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MORPHOLOGICAL STUDIES ON *HIRSCHMANNIELLA ORYZAE* (SOLTWEDEL) (NEMATODA : TYLENCHIDA)

By

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(With 11 Text-figures)

INTRODUCTION

The genus *Hirschmanniella* Luc and Goodey, 1963 is cosmopolitan in its distribution and an established parasite of paddy, banana, etc. Sanwal (1957) gave a good account of the morphology of one of its widely known species, *H. gracilis* (de Man, 1880). Sher (1968) gave an excellent revision of the genus which to-date includes 16 species. The species, *H oryzae* (Soltwedel, 1889) Luc and Goodey, 1963 is of common occurrence around paddy in this country and also in Japan, Formosa, Malaysia, Nigeria, Venezuela, San Salvador, Indonesia, Ghana etc. Taking into account the world wide distribution of the genus and its economic importance, it was proposed to undertake detailed morphological study of *H oryzae* which is the most common species of the genus in India. The morphological studies are important because these provide pertinent information on a particular species and its intra-specific variations ; this has a direct bearing on the taxonomy of the genus concerned and also on the related groups. The utility of a structure as taxonomic character can only be judged by adequate study of the morphology based on large number of specimens obtained from different populations.

The observations were made on fixed as well as on live specimens.

The authors are thankful to Professor S. Mashhood Alam, Head, Department of Zoology for providing laboratory facilities.

MATERIALS AND METHODS

Materials.—The specimens of *Hirschmanniella oryzae* for the present study were collected from the soil around roots as well as from within the roots of paddy, *Oryza sativa* L. from the following localities in the district Aligarh.

(i) 73 ♀♀ and 27 ♂♂ from Jamalpur north on 1.1.1968
(ii) 58 ♀♀ and 22 ♂♂ from Jamalpur east on 3.11.1967
(iii) 55 ♀♀ and 22 ♂♂ from Bhambhola on 26.10.1967
(iv) 56 ♀♀ and 21 ♂♂ from University Agriculture Farm (A) on 26. 10.1967
(v) 48 ♀♀ and 18 ♂♂ from University Agriculture Farm (B) on 3.11.1967
(vi) 45 ♀♀ and 20 ♂♂ from Qasimpur north on 4.3.1968
(vii) 57 ♀♀ and 20 ♂♂ from Qasimpur south on 4.3.1968
(viii) 56 ♀♀ and 21 ♂♂ from Zohra Bagh on 4.3.1968
(ix) 56 ♀♀ and 19 ♂♂ from CDF road field (A) on 25.2.1968
(x) 56 ♀♀ and 20 ♂♂ from CDF road field (B) on 18.3.1968

Methodology.—The specimens for the morphological study were stained with different techniques for obtaining various structural details. The gross morphology was studied on the specimens stained with the saturated solution of Picric acid. Intravital staining was done with different stains but the best results were obtained with Methylene blue. In particular, it was helpful in the study of excretory and digestive systems of the nematode. First, the specimens were kept for two hours in Methylene blue then fixed in Picro-carmine. Methyl red was also tried as intra-vital stain but it gave poor results.

Cuticle and associated structures.—For the study of Cuticle and its modifications, the method employed by Sanwal l.c. was followed except that the Silver nitrate was acidulated by adding a few drops of nitric acid. After the nematodes had assumed a dark brown appearance, they were transferred to a mixture of glycerine-alcohol (5 parts glycerine : 95 parts 30% alcohol) and then processed as usual. This method gives excellent details of cuticular structures including transverse striae, lateral fields, amphids, phasmids, oral aperture, anal opening, excretory pore, etc. It also stains the reproductive organs specially the nuclei of the oocytes, spermatocytes and that of the oesophageal glands. An attempt was made to study the nervous system by staining it with silver impregnation technique but poor results were obtained, more so, because the entire nervous system leaving aside the nerve ring and associated cells is very delicate and difficult to study in such a small nematode. For the study of sphincter muscles encircling the excretory bulb of the terminal excretory duct and outlet of the terminal duct some specimens were kept in a mixture of picric acid and A.F.A. for about two weeks.

For detailed study of the muscles the following method was found good :—First of all, living nematodes were stained with methylene blue and then fixed in picro-carmine. Later on, they were transferred to haematoxylin and finally to a mixture of aniline blue, orange red, and acetic acid (in equal proportions). After keeping the nematodes in this mixture for two weeks they were treated with

lactophenol. Specimens were then mounted in anhydrous glycerine by usual technique.

