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Aphelenchoides nechaleos n. sp. and *A. paranechaleos* n. sp. (Nematoda : Aphelenchoididae) from rice plants

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Summary – *Aphelenchoides nechaleos* n. sp. and *A. paranechaleos* n. sp., originally from rice stems from Sierra Leone and Vietnam respectively, are described and figured. Their general appearance and biology are very similar to those of *A. besseyi* Christie, 1942 but the females of the new species have a single, simple tail mucro and a longer post-vulval sac; the males have more robust spicules with a hooked tip and a simple tail mucro. The two new species are almost identical in appearance but differ slightly in female tail shape and curvature of the male spicules. Both new species swim vigorously in water, are readily cultured on fungi and can survive drying to some extent.

Résumé – Aphelenchoides nechaleos *n. sp. et* A. paranechaleos *n. sp. (Nematoda : Aphelenchoididae), associés au riz* – *Aphelenchoides nechaleos* n. sp. et *A. paranechaleos* n. sp. provenant à l'origine de tiges de riz de Sierra Leone et du Vietnam, respectivement, sont décrits et figurés. Leur aspect général et leur biologie sont très semblables à ceux de *A. besseyi* Christie, 1942, mais les femelles des deux espèces nouvelles ne possèdent qu'un seul mucron caudal, simple, et un sac post-vulvaire plus long; les spicules sont plus robustes et comportent une extrémité en crochet; le mucron terminal de la queue du mâle est semblable à celui de la femelle. Ces deux nouvelles espèces sont presque identiques, ne se séparant que par la forme de la queue de la femelle et la courbure des spicules. L'une et l'autre espèces nagent vigoureusement dans l'eau, peuvent être facilement élevées et, dans une certaine limite, survivre à la dessiccation.

Key-words : Taxonomy, nematodes, *Aphelenchoides,* rice.

An *Aphelenchoides* species from rice stems *(Oryza sativa)* has been held in culture at Rothamsted for several years. It originally occurred with *A. besseyi* and is so similar to that species in general appearance and biology that it was first thought to be a variant of that species. However, morphological studies, including those with the scanning electron microscope (SEM), and biochemical studies (Ibrahim *et al.,* 1994) indicate that it is a separate species. A population more recently obtained from rice stems from Vietnam is very similar in appearance to the Sierra Leone population but these two populations do not interbreed and they can be separated by slight morphological differences and also using biochemical techniques (Ibrahim *et al.,* 1994). The population from Sierra Leone is named *A. nechaleos* n. sp. and that from Vietnam is *A. paranechaleos* n. sp.

Materials and methods

Specimens of the two *Aphelenchoides* populations from rice stems from Sierra Leone and Vietnam were initially cultured on the fungus *Botrytis cinerea* on potato dextrose agar in Petri-plates; they have also been cultured on *Rhizoctonia cerealis* and the Sierra Leone population also reproduced on the fungus *Helminthosporium oryzae* which had been isolated from the host plant tissue.

Measurements and drawings were made mainly of specimens killed by heat and fixed and mounted in TAF; the holotypes were in glycerol. Specimens for permanent mounts were killed and fixed by adding hot TAF or F.P. 4:1 fixative, each plus two per cent glycerol; the fixative solution was allowed to evaporate over several weeks until the specimens were in glycerol in which they were mounted. Additional specimens were processed to glycerol by the rapid method of Seinhorst (1959) and others mounted in lacto-phenol. Specimens fixed in TAF or F.P. 4:1 were also used for studies with the SEM; some specimens were fixed in 4 % glutaraldehyde in 0.05 M potassium phosphate buffer at pH 6.8 using the microwave fixation technique of Jones and Ap Gwyn (1991). Fixed specimens were transferred to modified " Beem " capsules and dehydrated to 100 % ethanol; specimens were then critical point dried using CO_2, mounted onto stubs and coated with gold and examined with an Hitachi S-450 SEM at 20 KV. More details of the above fixatives/methods are given in Southey (1986).

