

Surface features in the taxonomy of *Pratylenchus* species

Donald C.M. CORBETT and Sybil A. CLARK

*Agricultural Research Council, 160 Great Portland Street, London,
and Rothamsted Experimental Station, Harpenden, Herts.*

SUMMARY

Surface features of eighteen species of *Pratylenchus* were examined by scanning electron microscopy. Head shape and annulation were found to be good taxonomic characters, and the species were separable into three groups by the pattern formed by the first head annule and oral disc (the « face »). The lateral field was found to be too variable for distinguishing species as were the depth and distance apart of transverse striae, except in one or two species. Shape of female tail and striations around the tail tip were stable, and helped to distinguish between species, but number of annules on the ventral surface of the female tail was similar for nearly all species examined.

RÉSUMÉ

Les caractères de la cuticule dans la taxonomie des espèces de Pratylenchus

Les caractères cuticulaires de dix-huit espèces de *Pratylenchus* ont été examinés au microscope électronique à balayage. Il a été observé que la forme et l'annélation de la tête étaient de bons caractères taxonomiques et les espèces peuvent être séparées en trois groupes d'après la configuration du premier anneau de la tête et du disque labial (la « face »). Excepté dans un ou deux cas, les champs latéraux, ainsi que la profondeur et l'écartement des stries transverses, étaient trop variables pour caractériser les espèces. La forme de la queue chez les femelles et les striations qui entourent l'extrémité de la queue sont stables et peuvent être utilisées pour différencier les espèces ; mais le nombre d'anneaux sur la face ventrale de la queue de la femelle était le même chez presque toutes les espèces examinées.

Loof (1978) in the most recent comprehensive review of *Pratylenchus* lists twelve diagnostic characters that have proved reliable for distinguishing species. Of these six are surface features and therefore amenable to investigation with the scanning electron microscope (SEM). These are : number of lip region annules, shape of lip region, depth and distance of transverse striae, structure of lateral field, shape of female tail and number of tail annules in the female. Corbett and Clark (1973) were the first to study the front surface of the head (or 'face') in *Pratylenchus* which can be seen only with the SEM but since their report this feature has not been used systematically, although one or two species have been illustrated. We bring together and examine for eighteen species (Tab. 1) the surface features that we think are most useful in distinguishing between *Pratylenchus* species.

Materials and methods

Most of the nematodes examined in our study were cultured axenically on excised maize roots in agar (Mountain, 1955) or on lucerne callus in agar (Krusberg & Blickenstaff, 1964). Many of the populations were derived from single females, but in one or two cases the specimens collected from field samples were used.

For examination under the SEM they were killed by heat and fixed in TAF (Courtney, Polley & Miller, 1955) or killed and fixed in hot FP 4 : 1 (Netscher & Seinhorst, 1969). Specimens were processed to Spurr's low viscosity epoxy resin, coated with gold (for details cf. Clark & Stone, 1975) and examined with a Cambridge Instruments Stereoscan MK. IIa microscope at accelerating voltages of 5 or 10 KV.

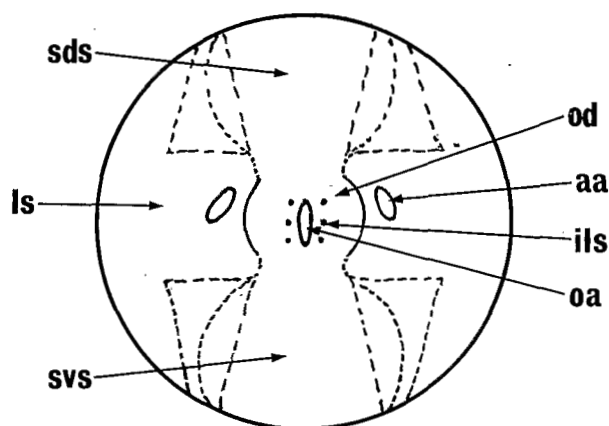


Fig. 1. Diagram of 'face' showing oral disc and variations of first head annule. aa = amphid aperture; ils = inner labial sensilla; ls = lateral segment; oa = oral aperture; od = oral disc; sds = sub-dorsal segment; svs = sub-ventral segment.

Table 1

Species of *Pratylenchus* examined and their origins

<i>Pratylenchus</i> species	Origin and Host
<i>andinus</i>	Potato, Bolivia
n. sp. 1	Oats, Bolivia *
<i>brachyurus</i>	Maize soil, Bolivia
<i>coffea</i>	Citrus, Florida
<i>crenatus</i>	Barley, England (two populations)
<i>fallax</i>	Barley, England
<i>goodeyi</i>	Bananas, England
<i>loosi</i>	Tea, Ceylon *
n. sp. 2	Alexandrian clover, Israel *
<i>neglectus</i>	Barley, England; spinach, Bolivia
<i>penetrans</i>	Arctaea, England; apple soil, New York, USA
<i>pinguicaudatus</i>	Wheat, England
<i>pratensis</i>	Meadow grass, England
<i>sefaensis</i>	Maize, Nigeria
<i>thornei</i>	Wheat, England; wheat, Bolivia
<i>vulnus</i>	Glasshouse roses, England
n. sp. 3	Cordyline, Scotland *
<i>zeae</i>	Maize soil, Bolivia; maize, Malawi; maize, Zimbabwe

* Type locality and type host

Results

The techniques used to prepare nematodes for the SEM frequently cause some distortion; in this study we have selected those which showed least collapse.

LIP REGION

The shape of the lip region showed well in SEM photographs and was characteristic for some species (Figs. 2, 3 and 4) but heads were more domed in profile than in prepared glycerine mounts seen with the light microscope; however they differ sufficiently between species to form a useful aid to identification. *P. brachyurus* (Fig. 2d) closely resembles *P. neglectus* (Fig. 3e) in shape but has a different face pattern. *P. coffea* and *P. loosi* are very similar with heads continuous with the body contour and resemble no other species (Fig. 2e, f). *P. crenatus*, *P. zeae* and *P. sefaensis* (Fig. 2j-l), *P. n. sp. 1* (Fig. 3f) and *P. fallax* (Fig. 4j) resemble each other in having dome shaped heads set off from the body; *P. thornei* and *P. n. sp. 2* have high heads almost continuous with the body contour (Fig. 3k, l) and *P. penetrans*, *P. andinus* and *P. pinguicaudatus* (Fig. 4f, k, l) have the same almost cap-like head outline set on top of a wider body. *P. n. sp. 3* (Fig. 3j) and *P. vulnus* (Fig. 4e) have high rounded heads and *P. goodeyi* (Fig. 3d) and *P. pratensis* (Fig. 4d) are flattened in front with rounded edges to the head outline.

