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A morphological and biochemical comparison of the four cyst nematode species, *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (Nematoda : Heteroderidae) known to attack rice *(Oryza sativa)*

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Summary – Populations of the four rice cyst nematode species (*Heterodera elachista* Ohshima, 1974, *H. oryzicola* Rao & Jayaprakash, 1978, *H. oryzae* Luc & Berdon Brizuela, 1961 and *H. sacchari* Luc & Merny, 1965) were compared using both morphological and biochemical techniques. All four species could be separated from each other using morphology, but problems arose in the identification of *H. sacchari* and *H. oryzae* due to similarities in certain morphological characters. Scanning electron microscopy of the lip region of the second stage juveniles showed that *H. elachista* was very different from the other species. *H. oryzicola* had a less elongated lip region than *H. oryzae* and *H. sacchari* which had similar lip regions. Non-specific esterase banding patterns allowed separation of all four species and helped to resolve the problems of morphological similarities. The use of morphology and biochemistry in identification and taxonomy is discussed.

Résumé – Comparaison morphologique et biochimique entre les quatre espèces de nématodes à kystes *(Heterodera elachista, H. oryzicola, H. oryzae et H. sacchari)* (Nematoda : Heteroderidae) connues pour attaquer le riz *(Oryza sativa)* – Des populations appartenant aux quatre espèces de nématodes à kystes du riz (*Heterodera elachista* Ohshima, 1974, *H. oryzicola* Rao & Jayaprakash, 1978, *H. oryzae* Luc & Berdon Brizuela, 1961 et *H. sacchari* Luc & Merny, 1965) ont été comparées en utilisant des critères morphométriques et biochimiques. Les quatre espèces peuvent être séparées les unes des autres en se fondant sur la morphologie, mais des problèmes existent concernant *H. sacchari* et *H. oryzae* dont certains caractères morphologiques sont très semblables. Des observations en microscopie électronique à balayage de la région labiale des juvéniles de deuxième stade montrent que *H. elachista* est très différent des trois autres espèces. *H. oryzicola* présente une région labiale moins allongée que celle de *H. oryzae* et *H. sacchari* lesquels sont très semblables sur ce point. Les profils des estérases non spécifiques permettent de séparer les quatre espèces et peuvent aider à surmonter le problème des ressemblances morphologiques. L'utilisation de la morphologie et de la biochimie pour l'identification et la taxonomie est discutée.

Key-words : Nematodes, *Heterodera*, rice, morphology, esterasic profiles.

Identification of *Heterodera* species has traditionally been based on the shape and size of the whole cyst, structures on and within the vulval cone and measurements of the second stage juveniles (Mulvey, 1957, 1960; Hesling, 1965). An additional aid to species identification has been the placement of species into groups (Mathews, 1971; Mulvey, 1972) based on vulval structures. The species under investigation here have all been placed into the "*schachtii*" group due to the ambifenestrate vulval cone top, long vulval slit and the presence of bullae and/or an underbridge within the cone.

From the original descriptions of the four species, the measurements showed some overlap between species (Table 1). All the second stage juveniles were reported to have three lateral lines and small phasmids in the anterior section of the tail. Identification of *Heterodera sacchari* Luc & Merny, 1965 on the presence of " finger-like " projections on the underbridge has shown consistancy between populations (Luc, 1974; Luc & Taylor, 1977). However, when studying a wide range of *Heterodera* species Mulvey (1972, 1974) confused specimens of *Heterodera oryzae* Luc & Berdon Brizuela, 1961 with *H. sacchari* due to contamination of material (Luc, pers. comm.). This caused problems when attempting to separate these two species for this project and so further work on morphology and alternate methods of identification were thought necessary.

From a review of the literature *H. sacchari* was originally reported from sugar-cane in Congo (Luc & Merny, 1965), but has been found to attack rice (Merny, 1970; Babatola, 1983) and to occur in a wide range of

countries (Swarup *et al.*, 1964; Jerath, 1968; Odihirin, 1975; Maqbool, 1981). *H. oryzae* was originally described on rice from the Côte d'Ivoire (Luc & Berdon Brizuela, 1961) but has also been reported from several different countries (Ou *et al.*, 1975; Rao & Jayaprakash, 1977; Taylor, 1978). However, there are doubts as to the validity of some of the identifications (Luc, pers comm.). There is a possibility of the two species being confused with each other and occuring as mixed populations. The close morphology of these two species could make accurate identification difficult.

Of the other two species known to attack rice, *Heterodera elachista* Ohshima, 1974 has been found only in Japan and *Heterodera oryzicola* Rao & Jayaprakash, 1978 in India.

Observations and measurements of important characters of the cyst, cyst cone and second stage juveniles, scanning electron microscopy of the lip region of the second stage juveniles and non-specific esterase banding patterns were used to compare the populations of rice cyst nematodes cultured at the CAB International Institute of Parasitology. Biochemical techniques were restricted to non-specific esterase staining using polyacrylamide gel electrophoresis (PAGE) as other researchers (Rumpenhorst, 1985) had shown that non-specific esterase banding patterns were distinctive between different cyst nematode species difficult to separate morphologically *(H. avenae* races and *H. mothi)*. Non-specific esterase banding patterns have also been used for *Meloidogyne* species (Janati *et al.*, 1982; Dalmasso & Bergé, 1983).

Materials and methods

Cyst cultures

The four cyst nematode species had been cultured on rice *(Oryzae sativa)* for at least three generations before comparisons were made. *Heterodera sacchari, H. oryzae* and *H. oryzicola* were cultured on rice cv. IR36. *H. elachista* was cultured on rice cv. Hata Sangaku as it reproduced poorly on IR36. *H. oryzicola* was obtained from Kerala State, India, *H. sacchari* and *H. oryzae* from the Côte d'Ivoire and *H. elachista* from Japan. The *H. oryzae* sample was found to be contaminated with *H. sacchari* and separation of the species proved difficult using just morphology. A series of single cyst cultures from cysts considered to be morphologically *H. oryzae* (no " fingerlike " projections on the underbridge) were isolated and maintained separately. One isolate (SCC6) was used for the morphological and biochemical comparison. The accuracy of the identification of *H. oryzae* SCC6 was tested using morphological and biochemical techniques.

