

Rhigonema trichocephalum sp. n. (Nematoda : Rhigonematidae), parasite of a millipede (Diplopoda : Spirobolida) from Myanmar

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Summary – A new species of parasite in the Rhigonematidae, *Rhigonema trichocephalum* sp. n., is described from the intestine of an unidentified spirobolid millipede (Diplopoda : Spirobolida) from Myanmar. The new species is characterized by the following combination of characters : female body length of about 6.6 mm; cuticular pilosity restricted to the anterior portion of the body; female genital tract having a long vagina divided into a thick-walled, muscular distal region followed by a thin-walled vaginal chamber, vulval flap present; vaginal diverticulum absent; female tail with a markedly salient posterior anal lip; male spicule tips surrounded by a cuticular membrane; three pairs of post-cloacal papillae located subdorsally or laterally as opposed to subventrally. Scanning electron micrographs of the cervical region and female tail region supplement the description.

Résumé – *Rhigonema trichocephalum* sp. n. (Nematoda : Rhigonematidae) parasite d'un mille-pattes (Diplopoda : Spirobolidae) provenant du Myanmar – Une nouvelle espèce de parasite appartenant aux Rhigonematidae, *Rhigonema trichocephalum* sp. n., est décrite provenant de l'intestin d'un Spirobolide (Diplopoda : Spirobolida) indéterminé récolté au Myanmar. Cette nouvelle espèce est caractérisée par la combinaison de caractères suivante : corps de la femelle long d'environ 6,6 cm; pilosité cuticulaire limitée à la partie antérieure du corps; tractus génital femelle comportant un long vagin divisé en une région distale musculaire à paroi épaisse suivie par une chambre vaginale à paroi mince; volets advulvaires présents; diverticulum vaginal absent; queue de la femelle pourvue d'une lèvre anale postérieure nettement saillante; trois paires de papilles post-cloacales situées subdorsalement ou latéralement, mais non subventralement. Des photographies au microscope électronique à balayage de la région cervicale et de la queue de la femelle complètent la description.

Key-words : Myanmar, *Rhigonema*, SEM, taxonomy.

During June, 1994 a spirobolid millipede was collected by one of us (D. Moore) during an unscheduled roadside halt about 20 km south of Pyay on the main route from Yangon to Pyay, in Myanmar (formerly known as Burma). The diplopod was subsequently killed and fixed in 70 % ethanol, the body being broken in several places to facilitate penetration by the fixative. Dissection of the millipede revealed several dozen specimens, mostly female or juvenile, of a new species of *Rhigonema* Cobb, 1898, together with a few female *Thelastoma* sp., in the lumen of the anterior region of the intestine.

The genus *Rhigonema* contains about 50 nominal species with a predominantly tropical and subtropical distribution, most species being described from South America, Africa, India and the Pacific region. All species are monoxenous gut parasites of diplopods, almost invariably from representatives of the Spirobolida or Spirostreptida, and appear to have had a long evolutionary association with this ancient group of arthropods

(Adamson & Van Waerebeke, 1985). The new species described here clearly belongs to the African/Indian group of species, the arrangement of the male copulatory papillae being typical of this group and distinct from that of the forms from the Americas.

Specimens for study by light microscopy were post-fixed in TAF and then transferred to a 5 % glycerol in water solution and processed to anhydrous glycerol over 4 or 5 days via a slow evaporation technique at 40 °C. Some specimens were used for studies of the *en face* view or dissected in order to elucidate details of the female genital tract structure. Several female specimens destined for scanning electron microscopy were post-fixed overnight in 1 % osmium tetroxide, dehydrated through a graded series of ethanol, critical point dried with CO₂, mounted on stubs and sputter coated with a 750 Å layer of gold. They were examined at an accelerating voltage of 5 or 10 kV. Measurements are given in the form : mean ± standard deviation (range).