The number of lip region annules varied between species and somewhat within species. For example, *P. neglectus* (Fig. 3e) normally with two head annules was found occasionally with a partial third annule; *P. crenatus* (Fig. 2j) usually with three head annules, occasionally with a fourth; *P. thornei* (Fig. 3k) with breaks and folds in head annules to give three or four depending on the viewing position or *P. pinguicaudatus* (Fig. 4l), normally with three annules reduced to two at one point on the head, or on other specimens having four head annules. The most variable species studied was *P. n. sp. 3* (Fig. 3j); most had four annules but many had three on one or both sides of the head, seen by light microscopy. Variation of this kind was found in most species studied, but variability was not so large as to invalidate number of lip region annules as a good distinguishing character: however it did emphasise the need to look at more than one specimen in a population to be certain of identifying the species. When combined, number of lip region annules and shape of the lip region constitute good taxonomic characters with the light microscope or the SEM.

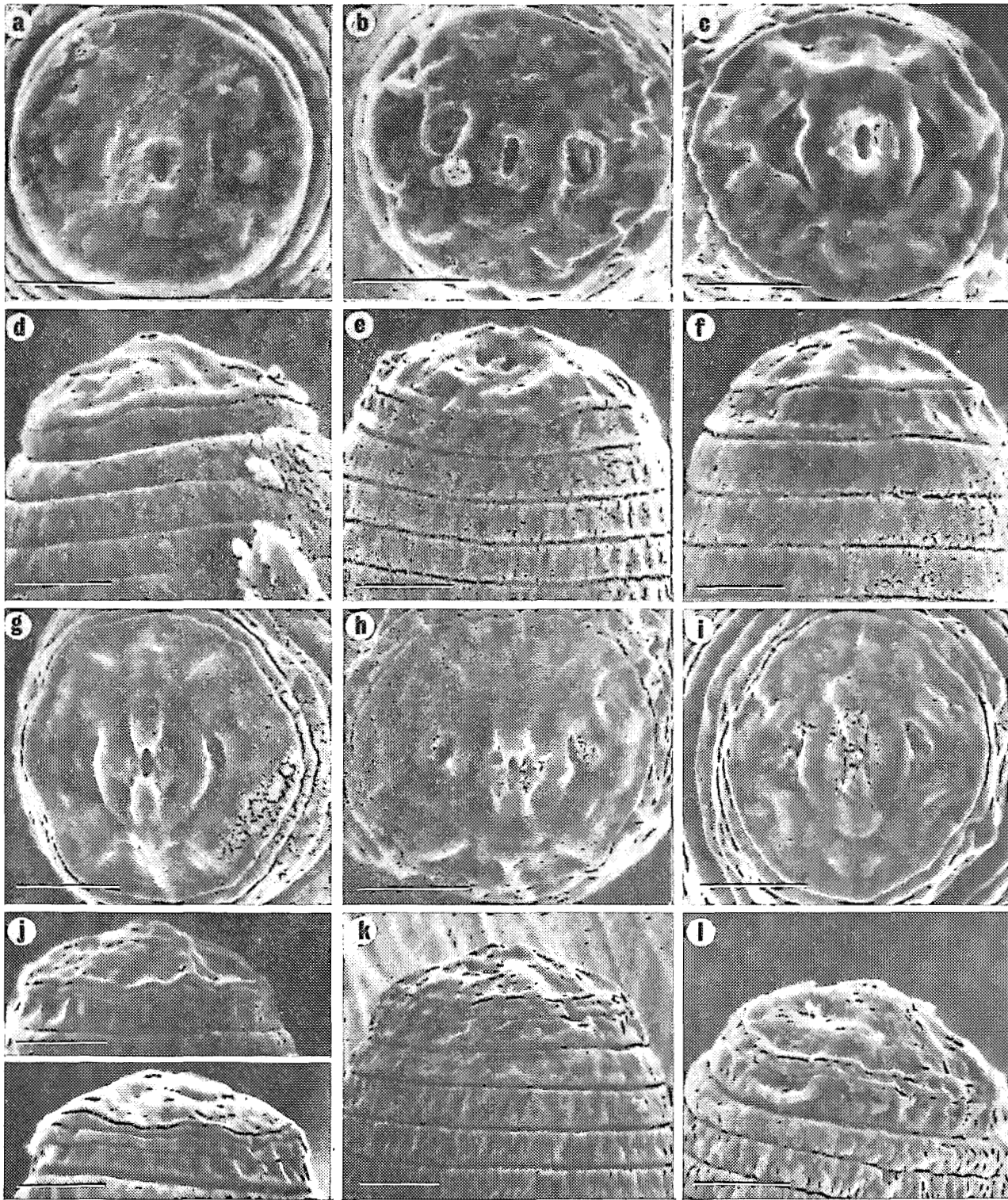


Fig. 2. *Pratylenchus* spp. heads. a-c, g-i : face view ; d-f, j-l : profile ; a, d : *P. brachyurus* ; b, e : *P. loosi* ; c, f : *P. coffeae* ; g, j : *P. crenatus* ; h, k : *P. zae* ; i, l : *P. sefaensis*. Scale bar = 2 μ m.