The life cycle of the Sierra Leone population was determined by placing 20 freshly laid eggs in a drop of water under a coverglass on *B. cinerea* in 5 cm diameter agar plates at 25 °C and observing 20 replicates daily for hatching and subsequent development to egg laying adults. the optimum temperature for reproduction was determined by inoculating similar *B. cinerea* plates with 50 specimens, mixed stages, per plate and incubating ten replicates at each temperature of 5, 10, 15, 20, 25, 30, 35 and 38 °C. After 3 weeks the fungus agar mat was chopped into 0.5 cm square pieces and extracted overnight on Baermann-type dishes (Southey, 1986) and the nematodes recovered counted.

Cross breeding between males of the Sierra Leone population and females of the Vietnam population, and vice versa, was attempted. A moulting, hence noninseminated, pre-adult female plus five males of the other population was placed in a drop of water on a cover glass which was then inverted onto a young agar plate culture of *B. cinerea*. Controls were a pre-adult female with five males of the same population; there were ten replicates of each crossing. The plates were kept at room temperature and extracted as above after 4 weeks to check for nematode reproduction.

Aphelenchoides nechaleos * n. sp.

(Figs 1A-L; 2 A-F, K-M)

Measurements

See Table 1.

Description

Female : Lip region distinctly offset with rounded sides and flattened anteriorly, 6-7 µm wide and 3 µm high; appears smooth under light microscope. When viewed with the SEM the lip region has six to eight very fine transverse annulations, the typical Aphelenchoid face pattern (Hooper & Clark, 1980) with a distinct labial disc encircling the labial papillae. The elongate body, usually straight when killed by heat, has fine transverse annulations slightly less than 1 µm wide interrupted by the lateral field which when viewed with the light microscope usually shows four evenly spaced lines in the middle third of the body but occasionally six fine lines are seen. However, when viewed with the SEM this species usually shows six lines in the middle third of the body, the outer and central bands being raised whereas the intervening bands are flattened but sometimes four lines are seen with the SEM. Stylet 10.5-11.5 µm long, the cone slightly shorter than the shaft. The base of the latter with small but distinct thickenings. Median oesophageal bulb rounded to slightly oval with the refractive thickenings usually placed centrally. Excretory pore and nerve ring one to two body widths posterior to the median bulb. Oesophageal glands over lap the intestine dorsally, indistinct in many specimens. Vulva at about two thirds of the body length from the anterior end. Reproductive tract with a single anterior ovary; there is a prominent post-vulval sac which usually extends just over half the distance from the vulva to the anus. Sperm are often present in the post-vulval sac, the uterus and the distal end of the oviduct. Tail convex-conoid, straight on specimens killed by heat, usually just over four anal body widths long with a simple, smooth, terminal mucro.

Male : Lip region, stylet and oesophagus similar to female. Tail end curls ventrally through 45 to 90° when killed by heat; tail with a simple terminal mucro; the usual three pairs of subventral papillae present. Spicules well developed; the dorsal limb smoothly curved in its proximal half but flattened to concave in the distal half and ending in a ventrally curved tip; the ventral limb appears much weaker than the dorsal limb and under the light microscope often appears to end some 2 µm from the curved tip of the dorsal limb. The rostrum and apex are moderately developed; a tangent drawn from the apex to the spicule tips is separated from the tangent from the apex through the rostrum by some seven to twelve radial degrees.

Type habitat and locality

From the stems of paddy rice plants (*Oryza sativa* L.) from experimental plots of the west African Rice Research Station, Rokupr, Sierra Leone.

Type specimens

Holotype (female) on slide No. 86/30/1 and paratype males and females on slides Nos 86/30/2-10 in the nematode slide collection of the Entomology and Nematology Department, Rothamsted Experimental Station. Paratypes also deposited with the CAB International Institute of Parasitology, St. Albans, Herts., England; The USDA Nematode Collection, Beltsville, Maryland 20705, USA; the Muséum National d'Histoire Naturelle, Paris, France.

Diagnosis and relationships

Aphelenchoides nechaleos n. sp. is characterized by the adults being over 600 µm long with a stylet about 11 µm long, the female with a prominent post-vulval sac extending over half the distance from the vulva to the anus and its tail straight and convex-conical with a simple terminal mucro. Males are common and functional; they have prominent spicules with the dorsal limb flattened to indented in its distal half and the tip curled ventrally. The lateral field of adults usually appears as four lines, occasionally six, under the light microscope but six lines are usually seen with the SEM; this variation seems to be due to contraction/expansion ot the two bands between the middle and outer bands. The

* *A. nechaleos* n. sp. is so named because of its active swimming nature and is derived from the Greek *necho* - I swim.

excretory pore is one to two body widths posterior to the median bulb. This species is a vigorous swimmer in water.

