

# *Nothocriconema shepherdae* n. sp. (Nematoda: Criconematidae) with observations on extracuticular layer formation

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## SUMMARY

*Nothocriconema shepherdae*, n. sp., closely related to *N. lamellatum* (Raski & Golden, 1966) De Grisse, 1967 is described from soil associated with herbaceous plants from Kenya. Females are 0.42-0.51 mm long with 48-56 body annules; stylet 72-81  $\mu\text{m}$  long; vulva on 5th or 6th annule from bluntly rounded terminus. Males have crenate bursa, slightly curved spicules 35-40  $\mu\text{m}$  long and three incisures in the lateral fields. Juvenile has 74 annules, ten longitudinal rows of scales bearing sharply pointed spines, stylet 48  $\mu\text{m}$  long and bluntly conoid tail. Entire surface of female appears covered with minute refractive dots when viewed with an oil immersion objective. Scanning electron microscopy showed that this appearance is due to an extracuticular incrustation which is mostly fragmented into minute polygonal pieces reminiscent of the subcrystalline layer of *Heterodera* spp. Studies of type material of the two species of *Amphisbaenema* Orton Williams, 1982, *Merocriconema braziliense* Raski & Pinochet, 1975 and *Cerchnotocriconema psephinum* Bernard, 1982 indicate that surface markings on those species are similar in nature to the extracuticular layer observed in *N. shepherdae* n. sp. The relationships of the genera *Amphisbaenema*, *Cerchnotocriconema* Bernard, 1982, *Merocriconema* Raski & Pinochet, 1975, *Nothocriconema* De Grisse & Loof, 1965 and *Paracriconema* Ebsary, 1981 are briefly discussed. These nominal genera are considered to be closely related but more extensive study is needed before their status can be fully assessed.

## RÉSUMÉ

*Nothocriconema shepherdae* n. sp. (Nematoda: Criconematidae)  
et observations sur une formation lamellaire extracuticulaire

Les auteurs donnent la description de *Nothocriconema shepherdae* n. sp., provenant de la rhizosphère de graminées du Kenya. Cette nouvelle espèce, proche de *N. lamellatum* (Raski & Golden, 1966) De Grisse, 1967 a les caractéristiques suivantes : femelles L = 0,42-0,51 mm ; R = 48-56 ; stylet = 72-81  $\mu\text{m}$  ; RV = 5-6 ; extrémité postérieure arrondie, tronquée ; mâles : bursa crénelée, spicules légèrement courbés et longs de 35-40  $\mu\text{m}$  ; champ latéral à trois incisures ; juvéniles : R = 74 ; dix rangées longitudinales d'écaillés cuticulaires portant des épines acérées ; stylet : 48  $\mu\text{m}$  ; queue conoïde-tronquée. En microscopie à lumière transmise (objectif à immersion), la surface entière des femelles apparaît couverte de petites taches réfringentes. La microscopie électronique à balayage a montré que cet aspect est dû à une sorte de croûte extracuticulaire, en général fragmentée en petites pièces polygonales, rappelant la couche subcrystalline des *Heterodera*. L'étude du matériel type des deux espèces d'*Amphisbaenema* Orton Williams, 1982, de *Merocriconema braziliense* Raski & Pinochet, 1975 et de *Cerchnotocriconema psephinum* Bernard, 1982 a montré que les ornements cuticulaires superficielles de ces espèces sont de nature semblable à la couche extracuticulaire observée chez *N. shepherdae* n. sp. Les relations entre les genres *Amphisbaenema*, *Cerchnotocriconema* Bernard, 1982, *Merocriconema* Raski & Pinochet, 1975, *Nothocriconema* De Grisse & Loof, 1965 et *Paracriconema* Ebsary, 1981 sont brièvement discutées. Ces genres sont considérés comme étroitement apparentés mais des observations supplémentaires sont nécessaires avant que leur statut ne puisse être fermement établi.

Six females, two males and a juvenile of a criconematid nematode were found in soil associated with the roots of herbaceous native plants from Mount Kenya, Kenya, which was sent to the Harpenden Laboratory of ADAS from the Royal Botanic Gardens, Kew. Microscopic examination showed that the entire body surface of all the females was covered with minute refractive dot-like structures giving the body a 'punctate' appearance. *Nothocriconema shepherdae* n. sp. is described below.

