Comparative Fine Structure of the Stomatal Region of Males of Meloidogyne incognita and Heterodera glycines[1]

J. G. BALDWIN and HEDWIG HIRSCHMANN[2]

Abstract: The fine structure of the stylet, "guiding apparatus," and protractor muscles of males of *Meloidogyne incognita* and *Heterodera glycines* is elucidated and compared. In both nematodes, the stylet cone is of greater electron density than the shaft. The cone is heterogeneous; shaft material extends into it for most of its length, whereas the shaft proper and knob regions are relatively homogeneous. The stylet lumen is round throughout its length in *H. glycines*, but in *M. incognita* changes from round, posteriorly, to irregular, triradiate, and oblong near the stylet tip. The various layers of the lining of the stylet shaft are continuous with those of the cuticular lining of the esophageal lumen. Similarly, cuticular layers of the stomatal lining and vestibule extension are continuous with the body wall cuticle. The stylet "guiding apparatus" is formed by linings of the stomatal opening, vestibule, and vestibule extension, together with transversely folded membranes, which extend further posteriorly and attach near the junction of shaft and cone. Noncontractile regions of the three stylet protractor muscles originate in the esophagus. Contractile portions extend anteriorly from the stylet knobs and branch into a total of 10 elements that attach near the basal ring of the cephalic framework and the vestibule extension. A second, noncontractile region is present at the anteriormost part of the protractor muscles in *H. glycines*. *Key Words:* Cyst nematode, root-knot nematode, stylet, stylet "guiding apparatus," stylet protractor muscles.

The stomatal region of the digestive system is very diverse in structure and function among different nematodes and, therefore, is important in taxonomy and phylogeny as well as in elucidating the biology of some genera with respect to feeding habits. Earlier studies of stomatal fine structure in plant-parasitic nematodes were primarily limited to the Dorylaimida in attempts to explain virus transmission by nematodes (4). Tylenchid stomata have been examined only recently (5, 6, 9, 15). Additional fine-structural studies are needed to better understand structure and eventually ontogeny of the tylenchid stylet with its guiding apparatus and musculature.

Although males of *Heterodera* and *Meloidogyne* species apparently do not feed (10, 14), light microscopy indicates that they

do not exhibit the degree of stomatal and esophageal degeneracy present among non-feeding adult nematodes of other genera [viz. *Radopholus, Tylenchulus, Cricone-moides* (12)]. Fine-structural studies may elucidate whether or not the stomatal region, as well as the remaining digestive system, of males of *Heterodera* and *Meloido-gyne* are functional feeding organs. Furthermore, electron microscope observations of the stoma of all forms of Heteroderidae may be important in clarifying relationships among its genera.

MATERIALS AND METHODS

Meloidogyne incognita (Kofoid and White) Chitwood and *Heterodera glycines* Ichinohe were isolated from North Carolina populations and propagated in the greenhouse on tomato (*Lycopersicon esculentum* Mill.) and soybean (*Glycine max* L. Merr.), respectively. Males were selected and prepared for observation as described previously (2, 3). Sections were examined with a Hitachi HS-8 electron microscope operated at 50 Kv.

OBSERVATIONS

STYLET AND "GUIDING APPARATUS"

M. incognita: The stomatostylet of males of the North Carolina population of *M. incognita* has an average length of 23 μm including knobs, shaft, and cone (Fig. 1A, 2). It changes in diam from about 5.5 μm at the knobs to 1.8 μm at the anterior end of the shaft (Fig. 3-8), and less than 0.7 μm at the tip of the cone (Fig. 13). The bases of the two subventral knobs extend slightly posteriorly to the dorsal knob, and point of attachment of the cuticle lining of the esophageal lumen (Fig. 5, 26). The major portion of knobs and shaft is composed of an electron-lucent material in which strands of slightly more dense substance are scattered (Fig. 3, 4). Although the stylet lumen is circular and central in the knob region and posterior half of the shaft (Fig. 5-7), it becomes irregularly shaped anteriorly in the shaft (Fig. 8). The lumen remains about 0.3 μm in diam throughout knob and shaft region.

The conical part of the stylet is some-what more smoothly surfaced than the shaft and is generally of greater electron density. Its base is first apparent as an outer sheath which surrounds the anterior end of the shaft and as an inner sheath which surrounds the lumen (Fig. 9). Further anteriorly, the enclosed shaft material is confined to longitudinal channels between faint radii of cone material, which extend from the stylet lumen to the periphery, and dense strands extend longitudinally through the channels (Fig. 10). As the cone narrows, shaft material is reduced to a single dorsal chamber, and the lumen changes from central and triradiate (0.3 μm diam) to ventral and oblong (0.14 x 0.23 μm) (Fig. 11). Finally, the lumen opens on the ventral side, and shaft material terminates so that the oval-shaped anteriormost part of the stylet is solid (Fig. 13).

The conical part of the stylet is located within a channel, designated here as *stomatal cavity*, which extends posteriorly from the external stomatal opening to the base of the cone. In the shaft region, membranes, modified as junctional complexes,
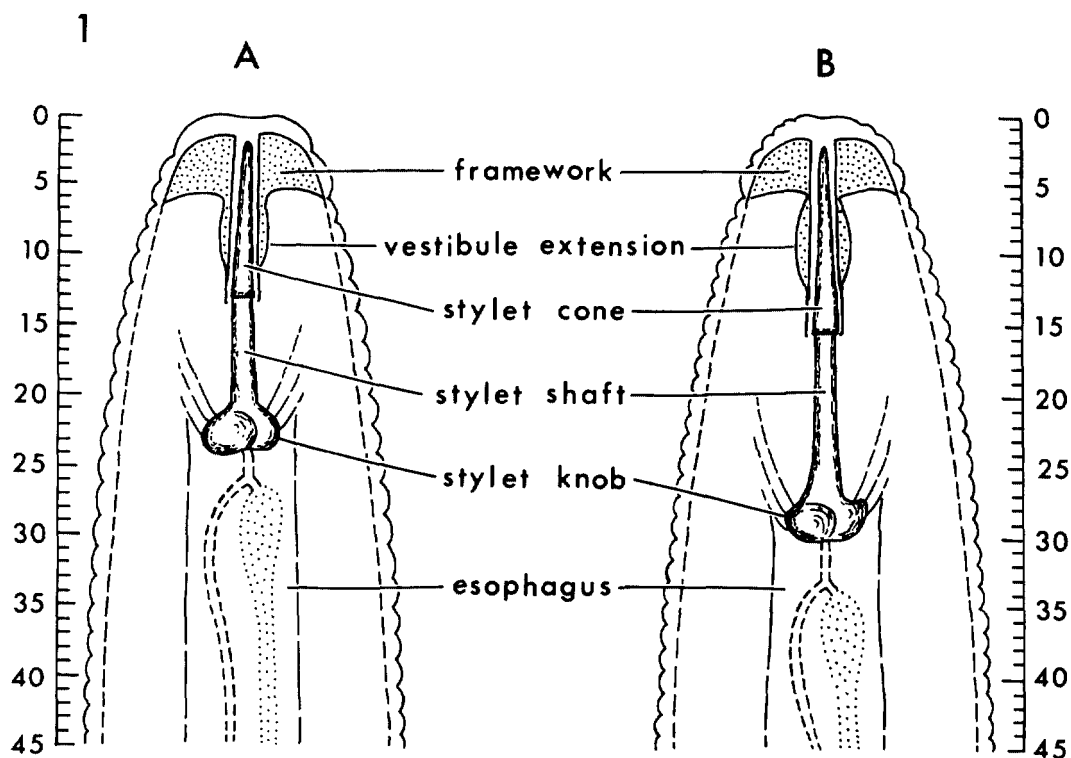

