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Morphology of cysts and second stage juveniles of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 from Norway

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Summary.- Morphological characters and morphometrics of cysts and second stage juveniles of two *Heterodera filipjevi* populations from Norway were studied and compared with published data on *H. filipjevi*. The results of the morphometric analysis extends the range previously reported for cyst characters. The study also provides new information on morphology of the female head, the cyst cuticle, the vulval cone and the lateral field of the second stage juvenile. The complementary data will facilitate the correct identification of *H. filipjevi* by morphology.

Keywords: *Heterodera filipjevi*, morphology, morphometrics, Norway.

Resumen.- Se estudian rasgos morfológicos y morfométricos de quistes y formas juveniles de segundo estadio de dos poblaciones de *Heterodera filipjevi* recolectadas en Noruega, y se comparan con los datos ya publicados sobre esta especie. Los resultados obtenidos del análisis morfométrico extienden el rango conocido de los caracteres en el caso de los quistes. Así mismo, se aporta información inédita sobre la morfología de la región labial de la hembra, la cutícula del quiste, el cono vulvar y el campo lateral del segundo estadio juvenil. La nueva información que ahora se proporciona facilitará la correcta identificación de *H. filipjevi* a partir de su morfología.

Palabras clave: *Heterodera filipjevi*, morfología, morfometría, Noruega.

INTRODUCTION

There are a number of species within the genus *Heterodera* which reproduce on graminaceous hosts in Europe (Williams & Siddiqi, 1972; Ritter, 1982; Rivoal & Cook, 1993; Evans & Rowe, 1998). *Heterodera* species in the *Heterodera avenae* group are distinguished from each other by small differences in detail rather than in gross morphology.

In Scandinavia *H. filipjevi* has previously been known as the "Gotland strain of *H. avenae*" (Andersson, 1973; Videgård, 1973). Studies have confirmed that the Gotland strain belongs to the species *H. filipjevi* (Ferris *et al.*, 1989; Valdeolivas & Romero, 1990; Subbotin *et al.*, 1996; Andrés *et al.*, 2001). In Norway the occurrence of *H. filipjevi* was recorded recently (Holgado *et al.*, 2004a) and this species seems to be an important pest of cereals in both Sweden and Norway (Andersson, 1973; Ireholm 1990, 1994; Holgado *et al.*, 2004a).

With the growing numbers of descriptions of additional species in the *H. avenae*- complex there is an urgent need for collecting more information on descriptive characters for *H. filipjevi*. The objectives of this study are to compare the morphology and morphometrics of two Norwegian population of *H. filipjevi* with published information for the species, and to provide complementary information on its morphology.

TABLE I. Measurements (in µm) of cysts and eggs of *Heterodera filipjevi* from Norway, and measurements reported by Madzhidov (1981) and Brzeski (1998).

Character	Sándefjord population 184		Sandefjord population 185		*H. filipjevi* (Madzhidov, 1981)		*H. filipjevi* (Brzeski, 1998)	
	n	mean (range)	n	mean (range)	n	mean (range)	n	mean (range)
Cyst								
Body length excl. neck	100	680 (458 – 874)	100	692 (455–869)	25	690 (490 – 830)	-	(490 – 900)
width	100	518 (306 – 747)	100	509 (253-657)	25	490 (340 – 620)	-	(360 – 580)
Length/width	100	1.3 (1.0 – 1.6)	100	1.4 (1.0 –1.8)	25	1.4 (1.1 – 1.6)	-	(1.3 – 1.8)
Vulval slit length	40	8.7 (7.6 – 10.8)	35	7.9 (6.0 – 9.3)	25	7.3 (6.3 – 8.4)	-	(6 – 12)
Fenestra length	40	48.1 (38.4 – 58.4)	35	47.8 (38.6 – 56.2)	25	51.5 (41 – 64)	-	(40 – 56)
Semifenestral width	40	25.3 (20.8-30.8)	35	23.3 (19.3 – 32)	25	27.5 (21 – 33)	-	(17 – 30)
Vulval bridge width	40	10.2 (7.2 – 12.4)	35	10.9 (8.0 – 13.1)	25	7.7 (6.3 – 9.4)	-	8
Fenestral length/width	40	1.9 (1.7 – 2.1)	35	2.0 (1.7 – 2.8)	-	-	-	(1.7 – 2.5)
Underbridge length	27	70 (53 – 85)	15	82 (60 – 110)	25	82.4 (72.5 – 101.5)	-	(60 – 108)
Underbridge width	10	7.7 (5.7 – 11.3)	15	6.5 (4,0 – 9,0)		16.8 (15.4 – 17.5)		
Eggs								
Length	100	131 (119.1 – 140.8)	100	125 (108 – 141)	-	137 (127 – 150)	-	(127 – 150)
Width	100	48 (44 – 54)	100	47 (40 – 51)	-	51.2 (46 – 54)	-	(46 – 54)

