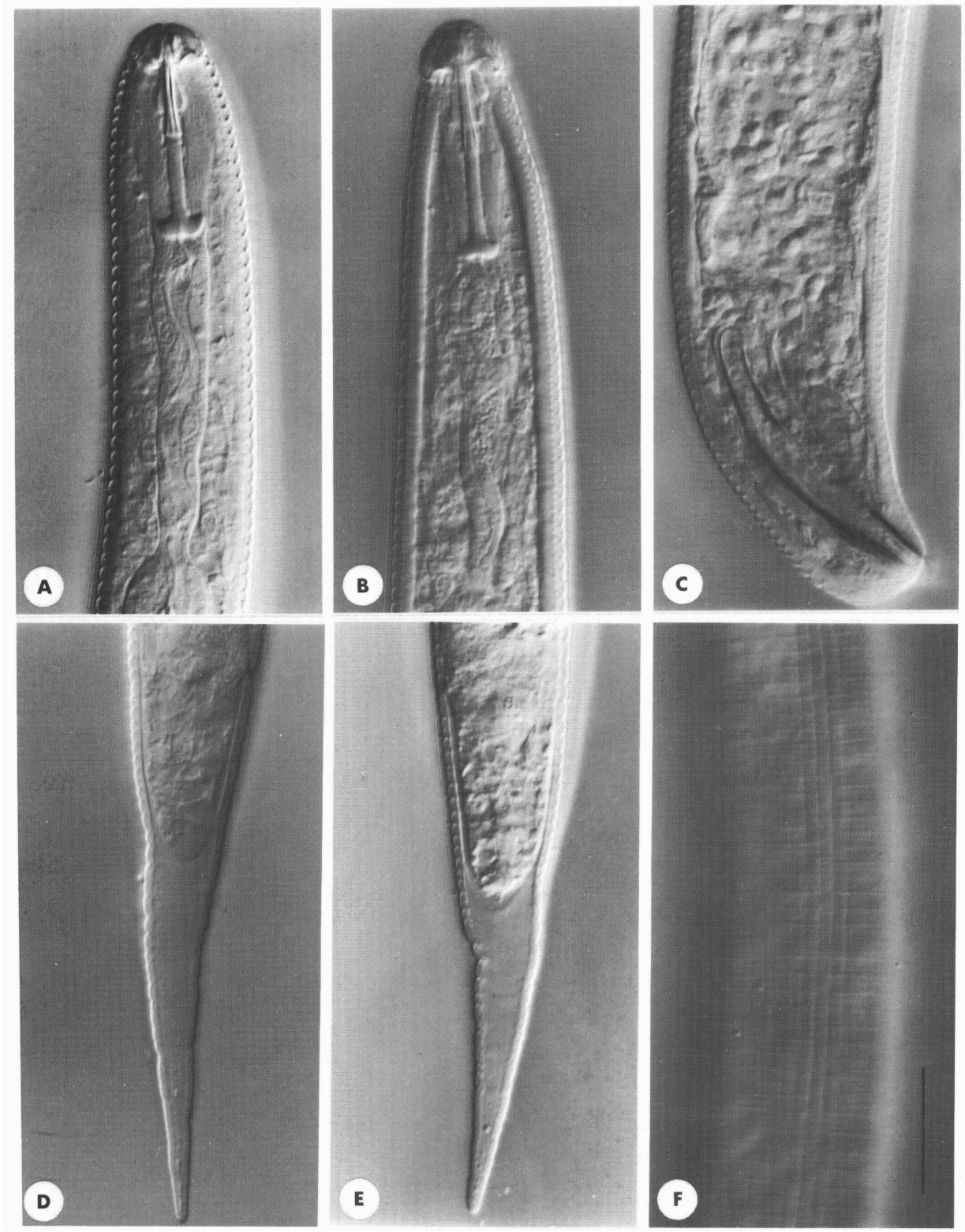

Fig. 3. *Heterodera pratensis* sp. n. A: Second-stage juvenile, anterior end; B: Male, anterior end; C: Male, posterior end; D, E: Second-stage juvenile, tails; F: Second-stage juvenile, lateral field in mid-body region (Scale bar - 15 µm).