***Rhigonema trichocephalum* * sp. n.**
(Figs 1-3)

MEASUREMENTS

Female (n = 10) : L = 6.65 ± 0.47 (5.56-7.23) mm; width = 308 ± 36 (237-335) μm ; oesophagus = 473 ± 16 (432-488) μm ; basal bulb (length \times width) = $128 \pm 6.4 \times 155 \pm 6.7$ μm ; head to vulva = 3.95 ± 0.32 (3.16-4.24) mm; anal body width = 118 ± 8 (107-133) μm ; tail = 260 ± 21 (234-296) μm ; uterine eggs (n = 10) = $82 \pm 3.6 \times 64 \pm 1.9$ μm ; a = 21.6 (19.5-27.5); b = 14.1 (12.0-15.0); c = 25.6 (21.9-29.2); c' = 2.2 (2.0-2.6); V = 59.4 (56.7-61.1).

Male (n = 6) : L = 4.71 ± 0.35 (4.14-5.08) mm; width = 228 ± 17 (208-250) μm ; oesophagus = 426 ± 13.5 (403-445) μm ; basal bulb (length \times width) = $115 \pm 4.9 \times 133 \pm 4.7$ μm ; anal body width = 108 ± 12 (88-120) μm ; tail (n = 5) = 196 ± 20 (163-211) μm ; left spicule (n = 5) = 281 ± 23.6 μm ; right spicule (n = 6) = 286 ± 22.5 μm ; a = 20.7 (19.5-21.7); b = 11.1 (9.7-11.8); c = 24.0 (19.6-35.9); c' = 1.8 (1.6-2.4).

Holotype (female) : L = 6.88 mm; width = 328 μm ; oesophagus = 488 μm ; head to vulva = 4.13 mm; anal body width = 114 μm ; tail = 254 μm ; a = 21.0; b = 14.1; c = 27.1; c' = 2.2; V = 59.9.

DESCRIPTION

Adults : Medium to long nematodes. Cephalic region heavily cuticularized and consisting of a broad cephalic cap followed by a narrower cephalic collar. Cephalic cap circular in *en face* view and bearing four mammilliform papillae, two subdorsal and two subventral with the amphids located laterally at the junction of the cap and the cephalic collar. Cuticle with fine transverse striae, anteriorly pilose with numerous, hair-like spines or microtrichs along the posterior margin of the striae. Pilose region restricted to the anterior part of the body, extending posterior to the cephalic collar for about 1.5 to 2 oesophagus lengths. Microtrichs very short and delicate, but longer (4-5 μm) and denser at the cephalic extremity. Oral opening triradiate, the dorsal and two subventral sectors being of equal development. Three jaw-like structures at the anterior end of the oesophagus, the dorsal and two subventral sectors being of equal development and bearing a number of teeth and cuticular ridges. Oesophagus typical of the genus, comprising a powerful, muscular, cylindrical corpus which is distally expanded; redundant isthmus; powerful basal bulb broader than long and tapering towards the oesophago-intestinal junction. Basal bulb anchored to the body wall by muscles attached to its broadest point and extending

to the body wall. Three cardia extending from the bulb into the intestine; intestinal cells packed with brown granules. Nerve ring encircling the corpus at about its mid-point. Excretory pore just anterior to the corpus/basal bulb junction; excretory duct vesiculate. Nine very prominent brown-tinted arcade cells forming a palisade around the oesophagus just posterior to its distal expansion.

Female : Vulva in the form of a broad, transverse slit covered by a long, flap-like extension of the anterior lip and located in the posterior half of the body. A brownish or amber coloured deposit usually found around the vulval opening and flap. Vagina long, anteriorly directed before flexing posteriorly and comprising a powerful sphincter muscle; a short, muscular, thick-walled *vagina vera* and a longer, thin-walled *vagina uterina* with weaker circular muscles. Vaginal diverticulum absent. Ovejector structure conforming to Type 2 of Adamson (1987). Spermatozoa visible in the vagina of several specimens. Two opposed, reflexed genital tracts, each with its own uterus. Genital tracts replete with numerous (100 or more), subovoid eggs with smooth, thick-walled shells. Contents of the uterine eggs showing no sign of differentiation. Tail of medium length, conoid at first and then tapering evenly to a spicate terminus. Posterior anal lip markedly salient, offset from the rest of the tail and with powerful *dilator ani* muscles attached.