FIG. 1. Diagram illustrating stomatal region of male of *Meloidogyne incognita* (A) and *Heterodera glycines* (B). Scale units are in micrometers and indicate distance from anterior extreme of nematode.
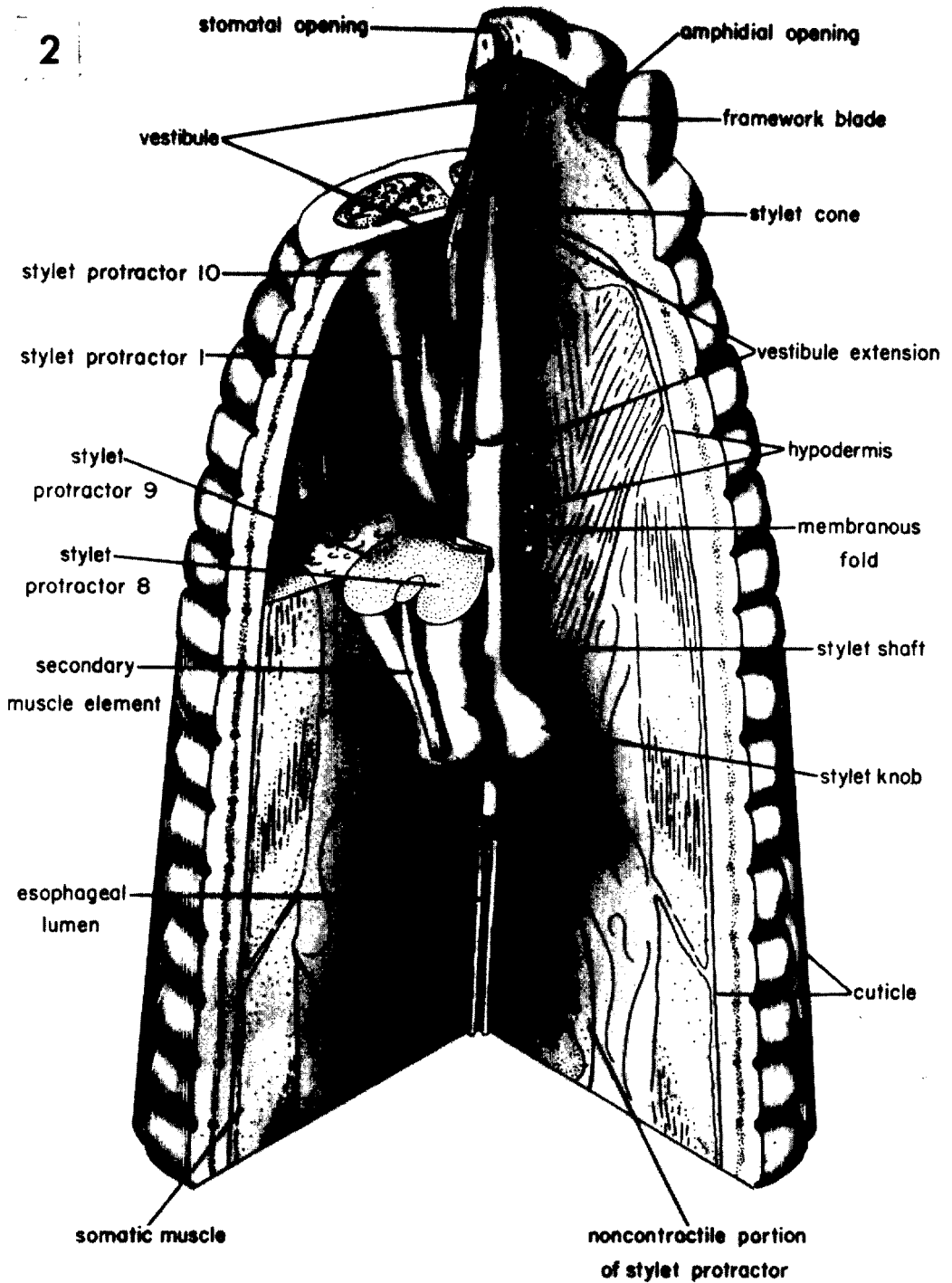
2

stomatal opening

amphidial opening

vestibule

framework blade

stylet cone

stylet protractor 10

stylet protractor 1

vestibule extension

hypodermis

stylet protractor 9

membranous fold

stylet protractor 8

stylet shaft

secondary muscle element

stylet knob

esophageal lumen

cuticle

somatic muscle

noncontractile portion of stylet protractor

FIG. 2. Three-dimensional, ventral-view diagram of cephalic region of *Meloidogyne incognita* male, showing relationship of stylet to "guiding apparatus" and protractor musculature. Basic structural relationships also generally apply to *Heterodera glycines male*.
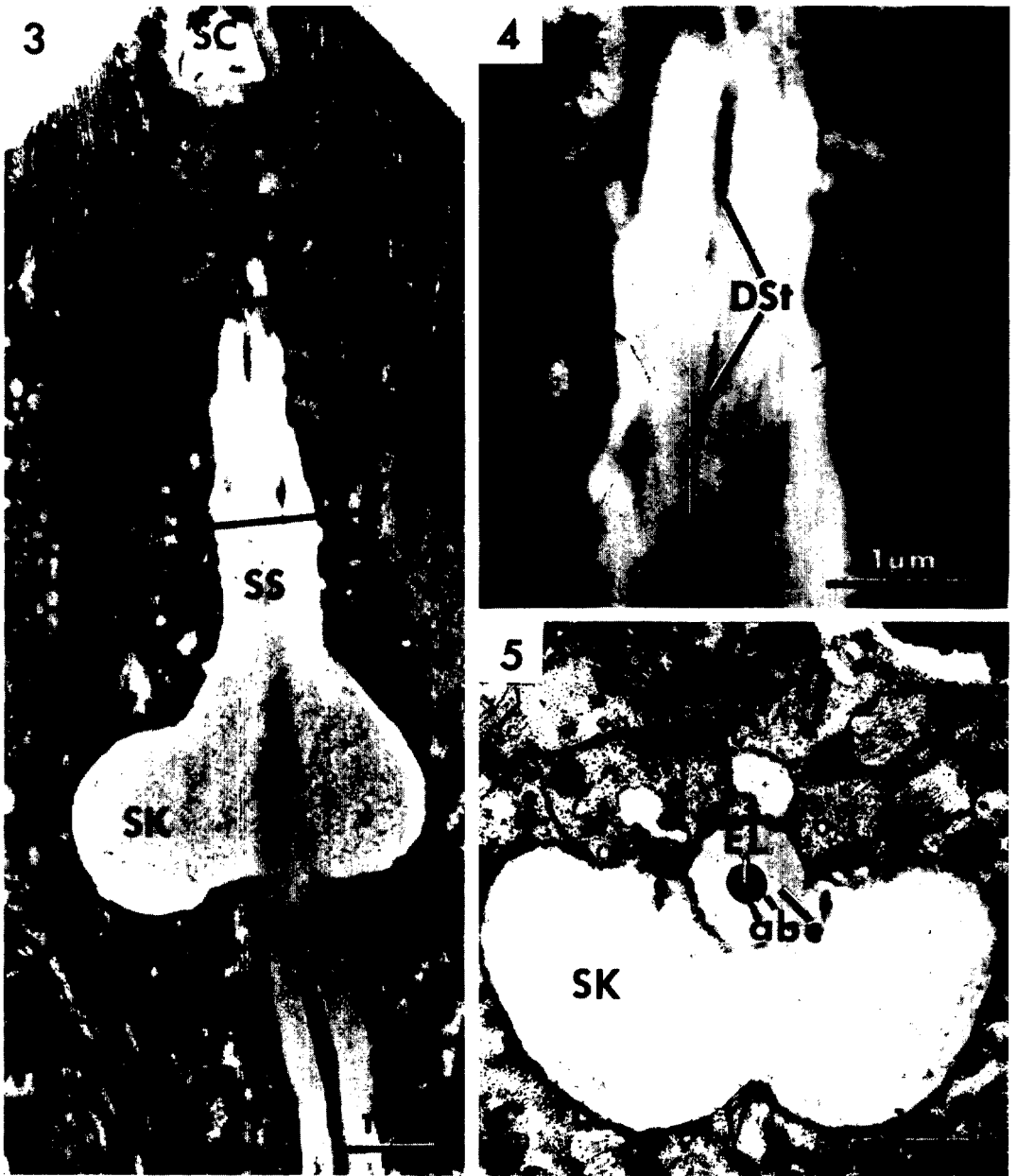
FIG. 3-5. 3) Longitudinal section through stylet of *Meloidogyne incognita*, showing shaft (SS), knobs (SK), and part of cone (SC). Rectangle enlarged in Fig. 4. SP, stylet protractor muscles. 4) Enlargement of rectangle in Fig. 3, showing portion of stylet shaft with membranous fold of "guiding apparatus" (arrows). DSt, dense strands; EMa, electron-lucent material. 5) Cross section through base of subventral stylet knobs (SK) in *M. incognita*, showing attachment of esophageal lumen (EL) with three layers: *a*, trilaminate inner layer; *b*, electron-lucent layer; *c*, dense layer (level 24.0, Fig. 1A).

extend posteriorly, fold inward, and attach anteriorly near the junction of stylet cone and shaft (Fig. 2, 3, 4). In cross section, these membranes appear as two circles surrounded by hypodermis (Fig. 2, 8), and the hypodermal area between the membranes is filled with electron-lucent material which is continuous with an outer layer of the vestibule extension (Fig. 2, 3, 4). The junctional complexes, together with stomatal linings, which include vestibule extension, vestibule, and stomatal opening, form what

has been generally designated by light microscopy as "guiding apparatus." Cross sections through the base of the shaft indicate that hypodermal tissue extends between protractor muscles and stylet (Fig. 2), and is bound to these surfaces by tight junctions and hemidesmosomes, respectively (Fig. 7).