Comparison of second stage juveniles was used to aid in identification but, as environment has been found to influence size of juveniles (Netscher & Pernès, 1971), valid comparison with the original descriptions was not possible. Certain characters have been shown to be helpful in separating species (Wouts & Weischer, 1977) and most of these have been used in this comparison.

Sampling of cysts

Soil and roots were sampled using a simple decanting and sieving technique (Southey, 1986). For morphological comparisons, 100 cysts of each species and isolate were removed and placed in distilled water. Samples of 25 while females were also removed and placed in 1.0 ml sterile deionised distilled water and stored at − 20 °C until used for biochemical comparison.

Morphological comparisons

Fifty mature cysts were placed in a drop of water on a cavity slide and body length (BL) and body width (BW) measured under a light microscope. The ratio BL/BW was calculated. Analysis of variance was used to determine if there were any significant differences between the four species ($p = 0.05$). Standard test of means was used to determine the difference between species ($p = 0.05$).

Cyst cones from 10 cysts were removed and mounted in glycerine jelly for examination and measuring. Length of vulval slit, width of vulval bridge, width of fenestra, length of fenestra and length and width of underbridge were measured. Analysis of variance was used to determine if there were any significant differences between the four species ($p = 0.05$). Standard test of means was used to determine significant differences between species ($p = 0.05$). Presence or absence of underbridge, bullae and " fingerlike " projections on the underbridge were also noted.

Twenty second stage juveniles were allowed to hatch from the dissected cysts and heat killed, fixed in TAF and transferred to hot lactic-phenol to be mounted in lactic-phenol using the wax seal method (Southey, 1986). Direct comparison between these measurements and those in the original descriptions could not be done due to the different mounting methods. Measurements were taken of body length, body width, stylet length, distance from anterior region to junction with œsophagus, distance from anterior region to base of œsophageal bulb, tail length, tail width and length hyaline portion of tail. The ratios a, b, b′, c and c′, as well as the ratio of hyaline portion/stylet length were calculated. Analysis of variance was used to determine if there were significant differences between the four species ($p = 0.05$). Standard test of means was used to determine the significant differences between species ($p = 0.05$). All statistical analysis involved using the computer statistical package NANOSTAT (Copyright 1987 M. J. R. Healy).

Scanning electron microscopy

To prevent excessive shrinkage, the second stage juveniles of the four different species were fixed using 4 % gluteraldehyde in 0.05 M potassium phosphate buffer at pH 7.4 using the microwave fixation technique of Jones

and Apgwynn (1991). The post fixing agent of 1 % osmium was then rinsed from the specimens several times using cold (4 °C) 0.05 M potassium phosphate buffer.

The juveniles were then transferred to Beem capsules and dehydrated to 100 % ethanol (Eisenback, 1985). The specimens were critical point dried using CO_2, mounted onto stubs with the lip region of the nematode uppermost and coated with gold (Southey, 1986). The specimens could then be examined in an Hitachi S-450 SEM microscope at 20 kV.

BIOCHEMICAL COMPARISON

Enzyme extraction

An additional population of *Heterodera sacchari*, collected from Cameroon (wild grasses) and cultured on rice cv. IR36, was used to test if non-specific esterases which separated different species were consistent for different populations of the same species. For each species, 25 to 30 white females were collected and stored in 1 ml of sterile distilled water in a 1.5 ml microcentrifuge tube at – 20 °C. Throughout the extraction procedure the samples were kept on ice. The samples were washed in distilled water and 40 µl extraction buffer (20 % glycerol, 2 % Triton X-100 and 0.01 g Bromophenol Blue) added. The nematodes were homogenised using a small plastic pestle and centrifuged at 5000 rpm for 10 min at 4 °C. The clarified supernatant was introduced immediately into the electrophoretic cell.

Electrophoresis

The esterase isoenzymes present in the nematode samples were determined by native polyacrylamide gel electrophoresis in a mini-slab gel apparatus (Atto, Tokyo). A 3 % acrylamide stacking gel and 7 % acrylamide separating gel were used. Nematode samples of 20-25 µl were injected into the electrophoresis cell. The buffer system was essentially that of Laemmli (1970) except that Triton X-100 was substituted for sodium dodecyl sulphate (SDS) in our experiments. A constant current of 15 mA was applied until the marker dye reached the separating gel when the current was increased to 20 mA. When the marker dye reached the base of the separating gel the power was turned off and the gel removed.

Enzyme staining

Esterase activity was estimated by incubating the gels at 37 °C for 15-30 min in a solution of 100 mg Fast Blue RR salt, 50 µg α-naphthyl acetate and 50 µg α-naphthyl butyrate dissolved in 5 ml acetone and made up to 100 ml with 0.2 M Tris-Chloride buffer pH 6.6. The reaction was stopped by adding 10 % acetic acid. Electrophoretic mobility (Rm) for each enzyme band was calculated as the ratio of its movement to that of the marker dye.

Results

MORPHOLOGICAL COMPARISONS

Cysts

Heterodera elachista was significantly smaller than all other species (Table 2) with *H. oryzicola* significantly larger than *H. elachista* and smaller than *H. oryzae*

Table 1. Measurements of cysts and second stage juveniles from the original description of *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (all measurements in µm).

