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Hexatylus viviparus (Nematoda, Tylenchida, Hexatyulina) : head skeleton morphology and a comparison of head symmetry with that of *Ditylenchus dipsaci* (Tylenchina)

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SUMMARY

Previous workers believed that among nematodes of the order Tylenchida, *Hexatylus* and some other neotylenchs have eight head sectors instead of the usual six. Scanning electron microscopy of the head of *Hexatylus* also shows a complex pattern of ridges that is difficult to interpret. Thin sections of the head studied by transmission electron microscopy clarified the structure and showed that the basic hexaradiate condition is still recognisable although it is modified into a twelve sectoried pattern. This is the result of bifurcation of each of the six radial partitions of the head skeleton to accommodate the forward extension of certain branches of the stylet protractor muscles. These branches insert on to the cuticle of the 'face' whereas in other tylenchs they insert on to the posterior surface of the head skeleton itself. The resulting skeletal framework in *Hexatylus* is very slender with very little sclerotisation. A transverse section shows not only the six sectors containing nerve tissue, the only tissue present in the head tip of other tylenchs, but also six smaller sectors containing muscle. The positions of the amphidial apertures and the cephalic, outer and inner labial sensilla are also elucidated. The implications of these observations for the taxonomy of this group are discussed.

RÉSUMÉ

Hexatylus viviparus (Nematoda, Tylenchida, Hexatyulina) : morphologie du squelette céphalique et comparaison de la symétrie céphalique avec celle de *Ditylenchus dipsaci* (Tylenchina)

Les travaux antérieurs laissaient penser que parmi les nématodes de l'ordre des Tylenchida le genre *Hexatylus* et quelques autres Néotylenchides présentaient huit secteurs céphaliques au lieu des six habituels. En microscopie électronique à balayage la portion céphalique d'*Hexatylus* montre un réseau complexe de rides difficile à interpréter. L'étude de sections ultra-fines de cette portion en microscopie électronique à transmission a permis de clarifier cette structure et de montrer que la structure hexaradiée de base est reconnaissable quoique modifiée en un système à douze secteurs. Ce dernier est dû à la bifurcation de chacune des six partitions radiales du squelette céphalique en relation avec les extensions antérieures de certaines branches des muscles protracteurs du stylet. Ces branches sont insérées sur la cuticule de la « face » des nématodes, alors que chez les autres Tylenchides ils le sont sur la face postérieure du squelette céphalique lui-même. Il en résulte que ce squelette est particulièrement mince et très peu sclérotisé. Les sections transversales montrent non seulement les six secteurs contenant le tissu nerveux (seul tissu présent dans l'extrémité céphalique des autres Tylenchides) mais aussi six petits secteurs contenant du tissu musculaire. La position des ouvertures amphidiennes et celle des papilles céphaliques et labiales (internes et externes) est, elle aussi, élucidée. Les implications de ces observations concernant la taxinomie du groupe sont discutées.

The stylet-bearing fungal-feeding nematode *Hexatylus viviparus* T. Goodey belongs to the order Tylenchida as do most plant-parasitic forms, but it shows several biologically interesting and systematically important differences from the others. Some of these differences (e.g. exclusively passive feeding and the associated structure and operation of the aliment-

ary tract) have been described (Doncaster & Seymour, 1974; Seymour, 1974; Shepherd & Clark, 1976). Nickle (1968) reviewed the morphology and taxonomy of *Hexatylus* and related genera in the Neotylenchidae and, following T. Goodey (1938) and Thorne (1941), included in his diagnosis of the genus *Hexatylus* an octagonal cephalic framework (see our