The vestibule extension is primarily a three-layered structure which extends from the posterior end of the cone to the vestibule of the cephalic framework (Fig. 2). The innermost layer, *1*, which is about 0.05 μm thick, is continuous with the outer layer of external cuticle. Layer *1* may be further resolved into a thin (0.02 μm) electron-lucent zone, *1a*, and a dense zone, *1b* (0.03 μm) (Fig. 9-11). A narrow stomatal space separates layer *1* from the stylet. The medial layer, *2*, is relatively dense and homogeneous. It varies from 0.14 μm in thickness at the base (Fig. 9) to 0.50 μm at the broadest part of the vestibule extension (Fig. 10). Similarly, the outer, less dense layer, *3*, is 0.07 μm thick at the base (Fig. 9) but broadens anteriorly to 0.40 μm (Fig. 11). Narrow invaginations of layer *1* may extend into the medial layer, *2* (Fig. 10, 11).

The cephalic framework (including the basal ring, framework blades, and vestibule) is anterior to the vestibule extension (Fig. 12). The basal ring of the framework is continuous with the basal layer of body wall cuticle, and the vestibule is continuous with the vestibule extension, posteriorly. Vestibule and framework blades are of greater electron density than the basal ring. Posteriorly, the six radial blades are slightly arched and extend, one dorsally, two subdorsally, one ventrally, and two subventrally, from vestibule to body wall (Fig. 12). Thus the framework is bilaterally, rather than radially symmetrical, because of the large size of the lateral sectors. Internally the vestibule is lined with layer *1* of the vestibule extension (Fig. 12, 13) which is anteriorly continuous with the body wall cuticle. The stomatal opening is a slit 1.3 μm long when the stylet is retracted (Fig. 14).

H. glycines: The stomatostylet of males of *H. glycines* is approximately 28 μm long and changes in diam from about 5 μm at the knobs (Fig. 15) to 1.8 μm at the anterior end of the shaft (Fig. 16), and tapers to a blunt end at the terminus of the cone.

Knobs and shaft, including the lumen-lining, are similar to those described for *M. incognita* (Fig. 15, 16); however, the conical portion and "guiding apparatus" in *H. glycines* are different from those of *M. incognita*. At the base of the cone, shaft material is present between outer and inner layers of the cone, but radii dividing the shaft chamber have not been resolved in this region in *H. glycines* (Fig. 17). However, anteriorly there is an indication of radii as well as dense strands (Fig. 18). Here the lumen moves ventrally, and inner and outer cone material eventually fuse to result in an incomplete ring of shaft material (Fig. 19) which is eventually reduced to a dorsal chamber (Fig. 20). The lumen which is round (0.2 μm diam or less) opens ventrally (Fig. 22), and the oval-shaped tip of the stylet is solid.

The "guiding apparatus" in *H. glycines* males consists of stomatal linings and membranes modified as junctional complexes which surround the anterior portion of the shaft. These junctional complexes are similar to those described for *M. incognita*, although in contrast to the latter, membranes of the fold are longitudinally folded (Fig. 16). The hypodermal region between the membranes of the fold is filled with granular substance which surrounds most of the vestibule extension and is continuous with its outermost layer (Fig. 16, 17).

The posterior portion of the vestibule extension has an electron-lucent outer layer, *3* (0.06 μm thick), which is not clearly demarcated from the granular substance (Fig. 17). Three or four evenly spaced, shallow evaginations of the stomatal cavity extend into the moderately dense layer, *2* (0.1 μm) of the vestibule extension (Fig. 17), and anteriorly become more pronounced (Fig. 18, 19). Here the innermost layer, *1* (0.48 μm) includes an internal lining consisting of a thin electron-lucent zone, *1a*, a dense layer *1b*, and a thin electron-lucent layer, *1c*. Layer *1b* is about 0.05 μm in thickness except at the apexes of the invaginations where it is very thin. Externally, the granular substance is greatly reduced, and the protractor muscles are only separated at their anterior point of attachment by a thin hypodermal layer and the basal lamina which encloses the protractors (Fig. 18). The vestibule extension
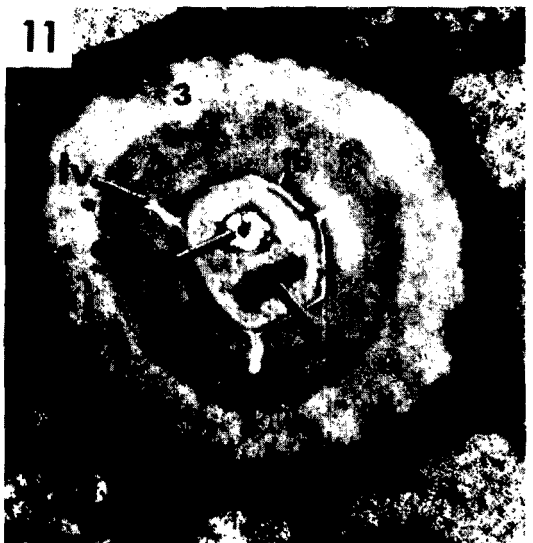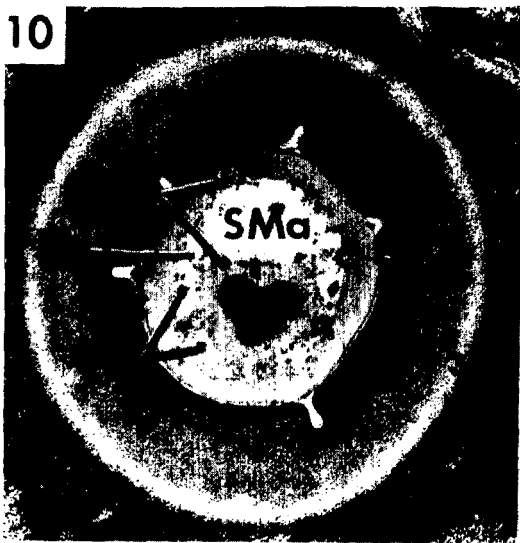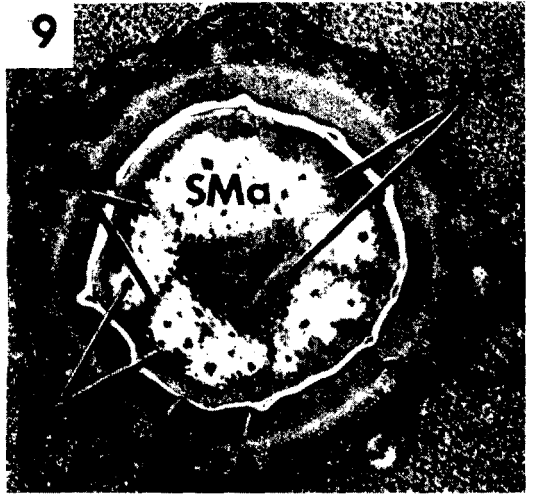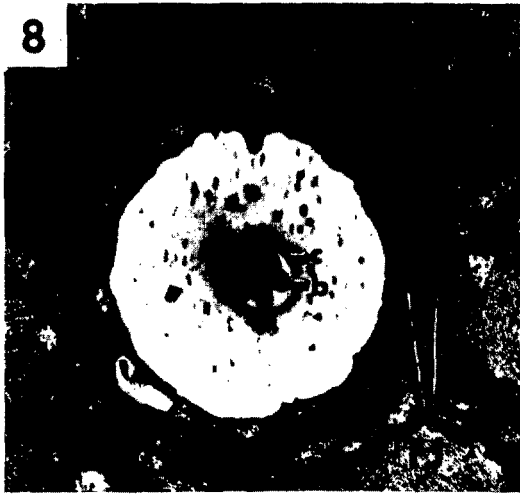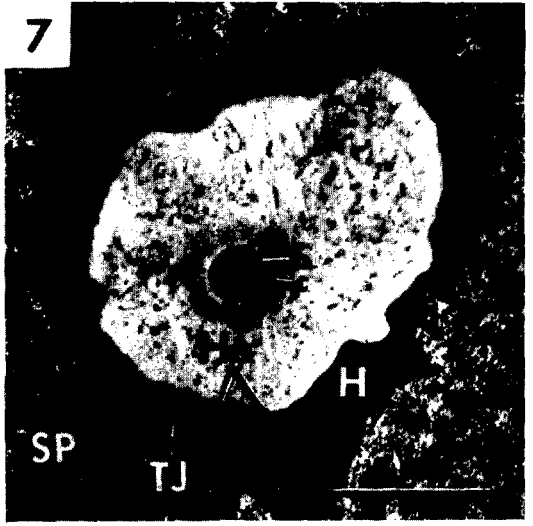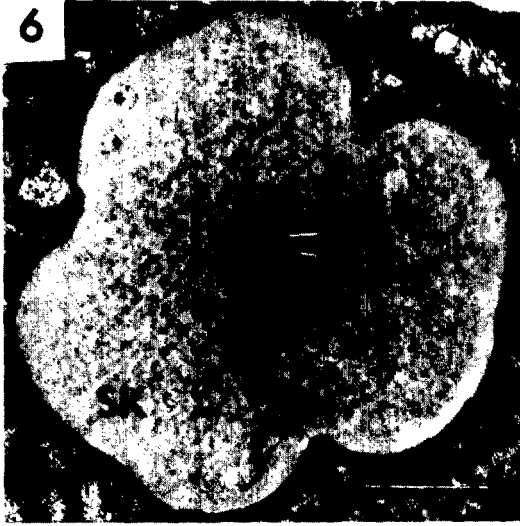
is bound to the hypodermis by numerous hemidesmosomes (Fig. 18). Anteriorly to the protractors, however, granular substance extends between the hemidesmosomes (Fig. 19, 20). The vestibule extension is reduced in diam as it approaches the framework, and layer *1a*, which is very thin and poorly resolved posteriorly (Fig. 19), broadens as it approaches the vestibule (Fig. 20). Layer *1a* appears to have an electron-dense internal boundary (Fig. 20-22). Stomatal space between vestibule extension and cone is much larger than in *M. incognita* (Fig. 9, 10, 18, 19).