## Material and methods

All specimens were fixed in TAF. For Scanning Electron Microscopy one female was dehydrated by 30 min immersion in each of 30%, 50%, 70%, 90% and two changes of absolute ethanol. The alcohol was then removed in a critical-point dryer, and the specimen mounted on a gum-coated stub and sputter-coated with a 750Å layer of gold. It was then examined under an ISI-60 SEM at the Commonwealth

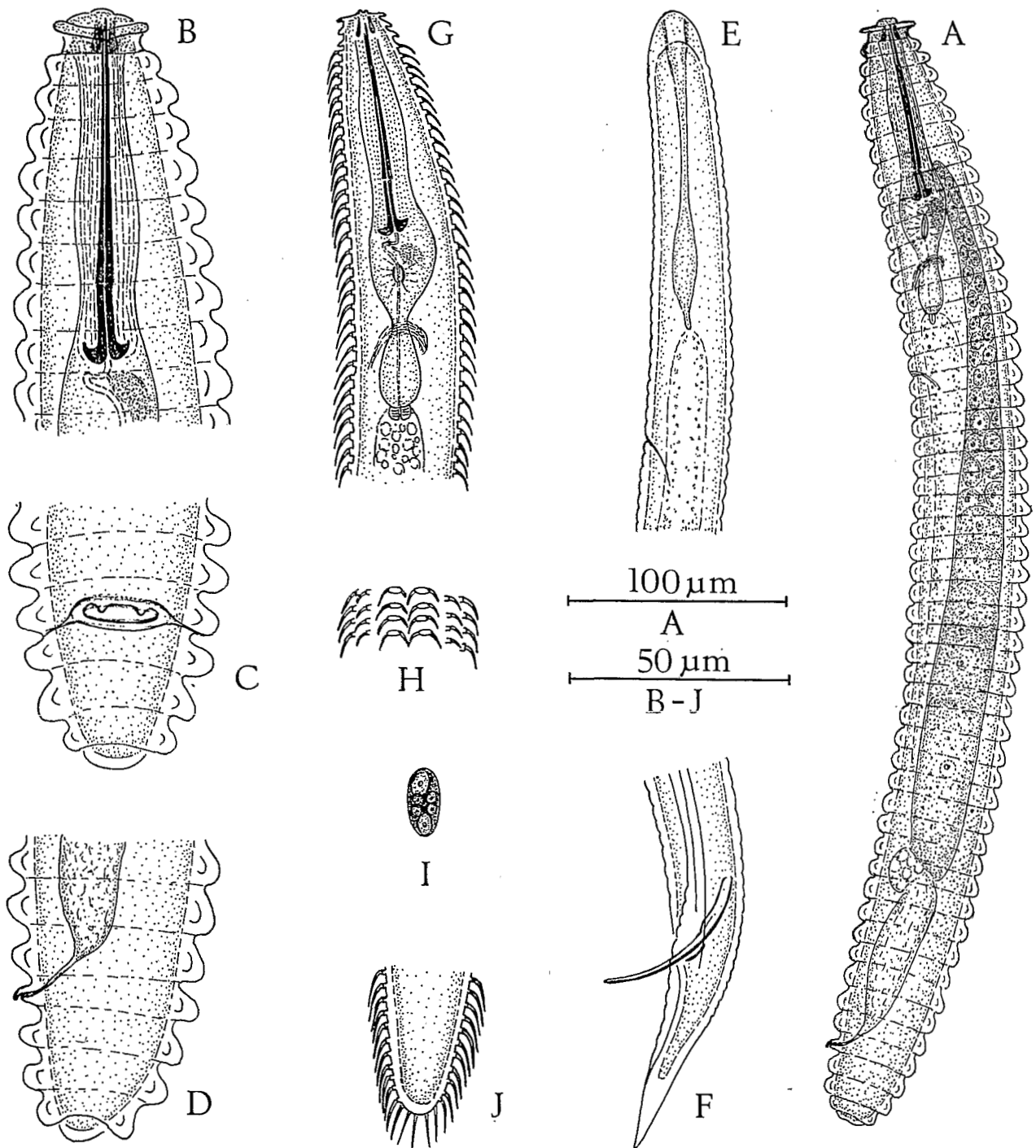


Fig. 1. *Nothocriconema shepherdae* n. sp. Female. A : entire animal ; B : Anterior end ; C : Tail end, ventral ; D : Tail end, lateral. Male. E : Oesophageal region ; F : Tail end. Juvenile. G : Oesophageal region ; H : Mid-body annules ; I : Genital primordium ; J : Tail end.