spherically rounded, almost two times wider than high, slightly set off, with 4-5 (exceptionally 6) discontinuous annules and a faint labial disc. Cephalic framework heavily sclerotized. Anterior cephalids two to three annules and posterior cephalids eight to ten annules behind lip region. Stylet strong, knobs rather small, rounded and slightly indented or almost flat anteriorly, sloping posteriorly. Median bulb oval, occupying about half of the body diameter, valves about 4 µm long. Oesophageal glands mostly rather slender and occupying about one fourth to one fifth of the body diameter. Hemizonid 1.5-2 cuticle annules long and 0-4 (occasionally more) annules anterior to excretory pore. Lateral field with four lines, the outer bands irregulary areolated over most of the body. Cuticle annules 2 µm wide at mid-body. Spicules arcuate, with tridentate terminus. Gubernaculum almost linear. Tail short and rounded, often appearing smooth at the end. Phasmid opening not observed.

Second-stage juveniles. Body of heat-killed specimens curved ventrad. Lip region slightly set off, flatly rounded (2.5 times wider than high), with 2 (-3) indistinct annules and a wide labial disc. Cephalic framework strongly sclerotized. Stylet strong, cone slightly shorter than the posterior part. Stylet knobs slightly concave or almost flat anteriorly, sloping slightly posteriorly. Stylet base 5.2-6.3 (5.8) µm wide and 2.5-3.1 (2.9) µm high. Almost no thickening of the cuticle posterior to lip region. Median bulb oval, generally occupying slightly more than 50% of the corresponding body width, valves 3.2-3.8 µm long. Oesophageal glands well developed, extending to 42 (37-48)% of body length from anterior end. Hemizonid 0-3 annules anterior to excretory pore, 1.5-2 body annules long; hemizonion 5-9 annules behind hemizonid, usually indistinct. Genital primordium situated at 56 (51-61)% of body length behind anterior end, with four distinct nucleate cells, 16 (13-19) µm long and 9.3 (7.5-10.5) µm wide. Lateral field with four lines, but outer lines mostly indistinct and outer bands completely areolated so that only the two inner lines are visible. Cuticle annules 1.8-2.0 µm wide at mid-body. Phasmids prominent, 2-4 body annules posterior to anus, with lens-like structure in cuticle. Tail gradually tapering to a narrow, rounded terminus, 7.5-10.7 µm wide at beginning of hyaline part and 1.6-2.5 µm wide at 3 µm from terminus. Hyaline portion occupying more than 60% of tail length, irregularly annulated; limit of pseudocoelom in tail rounded and centrally situated. Hyaline tail portion 3.5-5.6 (4.5) times longer than its maximum width.

Eggs. Cylindrical with rounded ends. Egg shell without visible markings. Juvenile folded four times within the egg.

Morphological characters of other populations. Populations from Östergaard, Germany, Putilovo and Kurilovo, Russia, were included in the morphological studies. Morphometrics of cysts and of second-stage juveniles are given on Tables 2 and 3. In other morphological characters these populations closely agree with those described for the type population.

Isoelectric focusing analysis. Samples of 9 German and 2 Russian populations and 1 Estonian population of *H. pratensis* sp. n. produced consistent banding patterns on IEF gels (pH range 3-7 and 3-10), which are distinctly different from those of the six other species of the *H. avenae* complex. As observed for other *Heterodera* species, there is some insignificant variation in protein patterns, mainly in intensity of bands, but the basic (typical) pattern is obvious and identical, so that all the populations studied could be considered members of the new species. The typical protein patterns for *H. pratensis* sp. n. and those of *H. arenaria, H. aucklandica, H. avenae, H. filipjevi, H. iri and H. mani* are shown in Figs. 4 & 5.

rDNA-RFLP analysis. The amplification of the ITS regions of each population of *H. pratensis* sp. n., *H. avenae* and *H. aucklandica* gave one fragment of approximately 1.06 kb. PCR products were not obtained in the negative control lacking a DNA template. Eight enzymes produced no intraspecific variation in restriction patterns of PCR products of the Lindhöft and the Putilovo populations and two enzymes, *Hin*fI and *Tru*9I, produced no variation between any of the studied populations of *H. pratensis* (data not shown). The RFLP patterns obtained with *Alu*I, *Hin*fI, *Ita*I, *Rsa*I and *Tru*9I clearly distinguished *H. pratensis* sp. n. from *H. avenae* and with *Cfo*I, *Hin*fI, *Ita*I, *Taq*I and *Tru*9I from *H. aucklandica*. The enzymes *Alu*I, *Cfo*I, *Rsa*I, *Taq*I distinguished *H. avenae* and *H. aucklandica* from each other (Fig. 6).