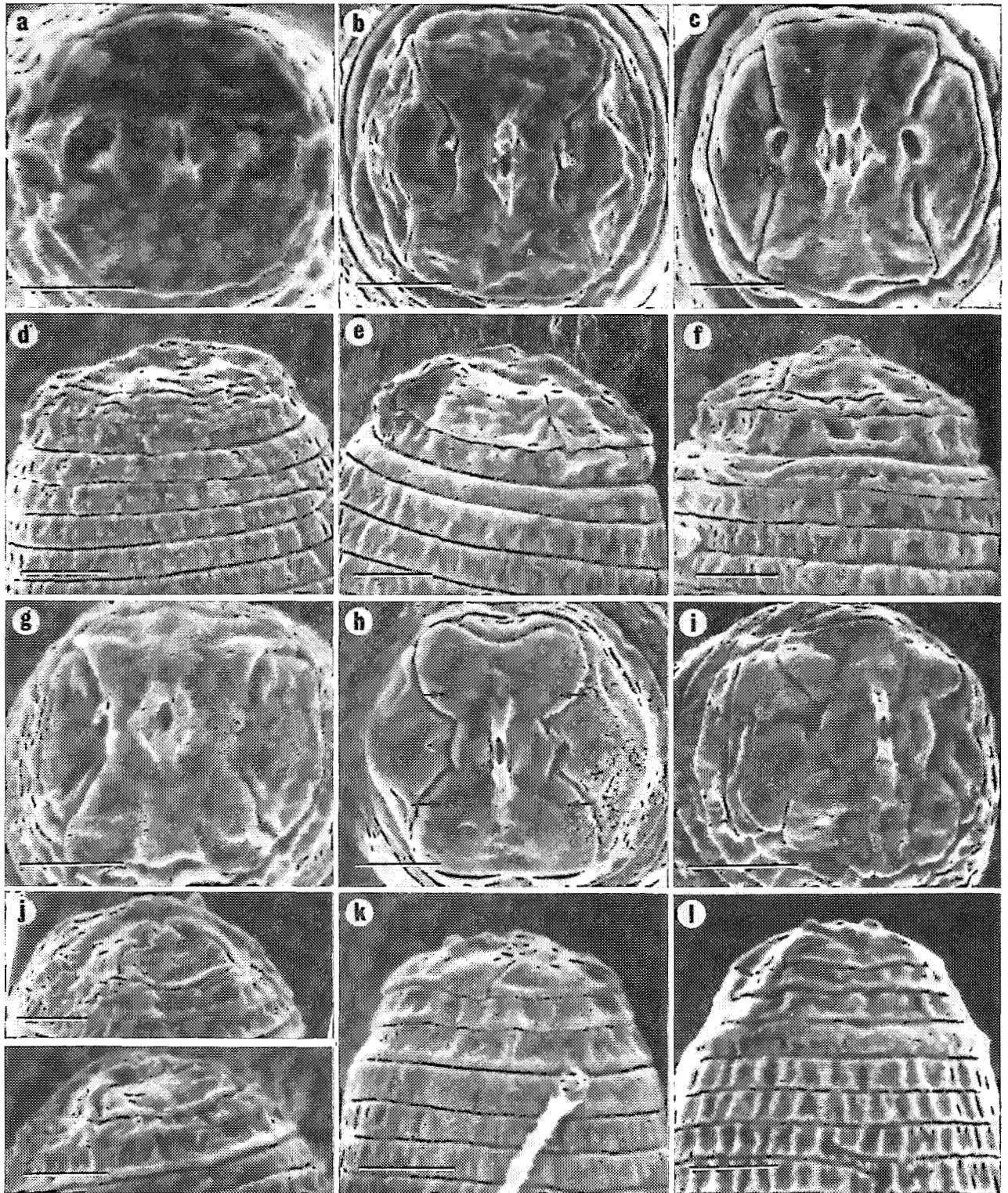


Fig. 3. *Pratylenchus* spp. heads a-c, g-i : face view ; d-f, j-l : profile ; a, d : *P. goodeyi* ; b, e : *P. neglectus* ; c, f : *P. n. sp. 1* ; g, j : *P. n. sp. 3* ; h, k : *P. thornei*, arrows in h point to possible location of cephalic sensilla ; i, l : n. sp. 2. Scale bar = 2 μ m.

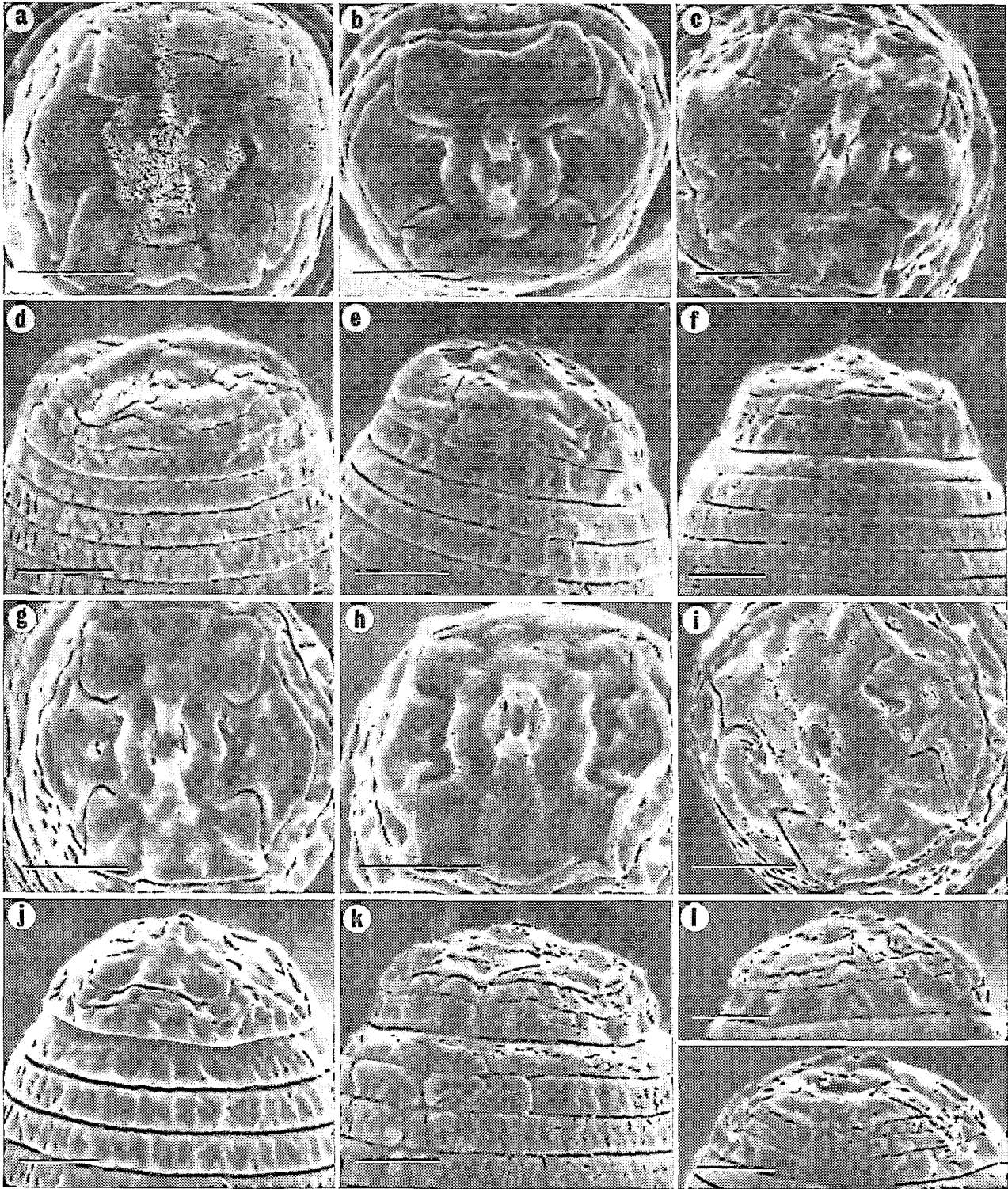


Fig. 4. *Pratylenchus* spp. heads. a-c, g-i : face view ; d-f, j-l : profile ; a, d : *P. pratensis* ; b, e : *P. vulnus*, arrows in b point to possible location of cephalic sensilla ; c, f : *P. penetrans* ; g, j : *P. fallax* ; h, k : *P. andinus* ; i, l : *P. pinguicaudatus*. Scale bar = 2 μ m.