MATERIALS AND METHODS

Two populations (no. 184 and 185) of *H. filipjevi* were collected in autumn 2001 from two different farms at Sandefjord in the county of Vestfold, southern Norway. The soil samples were air-dried and passed through a 5 mm sieve, and cysts were extracted using a fluidising column (Trudgill *et al.*, 1973). For morphological studies, white females and cysts containing fully developed second stage juveniles were used. Vulval cones were cut and prepared from mature cysts, and vulval structures were observed in cone tops mounted in glycerine jelly (Hooper, 1986). White females and eggs were mounted live in distilled water. Second stage juveniles, were killed by heating at +50°C in a glass tube containing a small amount of water in a microwave oven, after which they were cooled and fixed in TAF (Jones & Ap Gwynn, 1991). Permanent microscope slides were made of fixed second stage juveniles mounted in glycerine (Hooper, 1986). An interference contrast microscope LEITZ DMR equipped with the LEICA QWin "Image Processing and Analysis System" was used for observations and measurements. Air-dried cyst cones, and white females from the Norwegian populations were critical point dryed, and prepared for scanning electron microscopy (SEM). The vulval cones were cut free and mounted onto stubs with the fenestral area uppermost. The cones and whole females were coated with gold (Southey, 1986) before examination in a Philips XL40 SEM.