Type locality and hosts. Cysts with viable contents were collected 10 October 1986 from sandy soil (pH 7.8) from a pasture near the coast of the Baltic Sea at Lindhöft, east of Eckernförde, Schleswig-Holstein, Germany (UTM NF63).

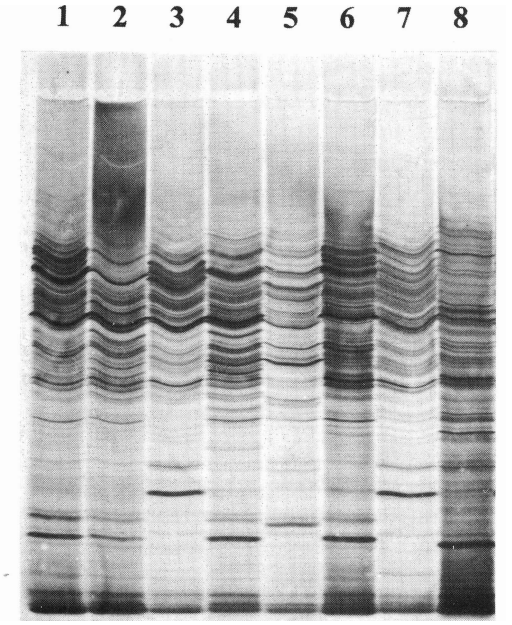

Fig. 4. IEF protein patterns of species of the *H. avenae* complex (Servalyt 3-7). Lanes: 1+6: *H. pratensis* (Östergaard, Germany); 2: *H. pratensis* (Assamalla, Estonia); 3+7: *H. avenae* (Taaken, Germany); 4: *H. pratensis* (Gandesbergen, Germany); 5: *H. iri* (Gandesbergen, Germany); 8: *H. filipjevi* (Pushkin, Russia).

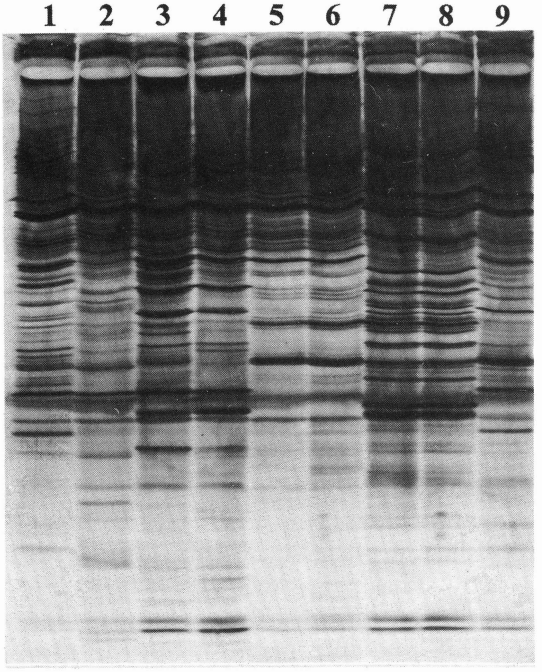

Fig. 5. IEF protein patterns of species of the *H. avenae* complex (Servalyt 3-10). Lanes: 1: *H. aucklandica* (Auckland, New Zealand); 2: *H. arenaria* (The Netherlands); 3: *H. pratensis* (Lindhöft, Germany); 4: *H. pratensis* (Östergaard, Germany); 5: *H. avenae* (Grafenreuth, Germany); 6: *H. avenae* (Taaken, Germany); 7: *H. filipjevi* (Baimak, Russia); 8: *H. filipjevi* (Pushkin, Russia); 9: *H. mani* (Hamminkeln, Germany).