Characters	*H. elachista*(1)	*H. oryzicola*(2)	*H. oryzae*(3)	*H. sacchari*(4)
CYSTS				
	n = 35	n = 25	n = 92	n = 100
Body length	406	484	571	654
	(350-560)	(414-520)	(310-810)	(380-1030)
Body width	315	314	457	445
	(250-450)	(220-348)	(220-690)	(280-830)
SECOND STAGE JUVENILES				
	n = 25	n = 50	n = 100	n = 100
Body length	367	392	440	480
	(330-405)	(370-428)	(373-507)	(420-530)
Stylet length	18.6	18	20.8	22
	(18-19.5)	(17-19)	(19.5-22.0)	(21-24)
Tail length	52.7	55		
	(44-57)	(50-60)	67-69*	49-60*
Hyaline region (h)	31.4	28	39.5	26
	(26-36)	(22-29)	(35-45)	(20-30)

* = due to difficulties in establishing position of anus, accurate measurement was difficult.
(1) : Ohshima, 1974; (2) : Rao & Jayaprakash, 1978; (3) : Luc & Berdon-Brizuela, 1961; (4) : Luc & Merny, 1965.

Table 2. Measurements (in μm) of whole cysts and the ratio of body length to body width (ratio a) (n = 50) of the four rice cyst nematodes *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari*.

Character	*H. elachista*	*H. oryzicola*	*H. oryzae*	*H. sacchari*	ANOVA
Body length	446.3 + 30.3 b (328-557)	500.5 + 29.4 c (344-639)	714.8 + 71.5 a (442-999)	735.0 ± 54.7 a (442-983)	*
Body width	322.6 + 26.0 b (229 - 449)	366.0 + 28.2 c (250-589)	426.6 + 53.8 a (229-655)	456.0 + 44.2 a (279-753)	*
Ratio a	1.39 + 0.09 b (1.1-1.9)	1.38 + 0.08 b (1.1-1.7)	1.71 + 0.12 a (1.1-2.3)	1.63 + 0.11 a (1.1-2.2)	*

Same letter after mean indicates no significant difference, standard t-test, d.f. = 98, P = 0.05 % level.
Body length excludes neck. * = significantly different, P = 0.05 %, analysis of variance (ANOVA), d.f. = 3,147.

Table 3. Measurement (in μm) of the cyst cones of the four rice cyst nematodes *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (n = 10).

Characters	*H. elachista*	*H. oryzicola*	*H. oryzae*	*H. sacchari*	ANOVA
L. vulval slit	37.5 + 2.8 a (26-46)	42.7 + 4.4 a (32-55)	50.7 + 4.0 b (41-65)	52.6 + 3.0 b (44-62)	*
W. vulval bridge	5.8 + 2.0 ab (2-16)	4.7 + 0.7 a (3-6)	12.4 + 1.9 c (6-16)	8.5 + 2.0 b (3-16)	*
L. fenestra	29.3 + 1.98 a (23-36)	26.5 + 3.60 a (16-44)	38.8 + 1.85 b (32-46)	40.1 + 3.76 b (29-46)	*
W. fenestra	32.0 + 2.50 b (26-39)	24.6 + 2.32 c (16-32)	51.6 + 5.20 a (36-69)	48.5 + 5.85 a (36-68)	*
L. underbridge	# 72.0 + 2.9 b (65-78)	## 79.2 + 8.6 b (61-104)	112.5 + 9.0 a (87-153)	129.6 + 10.0 a (111-172)	
W. underbridge	# 13.5 +3.2 c (10-23)	## 13.8 + 4.4 b (5-26)	53.3 + 3.5 a (39-62)	59.9 + 2.5 a (54-68)	
Underbridge	4/10	5/10	10/10	10/10	
Bullae	9/10	9/10	9/10	10/10	
" Fingerlike " projections	0/10	0/10	2/10	10/10	

Same letter mean indicates no significant difference, standard t-test, d.f. 18, P = 0.05 % level. # measurements from only 4 specimens.
= measurements from only 5 specimens. Ratio LFen/WFen = ratio of the length of fenestra by width of fenestra.
* = significantly different, P = 0.05 %, analysis of variance (ANOVA), d.f. = 3,27.

SCC6 and *H. sacchari*. There were no significant differences between *H. sacchari* and *H. oryzae* SCC6 with respect to total body length, width and ratio of body length to body width.

The four species could also be separated into the same groups on structures within the vulval cone. *H. sacchari* and *H. oryzae* SCC6 tended to have a much more prominent underbridge, with the central section enlarged into a plate-like structure. Fig. 1 shows the variation in shape between *H. sacchari* and *H. oryzae* SCC6 as well as the intraspecific variation found in *H. oryzae* SCC6. *H. elachista* and *H. oryzicola* had a much thinner and fragile underbridge which was often lost in older cysts and upon dissection. Within each pair there were few differences between measurements of structures on and within the vulval cone (Table 3). The presence of finger-like projections on the underbridge of all specimens of *H. sacchari* helped distinguish this species from *H. oryzae* S CC6. However, in some specimens of *H. oryzae* SCC6 there were structures which could have been identified as " fingerlike " unless specimens of *H. sacchari* had already been observed. Between *H. oryzicola* and *H. elachista* there was a marked difference in the shape of the fenestra (Fig. 2), with the length