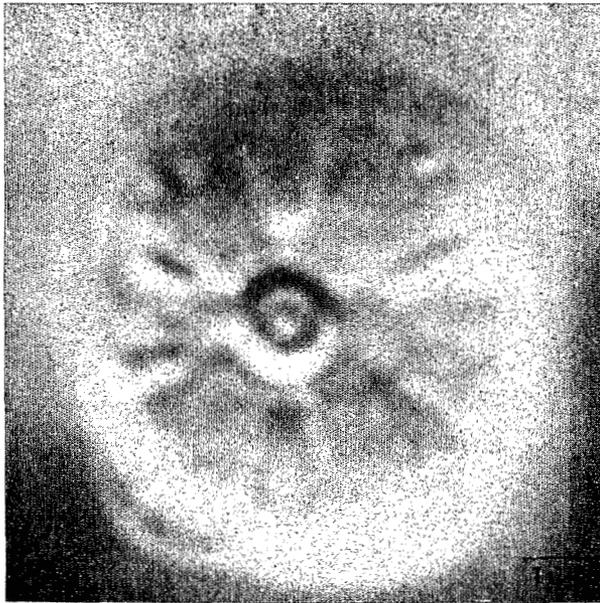
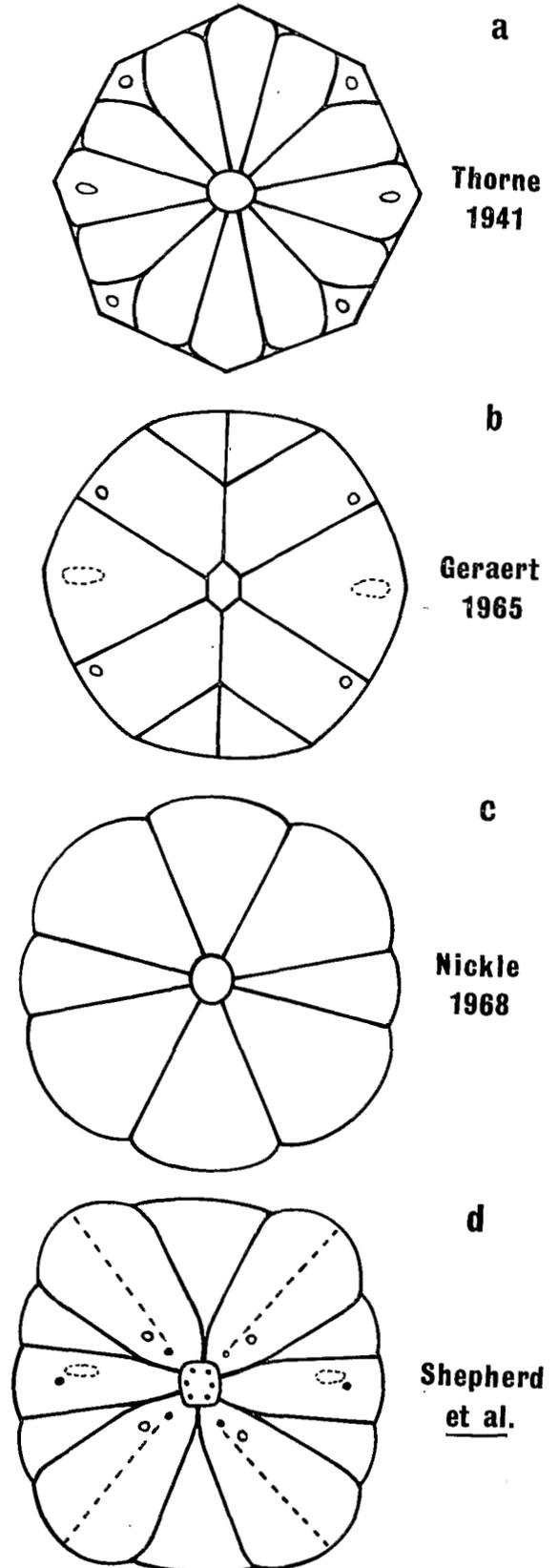


Fig. 1. *En face* view of the head of *H. viviparus* photographed with Nomarski illumination. $\times 7800$.

Fig. 2c), although Geraert (1965) had questioned this interpretation. Thorne thought the basic octagonal pattern was made up of sixteen sectors, twelve of fairly equal size and four smaller ones bearing the cephalic papillae (our Fig. 2a). Geraert's illustration (our Fig. 2b) suggested a variation on a hexagonal pattern. More recently Siddiqi (1980) also postulated a possible eight-sectored head symmetry in his new suborder Hexatylinea within the Tylenchida.

From examination of the *en face* pattern of *H. viviparus* with Nomarski differential interference contrast illumination (Fig. 1), it is understandable how all three of the interpretations (Fig. 2a-c) were arrived at, especially if the authors had mistaken the dorso-ventral for the lateral plane (the amphids are not clearly resolved by light microscopy). Scanning electron microscopy (SEM) of the head (Fig. 3a) gives the impression of a symmetry far more complex than the usual six-fold one of the Tylenchida (as in, for example, *Ditylenchus dipsaci* (Kühn) (Fig. 3b)) and of many other nematodes. Serial sections of the

Fig. 2. Diagrammatic representation of the *en face* view of the head of *H. viviparus* as seen by light microscopy and as interpreted by a : Thorne, 1941 ; b : Geraert, 1965 ; c : Nickle, 1968 ; d : Shepherd, Clark & Hooper (this work).