Chang's nerve stain (theonin 0.3 gm., formaldehyde 4% 10 cc and distilled water 90 cc) was found to be a good medium for the study of nervous system.

OBSERVATION

(For measurements and dimensions see Table 1 & 2)

General Body Shape

(Text-figs. 1 & 2)

The nemotodes are irregularly coiled, whitish to bluishgreen in colour while alive. In water, the nematodes start swarming in a few hours time. Their characteristic movement helps in quick detection under the stereoscopic binocular microscope. The nematodes upon fixation assume ventrally arcuate posture, slightly narrowing anteriorly above the level of oesophago-intestinal junction and sharply behind the anus. The width of body in the middle third is nearly uniform, except at vulva where it is maximum.

Head.—The head is slightly marked off from the body by a faint constriction, slightly hemispheroid in shape, 3 μ high and 8-9μ wide. It is supported by a well developed hexaradiate framework, formed by the fusion of six strong radially placed cuticularized ridges. The inner edges of these ridges at their extremities converge to form a tube-like structure which serves as a guide for the spear. The outer edges of these ridges form a disc-like basal plate (Text-fig. 5, D).

Lip region is rounded, flat at apex. *En face* view shows the usual six lips : two lateral, two dorso-lateral and two ventro-lateral. Each lip bears a minute papilla at its inner margin. In addition, there are four pairs of outer cephalic papillae, one pair on each dorso-lateral and ventro-lateral lips, the lateral lips having only one papilla on their outer margins but they also possess the amphidial apertures (Text-fig. 6, A).

Tail.—The tail is more or less cylindrical (Text-fig. 4, A-N) with blunt tip, usually with a distinct mucro. The dorsal surface of the tail is usually straight while the ventral side curves inward. Tail length varies from 70-120 μ (averaging 85 μ) or 4-6 anal body-widths (averaging 5 anal body-widths), possesses pore-like phasmids on the lateral sides in the posterior half of its length. The usual