In view of its association with rice stems and its swimming habit, *A. nechaleos* n. sp. might be confused with *A. besseyi*, the rice nematode. However *A. nechaleos* n. sp. is readily separated from *A. besseyi* in the female having a single, simple, tail mucro instead of three to four processes; also the post-vulval sac extends over half the vulva to anus distance whereas in *A. besseyi* it extends for less than a third of that distance (Franklin & Siddiqi, 1972). The spicules of *A. nechaleos* n. sp. differ from those of *A. besseyi* in having a more prominent apex and the dorsal limb being flattened distally with a curved terminus. Under the SEM *A. besseyi* adults of four different populations have a much more prominent labial disc than shown in *A. nechaleos* n. sp. (Fig. 2A, J).

The differentiation of *A. nechaleos* n. sp. from other species of *Aphelenchoides* with a female body length over 600 µm, a single tail mucro and four or six lateral lines is indicated in Table 2.

Biology

The vertical migration potential of *A. nechaleos* n. sp. is indicated by its ability to migrate within 20 h from the bottom to the top of a 50 cm long column of wet string inside a vertical polythene tube.

Table 1. *Morphometrics of* Aphelenchoides nechaleos *n. sp. and* A. paranechaleos *n. sp. (measurements in µm).*

	A. nechaleos			*A. paranechaleos*		
	Holotype female	Paratype females	Paratype males	Holotype female	Paratype females	Paratype males
n	1	20	20	1	20	20
Stylet	11	10.8 ± 0.08 (10.5-11.5)	10.5 ± 0.08 (9.5-11.0)	10.5	10.3 ± 0.08 (9.5-10.5)	9.5 ± 0.00 (9.5)
L	755	794 ± 17.0 (605-931)	719 ± 12.9 (601-812)	757	770 ± 12.6 (631-860)	679 ± 7.6 (625-735)
a	43	41 ± 0.7 (33-45)	41 ± 0.5 (35-44)	44	43 ± 0.6 (37-46)	45 ± 0.5 (40-50)
b'	12.8	11.5 ± 0.22 (8.6-13.0)	10.5 ± 0.1 (9.6-11.2)	12.9	10.7 ± 0.2 (8.6-13.2)	10.0 ± 0.1 (9.2-11.0)
b'	5.7	5.1 ± 0.11 (4.0-6.2)	5.1 ± 0.12 (4.2-6.2)	6.9	6.0 ± 0.2 (4.7-7.5)	5.9 ± 0.1 (4.9-6.5)
c	15.0	16.1 ± 0.22 (13.3-17.8)	17.3 ± 0.2 (15.5-19.8)	17.9	19.1 ± 0.3 (16.6-22.5)	18.4 ± 0.3 (15.5-20.7)
c'	4.4	4.2 ± 0.04 (3.9-4.6)	3.4 ± 0.04 (3.0-3.8)	3.7	3.5 ± 0.1 (3.2-4.0)	3.1 ± 0.1 (2.6-3.6)
G1	31	36 ± 1.1 (27-46)		30	40 ± 1.3 (30-52)	
V	67	66 ± 0.4 (61-68)		68	67 ± 0.5 (62-71)	
PVS/V-A%*	53	58 ± 1.8 (44-72)		42	46 ± 1.6 (34-61)	
T			54 ± 1.8 (38-68)			48.7 ± 1.4 (38-58)
S-DL**			16 ± 0.3 (15-20)			16 ± 0.2 (15-18)
S-VL			10 ± 0.2 (9-12)			10 ± 0.2 (8-11)
S-TB			7 ± 0.1 (6-8)			6 ± 0.1 (5-7)
S-D			15 ± 0.2 (13-17)			14 ± 0.2 (13-16)

* PVS/V-A% = post vulval sac length as a percentage of vulva to anus distance.
**S = spicule; DL = dorsal limb, VL = ventral limb, TB = tansverse bar, D = distance in straight line from apex to tip of dorsal limb.