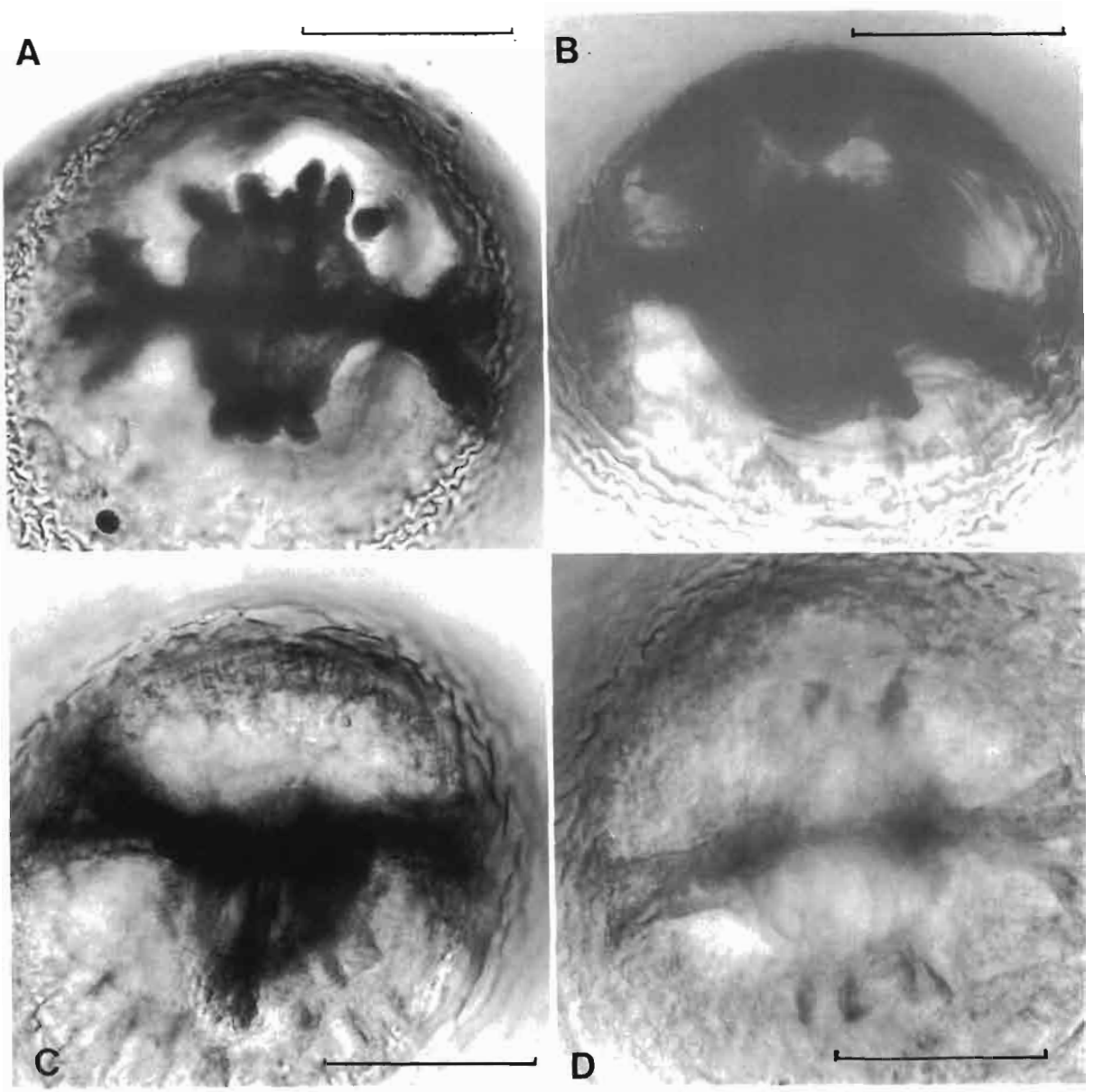

Fig. 1. Morphology of the underbridge of : A : *Heterodera sacchari;* B, C, D : *H. oryzae* SCC6 *(Scale bar = 50 µm).*

along the vulval bridge being much greater than the height in *H. oryzicola* compared to *H. elachista.* The fenestral region of *H. oryzae* SCC6, *H. sacchari* and *H. elachista* were similar.

Second stage juveniles

Many of the measurements of the second stage juveniles of all species and isolate were significantly different and showed no pattern as with the cyst and cyst cones (Table 4). Only tail length and hyaline portion of tail were not significantly different between species and isolate. The ratios also showed no pattern in significant differences between species (Table 5). Only the greater length of stylet of the second stage juveniles of *H. sacchari* and the more pointed appearance of the tail of *H. oryzicola* and *H. elachista* allow some diagnosis of species (Fig. 3). It is important when identifying species of *Heterodera* to consider cysts as well as second stage juveniles.

Scanning electron microscopy

Only the second stage juvenile lip patterns were investigated. For each species under investigation adjacent

Table 4. Measurements (in μm) of characters of the second stage juveniles of the four rice cyst nematode species *Heterodera elachista, H. oryzicola, H. sacchari* and *H. oryzae* (n = 20).

Character	*H. elachista*	*H. oryzicola*	*H. oryzae*	*H. sacchari*	ANOVA
Body length	402 ± 10.0 a (377-450)	440 ± 17.0 b (351-470)	554 ± 9.4 d (523-589)	592 ± 5.9 c (569-609)	*
Body width	16.4 ± 0.41 a (16-20)	16.5 ± 0.44 a (16-18)	16.3 ± 0.43 a (15-18)	19.4 ± 0.47 b (18-20)	*
Stylet length	19.2 ± 0.78 a (16-21)	20.2 ± 0.77 b (19-25)	20.5 ± 1.15 c (16-25)	24.1 ± 0.74 b (21-26)	*
Ant. end - junct. oes.	82.8 ± 3.92 a (66-99)	90.9 ± 6.16 b (79-119)	105.5 ± 9.5 c (73-139)	105.5 ± 8.44 b (66-132)	*
Ant. end - base oes.	124.6 ± 7.49 c (106-152)	136.9 ± 10.82 b (112-172)	178.0 ± 17.1 a (132-238)	160.0 ± 12.4 a (132-218)	*
Tail length	57.8 ± 2.94 a (47-70)	60.4 ± 3.65 a (41-75)	58.8 ± 2.25 a (49-66)	61.5 ± 3.00 a (49-69)	ns
Tail width	11.4 ± 0.77 a (10-15)	11.7 ± 0.56 a (10-13)	12.3 ± 0.72 a (10-15)	13.4 ± 0.83 b (11-15)	*
L/hyaline portion tail (h)	33.4 ± 1.79 a (28-41)	34.5 ± 1.95 a (29-41)	32.8 ± 1.30 a (26-44)	31.9 ± 2.37 a (28-39)	ns

Same letters after means indicate no significant differences, standard test of means, d.f. = 38, P = 0.05 %.
* = significantly different, P = 0.05 %, analysis of variance (ANOVA), d.f. = 3,57, NS = not significantly different.