Mycological Institute, Kew, and a HITACHI S-450 SEM at Rothamsted Experimental Station, Harpenden, U.K. Accelerating voltage was 20 kv in both cases.

Material for optical microscopy was processed to anhydrous glycerin by the slow method.

**Nothocriconema shepherdae** \* n. sp.

(Figs 1-3)

DIMENSIONS

*Female* (paratypes; n = 5) : L = 0.42-0.51 mm ; a = 8.7-9.6 ; b = 3.3-3.9 ; c = ? ; V = 92-93 ; stylet = 72-81  $\mu\text{m}$  ; R = 48-56 ; Rst = 9-11 ; Roes = 13-17 ; Rex = 17-20 ; RV = 5-6.

*Male* (paratypes; n = 2) : L = 0.33-0.34 mm ; a = 17-18 ; b = 5.1-5.7 ; c = 7.7-8.3 ; spicules = 35-40  $\mu\text{m}$  ; gubernaculum = 5-6  $\mu\text{m}$ .

*Juvenile* (paratype; n = 1) : L = 0.23 mm ; a = 7.3 ; b = 2.7 ; c = ? ; stylet = 48  $\mu\text{m}$  ; R = 74.

*Holotype* (female) : L = 0.48 mm ; a = 8 ; b = 3.7 ; c = ? ; V = 93 ; stylet = 78  $\mu\text{m}$  ; R = 53 ; Rst = 9 ; Roes = 15 ; Rex = 18 ; RV = 5.

DESCRIPTION

*Female* : Body short and stout, slightly ventrally curved upon killing, tapering to bluntly rounded extremities. Entire body surface covered with innumerable dot-like protuberances giving it a punctate appearance. These dots represent an extra-cuticular layer which is described below in detail (Figs 2, 3). Cephalic region has one annule 21-24  $\mu\text{m}$  wide, rounded but dorso-ventrally indented, directed anteriorly, well marked off from succeeding body annules which are rounded to slightly retrorse. Submedian lobes absent. First body annule 22-27  $\mu\text{m}$  wide, second 28-34  $\mu\text{m}$ , third 33-37  $\mu\text{m}$ , fourth 36-45  $\mu\text{m}$  ; annules in midbody 45-56  $\mu\text{m}$  wide, transverse striae 10-11  $\mu\text{m}$  apart. Margins of annules appear finely crenate due to protuberances of extracuticular layer. Detritus also present. Anastomoses of annules absent except in one paratype where several annules of posterior region of body are involved. Cuticle about 3  $\mu\text{m}$  thick. Stylet about 16% or 1/6th of body length, conus 55-63  $\mu\text{m}$  long or about 76% of stylet length, basal knobs 10-11  $\mu\text{m}$  across. Excretory pore post-oesophageal, 139-173  $\mu\text{m}$  from anterior extremity.

\* After Dr. Audrey M. Shepherd in recognition of the help received from her and other colleagues at Rothamsted Experimental Station in the study of the extracuticular structures in this species.

Vulva with overlapping anterior lips which may be poorly developed or prominent. Gonad large extending into oesophageal region. Spermatheca offset, non-axial. Anus not seen. Tail terminus bluntly rounded.

*Male* : Body curved ventrally upon killing. Cephalic region not set off, bluntly conoid and with indistinct annules. Oesophagus degenerate. Excretory pore post-oesophageal, 90  $\mu\text{m}$  from anterior extremity. Lateral fields 3-4  $\mu\text{m}$  wide, marked with three incisions. Spicules slightly ventrally curved, almost one tail length long. Bursa crenate, extending almost to tail tip. Tail 41-43  $\mu\text{m}$  long, elongate-conoid.