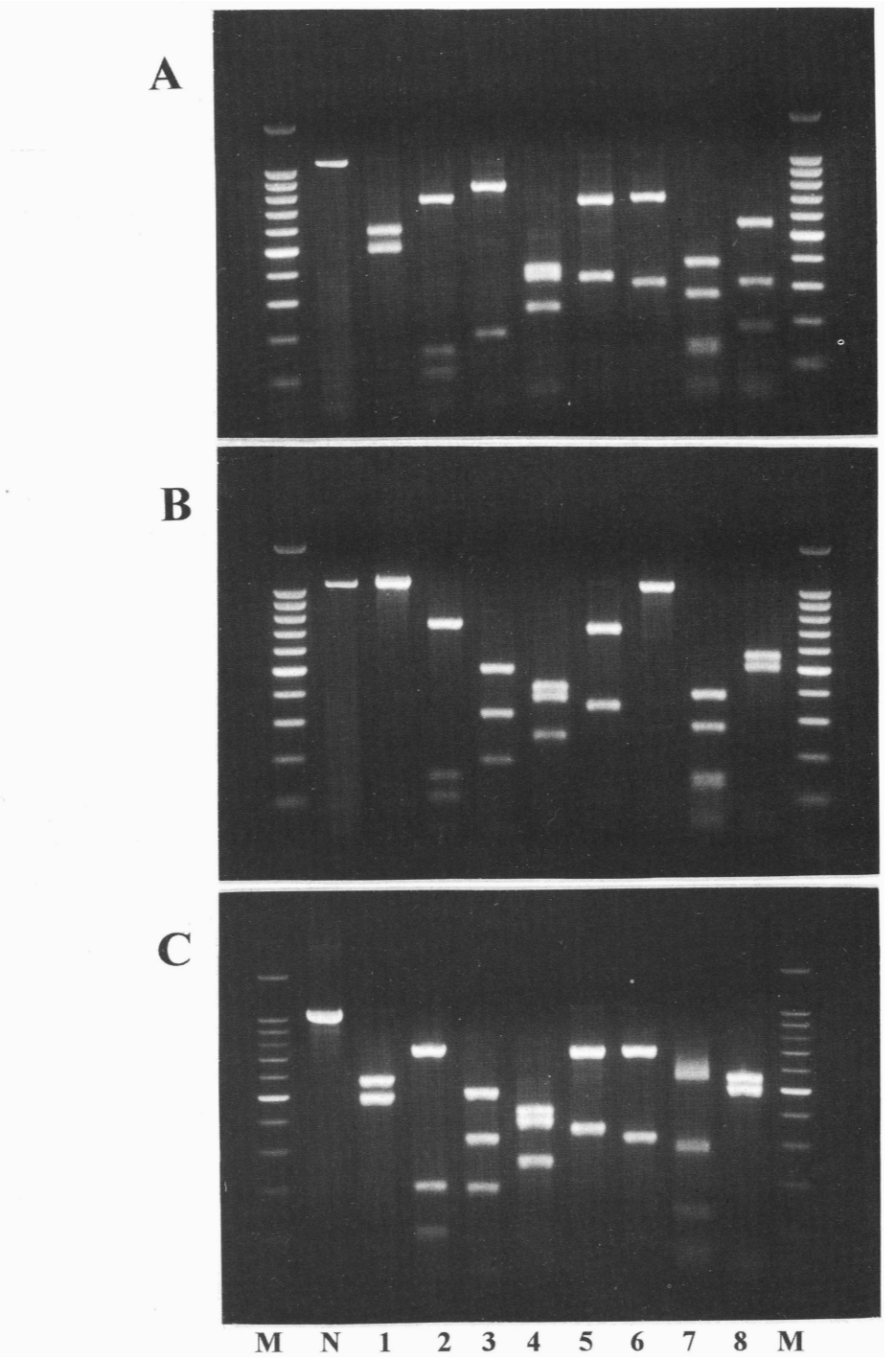

Fig. 6. Restriction fragments of amplified ITS regions of species of the *H. avenae* complex. A: *H. pratensis* sp. n. (type population); B: *H. avenae*; C: *H. aucklandica*. (Codes: Lanes M - 100 bp DNA ladder, N - unrestricted PCR product, 1 - *Alu*I, 2 - *Cfo*I, 3 - *Hin*fI, 4 - *Ita*I; 5 - *Pst*I; 6 - *Rsa*I; 7 - *Taq*I, 8 - *Tru*9I).