Table 1.—Measurements and Dimensions of *Hirschmanniella oryzae* ♀ ♀

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Dimensions and Measurements

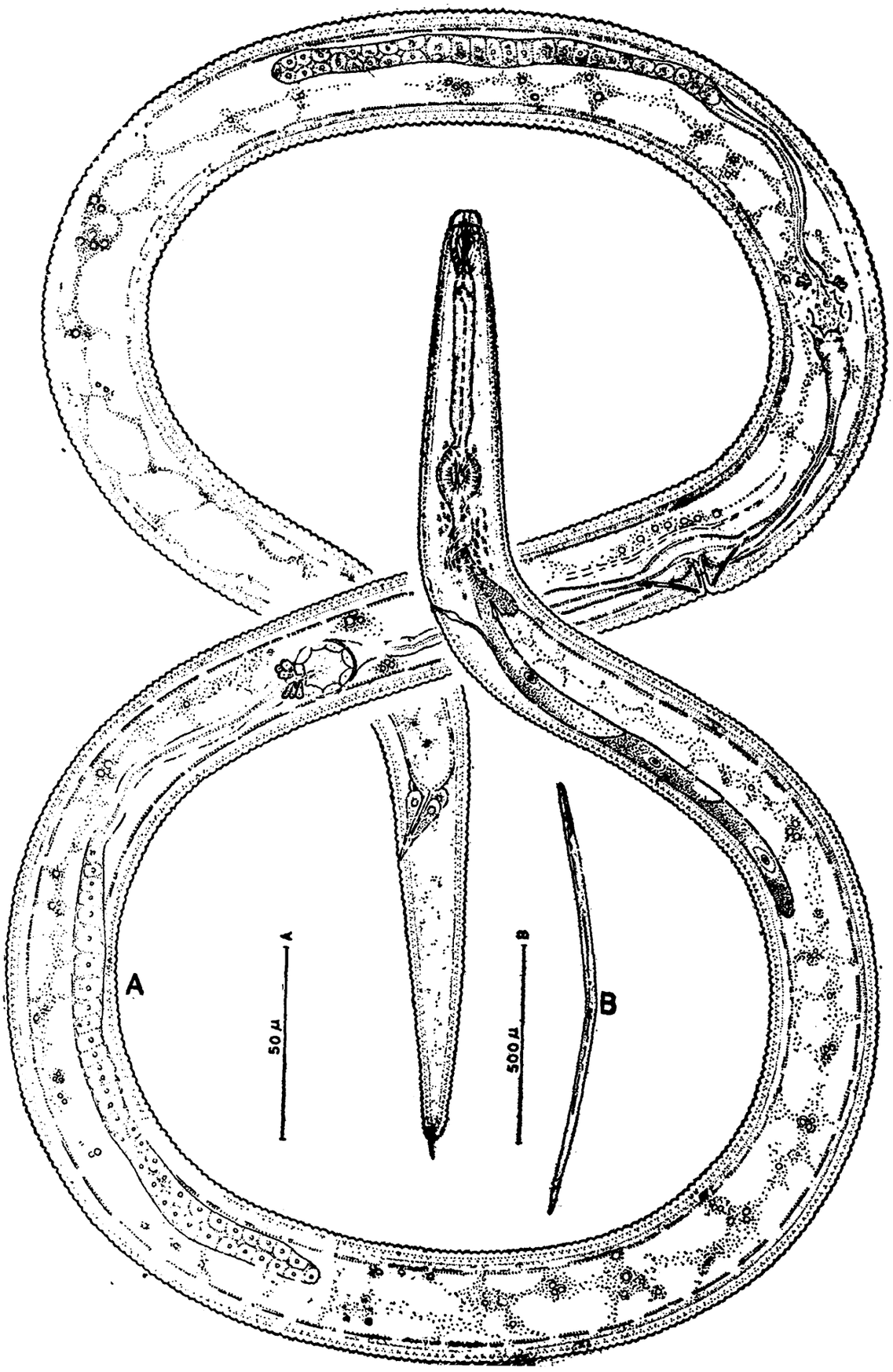
Measurements	Jamalpur North	Jamalpur East	Bhambhola	University Agriculture Farm A	University Agriculture Farm B	Qasimpur North	Qasimpur South	Zohra Bagh	Central Dairy Farm Road A	Central Dairy Farm Road B
LENGTH = 1.34 (1.00-1.59) (in mm.)	1.01-1.49	1.10-1.45	1.12-1.40	1.00-1.54	1.01-1.56	1.00-1.59	1.00-1.53	1.15-1.54	1.06-1.54	1.00-1.57
a = 52 (32-60)	46-53	43-60	39-56	39-58	32-60	40-60	37-56	40-59	41-58	43-57
b = 11.2 (7.6-13.1)	8.7-11.5	8.1-13.7	7.6-11.9	8.0-11.1	8.0-11.3	7.8-12.6	7.8-13.1	8.7-11.2	8.5-11.5	8.6-13.1
b′ = 5.0 (3.2-6.7)	4.1-6.4	5.1-6.7	4.1-5.7	4.1-5.6	3.3-5.4	3.4-5.9	4.1-6.6	3.7-6.3	3.9-6.1	4.3-6.1
c = 19 (13-27)	15-22	14-20	17-23	16-26	15-27	16-23	15-22	19-27	15-25	16-23
V = $^{24}53^{22}$ ($^{19-43}$46-67 $^{17-41}$)	48-57	46-56	48-67	46-61	48-58	48-53	48-51	46-57	46-63	48-53
G_1 = 24 (17-43)	19-22	17-23	19-43	18-23	19-38	17-29	19-27	18-32	18-29	19-24
G_2 = 22 (17-41)	18-20	17-22	20-41	16-21	15-29	18-23	16-33	17-26	18-23	16-21
Spear length = 17 μ (15-19 μ)	16-18 μ	15-18 μ	16-19 μ	15-17 μ	15-18 μ	15-19 μ	16-19 μ	16-19 μ	17-18 μ	15-19 μ
Metacorpus = 68 μ (56-85 μ)	56-80 μ	59-81 μ	59-83 μ	58-73 μ	59-85 μ	60-82 μ	59-81 μ	58-82 μ	59-86 μ	59-83 μ
Excretory pore	80-120 μ	83-110 μ	83-95 μ	87-113 μ	82-115 μ	85-117 μ	91-199 μ	93-120 μ	80-120 μ	83-111 μ
Dorsal oesophageal gland overlap	212-350 μ	210-320 μ	215-291 μ	214-330 μ	230-310 μ	250-319 μ	220-293 μ	215-281 μ	214-273 μ	220-333 μ
Vulval slit = 9 μ (8-10 μ)	9-10 μ	8-9 μ	8-10 μ	9-10 μ	9-10 μ	9-10 μ	8-10 μ	9-10 μ	8-10 μ	8-10 μ
Vagina length = 10 μ (8-12 μ)	10-12 μ	8-12 μ	8-11 μ	9-12 μ	8-12 μ	9-10 μ	10-12 μ	9-12 μ	8-11 μ	8-10 μ
Tail length = 85 μ (70-120 μ)	73-80 μ	73-80 μ	71-82 μ	73-91 μ	73-80 μ	73-89 μ	71-89 μ	73-95 μ	73-85 μ	73-120 μ
Tail striae = 50 (50-80)	50	50	50	50	50	50	50	50	50	50-80
Rectum = 16 μ (15-18 μ)	16-17 μ	16-18 μ	15-17 μ	15-18 μ	15-18 μ	16-17 μ	16-18 μ	16-18 μ	16-18 μ	15-18 μ
Anal body-width = 17 μ (15-24 μ)	17-20 μ	17-19 μ	16-22 μ	17-24 μ	16-22 μ	17-22 μ	17-23 μ	15-19 μ	17-18 μ	15-19 μ
Phasmids	13-52 μ	14-23 μ	16-22 μ	23-24 μ	16-23 μ	17-29 μ	23-26 μ	16-39 μ	16-33 μ	22-52 μ