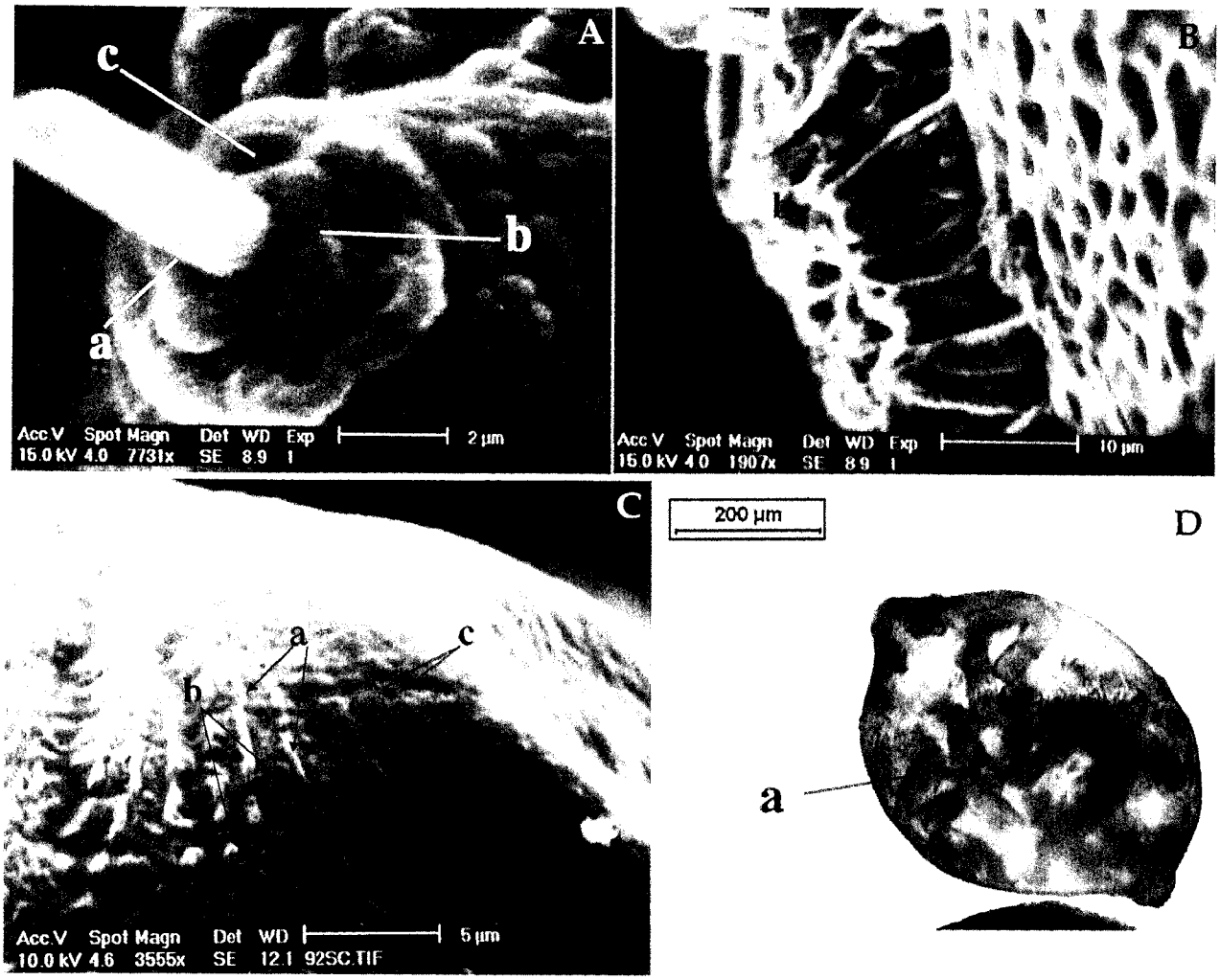

FIGURE 1. Morphology of *Heterodera filipjevi*. A: Scanning electron micrograph of the anterior end of a young female showing the protruded stylet (a), labial disc (b), and amphid opening (c). B: The cyst wall (a) is partially covered with a white sub-crystalline layer (b). C: The cyst wall has a zigzag pattern of ridges (a), irregularly arranged punctations (b), and pores (c). D: The cyst wall is often transparent with the outline of eggs (a) clearly visible.

RESULTS

Measurements of cysts, eggs and second stage juveniles of the two Norwegian populations of *H. filipjevi* are presented in Tables I and II. A cluster analysis of the morphological data showed more than 90% similarity between the two populations. The tables I and II also include data of this species reported by Brzeski (1998) and data from the original description of *Bidera filipjevi* by Madzhidov (1981).

White gravid females are pearly-white and lemon-shaped, with protruding neck and vulva cone. The cuticle bears a zigzag pattern that runs concentrically around the neck and vulval regions. The head is offset, with a squarish and prominent labial disc (Figure 1A). The female stylet has sloping knobs. Ovaries are paired and convoluted. The vulva is slit-like, protruding posteriorly. The anus is distinct. Eggs are cylindrical with rounded edges.