Subsequently the sampling material has been multiplied in the greenhouse on various grasses (*Poa annua, Festuca pratensis, Dactylis glomerata, Lolium perenne, Agrostis stolonifera, Cynosurus cristatus*). Attempts to rear the species on barley (var. 'City') and wheat (var. 'Tinos') failed.

Table 2. Morphometrics (in µm) of cysts and vulval areas of *Heterodera pratensis* sp. n.

Population	Lindhöft (Germany) type population	Östergaard (Germany)	Putilovo (Russia)	Kurilovo (Russia)
Cysts				
n	21	–	30	13
Length excl. neck	675±20 (530-800)	–	760±19 (480-960)	650±26 (510-825)
Width	570±17 (400-685)	–	565±15 (400-705)	490±15 (400-585)
Length/width	1.2±0.02 (1.1-1.4)	–	1.4±0.02 (1.2-1.6)	1.3±0.03 (1.2-1.6)
Vulval areas				
n	13	11	13	11
Vulval slit length	11.2±0.4 (8.9-12.9)	10.9±0.3 (9.9-13)	11±0.4 (9-13)	8.6±0.3 (7.5-11)
Vulval bridge width	6.9±0.3 (5.0-8.9)	6.6±0.4 (5.0–8.9)	7.0±0.5 (5.0-10)	9.9±0.3 (8.8–12)
Distance semifenestra – vulval slit	3.5±0.2 (2.0-5.0)	3.2±0.3 (2.0-5.0)	–	–
Mean semifenestral width	24±0.5 (20-28)	24±0.7 (20-28)	23±0.8 (18-28)	25 ± 0.4 (23-28)
Mean semifenestral length	20±0.4 (18-24)	20±0.7 (18-24)	–	–

Table 3. Morphometrics (in µm) of second-stage juveniles of *Heterodera pratensis* sp. n.

Population	Lindhöft (Germany) type population	Östergaard (Germany)	Putilovo (Russia)	Kurilovo (Russia)
n	30	33	20	20
Body length (L)	535±4 (490-575)	560±5 (500-600)	505±5 (470-530)	520±5 (465-565)
Oesophagus length	227±3 (205-250)	250±4 (205-280)	–	–
Anterior end to valve of median bulb (MB)	75±0.8 (66-88)	79±0.8 (72-91)	76±0.8(68-81)	77±0.9 (66-82)
Anterior end to excretory pore	104±0.7 (96-111)	102±0.8 (94-112)	105±1.2 (94-113)	108±1.0 (100-114)
Tail length	67±0.6 (60-73)	60±0.7 (51-69)	65±0.9 (59-70)	69±1.1 (53-77)
Hyaline part of tail length	42±0.6 (35-47)	38±0.7 (32-47)	42±0.7 (39-45)	45±0.8 (39-53)
Body width at mid-body	21±0.1 (20–23)	20±0.1 (20-23)	21±0.2 (20-22)	20±0.2 (19-21)
Body width at level of anus	15±0.1 (14-16)	15±0.1 (14-16)	15±0.1 (14-15)	14±0.1 (14-15)
Stylet length	24±0.1 (24-25)	25±0.2 (23-27)	25±0.2 (22-26)	24±0.2 (23-26)
Lip region width	9.8±0.1 (9.4-10)	–	9.5±0.1 (9.7-10.2)	9.2±0.02 (9.2-9.4)
Lip region height	3.9±0.04 (3.8-4.4)	–	4.1±0.1 (4.0-4.3)	4.1±0.1 (3.7-4.8)
DGO	5.1±0.1 (4.4-6.3)	–	6.0±0.2 (5.7-7.1)	6.1±0.2 (5.1-7.1)
a	25±0.2 (22-27)	28±0.3 (23-31)	25±0.3 (22-27)	27±0.4 (24-30)
b'	2.4±0.03 (2.1-2.7)	2.2±0.03 (2.0-2.8)	–	–
c	8.0±0.1 (7.5-8.7)	9.3±0.1 (8.3-10)	7.8±0.1 (7.3-8.5)	7.6±0.1 (6.9-8.8)
c'	4.3±0.1 (3.8-4.7)	4.1±0.1 (3.5-4.7)	4.3±0.1 (4.1-4.9)	4.8±0.1 (4.4-5.4)
L/MB	7.2±0.1 (6.4-7.9)	7.0±0.1 (6.4-7.9)	6.7±0.1 (6.2-7.2)	6.8±0.1 (6.3-7.9)