Male : Spicules paired, similar in size and appearance and ventrally arcuate. Dorsal limb thickened and slightly hamate proximally. Each spicule exhibiting cuticular sculpturing on the lamina for a substantial distance prior to the distal tip. Distal tip of each spicule virtually solid cuticle, apparently articulated with the dorsal and ventral shaft elements. Tip enveloped by a velum-like cuticular membrane more strongly developed along the dorsal margin [this feature was also recorded for *R. madecassum* by Van Waerebeke (1984) and appears to be present in the illustration of *R. seychellarum*, although not mentioned in the text by Adamson (1987)]. Spicule retractor muscles attached to the head of each spicule and extending anteriorly for about five cloacal body widths before diverging and attaching to the lateral body walls. Powerful spicule protractor muscles running to the subventral walls of the tail. Twenty three copulatory papillae disposed thus : four pre-cloacal pairs (the posteriormost of which being more prominent and closer together than the others) with a single ventromedian papilla located slightly more posterior on the anterior cloacal lip; seven post-cloacal pairs comprising four subventral pairs, one lateral or sublateral pair and two subdorsal pairs. Caudal alae absent. Tail short, conoid, dorsally convex and usually with a ventrally located spike about 42 ± 14.2 μm long (completely absent in one specimen, the tail terminus being irregularly rounded). Thin protoplasmic core extending into the tail spike.

* Derived from the Greek words *trichodes* and *peplos*, meaning hairy cloak or robe and referring to the pilose appearance of the worms.