Newly formed cysts are lemon-shaped and partially covered with a white sub-crystalline layer (Figure 1B). The cyst wall has ridges running in zigzag patterns, and irregularly arranged punctations and pores (Figure 1C). The cyst cuticle is golden to light warm brown and is almost transparent, with the outline of individual eggs clearly visible (Figure 1D). The cyst length was 455-874 µm and the width ranged between 253-747 µm. The vulval cone is bifenestrate with horseshoe shaped semifenestra (Figure 2A) and has an underbridge (Figure 2B). The vulval slit varies between 6.0 and 10,8 µm in length, and the width of the vulval bridge is 7.2-13.1 µm. The fenestral length ranges from 38.4 to 58,4 µm, and the width of the semifenestrae was 19.3-32.0 µm. The ratio between the fenestral length and width was in the range of 1.7-2.8. The dimensions of the underbridge were 53-110 µm in length and 4.0-11.3 µm in width. The bullae are weak to medium, distinct, and mostly globular in shape with a pale to medium brown colour. Their position and arrangement vary between focal planes. The second stage juvenile body length ranges from 455-557 µm, and the tail is tapering to a rounded tip. The length of the tail is 52.0-67 µm and its hyaline part measures 30-41 µm, corresponding to more than 50% of the total tail length (Figure 2E). The head is offset and usually with three annules, and the distance from the head to the valves of the median bulb is 59-79 µm. The lateral fields have four lines, of which the inner two are more distinct, and the outer bands are heavily areolated (Figure 2C). The stylet is robust with anchor shaped basal knobs (Figure 2D), and measures 22-25 µm in length. The ratio of the hyaline tail to the true tail is 1.2-1.7.

DISCUSSION

H. filipjevi, originally described from Tadzhikistan (Madzhidov, 1981), has subsequently been reported from several countries. Studies have shown that this species is widespread in the former USSR, and that "*H. avenae* – like" populations from Sweden, England and Germany, which for many years were known as the "Gotland strain", "pathotype 3 of *H. avenae*" or as "race 3", respectively, also belong to *H. filipjevi* (Valdeolivas & Romero, 1990; Sturhan & Rumpenhorts, 1996; Subbotin *et al.*, 1996; Andrés *et al.*, 2001).

Compared to data given in the original description (Madzhidov, 1981), data of Brzeski (1998) and to measurements reported by Subbotin *et al.* (1996) our study on the two Norwegian populations extends the range of cyst length, cyst width, vulval slit length, fenestral length, vulval bridge width, the ratio of fenestral length to width and the underbridge length. The underbridge of the Norwegian populations is very similar to that of a Spanish population of *H. filipjevi* (Valdeolivas & Romero, 1990; Andrés *et al.*, 2001).

The width of the underbridge in the Norwegian populations stands out as being much smaller than reported in the original description; the reason could be the steep slope of the cones, which makes it difficult to focus on the ends of the underbridge perhaps giving a false impression of the terminal focal plane.

The female head of *H. filipjevi* has a squarish labial disc, resembling the labial disc of *H. trifolii* (Baldwin & Mundo-Ocampo, 1991). Cook (1975) reported that cysts of "pathotype 3 of *H. avenae*" have a thick sub crystalline layer, and a similar layer was observed here, this is common in most young cysts of *Heteroderidae*. Complementary information of *H. filipjevi* presented in this paper also concerns the ridges, punctations and pores of the cyst cuticle. In the original description of *H. filipjevi* Madzhidov (1981) the illustration of the top of the vulval cone indicates that the bullae are distributed concentrically. Our observations demonstrated that the distribution of bullae is highly variable. Complementary