G_1 = Anterior gonad .. G_2 = Posterior gonad

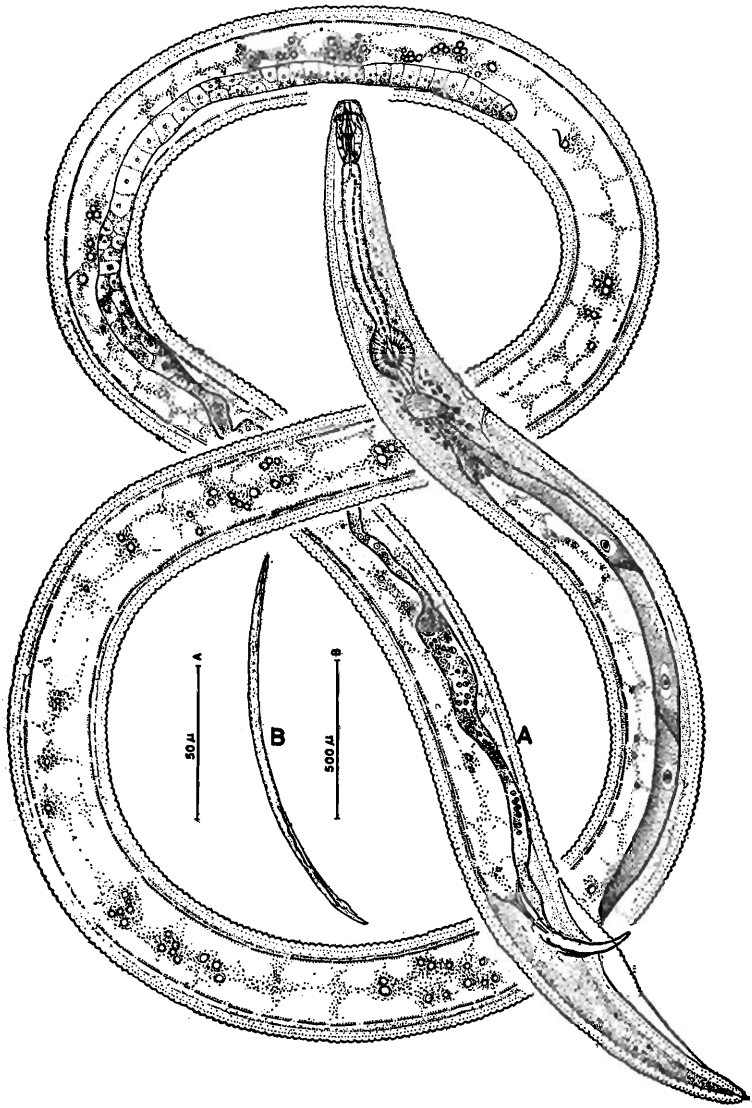
Table 2,—Measurements and Dimensions of *Hirschmanniella oryzae* ♂ ♂