*Juvenile* : Genital primordium indicates that it is the second stage. Entire body covered with spines borne on ten longitudinal rows of scales. Each scale has two sharply pointed backwardly directed spines on each side. Annules 3-4  $\mu\text{m}$  wide in mid-body. Oesophagus extending to 30 annules, stylet to 18th. Conus 35  $\mu\text{m}$  long, basal knobs 7  $\mu\text{m}$  across. Tail terminus bluntly conoid. Excretory pore and anus indistinct.

TYPE HABITAT AND LOCALITY

Soil around roots of assorted native herbaceous plants from Mount Kenya, Kenya. Material collected in April 1981.

TYPE SPECIMENS

Holotype female, two paratype females, two paratype males and one paratype juvenile deposited in the nematode collection of the Commonwealth Institute of Parasitology, St. Albans ; one paratype female at Rothamsted Experimental Station, Harpenden and another paratype female at ADAS, Harpenden Laboratory, Hatching Green, Harpenden, UK.

DIAGNOSIS

*Nothocriconema* with short, plump body, body surface of female covered with minute dot-like extracuticular layer, R = 48-56, stylet 72-81  $\mu\text{m}$  long, RV = 5-6, vulva with overlapping lips ; males with bursa and three lines in the lateral fields ; and juvenile with spines borne on ten longitudinal rows of scales.

RELATIONSHIP

*Nothocriconema shepherdae* n. sp. is closely related to *N. lamellatum* (Raski & Golden, 1966) De Grisse, 1967 but differs from it in having the first annule dorso-ventrally indented, on the absence of longitudinal markings ('scratches') on the annules and in the presence of numerous dot-like protuberances representing an extracuticular layer (first annule not