Only five authors include *en face* views of *Pratylenchus* in descriptions (Allen & Jensen, 1951; Corbett, 1969; Fortuner, 1973; Sher & Allen, 1953; Thorne, 1949). The head is very small, less than 8 μm across at the front, and when excised heads are viewed in transmitted light the heavy refractive head skeleton prevents easy discernment of features, especially at the surface. With the SEM the front of the head or 'face' is seen to be formed by the oral disc and first head annule (Fig. 1). All species of *Pratylenchus* have six small pores very close to the oval oral aperture: these are probably the inner labial sensilla (Coomans, 1979). On each side of the oral aperture, on the inner edge of the first head annule there is an amphid opening varying in size according to species, usually slightly dorsal to a line bisecting the oral aperture laterally. In many species the amphid opening is oblique, slanting inwards towards the dorsal surface. In some specimens small depressions can be seen on the outer portion of the sub-median segments of the first head annule; these may indicate the position of the cephalic sensilla (Fig. 3 h, 4 b) (Coomans, 1979).

The 'face' is undivided, or divided into sub-median and lateral segments, and the different arrangements fall into three groups. In Group 1, the nematodes have a plain undivided face with no division between sub-median and lateral segments, presumably due to fusion of the first head annule to the oral disc. These are: *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. goodeyi*, *P. loosi*, *P. sefaensis* and *P. zae* (Figs. 2a-c, g-i, 3a). In these nematodes there were differences in shape, size and orientation of the amphid apertures, but otherwise the structure of the 'face' was similar.

In other species examined, the area immediately surrounding the oral aperture was similar, but the outer part of the face was sub-divided into sub-median and lateral sectors. Group 2, consisting of *P. n. sp. 1*, *P. n. sp. 2*, *P. neglectus*, *P. thornei* and *P. n. sp. 3* has sub-median segments that are fused to the oral disc and are narrower at their inner extremity widening towards the outer edge of the face: they are separated from the larger lateral segments that complete the circular face, with amphid openings on their inner edges (Fig. 3b, c, g-i). Group 3, which includes *P. andinus*, *P. fallax*, *P. penetrans*, *P. pinguicaudatus*, *P. pratensis* and *P. vulnus*, has a more distinctive dumb-bell shaped pattern of the sub-median segments with slightly smaller lateral segments to complete the circle. The amphid apertures are again on the inner edges of the lateral segments (Fig. 4a-c, g-i). These face formations were consistent for different populations of several species: *P. penetrans* from the UK and the USA, *P. thornei* from UK and Bolivia, *P. neglectus*

from UK and Bolivia, *P. zae* from Bolivia, Malawi and Zimbabwe.

Three species have incisures in some part of the face pattern. *P. n. sp. 3* may have a diagonal incisure across the outer part of the lateral segment (Fig. 3g); in *P. n. sp. 2* the ventral sub-median segments are divided by an incisure running almost to the oral disc (Fig. 3i) and in *P. pratensis* there is an incisure in both sub-median segments parallel to the outer edge and midway between the oral aperture and the edge of the face (Fig. 4a). The significance of these incisures is at present unclear. In addition, the head annules in *P. pratensis* are wavy in outline.

DEPTH OF TRANSVERSE STRIAE AND WIDTH OF ANNULES

The body annules are separated from each other by transverse striae, the depth of which may appear to vary widely. Their distance apart corresponds to the width of the annules. Four species were examined under the light microscope: *P. crenatus*, apparently coarsely annulate had striae 0.50 to 0.67 μm deep and 1.3 to 1.4 μm apart; *P. goodeyi*, finely annulate, had striae 1.2 to 1.5 μm apart and 0.33 μm deep; *P. neglectus*, one of the most widely distributed and variable species, had striae 1.2 to 1.4 μm apart and 0.33 μm deep; and *P. thornei*, probably the most finely annulate, sometimes appearing to have a smooth cuticle, 1.3 to 1.6 μm apart and 0.22 to 0.33 μm deep.

Annulation varies as shown in Figures 5, 6 and 7; while the distance between striae can easily be measured in SEM photographs, the depth of striae cannot. Table 2 lists the width of annules of most of our species measured on SEM photographs at magnifications from 2 500 x to 12 200 x. The SEM photographs also make clear that differences in form of annulation occur, some e.g. *P. crenatus* and *P. zae* having rounded annules with deep striae between (Fig. 6d, e) and others e.g. *P. thornei* and *P. pinguicaudatus* being almost flat with shallow striae between them (Fig. 6j, p).

STRUCTURE OF THE LATERAL FIELD

This is a character much used to distinguish species of *Pratylenchus*. It is difficult to see clearly in transmitted light, despite the use of specialised techniques (De Grisse, 1961). The SEM, however, shows the lateral field up so clearly that it is now possible for the first time to evaluate its worth as a character to be used in identification.