The cephalic framework of *H. glycines* is radially symmetrical (Fig. 21). Six arched blades extend from the hexagonal vestibule to the basal ring, which is continuous with the body wall cuticle. The radial blades are dense and demarcated from the cuticle of the basal ring. The inner wall of the vestibule is lined with layer *1* of the vestibule extension which, anteriorly, is continuous with the outermost layer of the body wall cuticle. The stomatal opening is slit-like and about 1.3 μm long, when the stylet is retracted (Fig. 23).

STYLET PROTRACTOR MUSCLES

M. incognita: Males of this species have three stylet protractor muscle cells, each with a single nucleus which is located in a noncontractile portion. This portion extends about 9 μm posteriorly to the stylet knobs into the anterior esophagus (Fig. 24). The contractile portion of each muscle posteriorly attaches to a stylet knob (Fig. 24, 26, 28), and anteriorly, the muscles collectively branch into a total of 10 protractor elements (Fig. 27, 30-32). These protractors

attach to the basal lamina surrounding the vestibule extension and body wall near the basal ring of the cephalic framework (Fig. 34). The basal lamina, which encloses the esophagus, also collectively envelops the posterior portion of the contractile region of the protractors (Fig. 27), and anteriorly, it invaginates and eventually surrounds each muscle element at its anterior point of attachment (Fig. 33, 34).

The noncontractile portion of each muscle cell contains a large nucleus, numerous mitochondria, golgi, and β-glycogen (Fig. 24, 25). Anteriorly, near stylet knobs and base of the contractile region, fine filaments, occasional mitochondria, vesicles and sarcoplasmic reticulum become predominant (Fig. 26, 28). Here, junctional complexes occur among sarcolemmas (Fig. 26), and hemidesmosomes attach the muscles to the tops and sides of the knobs (Fig. 5, 6, 28). Further anterior to the knob region, both thick (sometimes hollow) and thin myofilaments fill the contractile region in a typical A band pattern (Fig. 29), but near the point of anterior attachment on vestibule extension and body wall by hemidesmosomes, the muscles again contain only thin myofilaments, sarcoplasmic reticulum, and occasional mitochondria (Fig. 33, 34).

Branching of the contractile regions into muscle elements begins slightly anteriorly to the knobs. As designated by Byers and Anderson (5), we have considered the dorsal muscle as *1-4*, according to the elements that branch from it, and the left and right subventral muscles as *5-7* and *8-10*, respectively (Fig. 27). In the region of the stylet shaft, elements *5* and *10* branch from the subventral muscles and extend to the dorsal side of the stylet (Fig. 30). Near the base of