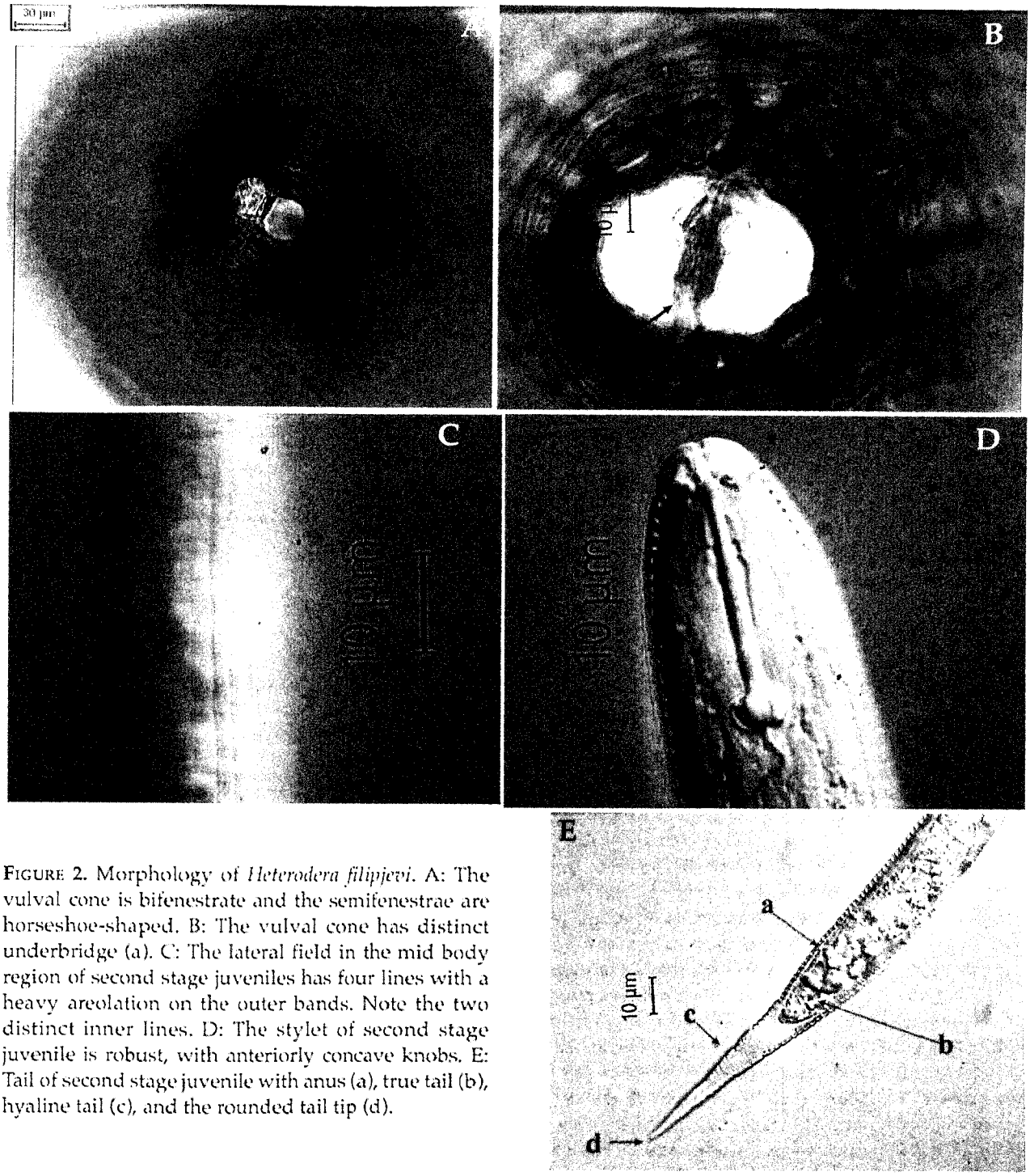

FIGURE 2. Morphology of *Heterodera filipjevi*. A: The vulval cone is bifenestrate and the semifenestrae are horseshoe-shaped. B: The vulval cone has distinct underbridge (a). C: The lateral field in the mid body region of second stage juveniles has four lines with a heavy areolation on the outer bands. Note the two distinct inner lines. D: The stylet of second stage juvenile is robust, with anteriorly concave knobs. E: Tail of second stage juvenile with anus (a), true tail (b), hyaline tail (c), and the rounded tail tip (d).

TABLE II. Measurements (in µm) of second stage juveniles of *Heterodera filipjevi* from Norway, and measurements reported by Madzhidov (1981) and Brzeski (1998).