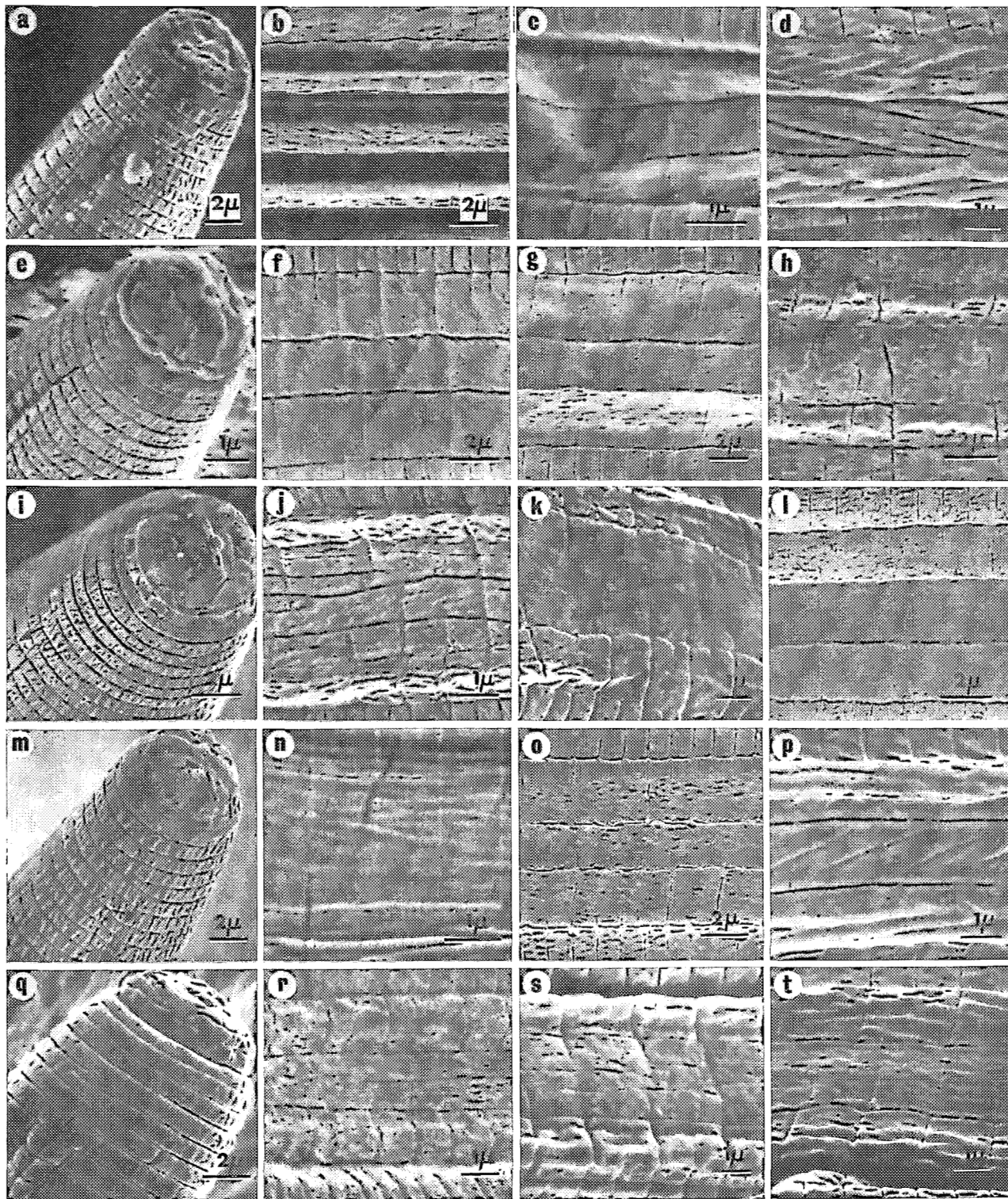


Fig. 5. *Pratylenchus* spp. lateral fields. a : *P. zae*, showing origin of lateral field ; b : *P. brachyurus* ; c, d : *P. loosi* ; e : *P. neglectus*, showing origin of lateral field ; f : *P. coffeae* ; g : *P. goodeyi* ; h, j, k : *P. neglectus* ; i : *P. thornei*, showing origin of lateral field ; l : *P. thornei* ; m : *P. n. sp. 2*, showing origin of lateral field ; n : *P. n. sp. 2* ; o : *P. pratensis* ; p : *P. vulnus* ; q : *P. penetrans*, showing origin of lateral field ; r : *P. penetrans* ; s : *P. fallax* ; t : *P. pinguicaudatus*.

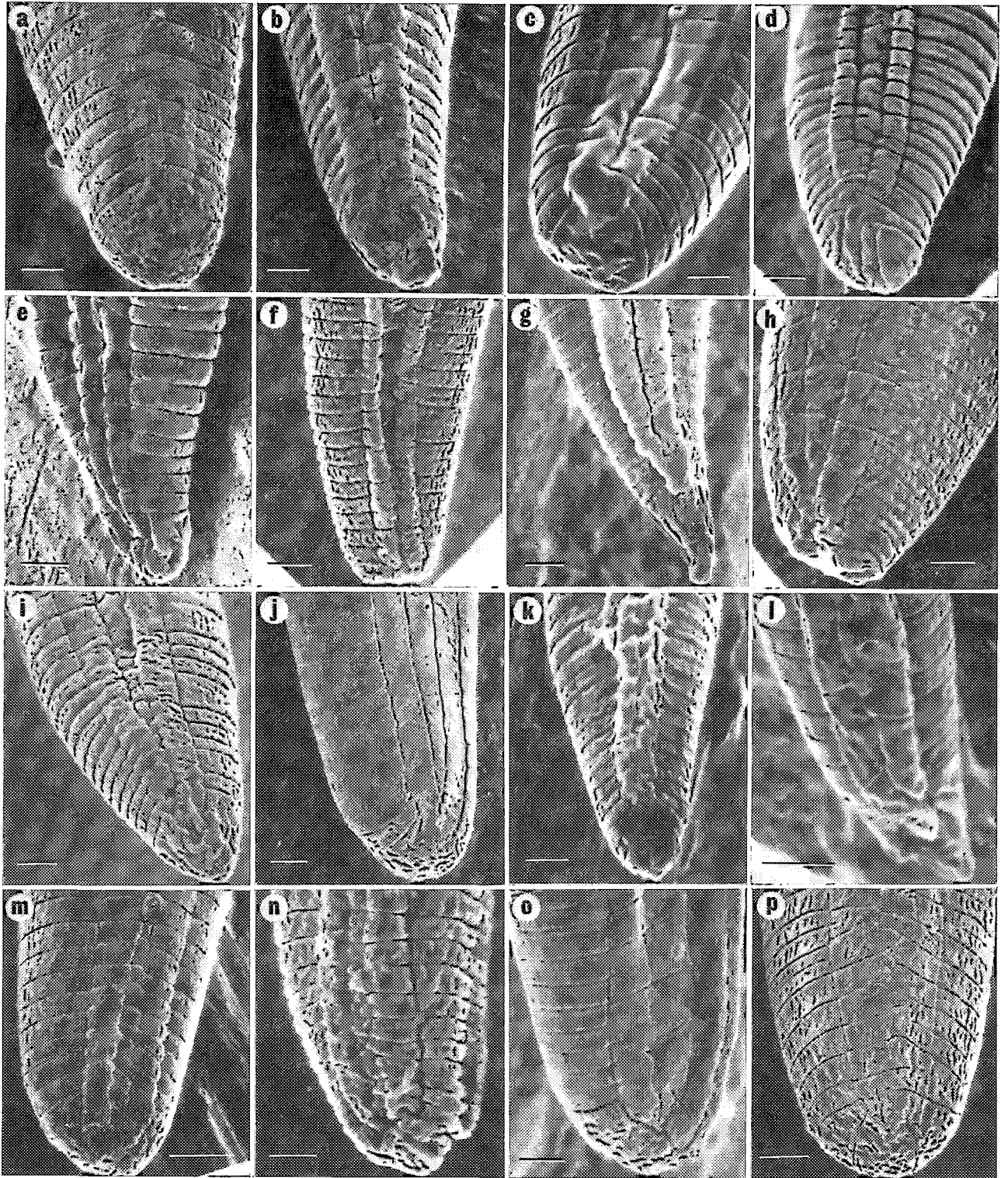


Fig. 6. *Pratylenchus* spp. tails. a : *P. brachyurus* ; b : *P. loosi* ; c : *P. coffeae* ; d : *P. crenatus* ; e : *P. zae* ; f : *P. sefaensis* ; g : *P. goodeyi* ; h : *P. neglectus* ; i : *P. n. sp. 1* ; j : *P. thornei* ; k : *P. pratensis* ; l : *P. vulnus* ; m : *P. penetrans* ; n : *P. fallax* ; o : *P. andinus* ; p : *P. pinguicaudatus*. Scale bar = 2 μ m.