←———⫷⫷⫷

FIG. 6-11. *6)* Cross section through stylet knobs (SK) of *Meloidogyne incognita* (level 23.0, Fig. 1A). *b, c,* as in Fig. 5; SL, stylet lumen. *7)* Cross section through stylet shaft and adjacent hypodermis (H) and stylet protractor muscles (SP) in *M. incognita* (level 21.0, Fig. 1A). *b, c,* as in Fig. 5; He, hemidesomosomes; TJ, tight junction. *8)* Cross section through anterior region of stylet shaft and surrounding tight junctions (TJ) in *M. incognita* (level 15.5, Fig. 1A). *b, c,* as in Fig. 5. Scale as in Fig. 7. *9)* Cross section through base of stylet cone and vestibule extension in *M. incognita.* Stylet cone composed of two rings of cone material (CMa) separated by shaft material (SMa), dense strands (DSt), and faint radii (Ra) (level 12.5, Fig. 1A). Layers of vestibule extension at this level: *1a,* thin inner layer; *1b,* thin inner dense layer; *2,* relatively thick moderately dense medial layer; *3,* outer less dense layer. Scale as in Fig. 7. *10)* Cross section through medial portion of vestibule extension in *M. incognita.* Stylet cone composed of two rings of cone material (CMa) with dense strands (DSt) and radii (Ra) present in shaft material (SMa) (level 9.0, Fig. 1A). *1b, 2, 3,* as in Fig. 9. Scale as in Fig. 7. *11)* Cross section through anterior portion of vestibule extension slightly beneath framework in *M. incognita* (level 7.0, Fig. 1A). *1b, 2, 3,* as in Fig. 9. Iv, invagination of vestibule extension; SL, stylet lumen; SMa, shaft material. Scale as in Fig. 7.
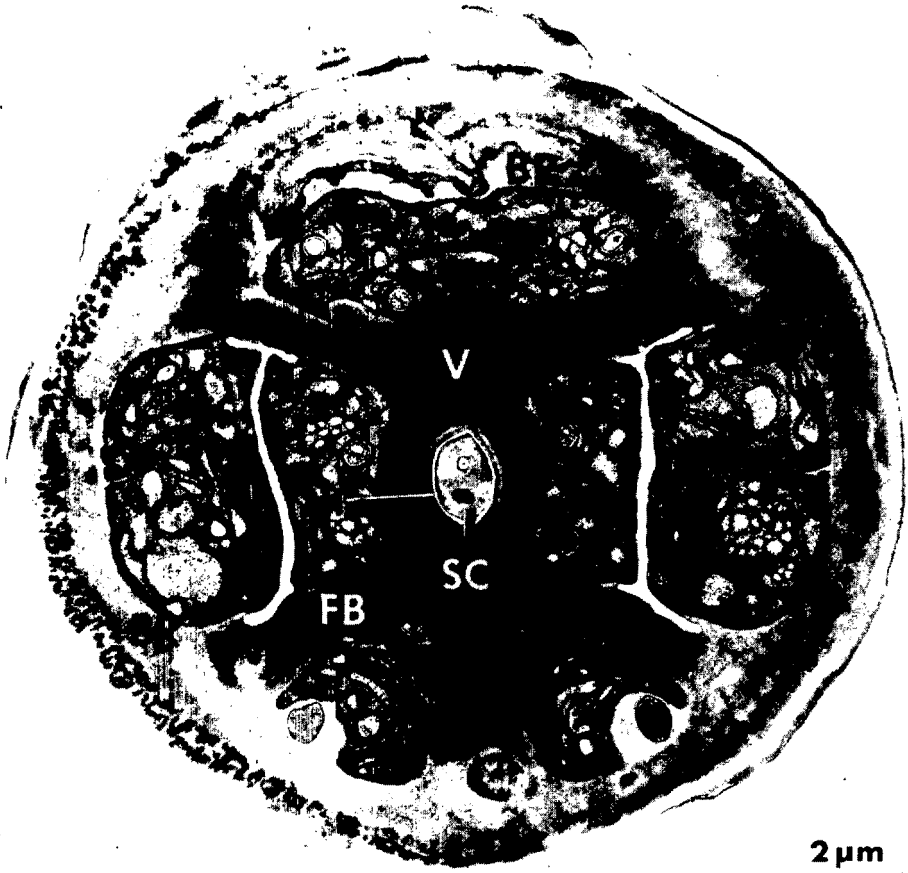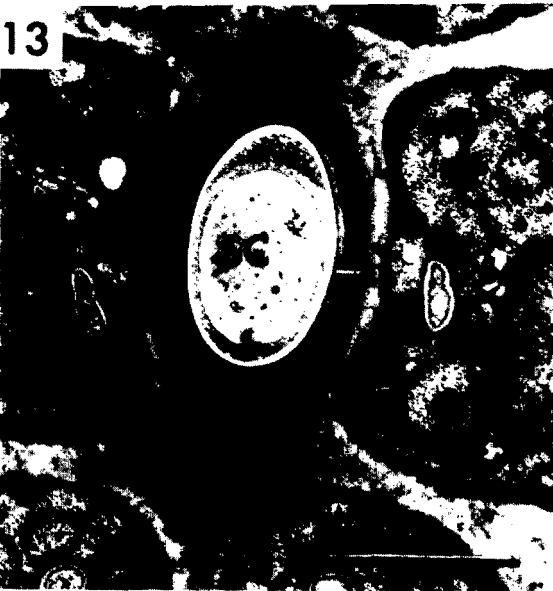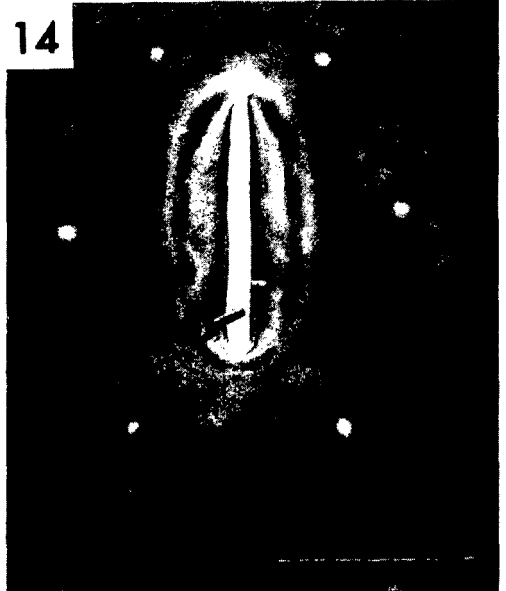
FIG. 12-14. *12)* Slightly oblique cross section through cephalic framework, including vestibule (V), framework blades (FB) and basal ring (BR) in *Meloidogyne incognita* (level 5.5, Fig. 1A). SC, stylet cone. *1* as in Fig. 9. *13)* Cross section through anterior portion of framework in *M. incognita*, showing vestibule (V) and anteriormost part of stylet cone (SC) (level 3.0, Fig. 1A). *1a* as in Fig. 9. *14)* Cross section through labial disc cuticle with stomatal opening (SO) in *M. incognita* (level 1.0, Fig. 1A). *1a* as in Fig. 9.

the vestibule extension, the dorsal muscle, *1-4,* divides into two equal elements (Fig. 31). Slightly anteriorly, the subventral elements split further into elements *6* and *7,* as well as *8* and *9,* which attach at the basal lamina exterior to the hypodermis of the body wall, and the basal lamina exterior to the hypodermis of the guiding apparatus, at the vestibule extension. Elements *5* and *10* also attach to the basal lamina, and dorsal elements, *1+2* and *3+4,* divide (Fig. 32).

In addition to the 10 major muscle elements, there are 4 secondary units, 2 subdorsal and 2 subventral, which posteriorly apparently attach to the esophageal lumen-lining and anteriorly terminate near the base of the vestibule extension (Fig. 2, 26, 27, 30, 31). These muscles are characterized by a distinct A band pattern of thick and thin myofilaments throughout their length.

H. glycines: Males of this species, as those of *M. incognita,* have three protractor muscles which anteriorly branch into 10 elements (Fig. 35-37) and four small separate secondary muscles that attach posteriorly to the esophageal lumen-lining (Fig. 35, 36). The noncontractile region of the muscles is generally similar to that of *M. incognita,* although the portion immediately posterior to and surrounding the knobs is filled with fine filaments and various forms of sarcoplasmic reticulum to a much greater extent than in *M. incognita* (Fig. 15, 35).

Further anteriorly, the muscles of *H. glycines* are surrounded by a basal lamina and attach adjacent to the vestibule extension as well as body wall (Fig. 37, 38). A second noncontractile region, which is filled with mitochondria and β-glycogen, exists at the anterior end of each muscle element (Fig. 38).
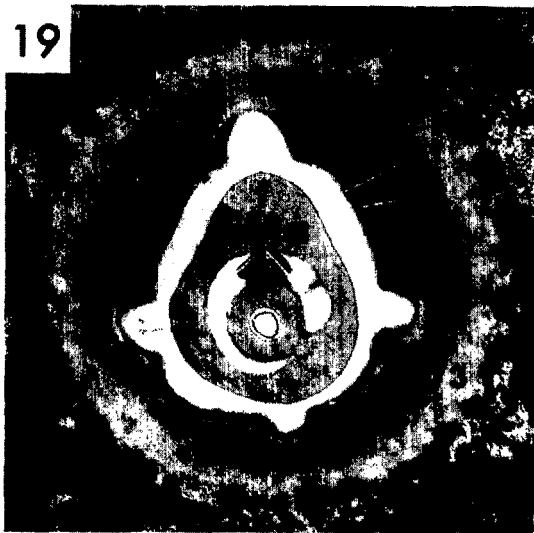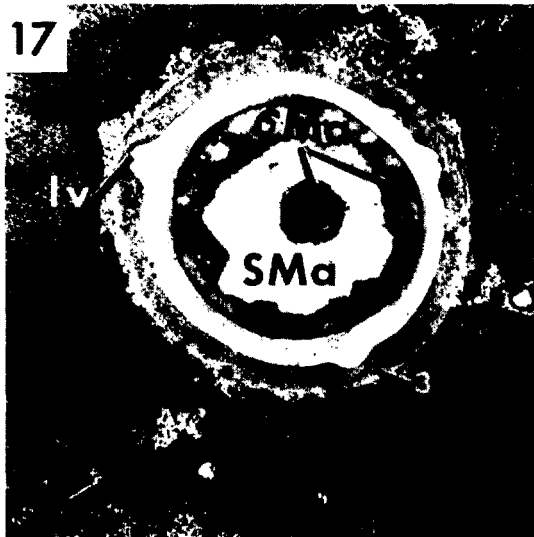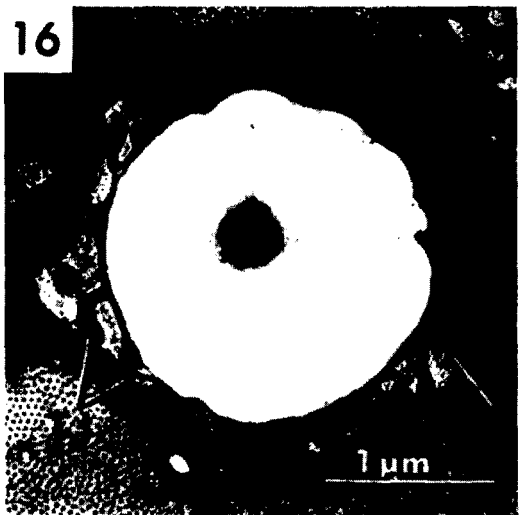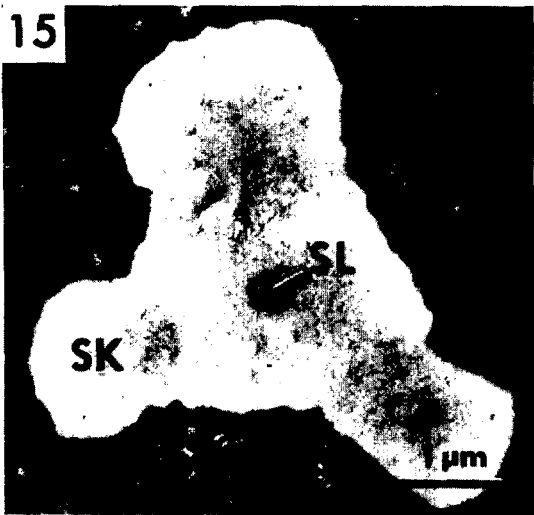
DISCUSSION