Dimensions and Measurements

Measurements	Jamalpur North	Jamalpur East	Bhambhola	University Agriculture Farm A	University Agriculture Farm B	Qasimpur North	Qasimpur South	Zohra Bagh	Central Dairy Farm Road A	Central Dairy Farm Road B
LENGTH = 1.45 (1.04-1.94) (in mm),	1.10-1.56	1.04-1.46	1.24-1.76	1.16-1.80	1.23-1.46	1.37-1.56	1.20-1.70	1.11-1.80	1.32-1.92	1.46-1.94
a = 54 (37-62)	37-48	37-53	39-54	39-62	41-56	53-59	52-56	43-58	43-60	43-62
b = 10.5 (7.0-12.8)	7.6-11.3	7.4-11.2	7.6-11.5	7.6-11.6	7.6-12.0	8.2-12.6	8.1-12.0	7.3-11.9	7.6-12.2	8.7-12.8
b′ = 4.9 (3.6-6.1)	3.9-5.4	4.1-5.3	3.9-4.9	3.8-5.3	3.9-5.6	4.1-6.0	3.9-5.9	4.3-5.0	4.1-5.3	4.3-6.1
c = 18 (15-22)	17-21	16-22	15-19	15-17	17-21	16-18	15-17	16-21	15-21	17-22
T = 33 (24-42)	26-36	28-39	27-41	28-33	29-38	27-42	29-38	30-42	24-36	29-39
Spicules = 25 μ (22-29 μ)	23-29 μ	22-29 μ	22-28 μ	24-29 μ	24-29 μ	22-29 μ	22-27 μ	22-26 μ	22-29 μ	22-29 μ
Gubernaculum = 8 μ (7-10 μ)	7-9 μ	7-10 μ	7-9 μ	7-9 μ	7-9 μ	7-9 μ	7-9 μ	7-9 μ	7-9 μ	7-10 μ
Caudal alae = 73 μ (58-80 μ)	58-73 μ	58-80 μ	58-80 μ	58-75 μ	58-79 μ	58-76 μ	58-80 μ	58-73 μ	58-80 μ	58-80 μ
Rectum = 22 μ (19-24 μ)	19-22 μ	19-23 μ	19-24 μ	19-24 μ	19-24 μ	19-24 μ	19-24 μ	19-24 μ	19-24 μ	19-24 μ
Phasmids	16-22 μ	17-25 μ	18-32 μ	16-35 μ	16-42 μ	16-43 μ	10-46 μ	15-43 μ	15-46 μ	15-48 μ



Text-fig. 1. *Hirschmanniella oryzae* (Soltwedel), A. Entire female. B. Actual shape of female.



Text-fig. 2. *H. oryzae*. A. Entire male. B. Actual shape of male.

number of tail striae is 50, but in few specimens it may have as many as 80 striae.

Cuticle and its Modifications

(Text-fig. 3)

The cuticle is almost uniformly thick throughout the body except at vulva, anus and at tail tip. It is about 1 μ thick on the general body surface, about 2 μ at vulva and anus while 2-3 μ at tail tip.

Transverse striations.—The head bears usually 6 fine, but distinct transverse striae placed at less than 1 μ apart. The number of head striae is constant, with the exception that rarely 5 striae may also be present. In the region of spear, the striae are of the same width as those on the head. Posterior to spear the striae become more and more distinct with the increase in the inter-strial width, becoming about 2 μ near the middle of the body. Posterior to middle this distance gradually decreases to about 1 μ near the tip of the tail. In few specimens, the inter-strial width remains uniform until the tail tip. In one specimen the inter-strial width was found to be of two different alternating thickness 1 & 2 μ on the body without any uniformity.

Mucro.—The cuticle at tail tip projects into a sharply pointed, peg-like 'mucro'. The length of the mucro varies from 1-3 μ. In some specimens, the mucro is inconspicuously developed or even absent ; this might have occurred due to some mechanical injury to the nematode.