Other localities. Additional populations identified as *H. pratensis* sp. n. were from the following localities in northern Germany: Östergaard near Gelting, Schleswig-Holstein, grassy vegetation at coast, sandy soil, pH 7.8. Missunde near Schleswig, grassy bank of Schlei river, peaty sand, pH 6.8. Benstaben near Bad Oldesloe, Schleswig-Holstein, permanent pasture, loamy sand. Otterndorf near Cuxhaven, permanent pasture, loamy fine-sand, pH 7.3. Freiburg/Elbe, meadow, loamy sand, pH 7.9. Stade, grassy vegetation at Elbe river, sandy soil, pH 8.1. Cranz near Hamburg, grassy vegetation at Elbe river, loamy sand, pH 8.0. Gandesbergen near Nienburg, grassy vegetation along Weser river, loamy sand, pH 6.7. In Russia *H. pratensis* sp. n. has been collected from the roots of false wheat, *Elytrigia repens* growing near an oat field, Putilovo, Leningrad region, sandy soil, and from grassland, basin of Mocha river, Kurilovo, Moscow region, loamy soil. A population from a barley field at Assamalla, Estonia, collected by E. Krall, was also identified

as *H. pratensis* sp. n.

Type material. Holotype cyst (cone top and second-stage juveniles ex holotype cyst), paratype cysts, females, males, second-stage juveniles and eggs deposited in the German Nematode Collection, Biologische Bundesanstalt, Münster, Germany. Additional paratypes are distributed as follows: Institute of Parasitology, Russian Academy of Sciences, Moscow; Zoological Institute, Russian Academy of Sciences, St. Petersburg; Rothamsted Experimental Station, England; U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland; Department of Nematology, University of California, Riverside; National Nematode Collection of New Zealand, Landcare Research, Auckland.

Differential diagnosis. In characters of cysts (lemon-shaped, short vulval slit, vulva region bifenestrate) and second-stage juveniles (phasmid with lens-like structure) *H. pratensis* sp. n. is similar to species in the *H. avenae* and *H. latipons* complexes. The species *H. latipons* and *H. hordecalis* are well distinguished by a strong underbridge in the vulva cone, absence of bullae (rarely developed), wide vulval bridge (>16 µm) and shorter second-stage juveniles (mean <500 µm). Among the species in the *H. avenae* complex (*s. str.*), *H. pratensis* sp. n. differs from *H. filipjevi*, *H. iri* and *H. mani* in the absence of an underbridge, in addition from *H. iri* in shorter juvenile tail (50-77 *vs* 77-103 µm), from *H. mani* in the shape of the stylet knobs of the juveniles (almost flat *vs* deeply concave) and from *H. filipjevi* by more slender and sharper tail terminus and longer hyaline part of tail (32-53 *vs* 28-45 µm).