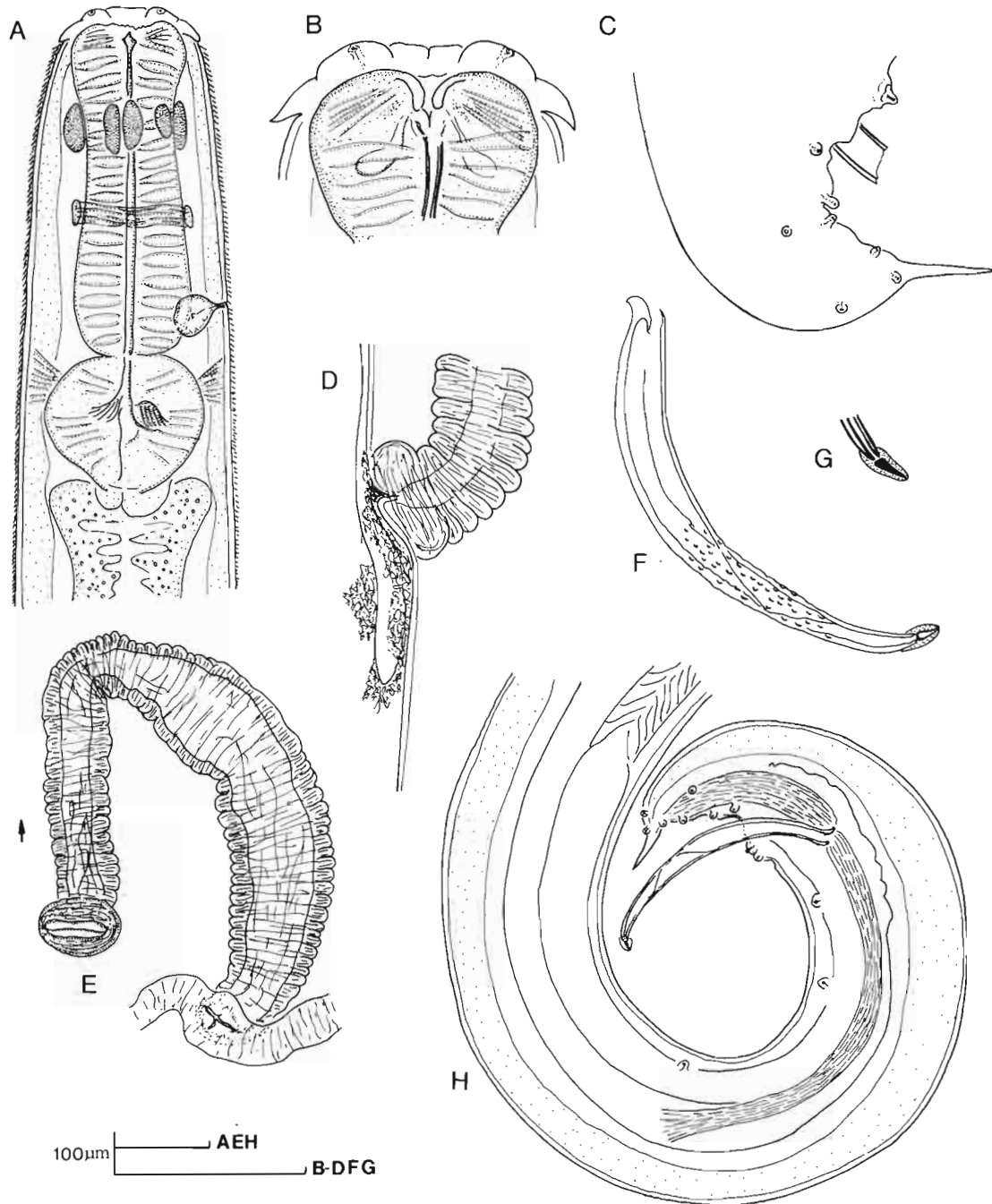


Fig. 1. *Rhigonema trichocephalum* sp. n. A: Female oesophagus; B: Cephalic region; C, H: Male tail; D: Vulval region; E: Vaginal structure; F: Spicule; G: Spicule tip and velum.

TYPE HOST AND LOCALITY

Posterior gut of an unidentified spirobolid millipede about 12 cm long collected by the roadside about 20 km south of Pyay on the Pyay/Yangon highway, Myanmar (formerly known as Burma), in June, 1994 by Dr. D. Moore.

TYPE MATERIAL

Holotype female, seven paratype females and four paratype males in the type collection of the International Institute of Parasitology, St Albans, Herts, UK (slide numbers T504/4/1 to T504/4/8); two paratype females and two paratype males in the type collection of the