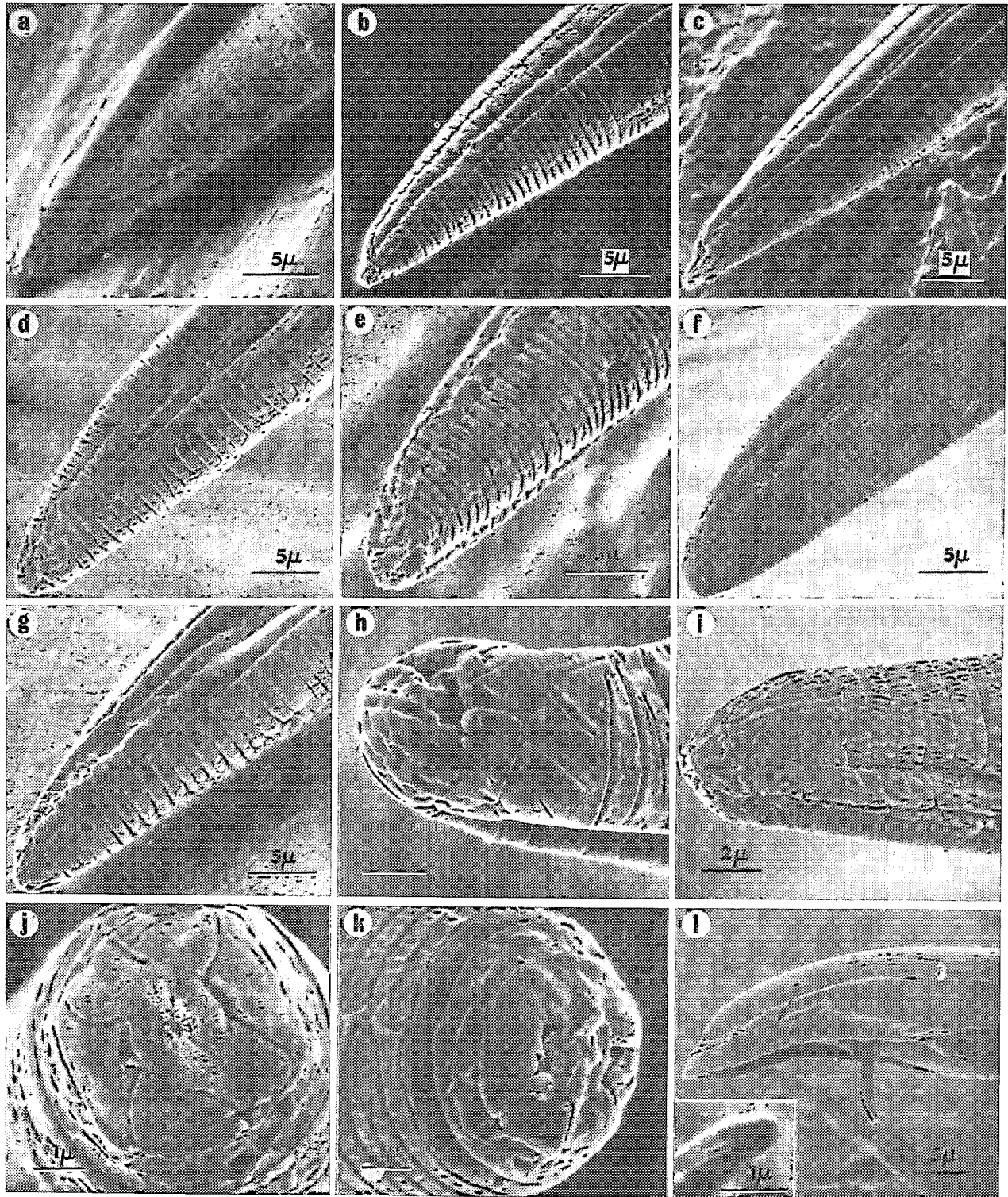


Fig. 7. a-g : *Pratylenchus* spp. tails. a : *P. loosi* ; b : *P. zae* ; c : *P. goodeyi* ; d : *P. n. sp. 1* ; e : *P. n. sp. 3* ; f : *P. pratensis* ; g : *P. penetrans* ; h, i : *Pratylenchus* spp. tail tips, showing tiling on upper surface ; h : *P. coffeae* ; i : *P. pinguicaudatus* ; j : *P. thornei* from Bolivia and k : *P. thornei* from Britain, showing pores near lip region ; l : *P. fallax* spicules, inset showing pores at tip.

Table 2

Width of annules in mid-body of species of *Pratylenchus*, measured from SEM photographs at point where annules join lateral field

<i>Pratylenchus</i> species	Annules width range (μm)	Annules width, mean (μm)
<i>andinus</i>	1.4-1.9	1.6
<i>brachyurus</i>	0.9-1.5	1.4
<i>coffea</i>	1.2-1.5	1.4
<i>fallax</i>	1.3-1.9	1.4
<i>goodeyi</i>	1.0-1.3	1.2
<i>loosi</i>	0.9-1.4	1.1
n. sp. 2	1.3-1.6	1.4
<i>neglectus</i>	1.0-1.8	1.6
<i>penetrans</i>	1.4-1.8	1.5
<i>pinguicaudatus</i>	1.2-1.6	1.4
<i>pratensis</i>	0.9-1.2	1.1
<i>sefaensis</i>	0.8-0.9	0.8
<i>thornei</i>	0.9-1.9	1.4
<i>zeae</i>	0.7-1.1	0.9

In *Pratylenchus* sp. the lateral field starts on the seventh to ninth body annule (Fig. 5 a, e, i, m, q) and for the greater part of its length has four lines, i.e. consists of three bands (Fig. 5 b-d, f-h, j-l, n-p, r-t). It ends on the tail, in many species continuing to the tail tip (Fig. 6). Across or within the bands there may be further lines which are said to be characteristic for each species, but which we found to vary greatly, possibly depending on age of the specimens examined.

We found that all species we studied had areolation of the outer bands (Fig. 5). Several species had partial or complete areolation of the middle band also: *P. brachyurus*, *P. loosi*, *P. neglectus* and *P. pinguicaudatus* (Fig. 5b, c, h, t). Two species (*P. loosi* and *P. vulnus*) are illustrated with oblique striae in the middle band (Fig. 5d, p): we also found these in *P. crenatus*, *P. n. sp. 1*, *P. n. sp. 3* and *P. zeae*.

The form of lateral field varied greatly within species, especially *P. neglectus*, and similarities were found between species; *P. neglectus* and *P. fallax* showed the same complex ornamentation of the middle band (Fig. 5j, s). Areolation across the lateral field is more frequent on the anterior part of the field (Fig. 5a, e, i, m, q) and behind the vulva,

especially on the tail (Fig. 6) than in the middle third of the nematode.

Few descriptions of species contain a sufficiently detailed description of the lateral field to enable it to be used as a differential character. Loof (1960) has discussed this in some detail.