Heterodera pratensis sp. n. most closely resembles *H. avenae*, *H. aucklandica* and *H. arenaria*, species without an underbridge in the vulval cone. It is distinguished from *H. arenaria* by the smaller cysts (averages: 650-760 *vs* 693-906 µm), eggs (120 × 44 *vs* 135-154 × 51-56 µm), second-stage juveniles (505-560 *vs* 587-663 µm), juvenile stylet (24-25 *vs* 28-29 µm), tail (60-69 *vs* 74-82 µm) and hyaline tail part (38-45 *vs* 48-53 µm). *Heterodera pratensis* sp. n. resembles *H. aucklandica* in most characters and dimensions; in second-stage juveniles the length of tail and hyaline tail end is slightly less (averages: 60-69 *vs* 75 µm and 38-45 *vs* 47 µm, respectively) and the tail end at 3 µm from terminus is wider (1.6-2.5 *vs* 1.2-1.5 µm); in the juveniles mostly only the two inner lines in the lateral field are distinct in *H. pratensis* sp. n., but four lines in *H. aucklandica*. In particular, *H. pratensis* sp. n. is very similar to *H. avenae*. The cysts are generally lighter brown and mostly more rounded (ratio L/W = 1.2-1.4 *vs* 1.5), the bullae are at different levels in the vulval cone and in general not immediately below the fenestrae. The spicules are longer in *H. pratensis* sp. n. (38-44 *vs* 33-38 µm) and their terminus is tridentate (bifid in *H. avenae*). The second-stage juveniles are distinguished from European *H. avenae* populations by the shorter stylet (average: 24-25 *vs* 26-28 µm) and slightly shorter hyaline part of tail (average: 38-45 *vs* 44-48 µm). [(Morphometrics of previously described species in part from Subbotin *et al.*, (1999)].

DISCUSSION

The new species *H. pratensis* sp. n. appears to be widely distributed in Europe. Because of its similarity, in particular, with *H. avenae* it has certainly often been "overlooked" and been identified as the common cereal cyst-nematode. Its possible role as a cereal parasite and its occurrence and distribution in agricultural soils has still to be studied.

The description of *H. pratensis* sp. n. has increased the number of species in the *H. avenae* complex within the *H. avenae* group to a total of seven; with the exception of *H. aucklandica* they are all known from Europe. There is evidence that at least one additional undescribed species is present in northern Europe; this also mainly occurs in grassland (Subbotin & Sturhan, unpubl.).

With the growing number of described *Heterodera* species distinction and reliable identification of species based on morphological characters alone is becoming increasingly difficult. Application of biochemical methods has provided new possibilities of species distinction, and new species are sometimes "detected" by such techniques. In the case of *H. pratensis* sp. n. isoelectric focusing of protein extracts supported evidence based on morphological observations that a "new" species was present. Inclusion of several "grassland populations" in the consecutive comparative studies provided consistent banding pattern for this group of populations, different from that of related species (Rumpenhorst, 1994; Sturhan & Rumpenhorst, 1996; Subbotin *et al.*, 1996). Recently developed DNA-based diagnostics of nematodes, especially based on the comparative analysis of the ITS1-5.8S-ITS2 region of ribosomal DNA, provide another attractive solution to problems associated with identification of many nematode species. The PCR-rDNA-RFLP method has already been suc-

cessfully used for the differentiation of other species of the *H. avenae* complex (Bekal *et al.*, 1997; Subbotin *et al.*, 1999). In our study of *H. pratensis* sp. n. five restriction enzymes produced rDNA-RFLPs that clearly distinguished this species from the closely related *H. avenae* and *H. aucklandica*.

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Gäbler C., Sturhan D., Subbotin S.A., Rumpenhorst H.J. *Heterodera pratensis* sp. n. - новая цистообразующая нематода из группы *H. avenae* (Nematoda: Heteroderidae).

Резюме. Приводится описание нового вида цистообразующих нематод из группы *Heterodera avenae* - *Heterodera pratensis* sp. n., обитающего на травянистых злаковых растениях. Новый вид широко распространен в луговых экосистемах Северной Германии и был также найден в России и Эстонии. *Heterodera pratensis* sp. n. надежно дифференцируется от других видов, относящихся к группе *H. avenae* методами изоэлектрического фокусирования белков и рестрикционного анализа рибосомальной ДНК (DNA-RFLPs). По своим морфологическим особенностям - отсутствию нижнего моста - новый вид наиболее близок к *H. avenae, H. aucklandica* и *H. arenaria*. Новый вид отличается от этих видов по форме и цвету цист, расположению булле и длине тела, гиалиновой части и хвоста личинок. Представлены диагностические спектры rDNA-RFLP для *H. pratensis* sp. n., *H. avenae* и *H. aucklandica*.