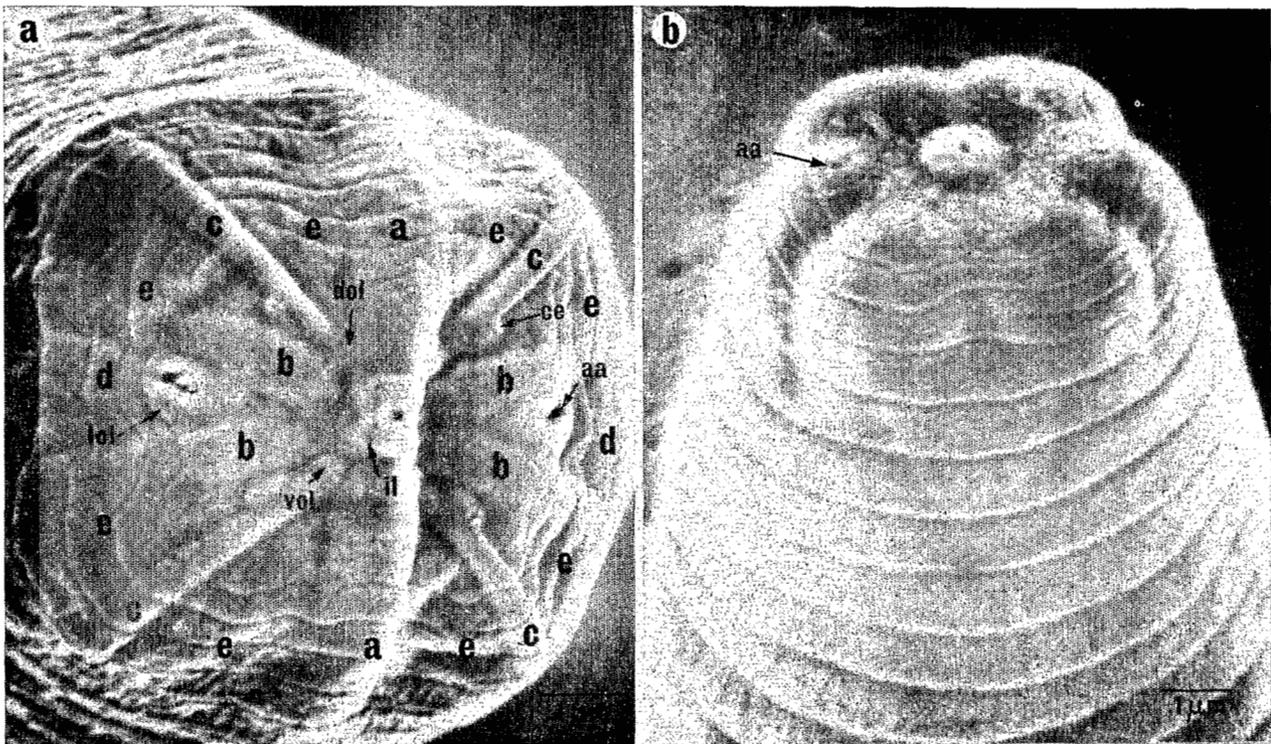


Fig. 3. a : SEM view of head of *H. viviparus*. dol = subdorsal-, lol = lateral- (approximate position), and vol = subventral outer labial papilla ; il = inner labial papilla ; ce = cephalic papilla ; aa = amphidial aperture. $\times 11\ 000$. Segments and/or ridges as follows : a (2) and b (4) = stylet protractor muscle insertions ; c (4) = cuticular ridges enclosing the neurons of the cephalic and outer labial sensilla ; d (2) = amphidial sectors ; e (8) = sectors containing neurons of accessory sensory receptors. b : SEM view of head of *D. dipsaci*. Positions of sensilla are unclear except for amphid (aa). $\times 9\ 300$.

head of *H. viviparus* studied with the transmission electron microscope (TEM) have clarified the structure of the head skeleton and the underlying tissues.

Materials and methods

Adult females of *H. viviparus* were maintained on agar plate cultures of *Botrytis cinerea* Pers. ex Fr. For TEM they were fixed for 24 h in 6 % glutaraldehyde in cacodylate buffer, the anterior excised and embedded in 1 % agar and fixation continued for a further 1 h. Post fixation was in 1 % OsO_4 in veronal acetate buffer for 3 h, and embedding in Spurr low viscosity resin (see also Shepherd & Clark, 1976). *D. dipsaci* specimens for comparison were fixed for 6 h then processed in a similar way. Serial sections about 60 nm thick were mounted on large-slot grids

with necoloidine film. Sections were pre-stained in KMnO_4 in phosphate buffer and stained in 3 % aqueous uranyl acetate and lead citrate.

For SEM both species were dehydrated in acetone and infiltrated with Spurr resin (Clark & Stone, 1975).