TAIL SHAPE AND ANNULATION

The shape and amount of annulation or crenation of the female tail is included in all descriptions of *Pratylenchus* species. The SEM pictures show, for the most part, that descriptions have been accurate. They also show that there is some variability but that the shape of the tail and presence or lack of annulation and its form around the tail tip are sufficiently characteristic for each species to be a good taxonomic character. Fig. 6 shows the tail tips of all the species we studied except *P. n. sp. 3* and *P. n. sp. 2* which are illustrated in Corbett (1982). Some wrinkling of the tail tip has occurred in all specimens but it is possible to distinguish this processing artefact from the 'crenation' around the tip characteristic of *P. crenatus*, *P. pratensis* and *P. fallax* (Fig. 6d, k, n). The tail shape described as characteristic for each species shows up well in SEM photographs, generally agreeing with the original authors' descriptions. The lateral field is particularly clear in most specimens; it extends to the tail tip in many species and seems to continue round the tail tip in *P. brachyurus*, *P. loosi*, *P. thornei* and *P. pinguicaudatus* (Fig. 6a, b, j, p) and stops short of the tail in *P. crenatus* (Fig. 6d). Where it is visible, the phasmid opens as a pore about midtail, and slightly to the ventral side of the lateral field. It is very conspicuous in some species, e.g. *P. vulnus* (Fig. 6l).

NUMBER OF TAIL ANNULES

Number of annules on the ventral surface of the tail between the anus and the tail tip is the final feature noted by Loof (1978) as having some value in identifying *Pratylenchus* spp. This annulation is easily counted under the compound optical microscope and very easily seen in the SEM (Fig. 7a-g). We found (Tab. 3) that tail annule number varied widely within species and there was some variation between species. Mean number of annules varied from 15 for *P. pseudopratensis* (range 12-19) to a mean of 27 annules for *P. sefaensis* (range 24-34) at the extremes of the range, therefore tail annules can aid in distinguishing species. However, most species have a mean number of tail annules that

falls within the range 18-24 annules, with ranges that overlapped much. It is impossible to differentiate between these species using number of tail annules as a character. It was found during the examination that the width of tail annules varied widely (Fig. 7a-g).

Table 3

Number of annules from the tail tip on which phasmids are found, and number of annules on ventral surface of tails of *Pratylenchus* spp.

<i>Pratylenchus</i> species	Phasmid annule	Number of annules	Mean
<i>alleni</i>	—	15-19 *	17 *
<i>andinus</i>	8-12	17-20	18
n. sp. 1	9-15	13-24	18
<i>brachyurus</i>	8-10	15-21	18
<i>coffea</i>	9-13	17-22	20
<i>convallariae</i>	—	16-20 *	19 *
<i>crenatus</i>	—	20-24 *	22 *
<i>fallax</i>	—	16-26 *	21 *
<i>flakkensis</i>	—	18-24 *	21 *
<i>goodeyi</i>	10-14	20-27 * ; 19-24	24 * 22
<i>lossi</i>	11-15	19-26	23
n. sp. 2	—	15-22	18
<i>neglectus</i>	8-12	16-19 * ; 11-19	17 * 16
<i>penetrans</i>	8-12	15-27 * ; 17-25	20 * 21
<i>pinguicaudatus</i>	8-12	18-25	21
<i>pratensis</i>	—	20-26 *	24
<i>pseudopratenis</i>	—	12-19 *	15 *
<i>scribneri</i>	—	21-25 *	23 *
<i>sefaensis</i>	12-18	24-34	27
<i>thornei</i>	—	19-26 *	23 *
<i>vulnus</i>	—	22-26 *	24 *
n. sp. 3	12-15	16-20	18
<i>zeae</i>	—	25-27 *	26 *

* Data from Seinhorst (1968).

MALES

We studied males in the following species in which they occur commonly : *P. coffea*, *P. fallax*, *P. goodeyi*, *P. lossi*, *P. n. sp. 2*, *P. penetrans* and *P. vulnus*. In all species the males had the same

'face' pattern and ornamentation of the lateral field in the fore and mid-body regions as was found in females of the same species. The bursa appears to be a rearward and ventrad extension of the lateral field. The phasmid appears as a small pore opening on the external surface of the bursa connecting to the body through a short tube within the bursa (Fig. 7l). We found two pores at the extremity of the spicule (Fig. 7l, inset), mentioned, but not illustrated, by Wen and Chen (1976).

OTHER SURFACE FEATURES

Phasmids show clearly in SEM photographs and are usually found in the middle of the lateral field about half way along the tail of females. The number of annules from the phasmid to the tail tip counted along the ventral surface is given for a number of species in Table 3. As this tended to vary approximately as the number of tail annules, we do not think it any better as a distinguishing character.

There is uncertainty about deirids, small pore like structures usually on the lateral field in the oesophageal region. Sher and Allen (1953) described the genus as having deirids, but Roman and Hirschmann (1969) could not find them on six species they studied. Loof (1978) doubts their presence in *Pratylenchus*, and we did not find them in the eighteen species we studied. It is probable therefore that they do not occur in *Pratylenchus*.

We found in one or two species, notably *P. thornei*, that there were pores on or near the head. In the Bolivian population of *P. thornei* a large pore opened usually in the basal head annule on the ventral side. A similar pore was found in the UK population of this species, but it more usually occurred on the first or second body annule than on the head (Fig. 7j, k). We do not know what function they serve nor do we know if they connect with structures or organs in the nematode.

We also found in two species, *P. coffea* and *P. pinguicaudatus*, that the body annulation was interrupted near the tail tip (Fig. 7h & i) by some longitudinal incisures that gave a tiled appearance to this region. It was an inconstant feature and not noted in other species.

Discussion

Lip region annulation as a character for separating species was first introduced by Allen and Jensen (1951) and has become important in diagnosis. We have confirmed and illustrated the variability found within the species but consider that the number

of lip annules is constant enough to form a good specific taxonomic character. However, the variability we found does emphasise the need to examine several in a population to be certain of identifying the species.

Shape of the lip region we also found to be characteristic for certain species, e.g. *P. brachyurus*, *P. thornei*, although this feature calls for very well prepared specimens for SEM photography; it may be better appreciated under the light microscope where the head is seen in profile in transmitted light and the head skeleton, which gives the head its shape, can also be seen. Taken together with number of lip region annules this makes a very good taxonomic feature.

In SEM photographs the front of the head (face) differs completely from earlier descriptions made in transmitted light and provides an interesting new character. The species we have examined fall at present into three groups on the form of the 'face', although examination of more species may show there to be only two groups, with no discontinuity in face pattern between the types present in Groups 2 and 3. Work in other nematode groups (Hooper & Clark, 1980; Stone, 1975) suggests that form of the head provides clues to phylogeny. In *Pratylenchus* morphological similarity has been the main criterion of closeness of relationships. In the species we have looked at it is difficult to relate face patterns we have found to presumed relationships. In Group 1 *P. coffeae* and *P. loosi* are morphologically very similar but are unrelated to any of the others in that group. There is no other evidence to relate *P. crenatus* to *P. coffeae* or *P. loosi*, and *P. brachyurus* and *P. zaeae* are morphologically as dissimilar as any two species in the genus: *P. crenatus* (Group 1) more closely resembles females of *P. fallax* (Group 3) than any other species. In Group 2 also there seems little in common in the morphology of the species. In Group 3 *P. fallax* and *P. penetrans* are similar morphologically. Work by Tarte and Mai (1976) suggests that the features used to distinguish these species, in particular tail shape and annulation are unreliable and one species may be a morphological variant of the other. However, Perry, Plowright and Webb (1980) failed to obtain fertile offspring from crosses of *P. penetrans* and *P. fallax* and concluded that they were separate species. *P. andinus* and *P. pinguicaudatus* closely resemble each other in this group, but differ slightly in 'face' pattern and in other respects. The others in this group are unrelated on morphological grounds. With observations of 'face' patterns in more *Pratylenchus* species true phylogenetic relationships may emerge. We found some variability in pattern within populations of the same species and between populations.