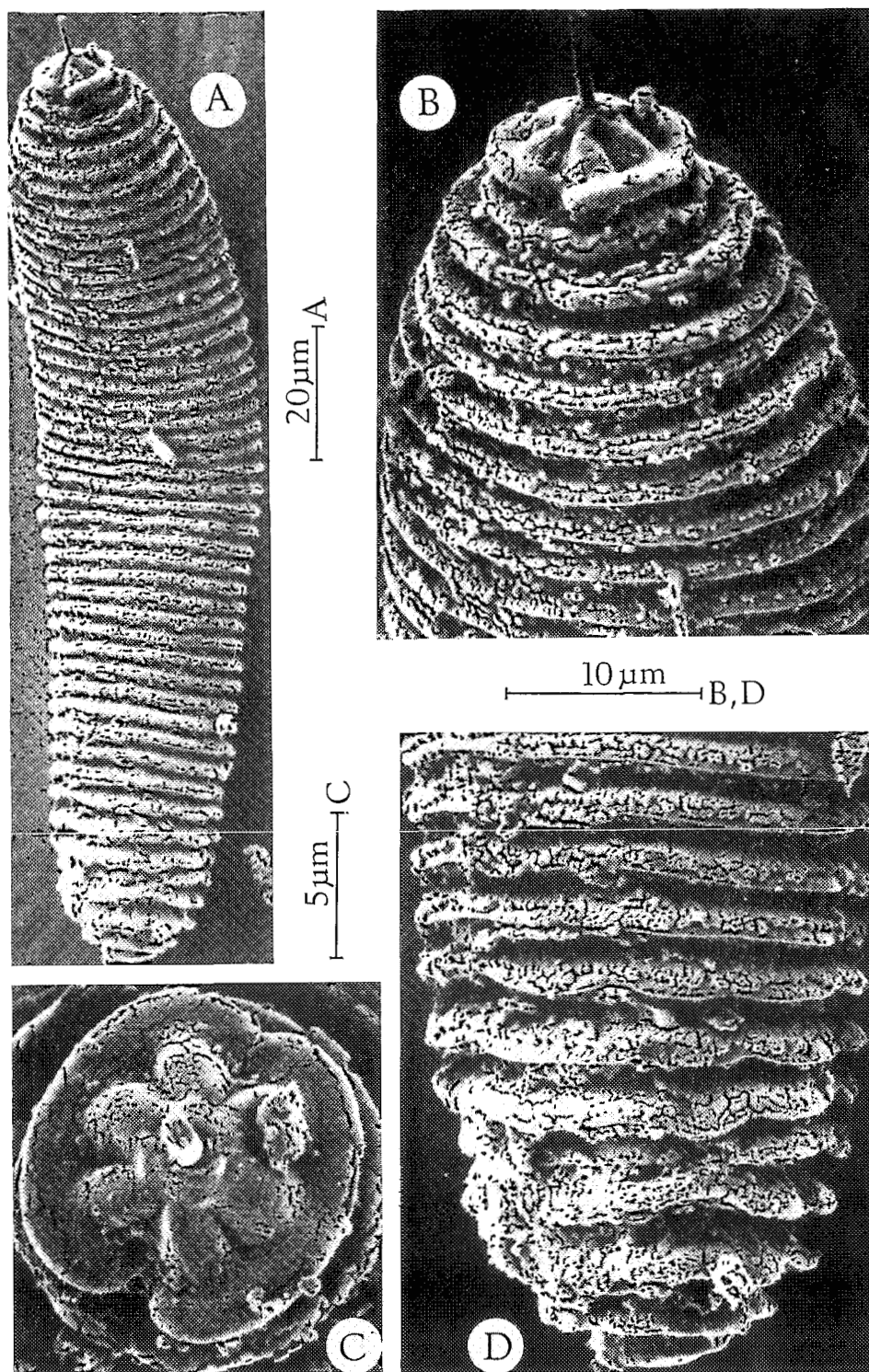


Fig. 2. *Nothocriconema shepherdae* n. sp. Female. A : Entire animal ; B : Anterior end ; C : *En face* view ; D : Tail end.