Table 5. Comparison of de Man's ratio's of second stage juveniles of *Heterodera elachista, H. oryzicola, H. sacchari* and *H. oryzae* (n = 20).

Ratio	*H. elachista*	*H. oryzicola*	*H. oryzae*	*H. sacchari*	
a	24.6 ± 0.88 a (21.3-28.1)	26.8 ± 1.42 b (19.5-30.2)	34.0 ± 1.03 d (30.1-39.3)	30.6 ± 0.87 c (28.4-33.8)	*
b	4.9 ± 0.31 b (3.8-6.3)	4.9 ± 0.20 ab (3.8-5.7)	5.4 ± 0.47 a (3.9-7.2)	6.6 ± 0.58 c (4.6-8.8)	*
b′	3.3 ± 0.24 b (2.5-4.1)	3.2 ± 0.18 b (2.4-3.8)	3.2 ± 0.30 b (2.3-4.4)	3.7 ± 0.27 a (2.3-4.5)	*
c	7.0 ± 0.37 a (5.4-8.4)	7.3 ± 0.32 a (6.4-8.8)	9.4 ± 0.30 b (8.6-10.7)	9.7 ± 0.48 b (8.4-11.9)	*
c′	5.1 ± 0.23 a (3.9-5.9)	5.2 ± 0.28 a (4.0-6.1)	4.8 ± 0.32 ab (3.8-6.0)	4.6 ± 0.20 b (3.8-5.2)	*
h/stylet	1.7 ± 0.12 a (1.4-2.6)	1.7 ± 0.12 a (1.1-2.2)	1.6 ± 0.11 a (1.4-2.2)	1.3 ± 0.11 b (1.0-1.9)	*

Same letters after mean indicate no significant difference, standard test of means, d.f. = 38, P = 0.05 % level. H = hyaline portion of tail. L = length.
* = significantly different, analysis of variance (ANOVA), P = 0.05 %, d.f. = 3,57.

submedial lips were fused with one another and with the labial disc. Only *H. elachista* retained some slight indentation between adjacent submedial lips. *H. oryzae* SCC6 and *H. sacchari* shared the character of submedial lips modified in shape so that they were the same width as the labial disc and elongate in the dorsal-ventral axis. In *H. elachista* and *H. oryzicola* the submedial lips were wider that the labial disc and they were only slightly elongate in *H. oryzicola* (Fig. 4). There were some abnormally shaped lip regions in all species but these were not common and mainly involved fusion of different parts of the lips. According to the Stone (1975) group-

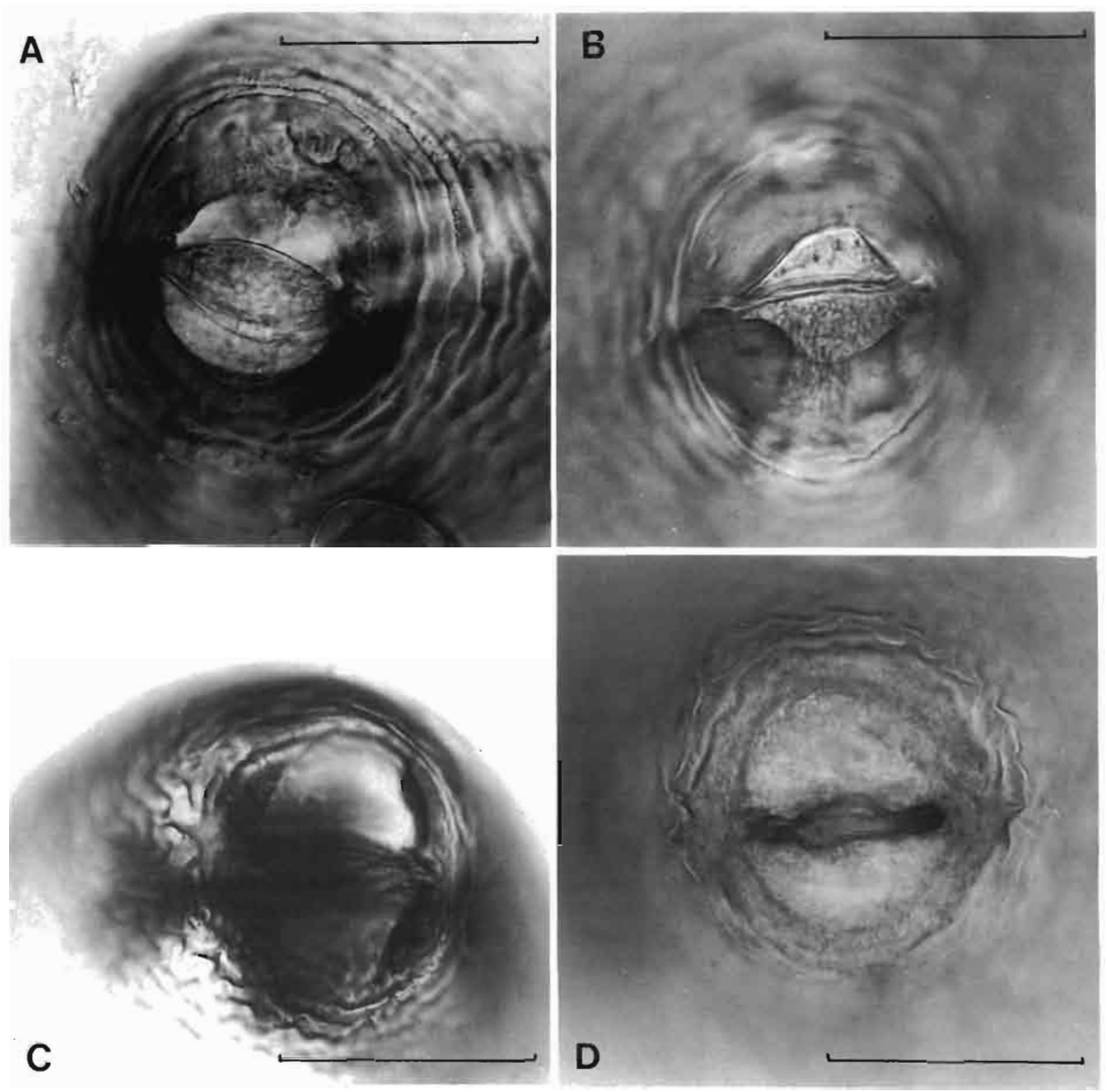

Fig. 2. Morphology of the cyst cone tops of : A : *Heterodera elachista;* B : *H. oryzicola;* C : *H. sacchari;* D : *H. oryzae* SCC6. *(Scale bar = 50 µm).*

ing of lip region *H. oryzicola, H. oryzae* and *H. sacchari* all fall within the *"avenae"* group and *H. elaschista* within the *"goettingiana"* group.