However, a basic pattern was a determinable characteristic for each species, and could be used reliably to differentiate them. This character is so reliable that mixtures of closely similar species could be distinguished using the SEM before light microscopy revealed that more than one species was present.

Anderson and Townshend (1980) examined populations of *P. penetrans* obtained from several different hosts and related differences in face patterns to host differences. We found at the outset of our study that processing for SEM viewing after specimens had been processed to glycerine for permanent mounting on slides led to collapse of the head cuticle around the framework, and the consequent stretching of the cuticle distorted the pattern, frequently giving rounded margins to the sub-median segments. Our results show that the variation between populations is not as great as Anderson and Townshend (1980) found with their glycerine processed specimens, and the similarity between the patterns we found for *P. coffeae*, *P. fallax*, *P. neglectus*, *P. penetrans*, *P. thornei* and *P. vulnus* and those recorded by other researchers working with populations collected elsewhere (Momota, 1979; Sher & Bell, 1975; Townshend & Anderson, 1976) shows that the patterns are invariant enough to be good taxonomic features. However, we believe a study of the influence of host and other external conditions on head pattern should be carried out on several species to determine the amount of variability that can be expected.

It is difficult to postulate the anatomical features of the 'face'. De Grisse's (1977) photographs and drawings of *P. penetrans* showed deep divisions between an oral disc and lateral plates. Loof (1978) duplicated his findings, including the assertion that only four of the six inner labial sensilla are on the front surface of the oral disc. We believe De Grisse (1977) misinterpreted folds caused by collapse of the head cuticle around the head skeleton as divisions of the face. Our photograph of the uncollapsed head of *P. penetrans* shows an oral aperture with six adjoining sensilla; we found these to be common to all the species of *Pratylenchus* studied. We show photographs of *P. thornei* and *P. vulnus* that suggest that the four cephalic sensilla lie on the sub-median segments just outside the circle of the oral disc but do not terminate in pores like the inner labial sensilla. Stone (1975) called the sub-median fused segments of *Heterodera* juveniles sub-median lips and the structure bearing the amphids the lateral lips. Hooper and Clark (1980) spoke of a cephalic plate pattern in their studies of Aphelenchoidea. For convenience and until it is proven otherwise, we prefer to regard the oral disc as that part in the centre of the 'face' extending out to near the amphids

and to which are fused to form different patterns the sub-median and lateral segments of the first head annule, the whole forming a cephalic plate.

Mean depth of striae varied from 0.22 μm to 0.67 μm in four species, a very small amount despite the apparent large difference between species in depth of striae, which is usually associated with annule shape. This difference, less than 0.5 μm , is smaller than the probable error in measuring. It was not possible to measure it in SEM photographs. It is unrealistic to use this difference quantitatively but it has usefulness as a subjective character.

Width of annules was easily measured on SEM photographs at magnifications of 2 500 \times to 12 200 \times and should be more accurate than measurements made under the light microscope. Annule width in fourteen species was unexpectedly variable. This was found to be due to the presence in several species of double annules — annules of normal width with an extra line in the middle (Fig. 5j, r). This together with the almost complete overlap of the range of annule widths in all species (except *P. sefaensis*, very narrow annules) makes this a character of little use in distinguishing species.

Structure of the lateral field has received much emphasis in *Pratylenchus* taxonomy, despite the difficulty of observation and the form of the lateral field has been used as the principal differentiating feature in *P. hexincisus*. Our work suggests that the lateral field in *Pratylenchus* basically consists of four lines and that a variety of ornamentation of the lateral field is found in so many species as to make its form a poor distinguishing character. For example, oblique striae in the lateral field have been used as supplementary differentiating characters and Loof (1960) found *P. neglectus*, *P. penetrans*, *P. thornei*, *P. loosi* and *P. coffeae* to have them. Other workers have discovered this character in *P. n. sp. 1*, *P. n. sp. 3*, *P. neglectus* and *P. hexincisus* (Corbett, 1982; Fortuner, 1973; Sher & Allen, 1953; Taylor & Jenkins, 1957; Townshend & Anderson, 1976). We found other ornamentation, and especially found areolation of the outer bands of the lateral field to be common. Examination in the light microscope showed *P. neglectus* to have four, six or seven lines in the lateral field in mid-body, and *P. crenatus* was often found to have six lines. We were able to examine paratype specimens of *P. hexincisus* and found them to have basically four lines in the lateral field, in common with other species, with oblique striae in mid band in some specimens, and in others a broken line in mid band to give five lines: we did not find six lines in the paratypes, which was one of the main features on which the species was erected. We consider therefore, that the status of *P. hexincisus* has to be re-examined,

preferably with the aid of the SEM, and consider that because of the intraspecific variability we found in the lateral field this is too variable to be of use as a good taxonomic character in differentiating *Pratylenchus*.

Tail shape varies between species quite considerably and although there is variation within all species it is possible to recognise a shape of tail characteristic for most species (Fig. 6). Some have striations around the tail tip (Fig. 6d) or more irregular crenations (Fig. 6n), that are distinguishable from the wrinkling that occurs as a processing artefact. As far as it was possible to tell with the specimens we photographed tail shape is a relatively stable character. However, Tarte and Mai (1976) and Townshend, Tarte and Mai (1978) have shown that this is not sufficiently stable in *P. penetrans* to enable distinction between it and *P. fallax*. This character needs to be re-examined in specimens raised in controlled conditions for these two species, and also in others where tail crenation is an important character distinguishing closely similar species.

In at least two species (*P. coffeae* and *P. pingui-caudatus*; Fig. 7h & i) an unusual form of break in annulation just above the tail tip occurred to produce a 'tiled' appearance. This feature may be confined to these species, in which case it could form a useful accessory character. Other species should be examined for its presence.

Number of annules on the ventral surface of tails has been used by Seinhorst (1968), and endorsed by Loof (1978), to differentiate species of *Pratylenchus*. We found much intraspecific variability in this character with many species having between thirteen and 24 annules on the ventral surface of the tail (Tab. 3). Although one or two species differed from the norm in the number of annules on the tail, we think that it is not a useful character in differentiating species.

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