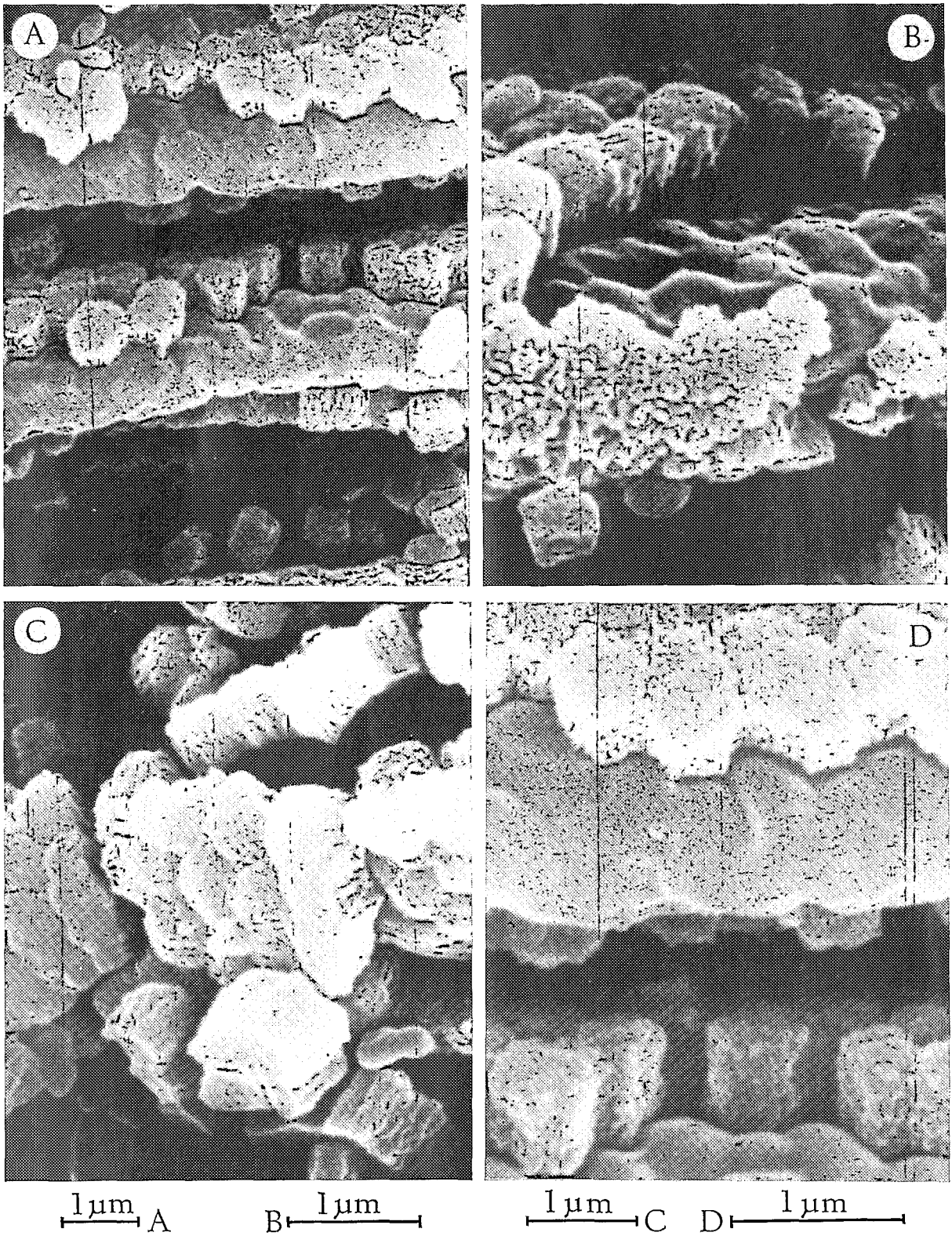


Fig. 3. *Nothocriconema shepherdae* n. sp. A-D : SEM showing the extracuticular layer (platelets) and the body surface of the female at different magnifications.



indented, longitudinal markings present on the surface of annules and extracuticular layer absent in *N. lamellatum*).

### Study of extracuticular structures

The surface of one female *N. shepherdae* has been examined at various magnifications under the scanning electron microscope (Figs 2, 3). The micrographs show that the dot-like or punctate appearance observed under the light microscope is due to an extracuticular incrustation, covering the entire body surface, which is cracked and fragmented into minute polygonal pieces of more or less uniform size. This extracuticular layer is strikingly reminiscent of the subcrystalline layer (SCL) of certain *Heterodera* species, whose nature was elucidated by Brown *et al.* (1971). The layer on *N. shepherdae* n. sp. females, however, differs from the SCL of *Heterodera* in several respects: *i*) the polygonal fragmentae are of a much smaller order of magnitude (about 45-120  $\mu\text{m}$  in *Heterodera* spp. and about 0.5-1  $\mu\text{m}$  in *N. shepherdae* n. sp.; *ii*) they appear to be composed of subspherical granules which tend to be aligned in rows perpendicular to the cuticle surface, whereas in *Heterodera* the SCL consists of convoluted laminae or flakes, also typically (e.g. in *H. avenae*) perpendicular to the surface; *iii*) in several *Heterodera* spp. the SCL has been shown to have two sub-layers but no such subdivision has been observed in *N. shepherdae*.

Brown *et al.* (1971) showed that the SCL in *Heterodera* consisted of a wax-like material mainly tetracosanoic acid, apparently associated with a yeast-like fungus on the cuticle surface of the female and postulated that the fungus converts excess sugars excreted by the nematode to long-chain fatty acids which are in turn partially converted to the calcium salt, probably by soil calcium.

With the material available it was not possible to determine the chemical nature of the extracuticular layer in *N. shepherdae*. However, the SE micrographs do not show any clear evidence of fungi or other organisms beneath the layer but what appear to be scattered bacterial cells on the outer surface are visible in some of them (Fig. 3).

Raski and Golden (1966) have described the posterior edges of the annules of *Criconemoides* (= *Nothocriconema*) *lamellatum* as having block-like platelets formed from a brittle layer on the cuticle. The authors were not sure of the exact nature of these platelets and thought they were either broken away pieces of the cuticle or a separate deposit outside the cuticle. Study of a paratype female of this species did not show structures similar to those found in *N. shepherdae*. On *N. lamellatum* the appearance of the posterior

margins of the annules appears to be due to the 'wrinkles' or 'scratches' of the cuticle seen in profile. Scanning electron microscopy would be needed to determine the exact nature of these markings.