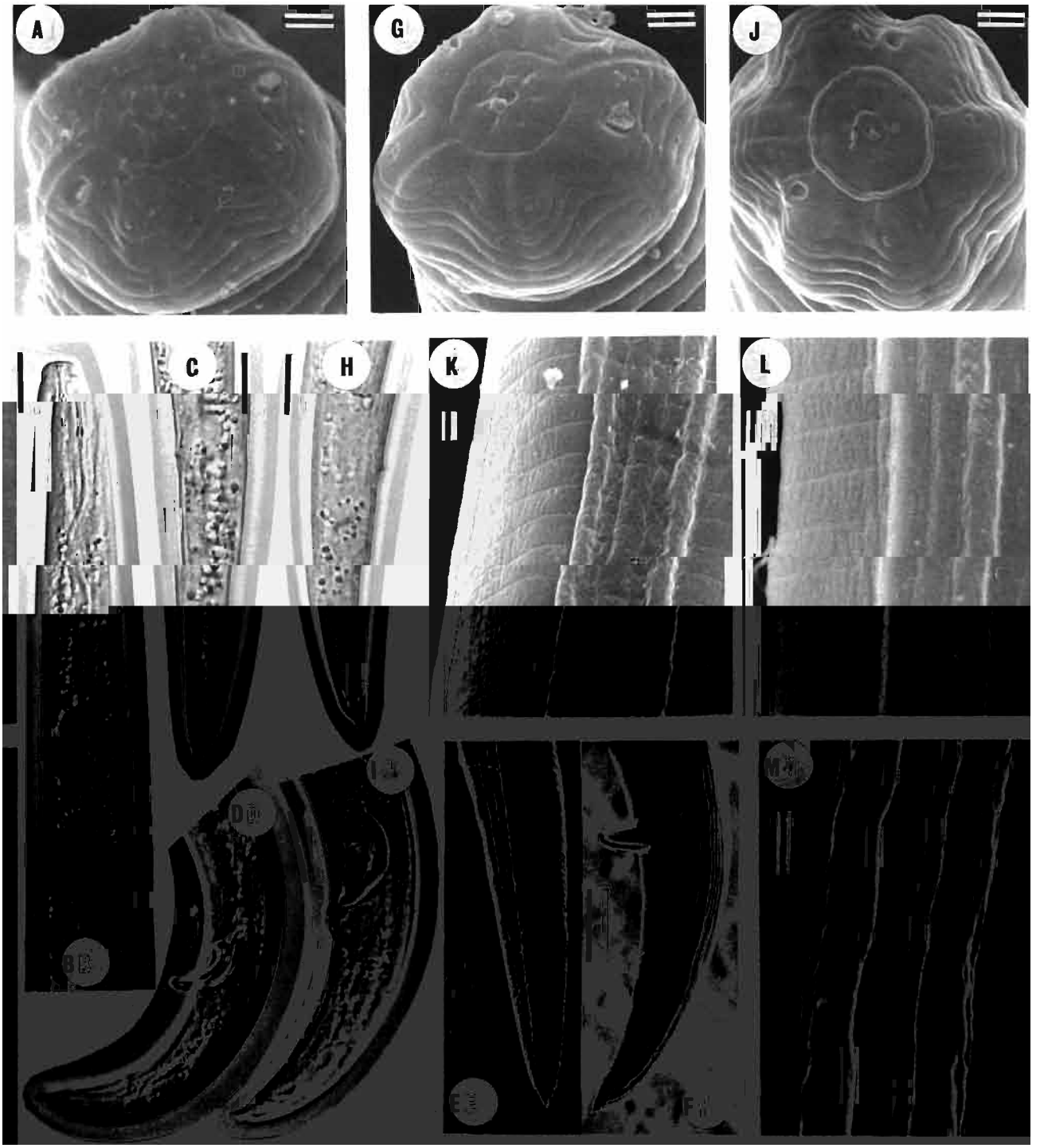

Fig. 2. Aphelenchoides nechaleos *n. sp. A : SEM of female face; B : Female lateral; C : Female tail; D : Male tail; E, F : SEMs of female and male tails.* – A. paranechaleos *n. sp. G : SEM of female face; H : Female tail; I : Male tail.* – A. besseyi : *J : SEM of female face.* – *K-M : SEMs of variations in lateral field as seen in mid body region of females of both* A. nechaleos *n. sp. and* A. paranechaleos *n. sp.* (Scale bars : A, G, J, K-M = 1 µm; B-F, H-I = 10 µm).