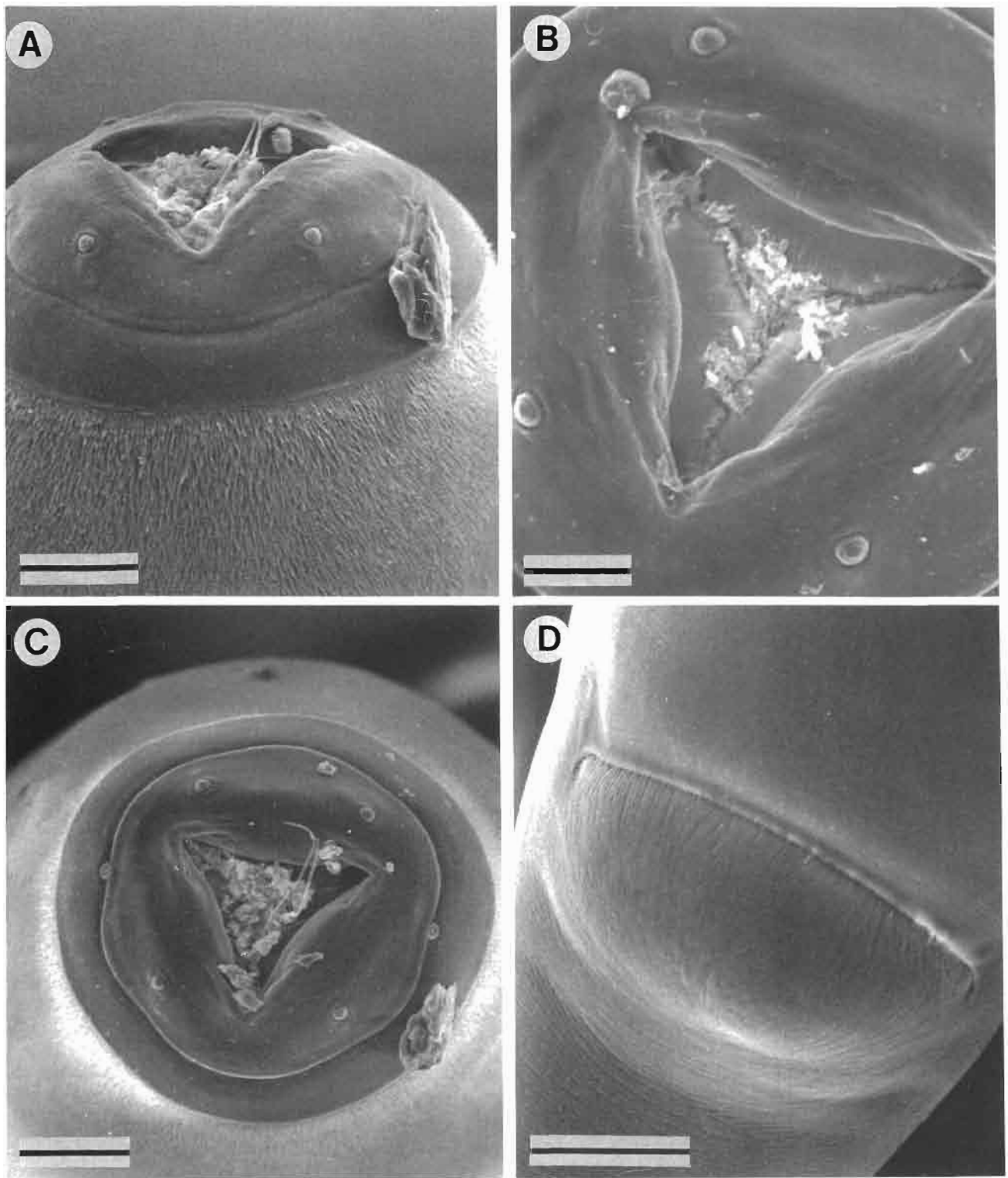


Fig. 2. *Rhigonema trichopeplum* sp. n. : SEM studies of female. A : Cephalic region; B : Oral cavity; C : En face; D : Salient posterior anal lip. (Scale bar : A, C = 20 μ m; B = 10 μ m; D = 35 μ m.)