Observations and discussion

The head of *H. viviparus* is squarer, both *en face* and in profile, than that of most other tylenchs. This is mainly because *i*) the stylet protractor muscles attach anteriorly not only to the head skeleton itself, as in most tylenchs, but also to the body wall cuticle of the front of the head (the 'face') (Fig. 1a-c) ; and *ii*) the capacity of the subdorsal and subventral sectors (*i.e.* those bearing the cephalic papillae) is

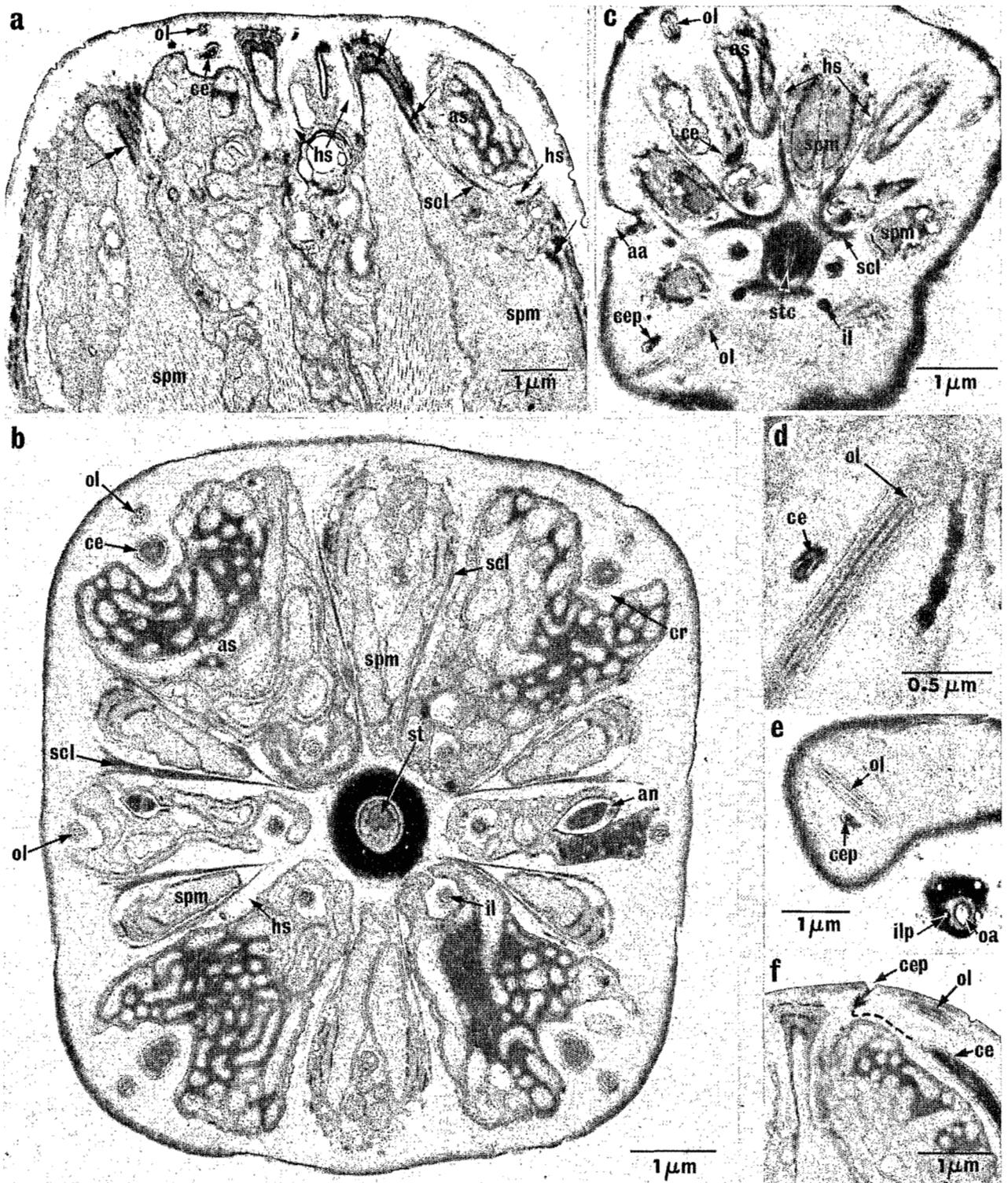


Fig. 4.