Stylets of several Tylenchida have been examined (5, 6, 9, 15) in addition to those of *H. glycines* and *M. incognita* of the present study. From these investigations certain generalizations can be made about the tylenchid stylet: in each case, the cone is of greater electron density than the shaft; cone material internally lines the lumen, and externally covers an anterior extension of the

shaft. Shaft and knob regions, on the other hand, are relatively homogeneous, although several layers of varying density may line the lumen. The stylet lumen appears to be round in the genera examined, except in *M. incognita* in which it varies from round, posteriorly, to irregular, triradiate, and oblong near the tip.

The "guiding apparatus" of *M. incognita* and particularly *H. glycines,* is similar to that described in *Tylenchorhynchus dubius* (Bütschli) Filipjev (5), and illustrated in *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven (6). These species have a vestibule extension similar to that described in *Ditylenchus dipsaci* (Kühn) Filipjev, although the stomatal lining of *T. dubius,* as well as *M. incognita* and *H. glycines,* connects near the junction of stylet cone and shaft rather than to a region near the knobs as suggested for *D. dipsaci* (15). Points of attachment and folds of the membranes in the stylet shaft region and possible folds of the posterior portion of the vestibule extension seem to be homologous with "guide rings" visible by light microscopy. However, the established terms "guide ring" and "guiding apparatus" may be inappropriate since they do not relate to the function of these structures.

The cuticular vestibule extension, which attaches near the junction of stylet cone and shaft, must fold or shorten during stylet protraction. It is possible that the relatively thin posterior portion of the vestibule extension folds as the stylet is protracted. Furthermore, invaginations of the stomatal space into the thick anterior portion of the vestibule extension allow for expansion, and thus shortening, to accomodate the protracted stylet. Partial attachment of stylet protractor muscles to the anterior vestibule extension may further tend to pull this portion outward as protractors contract. Hypodermal tissue and membranes surrounding the posterior part of the vestibule extension and stylet shaft, including granular substance and the membranous fold filled with electron-lucent material, are apparently sufficiently pliable to allow movement of the stylet.

The fine structure of the stylet protractor muscles of *H. glycines* and *M. incognita* males is similar to that of *D. dipsaci* (15),

27313539434852566064687276808488

93

ored97

lor101ored105 tended109113

ored

ored117

ored121

ored125129

P. penetrans (6), and *T. dubius* (5), and is probably similar throughout the Tylenchida. The smaller number of muscle elements observed with the light microscope in various Tylenchida (7, 8, 13) is apparently due to inadequacy of the light microscope in resolving plasmalemmas between closely apposed elements.

H. glycines and *M. incognita* differ from *D. dipsaci* in that the protractor muscles of *D. dipsaci* do not attach to the vestibule extension (15). *H. glycines* has a much more elaborate attachment system to the stylet knobs, with filaments extending further posteriorly than in *M. incognita*. Furthermore, the stylet musculature is more nearly symmetrical in *H. glycines* and differs by the presence of an anterior noncontractile region. The extra reserve of glycogen in this region would be a quick source of energy, which might be required for protruding the larger, more heavily sclerotized stylet of *H. glycines*. No stylet retractor muscles were detected. However, the elasticity of the esophagus is probably sufficient as a stylet retracting mechanism.

We have noted that the protractor muscles arise from noncontractile regions which are an integral part of the esophagus, and that the basal lamina which lines the esophagus also encompasses the protractor muscles. These observations, combined with the fact that the stoma terminates at the posterior end of the cone, and that the lumen-lining of stylet knobs and shaft is continuous with that of the esophagus, suggests that the shaft is more closely related to the esophagus than to the stoma, and

perhaps has developed both ontogenetically and phylogenetically from this structure, in contrast to other theories (1, 11). If additional investigations substantiate that the shaft is an integral part of the tylenchid esophagus, the spears of Tylenchida and Dorylaimida would be more similar than previously considered.

LITERATURE CITED

1. ANDRASSY, I. 1962. Über den Mundstachel der Tylenchiden (Nematologische Notizen, 9). Acta Zool. Hung. 8:241-249.
2. BALDWIN, J. G., and H. HIRSCHMANN. 1973. Fine structure of cephalic sense organs in Meloidogyne incognita males. J. Nematol. 5:285-302.
3. BALDWIN, J. G., and H. HIRSCHMANN. 1975. Fine structure of cephalic sense organs in Heterodera glycines males. J. Nematol. 7: 40-53.
4. BIRD, A. F. 1971. The structure of nematodes. Academic Press, New York. 318 p.
5. BYERS, J. R., and R. V. ANDERSON. 1972. Ultrastructural morphology of the body wall, stoma, and stomatostyle of the nematode, Tylenchorhynchus dubius (Bütschli, 1873) Filipjev 1936. Can. J. Zool. 50:457-465 (7 plates).
6. CHEN, T. A., and G. Y. WEN. 1972. Ultrastructure of the feeding apparatus of Pratylenchus penetrans. J. Nematol. 4:155-161.
7. COOMANS, A. 1962. Morphological observations on Rotylenchus goodeyi Loof and Oostenbrink, 1958. II. Detailed morphology. Nematologica 7:242-250.
8. COOMANS, A., and J. VAN BEZOOIJEN. 1968. Observations on the anterior body region of Helicotylenchus pseudorobustus. Nematologica 14:146-148.
9. DE GRISSE, A. T. 1972. Electron microscope studies of the stylet, stylet sheath and stylet muscle structure of Macroposthonia xenoplax

FIG. 15-20. *15)* Cross section through stylet knobs (SK) of *Heterodera glycines* (level 28.0, Fig. 1B). SL, stylet lumen. *16)* Cross section through anterior region of stylet shaft and folded tight junctions (TJ) which form guiding membranes in *H. glycines* (level 18.0, Fig. 1B). GS, granular substance. *17)* Cross section through base of stylet cone and vestibule extension in *H. glycines*. Stylet cone composed of two rings of cone material (CMa) separated by shaft material (SMa) (level 15.0, Fig. 1B). Layers of vestibule extension at this level: *2,* moderately dense layer; *3,* electron-lucent layer; GS, granular substance; Iv, invagination of vestibule extension. Scale as in Fig. 16. *18)* Cross section through medial portion of vestibule extension in *H. glycines*, showing faint radii (Ra) and dense strands (DSt) in region of shaft material (SMa) (level 10.0, Fig. 1B). Layers of vestibule extension as in Fig. 17; additional layers are: *1b,* thin dense inner layer; *1c,* thin medial electron-lucent layer. Stylet protractor muscle (SP) is surrounded by basal lamina (BL) where it contacts hypodermis (H) which surrounds vestibule extension. He, hemidesmosome; Iv, invagination. Scale as in Fig. 16. *19)* Cross section through stylet cone and vestibule extension anterior to attachment of protractor muscles in *H. glycines* (level 8.0, Fig. 1B). Shaft material (SMa) reduced to a partial ring. Thin innermost layer *1a* of vestibule extension visible; other layers as in Fig. 17 and 18. GS, granular substance. Scale as in Fig. 16. *20)* Cross section through stylet cone and vestibule extension just beneath framework in *H. glycines*, showing broadened layer *1a* in vestibule extension, chamber with shaft material (SMa) and stylet lumen (SL) (level 6.5, Fig. 1B). Layers of vestibule extension as in Fig. 17-19. Scale as in Fig. 16.

and M. rustica. Page 17 *in* Rep. 11th Int. Symp. Nematol., Reading, 3-8 September 1972.