Raski and Pinochet (1975) figured, but did not describe, plate-like markings on the annules of *Mero-criconema braziliensis* Raski & Pinochet, 1975. Recently, Orton Williams (1982) has proposed a new genus, *Amphisbaenema*, based on two new species of criconematids having the 'outer layer' of cuticle broken up into numerous platelets of varying sizes and shapes. This feature of the cuticle was regarded by him as the chief diagnostic character of the genus because it was considered unique in Criconematidae. The author also transferred *Nothocriconema lamellatum* to *Amphisbaenema* but he was not aware that this species had already been made type of the genus *Paracriconema* Ebsary, 1981. As mentioned above, the so-called 'platelets' reported in *N. lamellatum* are most likely features of the cuticle itself. However, examination of paratypes of *Amphisbaenema paradoxiger* Orton Williams, 1982 and *A. amicum* Orton Williams, 1982 indicates that the platelets described by Orton Williams are similar in nature to those now described in *Nothocriconema shepherdae* apart from the differences in size and possibly shape; SEM is needed to confirm this conclusion. Under oil immersion objective in the light microscope the dots or platelets appear very small and numerous on the body surface of *N. shepherdae*, slightly larger and clearly polygonal on *A. paradoxiger*, and fewer but relatively much larger and more plate-like on *A. amicum*, giving the surface of the latter a reticulate appearance. The paratype female of *Mero-criconema braziliensis* has been examined and shows extracuticular platelets of almost the same size, number and arrangement as *A. amicum*. Bernard (1982) described and illustrated irregular plates on anterior and posterior edges of the annules of adult females of *Cerchnotocriconema psephinum* Bernard, 1982. Examination of paratypes confirmed their presence but SEM would be needed to determine whether they are comparable to the structures on *N. shepherdae*.

### Discussion

In *Amphisbaenema paradoxiger* the labial annule is small, merges with the lip region and because it is coated with extracuticular layer, appears almost spherical. In the shape of the lip region, *N. shepherdae* is more closely related to *N. lamellatum* but in the formation of an extracuticular layer it is more like *Amphisbaenema paradoxiger*. *Amphisbaenema amicum* has a slightly differently shaped lip region and larger plates of extracuticular material in both of

which features it is strikingly similar to *M. braziliensis*. Our study has shown that these structures, which appear as platelets or punctate markings under the light microscope, are not part of the body of the nematode, therefore much taxonomic importance cannot be attached to them. This makes the validity of the genus *Amphisbaenema* questionable. The taxonomy of criconematid nematodes is at present in a state of flux (cf. Luc & Raski, 1981). The five species examined by us : *Nothocriconema shepherdae*, *Paracriconema lamellatum*, *Amphisbaenema paradoxiger*, *A. amicorum* and *Merocriconema braziliensis* show many similarities, for example, small size with few annules, simple oval slit-like vulva, bluntly rounded female tail, and similar males and juveniles, if known. However, there are differences in the shape of the lip region (spherical, sloping backwards or slightly anteriorly directed) and in the nature of the annules (smooth, crenate or with cuticular outgrowths). *Cerchnotocriconema psephinum* is also somewhat similar to the above species but the female tail is conical pointed and males are unknown. *P. lamellatum* is the only species which does not appear to have extra-cuticular structures, but otherwise it seems morphologically very close to *N. shepherdae* and *A. paradoxiger*. *A. amicorum* and *M. braziliensis* have almost the same type of lip region and extracuticular structures but differ in the absence of cuticular outgrowths on the annules of the former and their presence on the latter. The above similarities indicate that *Paracriconema*, *Amphisbaenema*, *Cerchnotocriconema* and *Merocriconema* are closely related to *Nothocriconema* as defined by Andr assy (1979).

Virtually nothing is known of the nature and origin of extracuticular structures in criconematids and little of their range of occurrence and diversity of form. Since they are found in six species in five genera the significance of such formations in the taxonomy and relationships of these nematodes needs further study and the status of these genera needs critical re-appraisal.

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