Fig. 4. TEM sections through the head of *H. viviparus*. a : L.S. showing very light construction of head skeleton (hs) and stylet protractor muscles (spm) attaching (at double arrows) to cuticle of face and body wall as well as to skeletal partitions. as = accessory sensillum ; ce = cephalic sensillum ; ol = outer labial sensillum ; scl = sclerotised region of skeleton, $\times 10\ 800$; b : T.S. showing slender spars of head skeleton (hs), the six each bifurcating to give twelve spars, with a very thin sclerotised layer (scl) at their centre. Six sectors contain stylet protractor muscles (spm). cr = cuticular ridge ; st = stylet. $\times 13\ 500$; Other abbreviations as in a ; c : slightly oblique T.S. at tip of head, showing head skeleton (hs) with light sclerotisation (scl), and stylet protractor muscle blocks (spm). The path of the left subdorsal cephalic sensillum (ce) turns off laterally at its tip. The tip of the left subventral outer labial sensillum (ol) is also shown. aa = amphidial aperture ; as = accessory sensillum ; cep = pore of cephalic papilla ; il = inner labial sensillum ; stc = stomatal cavity. $\times 13\ 000$; d : Tips of cephalic (ce) and outer labial (ol) sensilla at greater magnification. $\times 30\ 000$; e : T.S. tip of head, showing the oral aperture (oa) surrounded by pores of inner labial papillae (ilp), also the pore of a cephalic papilla (cep) alongside the outer labial sensillum (ol). $\times 11\ 250$; f. L.S. tip of head, showing the path of the cephalic sensillum recurving and opening through a pore at the surface. $\times 12\ 150$.

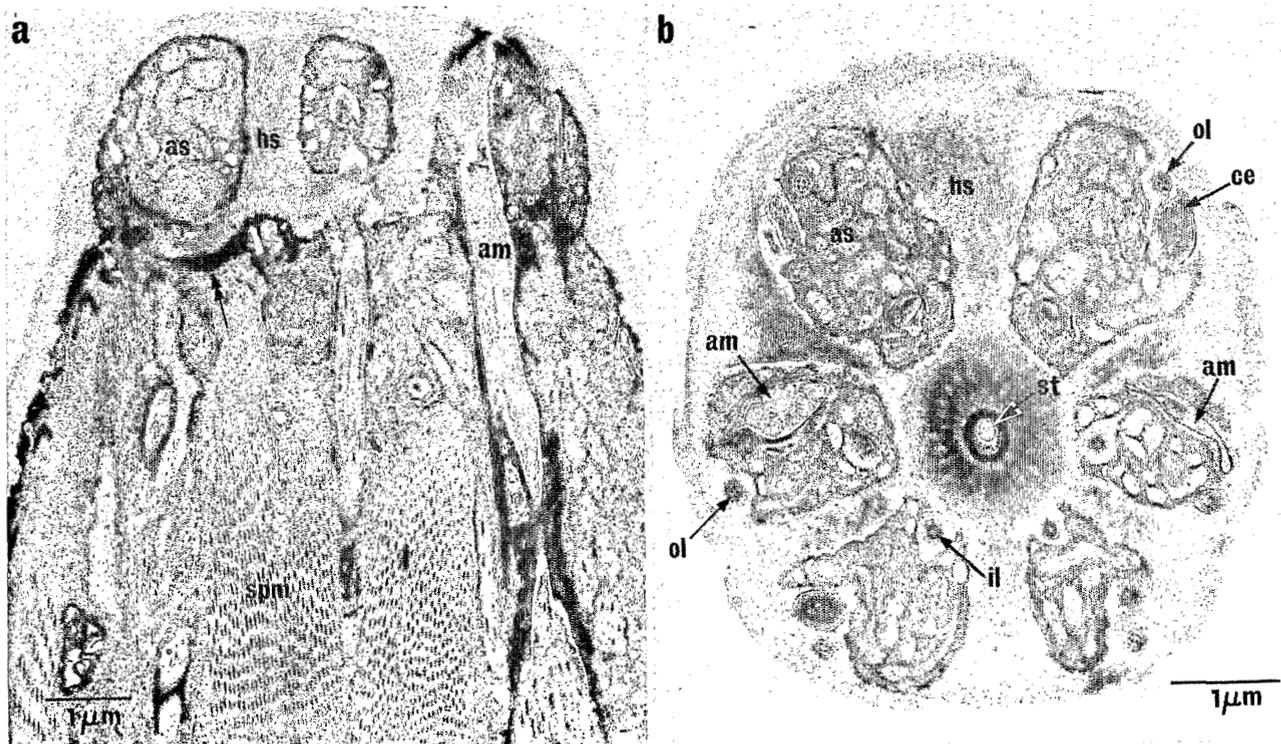


Fig. 5. TEM sections through the head of *D. dipsaci*. a : L.S. showing head skeleton (hs) with stylet protractor muscle (spm) attaching at double arrow. am = amphid ; as = accessory sensillum. $\times 11\ 250$; b : Slightly oblique T.S. showing heavily sclerotised head skeleton with six radial partitions. am = amphid ; as = accessory sensillum, ce = cephalic sensillum ; hs = head skeleton ; il = inner labial sensillum ; ol = outer labial sensillum ; st = stylet. $\times 14\ 000$.