Table 2. *Characters differentiating related* Aphelenchoides *species from* A. nechaleos *n. sp.*

A. blastophthorus Franklin, 1952	Longer stylet (15-16 μm) with prominent knobs; larger spicules - dorsal limb 30 μm.
A. brassicae Edward & Misra, 1969	Excretory pore opposite median bulb base; female tail shorter (c′ = 3) with longer mucro; spicules more smoothly curved.
A. haguei Maslen, 1979	Female tail ventrally concave with longer mucro; spicules more smoothly curved with more prominent apex.
A. hamatus Thorne & Malek, 1968 [after Vovlas 1982]	Stylet longer (12-13 μm); female tail ventrally curved with ventral mucro; spicules larger – dorsal limb 26-28 μm.
A. helophilus (de Man, 1880) Goodey 1933 [after Goodey, 1928]	Female body length over 1 mm; stylet 14-16 μm with prominent knobs; spicules smoothly curved.
A. lanceolatus Tandon & Singh, 1974	Lip region continuous; stylet longer (12.5-13 μm); female body fatter (a = 33); shorter post-vulval sac; spicules smoothly curved.
A. lichenicola Siddiqi & Hawksworth, 1982	Female body shorter (L = 610 μm), female tail shorter (c′ = 3.5); spicules characteristically swollen near distal end of dorsal limb.
A. lilium Yokoo, 1964	Excretory pore a body width posterior to nerve ring; longer stylet (12.5 μm); shorter post-vulval sac; female tail ventrally curved; spicules smoothly curved.
A. saprophilus Franklin, 1957	Shorter female body (L = 546 μm); ventrally curved female tail; larger spicules – dorsal limb 23 μm.
A sexlineatus Eroskenko, 1967	Shorter stylet (9 μm); shorter female body (L = 605-645 μm); longer post vulval sac; female tail with longer mucro.
A. submersus Truskova, 1973	Lip region narrower than adjacent body; female tail more curved ventrally; excretory pore anterior to median bulb.
A. suipingensis Feng & Li, 1986	Female with fatter body (a = 32); excretory pore opposite median bulb base; female tail ventrally curved with hair-like mucro.
A. tumulicaudatus Truskova, 1973	Lip region not offset; post vulval sac shorter; female tail with characteristic terminal swelling.

When allowed to dry slowly over 5 days in a dish at room temperature on a clump of cotton wool, a few (c. 1 %) *A. nechaleos* n. sp. adults and juveniles revived on rewetting after being dry for 20 weeks.

In temperature range studies *A. nechaleos* n. sp. reproduced well on *B. cinerea* on agar plates at 22, 25, 30 and 35 °C, somewhat less on plates at 15 and 20 °C and not at all on those at 5, 10 and 38 °C; eggs hatched in 3 days and the generation time, egg to laying of new eggs, was 6-7 days. These nematodes are motionless in water at 5 °C and survive in water at that temperature for several weeks.

Aphelenchoides paranechaleos * n. sp.

(Figs 1 M-R; Fig. 2 G-I, K-M)

MEASUREMENTS

See Table 1.

* In view of the similarity of this species with *A. nechaleos* n. sp., it is called *paranechaleos, para* being Greek for close.

DESCRIPTION

Female : General appearance especially regarding lip region, stylet, oesophagus and excretory pore the same as for *A. nechaleos* n. sp. The reproductive tract is also similar but the post vulval sac is slightly shorter, occupying 34-61 (46 %) of the vulva to anus distance. The conoid tail is straight on specimens killed by heat, the dorsal contour is convex but ventrally it is flat to slightly concave; tail 3.2 to 4 (3.5) anal body widths long ending in a simple mucro which tends to be in line with the ventral contour rather than terminal. The lateral field appears as four evenly spaced lines in the middle third of the body when viewed with the light microscope but four and six lines have been seen with the SEM.