BIOCHEMICAL COMPARISONS

All four species showed clear differences in non-specific esterase banding patterns (Fig. 5). The esterase banding pattern of *H. elachista* showed no similarity with any of the other species. Both populations of *H. sacchari* (Cameroon and Côte d'Ivoire) had similar banding patterns. *H. oryzae* SCC6 had only two bands (Rm = 0.41 and 0.44) in common with *H. sacchari* which indicates that separation of the species from a mixed population using a single cyst had occurred. *H. oryzicola* had a different band in common with *H. sacchari* (Rm = 0.50) and none in common with *H. oryzae* SCC6.

SHORT DESCRIPTION OF SPECIES

H. elachista : – small cysts with long vulval slit, well developed rounded semifenestra, few bullae and weak to

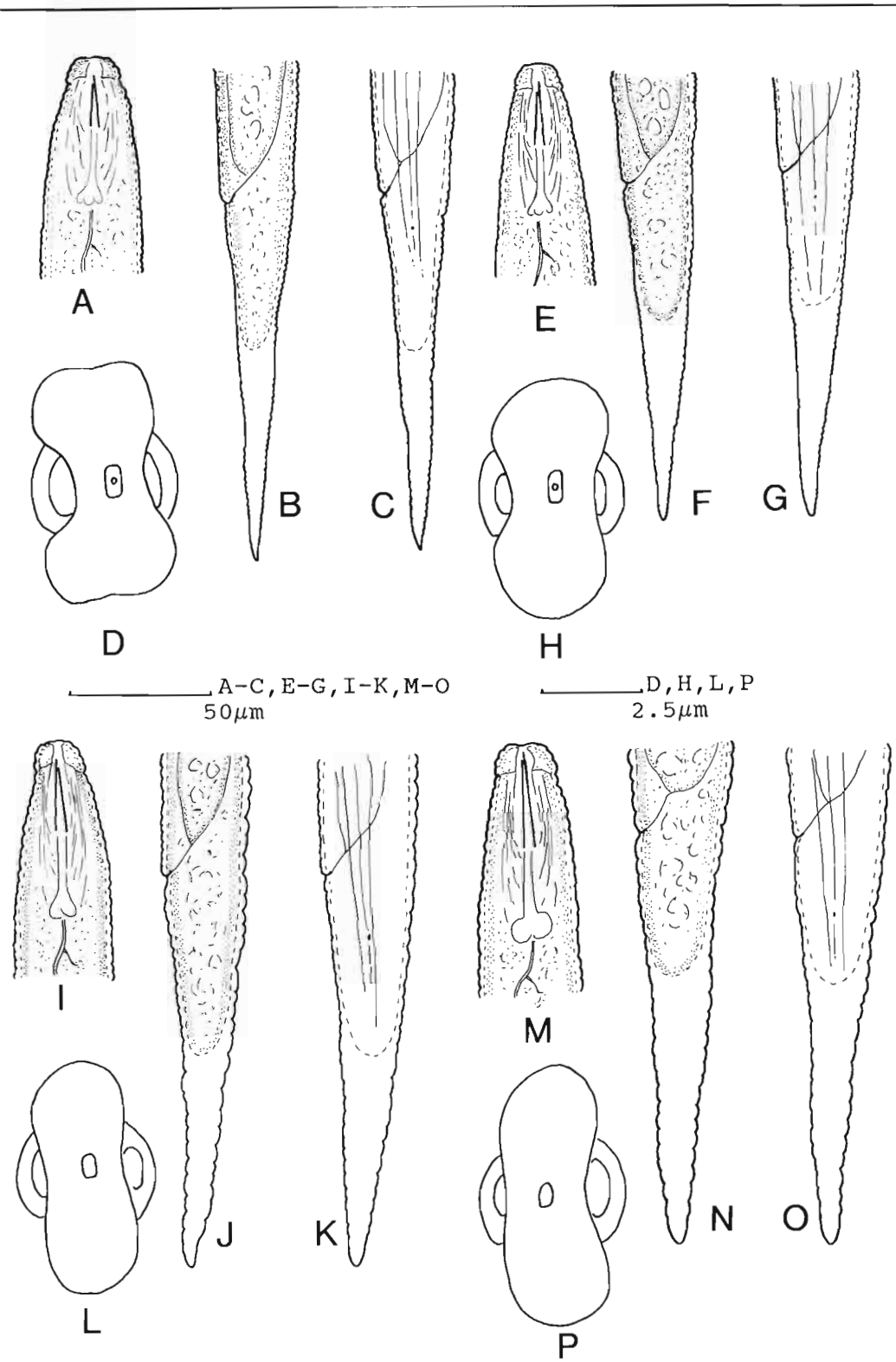

Fig. 3. Second stage juveniles of *Heterodera elachista* (A-D), *H. oryzicola* (E-H), *H. oryzae* SCC6 (I-L) and *H. sacchari* (M-P). Lip and head region (A, E, I, M); Tail region, internal (B, F, J, N); Tail region, external (C, G, K, O); *En face* view of lip region (D, H, L, P).