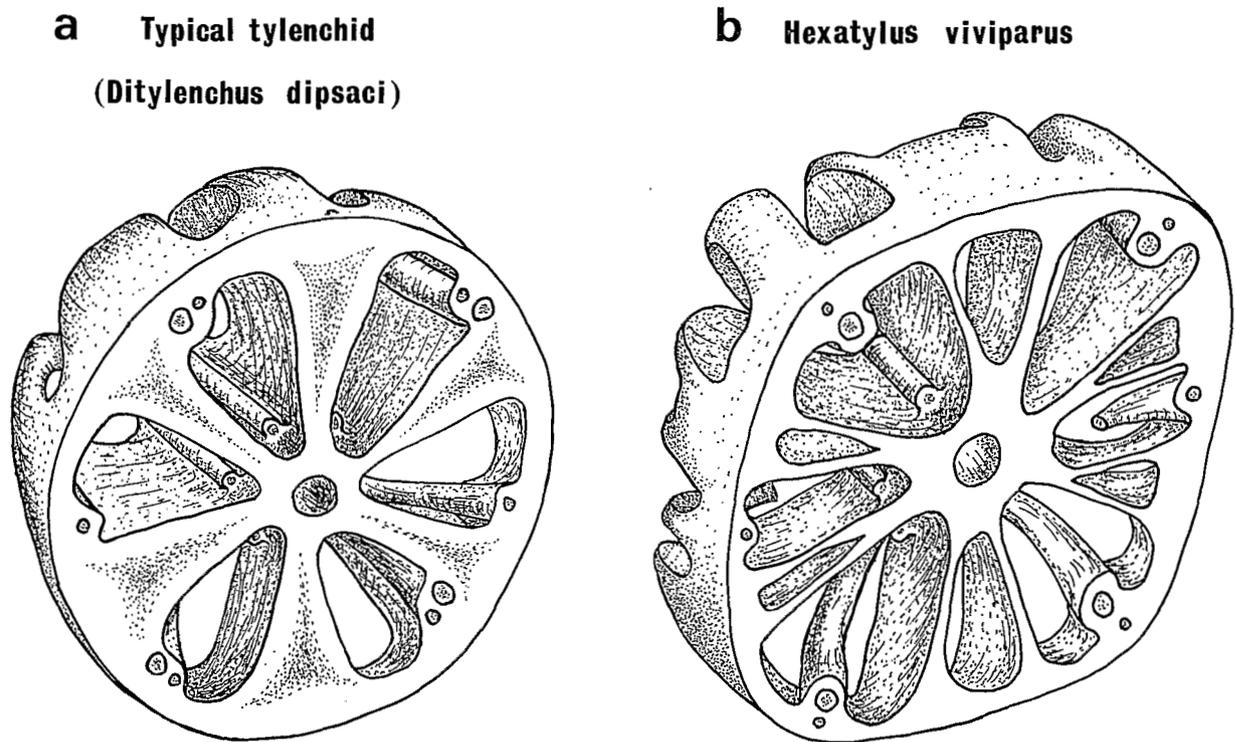


Fig. 6. Diagrammatic representation of head skeletons of a : a typical tylenchid (e.g. *D. dipsaci*) and b : *H. viviparus*.

enlarged to house the greater amount of sensory tissue (accessory receptors) in this species. The head skeleton is correspondingly modified to accommodate this tissue and, more especially, the extended muscles.

The tylenchid head skeleton appears in transverse section like a wheel, the stoma lining corresponding to the hub and the arms of the skeleton radiating out from this like spokes (Fig. 5b). The radial elements are the solid sclerotised partitions (Fig. 6a) that not only serve as surfaces for muscle attachment but also form the protective casing around the nervous tissue packed into the tip of the head. In most tylenchs there are six such partitions. In Figs 3b, 5a, b, 6a and 7a, *D. dipsaci* is taken as representing the typical tylenchid head symmetry. This and other tylenchid and aphelenchid heads are illustrated elsewhere (Yuen, 1967; De Grisse, 1977). The stylet protractor muscles are inserted anteriorly mainly on the broad posterior surfaces of the radial partitions (Figs 5a, 6a) which are slightly concave. The head skeleton itself consists of an electron translucent, finely fibrillar material that is continuous with and similar in appearance to the innermost layer of the cuticle of the head tip and of the stoma lining. In

the cuticle behind this region this layer is either very thin or absent. The skeletal framework is strengthened by an electron-dense, 'sclerotised' material at the core of each partition. This sturdy skeleton reinforces the structure, and also reinforces the impression of hexaradial symmetry of the tylenchid head as seen by light microscopy or SEM (Fig. 3b).