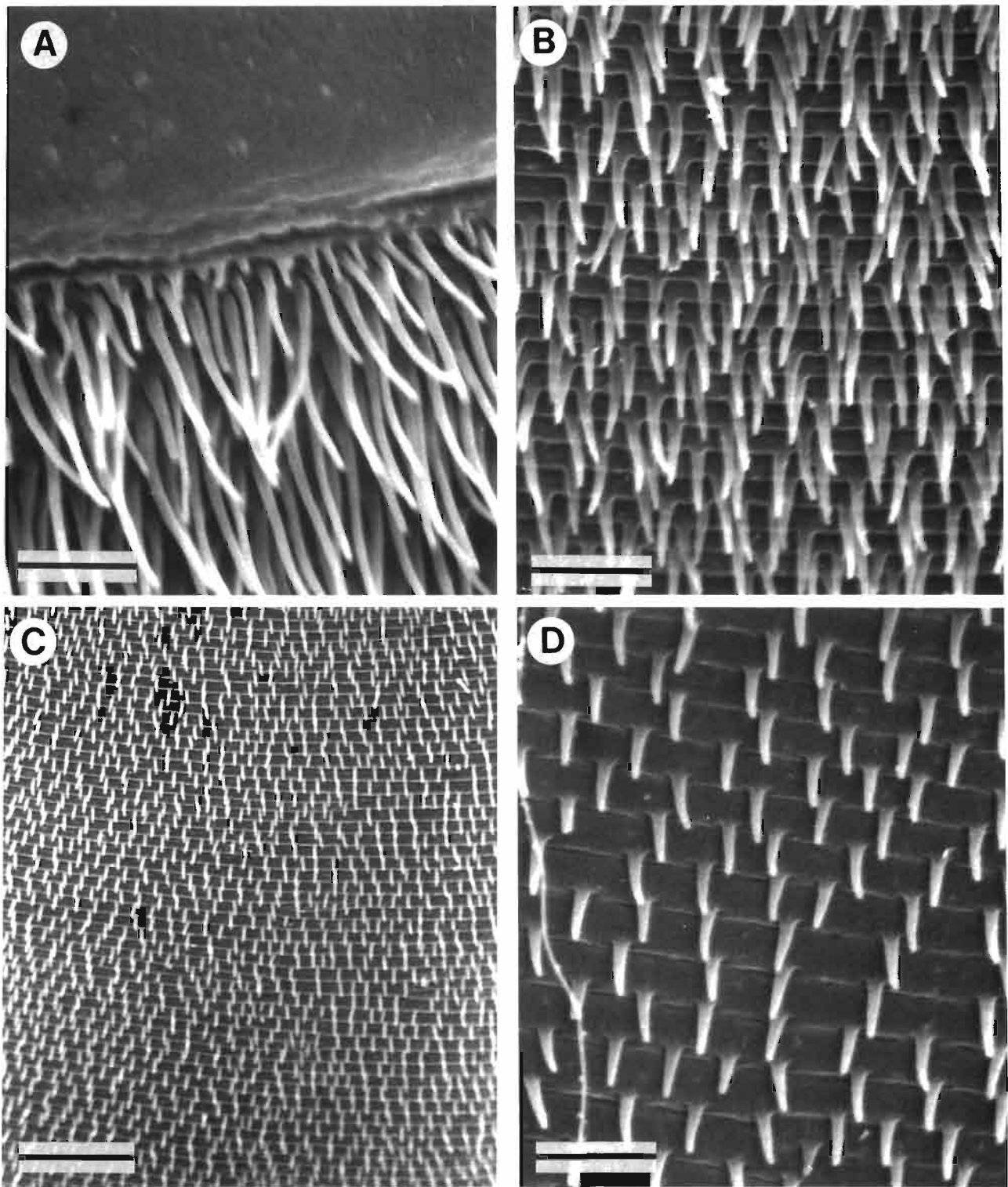


Fig. 3. *Rhigonema trichopeplum* sp. n. : SEM studies of female microtrichs. A : Cephalic collar region; B : Mid-oesophageal region; C, D : Just anterior to the point where the pilose region terminates. (Scale bar : A, B, D = 2 μ m; C = 8 μ m.)

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DIAGNOSIS AND RELATIONSHIPS

Rhigonema trichocephalum sp. n. is characterized by the following combination of characters: male and female body length of about 4.7 mm and 6.6 mm respectively; very short cuticular pilosity restricted to the anterior-most portion of the body; vulva covered by an extensive flap; female genital tract having a long vagina divided into a thick-walled, muscular distal region followed by a thin-walled vaginal sac; vaginal diverticulum absent; female tail with a salient posterior anal lip; male spicules similar, about 285 μm long with a small terminal velum; three pairs of post-cloacal papillae located sublaterally to subdorsally.

Rhigonema trichocephalum sp. n., in having a long vagina differentiated into two parts and in lacking a vaginal diverticulum, belongs to the Type 2 ovejector group proposed by Adamson (1987). In addition, the males have three pairs of the post-cloacal papillae located either subdorsal, sublateral or lateral in position, a feature found exclusively in those nominal species from the African and Indian regions (in the species from the Americas, all the post-cloacal papillae are subventral). Taking these features in combination, the following species need to be compared to *Rhigonema trichocephalum* sp. n.: *R. cristatai* (Gupta & Kumar, 1975) Van Waerebeke, 1984; *R. disparovis* Van Waerebeke, 1991; *R. erringtoni* Van Waerebeke, 1986; *R. madecassum* Van Waerebeke, 1984; *R. neyrae* Singh, 1955; *R. pachybolii* Adamson, 1983; *R. raoi* Adamson, 1983; *R. seychellarum* Adamson, 1987. Of these, the closest species appear to be *R. madecassum* from Madagascar and *R. pachybolii* from Gabon.