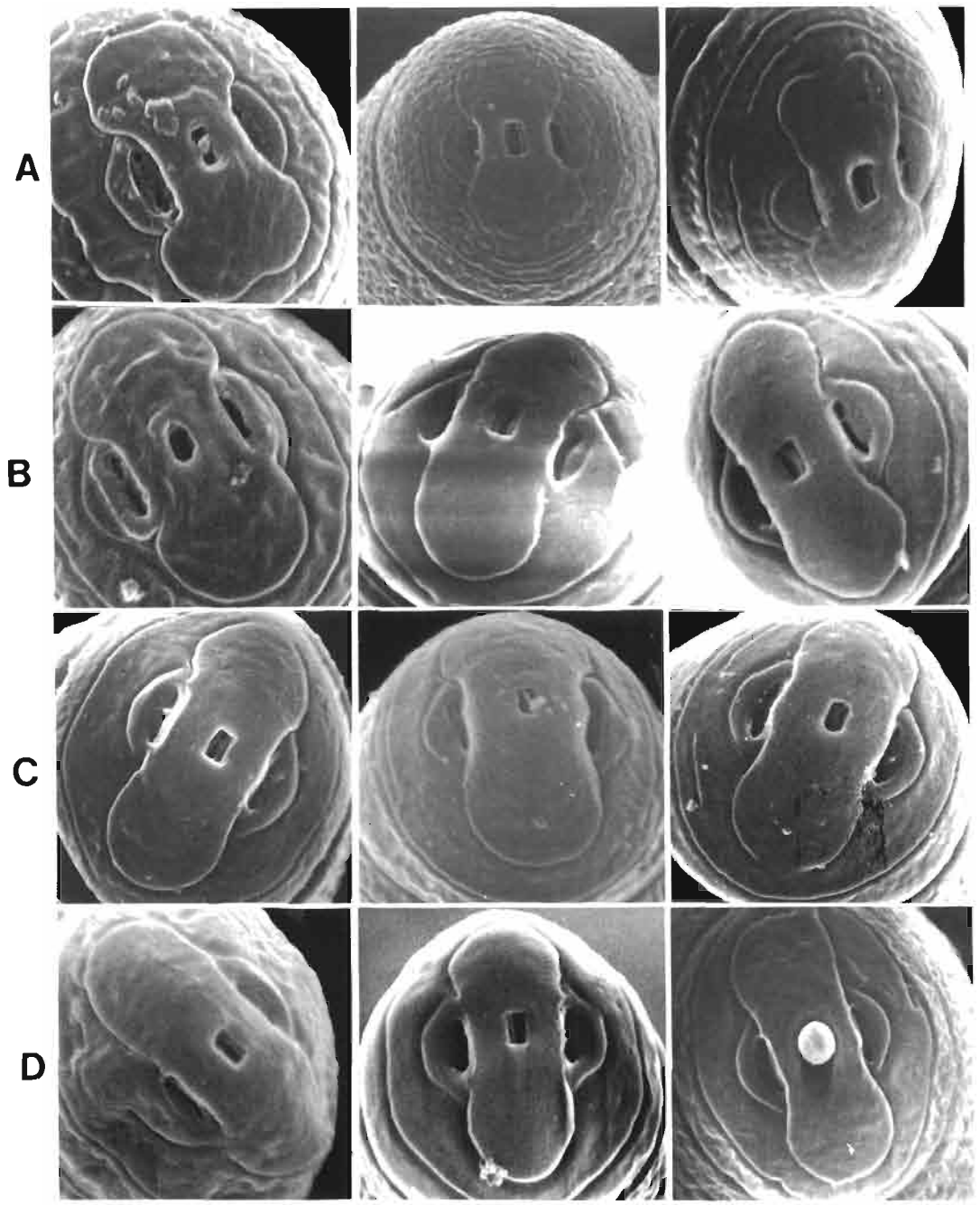

Fig. 4. Scanning electron micrographs of the lip region of the second stage juveniles. A : *Heterodera elachista;* B : *H. oryzicola;* C : *H. oryzae* SCC6; D : *H. sacchari (Scale bar = 2.6 µm).*

absent underbridge (" *schachtii* " group). *En face* view of lip region of second stage juveniles shows some slight indentation between adjacent submedial lips. Esterase banding patterns show three bands – Rm = 0.14, 0.19, 0.24 (estimated from Fig. 5).

H. oryzicola : – small cysts with long vulval slit, small, ovoid semifenestra, many bullae and weak to absent underbridge (" *schachtii* " group). *En face* view of lip region of second stage juveniles without identation and the submedial lips wider than labial disc and only slightly