Male : Generally similar to *A. nechaleos* n. sp. Lateral field with four lines. Spicules well developed with a moderate apex and rostrum. The dorsal limb is smoothly curved in its proximal half but is flattened to indented distally and curls ventrally at its terminus. The ventral limb is much thinner than the dorsal limb and often when viewed with the light microscope it appears to end distally some 2 μm from the tip of the dorsal limb (Fig. 1 Q & R, 2 I). A tangent drawn from the apex to the

rostrum usually passes through the tip of the spicules. The tail end curls ventrally through 45 to 90° when killed by heat, the conoid tail is dorsally convex, ventrally concave about three anal body widths long with the usual three pairs of subventral papillae.

TYPE HABITAT AND LOCALITY

From stem tissue of rice plants, local cultivar Chet Som, from the village of An Brih near Can Tho, Hau Giang Province, Vietnam.

TYPE SPECIMENS

Holotype (female) on slide No. 86/31/1 and paratype males and females on slides Nos. 86/31/2-10 in the nematode slide collection of the Entomology and Nematology Department, Rothamsted Experimental Station. Other depositions the same as listed for *A. nechaleos* n. sp.

DIAGNOSIS AND RELATIONSHIPS

Although very similar in appearance, *A. paranechaleos* n. sp. can be separated from *A. nechaleos* n. sp. in the females having a generally shorter tail c' 3.2-4 (3.5) *vs* 3.9-4.6 (4.2), also the tail contour is dorsally convex but flat to concave ventrally (cf Fig. 1E-G with M-O; 2c with 2H) whereas it is usually convex ventrally in *A. nechaleos* n. sp.; the male tail is somewhat shorter, c' 3.1 *vs* 3.6 (cf Fig. 1J with 1P). The curvature of the limbs of the male spicules is greater in *A. paranechaleos* n. sp. so that when the tangent from the apex to the rostrum is projected ventrally it passes through the tip of the spicules whereas in *A. nechaleos* n. sp. it misses the tip by several radial degrees. Although so similar to *A. nechaleos* n. sp., *A. paranechaleos* n. sp. did not interbreed with it on fungal plates; also their biochemical profiles are very different (Ibrahim *et al.* 1994) hence they are considered to be separate species.

In view of their morphological similarity the differences given in the diagnosis and relationships for *A. nechaleos* n. sp. also apply, to some extent, to *A. paranechaleos* n. sp. However, the somewhat shorter tail of *A. paranechaleos* n. sp. makes it closer to *A. brassicae* and *A. lichenicola*. It differs from *A. brassicae* in the more posterior position of the excretory pore, narrower female tail and different shaped spicules and from *A. lichenicola* in a generally longer body, 770 *vs* 610 µm, a simpler tail mucro and the male with different shaped spicules. It differs from *A. haguei* in a generally shorter female tail, c' 3.5 *vs* 4.4, with a shorter mucro and having less smoothly curved spicules with a terminal hook.

BIOLOGY

None of the ten replicates of a pre-adult female of *A. nechaleos* n. sp. with five males of *A. paranechaleos* n. sp. and vice versa gave viable progeny whereas the self matings yielded 9/10 replicates with good populations for *A. nechaleos* n. sp. and 10/10 for *A. paranechaleos* n. sp. This not only indicates that the two species are reproductively isolated but also indicates that their reproduction is obligatory amphimictic. Specimens of *A. paranechaleos* n. sp. swim vigorously in water and can survive slow drying to some extent. They readily reproduce on fungal-agar plates of *B. cinerea* or *R. cerealis*.

DISCUSSION

These two new species are so similar in morphology that it is indeed difficult to separate them using light microscopy. However, they are reproductively isolated and their esterase and protein patterns differ more from each other than do some other recognised *Aphelenchoides* species (Ibrahim *et al.* 1994). To some extent, the proposed separation creates « biochemical species » but the morphological differences between many other *Aphelenchoides* species are so small that the support of biochemical distinction has become desirable. Although there are few biochemical studies on Aphelench nematodes, de Guiran *et al.* (1985) showed that enzyme electrophoresis helped to distinguish closely related species of *Bursaphelenchus*. As reviewed by Williamson (1991), biochemical techniques are now widely used to justify the separation of morphologically similar species in various genera. In view of the vigorous swimming habit of these two new species and their association with rice stems it is possible that in the past they could have been mistaken for *A. besseyi*. However, as indicated earlier, they are readily distinguished morphologically; also they differ biochemically (Ibrahim *et al.*, 1994). Studies (by S.K.I.) are continuing to determine whether these two new species have any effect on the growth of rice plants.

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