After processing for SEM, tissue shrinkage in the head of *H. viviparus* discloses a complex pattern of ridges (Fig. 3a) quite different from that in *D. dipsaci* (Fig. 3b). This pattern, like that seen with light microscopy (Fig. 1), is difficult to interpret until the underlying structure is revealed in section in the TEM (Fig. 4a-c) for comparison with SEM (Fig. 3a). The TEM sections (Fig. 4a, b) show that there is a very light skeletal framework in which the basic six radial partitions are each penetrated from back to front by a large hole, like the foramina in vertebrate skeletal structures (Figs 6b, 7b). Thus, instead of the stylet protractor muscles inserting on to the concave rear surface of a partition as in *D. dipsaci* (Figs 5a, 6a), some branches insert on to the body wall posterior to the head skeleton and others pass right through the foramina and insert on to the cuticle of the face. This

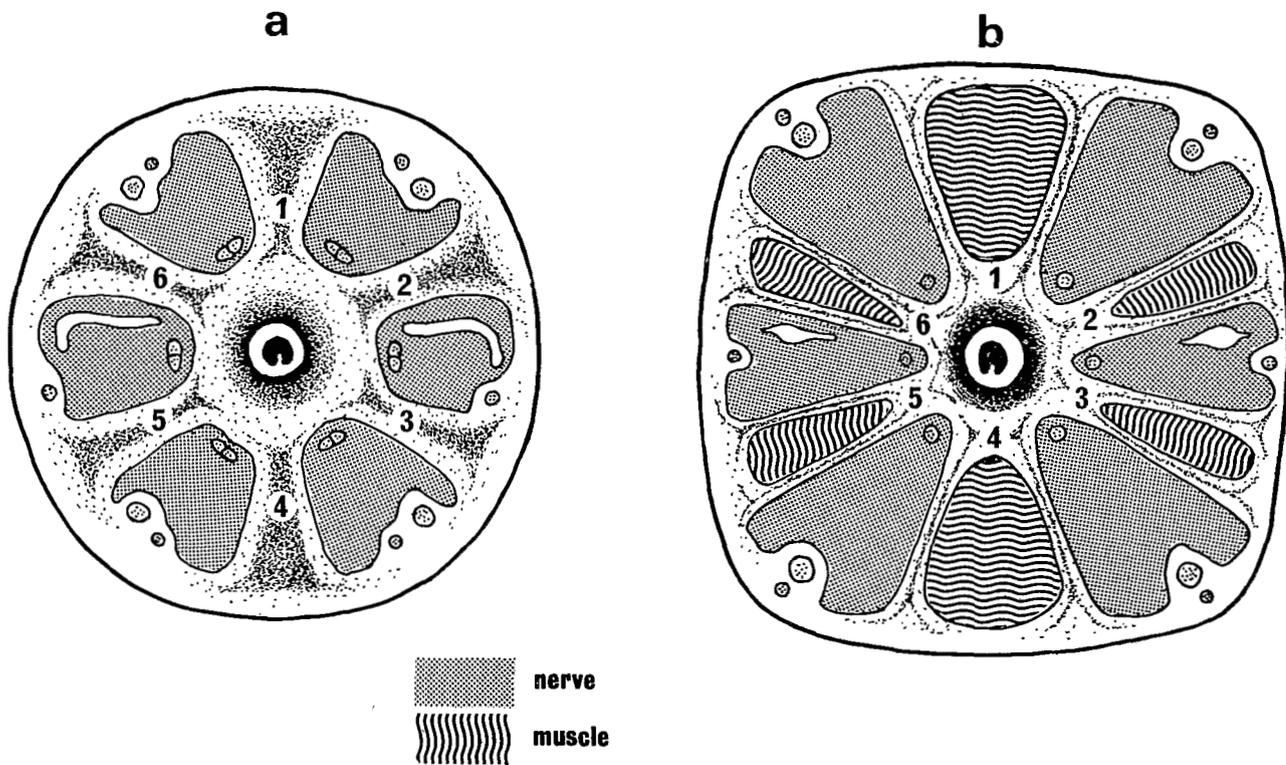


Fig. 7. Diagram of a T.S. through the head tip of a, a : typical tylenchid (e.g. *D. dipsaci*) and b : *H. viviparus* showing the head skeleton and the sectors containing nerve tissue only in a, and nerve and muscle in b.