Characters	Sandefjord population 184		Sandefjord population 185		*H. filipjevi* (Madzhidov, 1981)		*H. filipjevi* (Brzeski, 1998)
	n		n		n		n	
Lateral lines	30	4	30	4		-	4
		mean (range)		mean (range)	mean (range)		mean (range)
Body length	30	520 (490 – 557)	30	490 (455 – 532)	45 510 (430 – 580)	-	530 (480- 580)
Greatest body width	30	19.8 (19.5 – 21.5)	30	19.5 (18,0 – 22,0)	45 22.9 (21 – 24.5)	-	
a	30	26.2 (24,0 – 29,0)	30	25.1 (22.5 – 29,0)	23.6 (21 – 25)	-	25 (22 – 30)
Stylet length	50	24.1 (22.3 – 25.5)	50	23.3 (22,0 – 24.5)	45 26.5 (21.7 – 30.8)	-	25 (23 – 28)
Head to median bulb	30	73,0 (68,0 – 79,0)	30	66.9 (59.9 – 73,0)	69.6 (57 – 84)	-	-
Hyaline tail length	30	36,0 (31,0 – 41,0)	30	35,0 (30.5 – 41,0)	34.8 (31 –39)	-	36 (28 – 45)
True tail length	30	59.3 (54.5 – 67.5)	30	57.5 (52,0 – 60,0)	57.1 (49 – 63)	-	59 (52 – 68)
Hyaline tail length/ Stylet length		1.5 (1.2 – 1.7)		1.5 (1.3 – 1.7)	- 1.3 (1.0 – 1.6)	-	-

information on the morphology of the lateral fields of second stage juveniles, and the areolation of the outer bands, presented for the first time.

With regard to measurements of 2nd stage juveniles the dimensions of the two Norwegian populations correspond well with published data on *H. filipjevi* (Madzhidov, 1981; Subbotin *et al.*, 1996; Brzeski, 1998).

It is interesting to note that morphometric data on cyst characters of the Norwegian populations are more divergent in comparison with other populations than is the case for 2nd stage juveniles characters. Many of the cyst measurements recorded for the Norwegian populations extend the lower range of previously reported values. This could partly relate to variations in the local growth conditions for the females that would affected cyst / female morphometrics rather than juvenile characters. *H. filipjevi*, which seems to be widely distributed in the Palearctic region (Subbotin *et al.* 1996), will experience a magnitude of climatic conditions ranging from a hot continental climate (like in the central parts of Russia) to a mild coastal climate (like in Norway).

The cyst colour, overall fenestral length and the presence of an underbridge show that *H. filipjevi* closely resembles *H. mani* as described by Mathews (1971). However, IEF studies (Holgado *et al.*, 2004b) have shown that the Norwegian populations of *H. filipjevi* and *H. mani* Irish type population (kept at Rothamsted) have different protein profiles. Other electrophoresis studies have also demonstrated differences between *H. filipjevi* and *H. mani* (Sturhan & Rumpenhorst, 1996; Andrés *et al.*, 2001). Mathews (1971) reported that *H. mani* could not reproduce on cereals, while Cook (1982) reported that some *H. mani* populations could. This indicates that *H. filipjevi* probably cannot be separated from *H. mani* by its host range, and that more studies are needed to separate *H. mani* and *H. filipjevi* by morphological characters, biotests or both.

H. filipjevi and *H. avenae* can be separated on the basis of the morphology of the cyst and cyst cone (Holgado *et al.*, 2004a) e.g. *H. filipjevi* has a well developed underbridge, whereas the underbridge in *H. avenae* is weakly developed or absent. Bullae in *H. filipjevi* are weak to medium, distinct, mostly globular,

and pale to medium brown in colour, whereas they are strong, dark brown, numerous, clearly distinct and variable in shape in *H. avenae*.

The cyst wall of *H. filipjevi* is light in colour whereas *H. avenae* cysts have a dark brown to black cyst wall. Eggs of *H. filipjevi* are easily observed through the cyst wall, unlike those of *H. avenae*.

H. filipjevi is an important nematode in cereal producing areas, causing yield reduction in several countries (Ireholm, 1990; Subbotin *et al.*, 1996; Sturhan, 1996; Rumpenhorst *et al.*, 1996; Damadzadeh & Ansaripour, 2001; Holgado *et al.*, 2004a). Its biology and host preferences seems to differ considerably from those of *H. avenae*. Hence, in order to devise appropriate and effective methods of control, the correct identification of *H. filipjevi* is essential. The complementary information on morphological characters given in this study will facilitate the identification of *H. filipjevi* by morphology.

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