10. FRANKLIN, M. T. 1965. Meloidogyne—Root-knot eelworms. Pages 59-88 *in* J. F. Southey, ed. Plant nematology. Her Majesty's Stationery Office, London.

11. GOODEY, J. B. 1963. Speculations on the identity of the parts of the tylenchid spear. Nematologica 9:468-470.

12. GOODEY, T. 1963. Soil and freshwater nematodes. Second edition by J. B. Goodey. London, Methuen and Co., 544 p.

13. HIRSCHMANN, H. 1959. Histological studies on the anterior region of Heterodera glycines and Hoplolaimus tylenchiformis. Proc. Helminthol. Soc. Wash. 26:73-90.

14. SHEPHERD, A. M. 1965. Heterodera: Biology. Pages 89-102 *in* J. F. Southey, ed. Plant nematology. Her Majesty's Stationery Office, London.

15. YUEN, P. H. 1967. Electron microscopical studies on Ditylenchus dipsaci (Kühn). I. Stomatal region. Can. J. Zool. 45:1019-1033 (11 plates).

FIG. 21-23. *21)* Cross section through cephalic framework, including framework blades (FB) and basal ring (BR) in *Heterodera glycines* (level 5.5, Fig. 1B). Layers of vestibule as in vestibule extension, Fig. 17-20. *22)* Cross section through anterior portion of framework in *H. glycines*, showing tip of stylet where lumen (SL) turns ventrally toward its opening (level 3.5, Fig. 1B). Layer *1a* of vestibule as in vestibule extension, Fig. 17-20. *23)* Cross section through labial disc cuticle with stomatal opening (SO) in *H. glycines* (level 1.0, Fig. 1B).
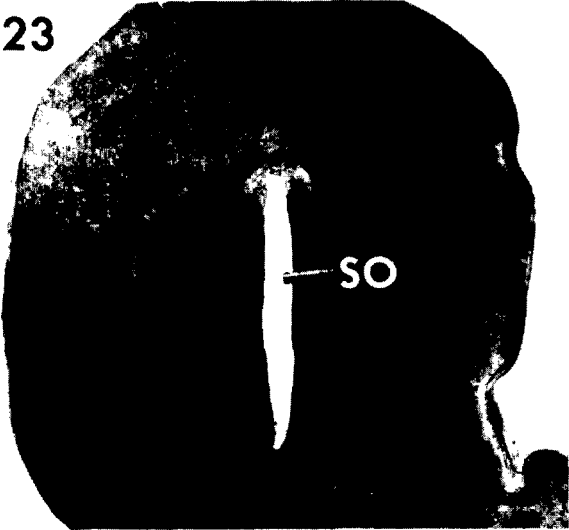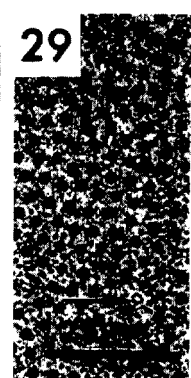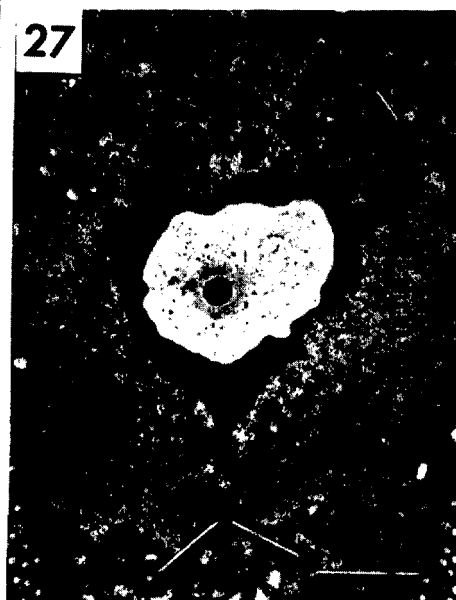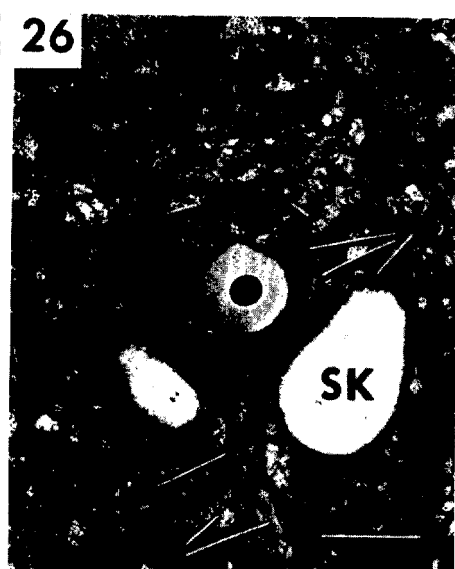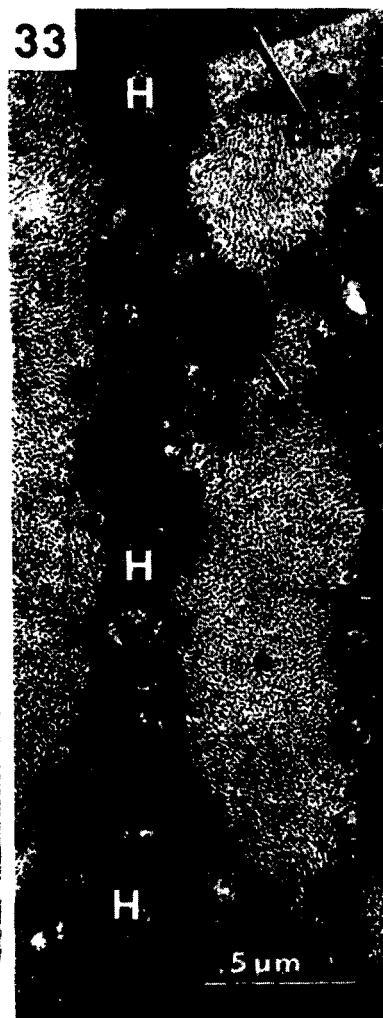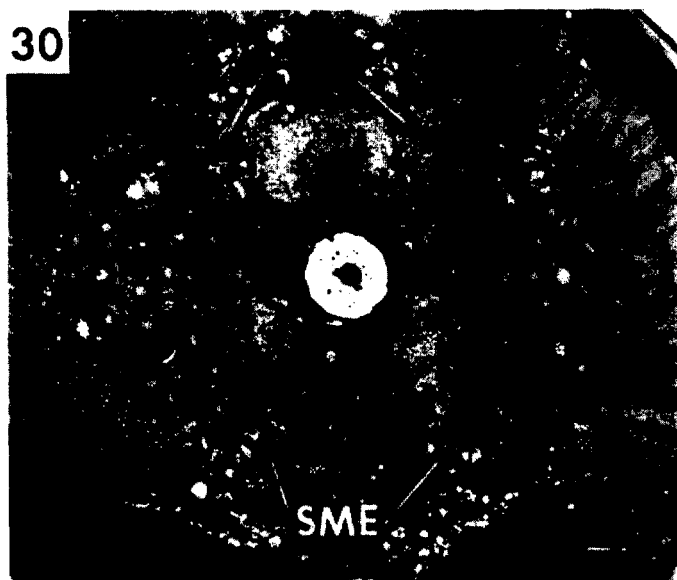
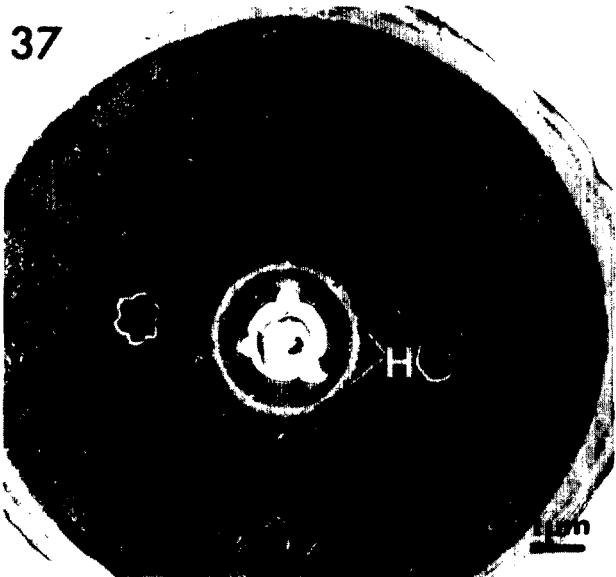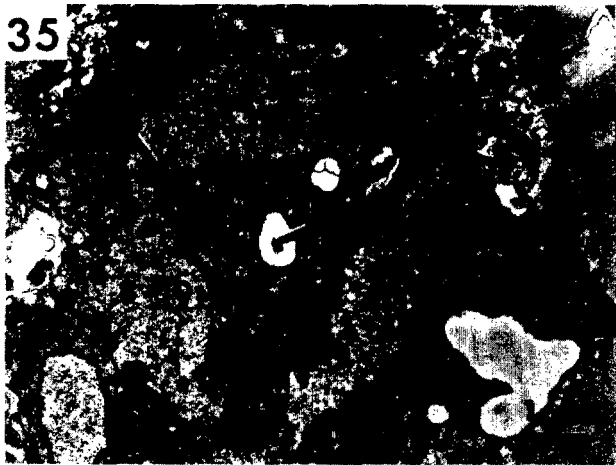35