arrangement has the effect of producing twelve very thin partitions or trabeculae, each with an extremely thin layer of sclerotised material at its centre (Figs 4a, b ; 7b). Also there are twelve sectors, not the customary six, and the framework encloses not just nerve tissue as in *D. dipsaci* (Figs 5b ; 7a) but both nerve and muscle tissue (Figs 4b, c ; 7b). In L.S. the attachment of the stylet protractor muscles to the facial cuticle and also to the side walls of the head and skeletal partitions (at double arrows in Fig. 4a) is clearly seen. In the light micrograph and the SEM picture, these muscle attachments form the dorsal, ventral and the dorso- and ventro-lateral segments (Fig. 1) or ridges (Fig. 3a). There is no demarcation of a head cap where the skeleton meets the body wall (Fig. 4a) as there is in *D. dipsaci* (Fig. 5a).

The ridges running from the centre to each corner forming the very distinctive cross-shaped pattern seen in Fig. 3a are the cuticular (or skeletal) ridges protruding into the "corner" cavities and carrying the sensory dendritic processes of the cephalic and outer labial receptors (Fig. 4b). Somewhat similar ridges are present in *D. dipsaci* (Fig. 5b) but the over-

all rigidity of the head skeleton evidently prevents their becoming so pronounced after the preparative treatment. Moreover, in *H. viviparus* the outer labial and the cephalic receptor processes lie on the same radius (the labial to the outside), so that the ridge is higher (Fig. 4b) than that in *D. dipsaci* where the receptors lie side by side in a lower but wider ridge (Fig. 5b). In *H. viviparus*, towards the tip of the cephalic receptor its channel makes a sharp lateral turn (Fig. 4c) to allow the process to recurve immediately beneath the cuticular surface (Fig. 4f). The very tip is thus brought alongside the outer labial receptor (on the side towards the amphid) (Fig. 4d, e), and is exposed through a pore (Figs 3a, 4f). The outer labial receptors end in a small cavity beneath the cuticular surface, four of them midway between the cephalic papillae and the stoma (Figs 3a ; 4c-e) ; the tips of the two lateral ones lie further out than these four, near the outer corner of the amphidial aperture, not outwardly detectable but in the approximate position indicated in Fig. 3a. The six inner labial papillae all open through pores on the labial disc around the stoma.

Thus the basic hexaradiate condition is still traceable in the head of *H. viviparus*, with the six sectors containing nerve tissues as in, for example, *D. dipsaci*, and the usual arrangement of sensilla. However it is modified into a twelve-sectored pattern by the bifurcation of each of the six skeletal partitions to accommodate the anterior projection of the stylet protractor muscle branches. The functional significance of this arrangement is not yet clear but it may be associated with the unusual feeding habits of this species. Other Neotylenchidae said to have an octagonal head skeleton when viewed with the light microscope may also prove to have similar adaptations, although this needs to be confirmed.

In other species with a cephalic framework the protractor muscles attach either to the framework, to the body wall just behind the head cap, to the cuticle of the stoma lining or to combinations of these regions (Wright, 1976). In those species lacking a sclerotised framework it appears that some of the protractor muscles, or some of the muscles controlling the buccal capsule, attach to the head tip, although published observations are few. Some of these latter species have cuticular "plates" (Wright, 1976), "bridges" and other excrescences (Lippens *et al.*, 1974) in the tip of the head but it is not clear whether these serve a similar function to the cephalic framework of the tylenchid species. Chitwood and Chitwood (1950) and Hooper (1978) suggest that the tylenchid head skeleton may have evolved as a modification of the cheilostom wall of more primitive forms.

Siddiqi (1980) defined the new suborder Hexatylyna, type superfamily Neotylenchoidea, with the type genus *Hexatylylus*, as having pore-like amphids close to the oral aperture. However, our studies show that the amphidial apertures are widely spaced, and outside the range of the pores of the cephalic papillae (Fig. 3a). Siddiqi's restriction of the Neotylenchoidea to groups with an "eight-sectored cephalic region" does not accord with our observations that *Hexatylylus* has a twelve-sectored cephalic region modified from the basic six-sectored one, although it can readily be understood why Siddiqi and the earlier workers interpreted the structure as they did.

These observations cast doubt on the validity of the interpretation of an eight-sectored cephalic framework within the Neotylenchoidea and elucidation by electron microscopy is desirable as material becomes available.

We propose that in the meantime the definition of the suborder Hexatylyna and superfamily Neotylenchoidea be amended to include a cephalic supporting framework with six to twelve sectors and eight to twelve sectors respectively. Details of the position of the amphid apertures of many Hexatylyna are not

known and as our observations of *Hexatylylus* show a different position from that interpreted by light microscopy it would seem that this character is not very reliable and should be excluded from the definition of Hexatylyna.

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