R. trichocephalum sp. n. differs from *R. seychellarum* by: tail shape in both sexes (conoid-spicate *vs* conoid); female possessing a salient posterior anal lip; smaller eggs (82 \times 64 *vs* 103 \times 81 μm); the disposition of the male pre-cloacal papillae. From *R. erringtoni* by: longer body in both sexes (female 5.56-7.23 *vs* 4.27-5.17 mm, male 4.14-5.08 mm *vs* 3.31-3.60 mm); tail size and shape in both sexes (short conoid-spicate *vs* much longer with a medium conoid anterior section followed by a very long spicate portion); vulval flap (present *vs* absent). From *R. disparovis* by: uterine eggs all of one size *vs* two distinct sizes; vulval flap (present *vs* absent); fewer male copulatory papillae (23 *vs* 27); shorter spicules (260-325 *vs* 389-458 μm); no fine spines in the anterior buccal region. From *R. madecassum* by: shorter body (female 5.56-7.23 *vs* 7.68-8.38 mm, male 4.14-5.08 *vs* 6.68-7.51 mm); vulval flap (present *vs* absent); female tail (conoid-spicate *vs* conoid); shorter, more equally sized, spicules. From *R. neyrae* by: shorter body length (female 5.56-7.23 *vs* 7.8-9.5 mm, male 4.14-5.08 mm *vs* 5.2-6.9 mm); vulval flap (present *vs* absent); male tail

shape. From *R. pachybolii* by: male tail shape (conoid-spicate *vs* conoid); spicule tips surrounded by a velum; spicules sculptured in appearance as opposed to smooth; longer female tail (234-296 *vs* 165-179 μm). *R. cristatai* is herein regarded as *species inquirenda* as the original description is inadequate, lacking in too many essential parameters to allow detailed comparison, yet appears to differ in lacking the prominent vulval flap found in *R. trichocephalum* sp. n.; Gupta and Kumar (1975) mentioning only a "... retrose, somewhat papilliforme anterior lip" and illustrating a slightly backwardly directed open vulva with no flap. *R. raoi* reportedly has a vagina which is entirely directed posteriorly and the tail is differently shaped, being conoid and lacking a spicate terminus.

Acknowledgements

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References

- ADAMSON, M. L. (1983). *Obainia gabonensis* n. gen., n. sp. and *Rhigonema pachybolii* n. sp. (Rhigonematidae; Nematoda) from *Pachybolus* sp. (Pachybolidae; Spirobolida; Diplopoda; Myriapoda) in Gabon. *Bull. Muséum nat. Hist. nat.*, 4^e sér., 5: 531-542.
- ADAMSON, M. L. (1987). Rhigonematid (Rhigonematida: Nematoda) parasites of *Scaphiostreptus seychellarum* (Spirostreptida; Diplopoda) in the Seychelles with comments on the ovejector structure in *Rhigonema* Cobb, 1898. *Can. J. Zool.*, 65: 1889-1897.
- ADAMSON, M. L. & VAN WAEREBEKE, D. (1985). The Rhigonematida (Nematoda) of diplopods: reclassification and its cladistic representation. *Annals Parasit. hum. comp.*, 60: 685-702.
- GUPTA, S. P. & KUMAR, P. (1975). On a new avian nematode, *Dudekemia cristatai* sp. nov. from a crested lark *Galerida cristata* (Linnaeus) from Lucknow. *Curr. Sci.*, 44: 861-862.
- SINGH, K. S. (1955). Two new species of nematodes from a millipede from India. *Revta ibér. parasit.*, Tomo Extraordinario: 35-44.
- VAN WAEREBEKE, D. (1984). *Rhigonema madecassum* n. sp. (Rhigonematidae: Nematoda) parasite de Diplopode à Madagascar: description et étude de la spermiogénèse. *Revue Nématol.*, 7: 267-282.
- VAN WAEREBEKE, D. (1986). *Rhigonema erringtoni* n. sp. (Rhigonematidae: Nematoda) parasite de Sphaeroteriüda (Diplopoda) de la presqu'île de Malacca. *Revue Nématol.*, 9: 115-117.
- VAN WAEREBEKE, D. (1991). *Rhigonema disparovis* n. sp. (Rhigonematidae, Nematoda) parasite de *Pachybolus laminatus* (Diplopoda) en Côte d'Ivoire. *Revue Nématol.*, 14: 95-100.