36

SME

37

H

1µm

38

SM

.25µm
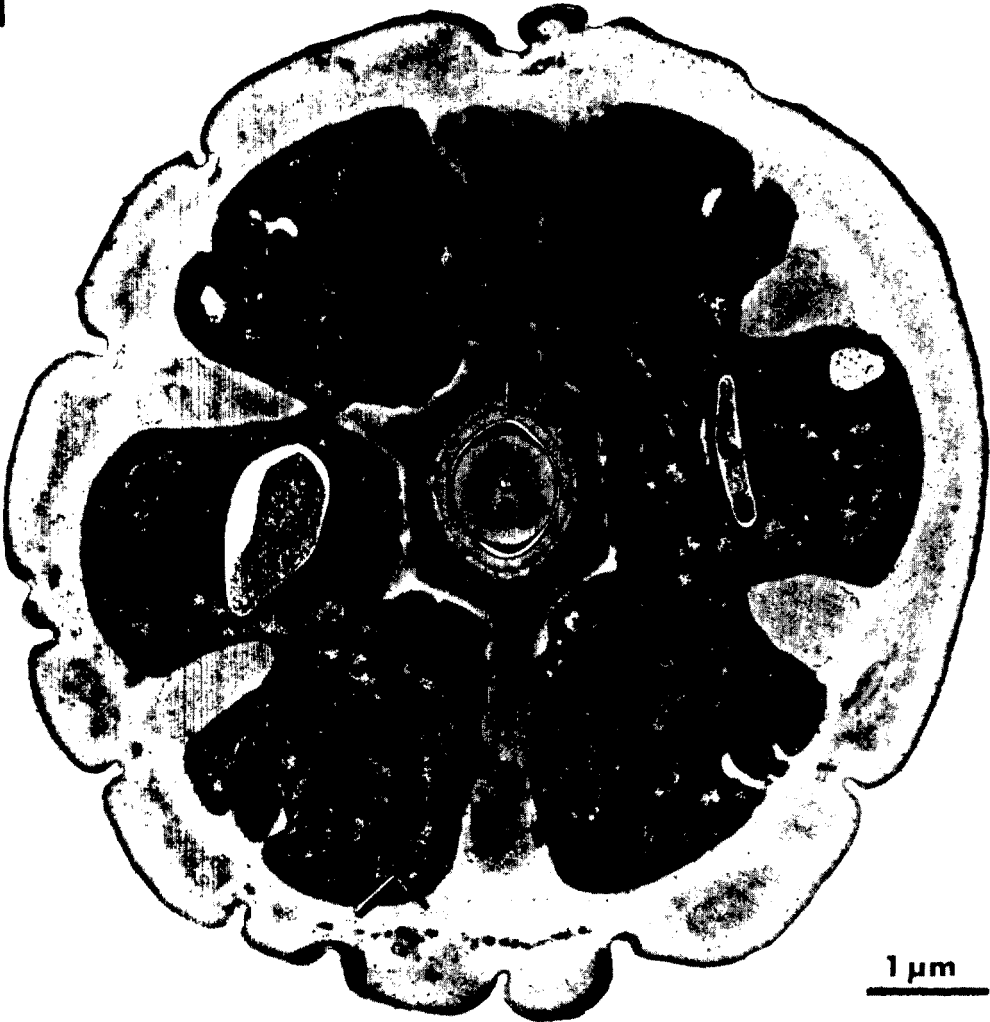
FIG. 24-29. *24)* Longitudinal section of *Meloidogyne incognita* with stylet protractor muscles (SP), including sarcoplasmic portions with nuclei (Nu). Rectangle enlarged in Fig. 25. DGO, dorsal gland orifice; Go, golgi apparatus; EL, esophageal lumen; SK, stylet knob; SS, stylet shaft. *25)* Enlargement of sarcoplasmic portion of stylet protractor muscle with organelles. ER, endoplasmic reticulum; Gl, β-glycogen; Go, golgi apparatus; M, mitochondrion. *26)* Cross section through base of subventral stylet knobs (SK) in *M. incognita,* showing attachment of stylet protractor muscles *(1-4, 5-7, 8-10)* and secondary muscle elements (SME) (level 24.5, Fig. 1A). JC, junctional complex; Vs, vesicles. *27)* Cross section through base of stylet shaft and contractile portion of stylet protractor muscles *(1-4, 5-7, 8-10)* in *M. incognita* (level 21.0, Fig. 1A). SME, secondary muscle elements. *28)* Cross section through region of stylet knob (SK) in *M. incognita,* showing hemidesmosome (He) connections to protractor muscle (level 23.0, Fig. 1A). *29)* Cross section through thick and thin myofilaments of stylet protractor muscle with some thick myofilaments hollow (arrows).

FIG. 30-34. *30)* Cross section through anterior region of stylet shaft in *Meloidogyne incognita,* showing stylet protractor muscles *(1-4, 5, 6+7, 8+9, 10)* (level 15.5, Fig. 1A). SME, secondary muscle elements. *31)* Cross section through base of stylet cone in *M. incognita,* showing stylet protractor muscles *(1+2, 3+4, 5, 6, 7, 8, 9, 10)* (level 12.5, Fig. 1A). *32)* Cross section through anterior portion of vestibule extension in *M. incognita,* showing stylet protractor muscles *(1, 2, 3, 4, 5, 6, 7, 8, 9, 10)* (level 8.0, Fig. 1A). *33)* Cross section through stylet protractor muscle *(6)* near its anterior terminus, with only fine filaments and an occasional mitochondrion (M) in *M. incognita* (level 7.0, Fig. 1A). H, hypodermis; BL, basal lamina. *34)* Cross section immediately posterior to basal ring of cephalic framework in *M. incognita* with attachment of protractor muscle *(7)* near vestibule extension (VE) and somatic muscles (SM) (level 6.0, Fig. 1A). BL, basal lamina.

FIG. 35-38. *35)* Cross section through stylet protractor muscles *(1-4, 5-7, 8-10)* of *Heterodera glycines* with fine filaments which occur posterior to stylet knobs (level 30.5, Fig. 1B). EL, esophageal lumen; SME, secondary muscle element. *36)* Cross section through stylet protractor muscles *(1+2, 3+4, 5, 6+7, 8+9, 10)* of stylet shaft in *H. glycines* (level 24.0, Fig. 1B). SME, secondary muscle elements. *37)* Cross section through stylet protractor muscles *(1, 2, 3, 4, 5, 6, 7, 8, 9, 10)* in region of stylet cone in *H. glycines* (level 10.0, Fig. 1B). H, hypodermis. *38)* Cross section through anterior portion of stylet protractor muscle *(6)* in *H. glycines* with cytoplasmic region. Arrow indicates thin myofilaments present near points of attachment (level 10.0, Fig. 1B). BL, basal lamina; M, mitochondrion; Gl, β-glycogen; SM, somatic muscle.