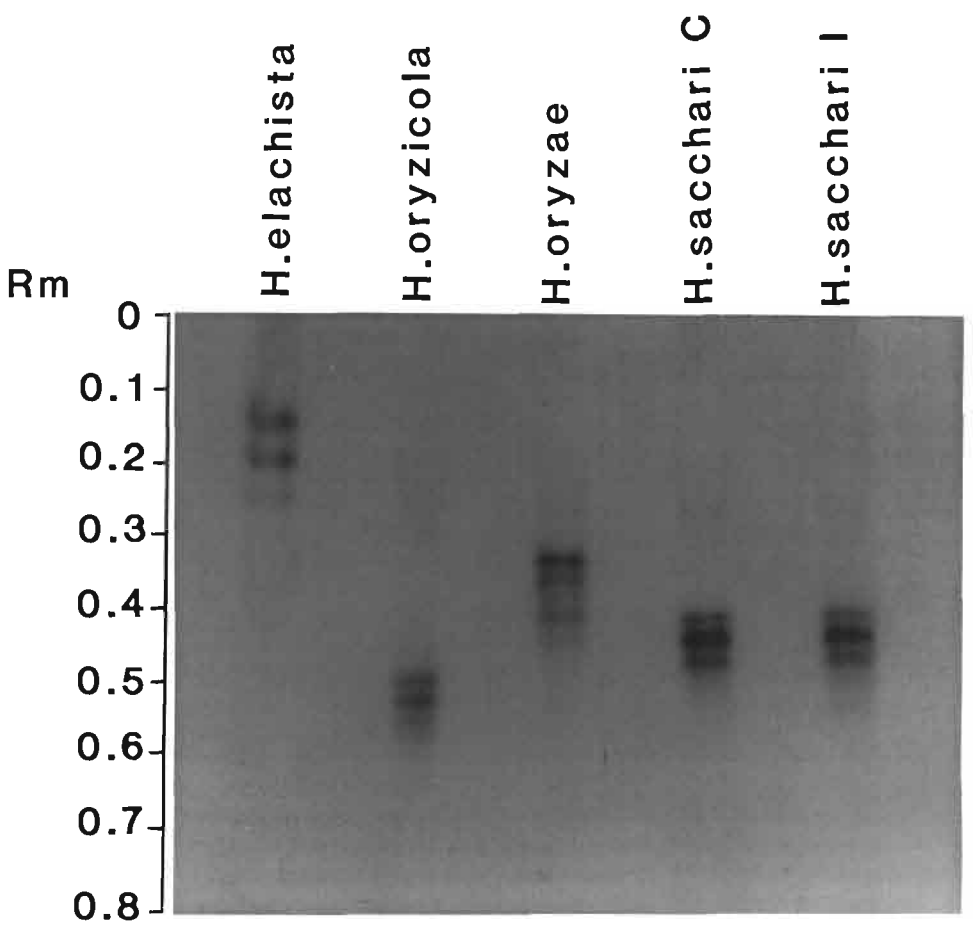

Fig. 5. Photograph of non-specific esterase banding patterns of the four rice cyst nematode species *Heterodera elachista, H. oryzicola, H. oryzae* SCC6 and *H. sacchari* (C = Cameroon pop.; I = Côte d'Ivoire pop.).

elongate. Esterase banding patterns show four bands – Rm = 0.50, 0.52, 0.55, 0.58 (estimated from Fig. 5).

H. oryzae SCC6 : – large cysts with long vulval slit, rounded semifenestra, bullae present and well developed underbridge without " finger-like " projections (" *schachtii* " group). *En face* view of lip region of second stage juvenile with submedial lips same width as the labial disc and elongate in the dorsal-ventral axis. Esterase banding pattern show four bands – Rm = 0.33, 0.37, 0.41, 0.44 (estimate from Fig. 5).

H. sacchari : – large cysts with long vulval slit, rounded semifenestra, bullae present and a well developed underbridge with " fingerlike " projections (" *schachtii* " group). *En face* view of lip region of second stage juveniles with submedial lips same width as the labial disc and elongate in the dorsal-ventral axis. Esterase banding pattern show four bands – Rm = 0.41, 0.44, 0.48, 0.50 (estimated from Fig. 5).

Conclusion and discussion

The four species could be separated into two groups based on the morphology of the cyst and cyst cone. *Heterodera sacchari* and *H. oryzae* SCC6 had large, ovoid cysts with a well developed underbridge; *H. elachista* and *H. oryzicola* had small, rounded cysts and a weakly developed or absent underbridge. Identification of species within these pairs was possible as *H. elachista* was generally smaller than *H. oryzicola* and *H. oryzae* SCC6 tended to be smaller than *H. sacchari*. Presence of " fingerlike " projections was not always a good character for separating *H. sacchari* and *H. oryzae* SCC6 as some specimens of *H. oryzae* SCC6 examined were found to have small protuberances on the underbridge which could be misinterpreted as " fingerlike ".

Of the four species compared using scanning electron microscopy, only *H. oryzicola* had not previously been investigated. *H. elachista* had been investigated by Mo-

moto and Ohshima (1976) and *H. sacchari* and *H. oryzae* by Stone (1975). *H. elachista* retained some slight indentation between submedial lobes and the submedial lips were wider than the labial disc. In *H. oryzicola,* the submedial lips were wider than the labial disc and only slightly elongate. *H. oryzae* SCC6 and *H. sacchari* had similar lip patterns with the submedial lips modified in shape so that they were the same width as the labial disc and elongated in the dorsal-ventral axis.

Esterase isoenzymes were found to be very useful in separating the four species and consistent between two populations of *H. sacchari.* The patterns could be used to show that the separation of *H. oryzae* SCC6 using single cyst cultures was successful. *H. elachista* was clearly different from all other species with the other three species having at least one band in common.

The use of groups based on the morphology of the cyst cone is important when identifying species of cyst nematodes mainly due to the need to separate the large number of species of *Heterodera* (over 50) into easily manageable groups. However, the use of these groupings to establish phylogenetic relationships between species may not be valid as the homology of cyst cone structures and their value in defining monophyletic groups has yet to be investigated.

Looking at the SEM of lip patterns of second stage juveniles of a wide range of species (Stone, 1975) the shape was useful in separating genera but not as useful in grouping or separating species. Species which differ in their lip patterns may not have correlated differences in cyst cone morphologies and visa versa. A closer examination of the species of *Heterodera* presently included in the "*schachtii*" and "*goettingiana*" group is required to determine if an additional grouping of species for those that have bullae and weak to absent underbridges should be proposed (e.g. *H. cyperi, H. elachista, H. oryzicola, H. mothi*).

In conclusion, morphological characters were able to separate the populations and isolates of the four rice cyst nematode species under investigation here. As only one isolate of *H. oryzae* (SCC6) was used in this study, further work using a range of isolates of both *H. oryzae* and *H. sacchari* (Côte d'Ivoire) is needed to determine the range of variability within and between species. PAGE and SEM techniques were able to supplement the classical morphological identification process, with PAGE being of particular importance. Protein and enzyme ana-
lyses have been shown to be useful tools for backing up morphological identification. They could also develop into useful taxonomic tools if it was possible to characterise those proteins or enzymes involved directly with developmental and pathological processes (Bergé & Dalmasso, 1985).

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