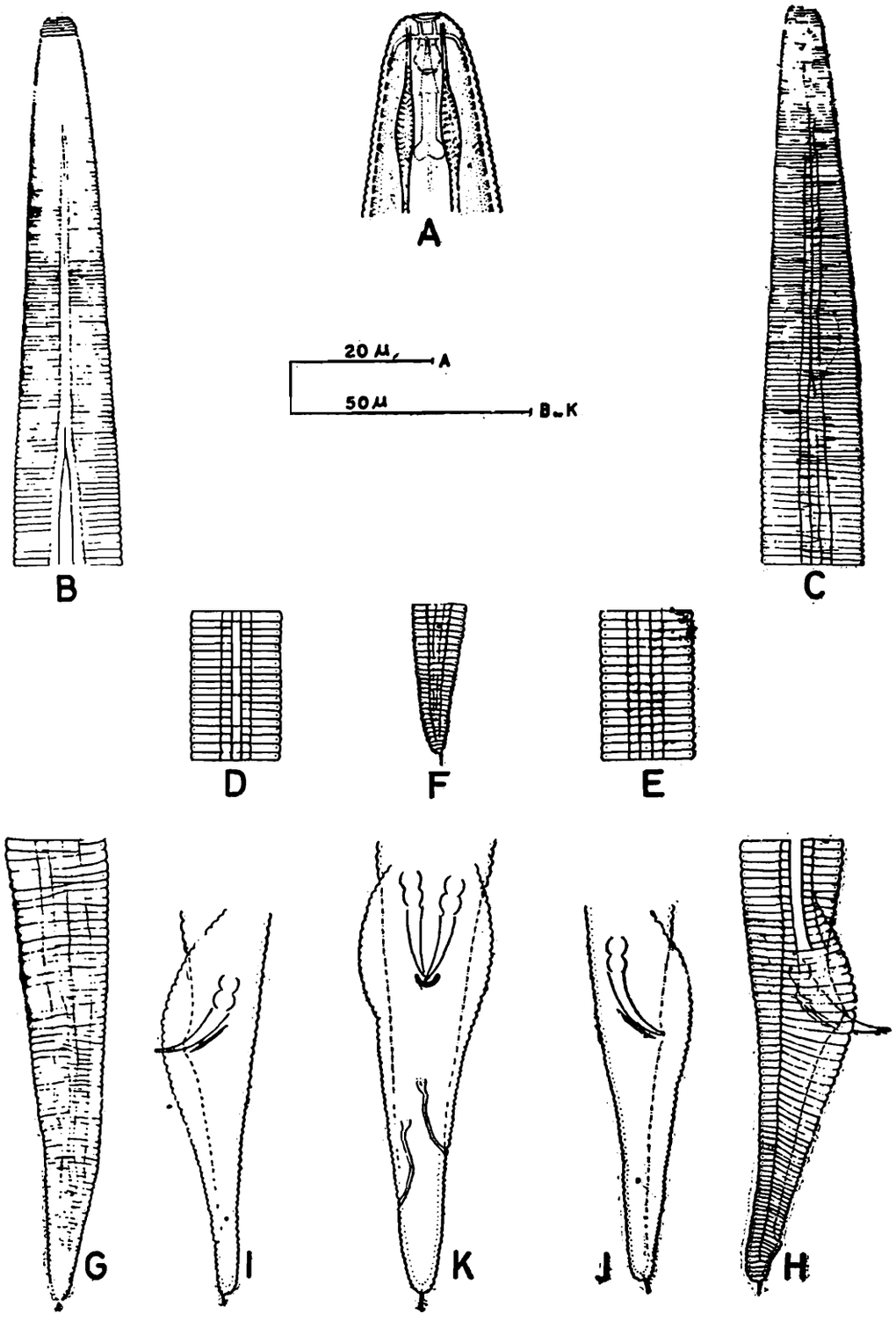
Lateral fields.—The lateral sides of the body are marked with four longitudinal incisures (lateral lines) overlying the lateral hypodermal chords. These markings are more prominent in the middle part of the body. They originate just behind the level of spear base as a single depression in the cuticle, run as single line until the middle of procorpus and then are joined by the second incisure, the third one originates slightly posterior to the second incisure. These three incisures follow a parallel course up to the median bulb. Afterwards, the middle one (first incisure) splits up into two at the level of nerve ring, and the number of incisures become four. In a cross section (Text-fig. 4, D) at the level of metacorpus, the lateral sides show two strong ridges and three grooves formed by the three incisures. In the region of nerve ring and posteriorly across section (Text-fig. 6, G-K) shows three strong ridges with four depressions formed by the four incisures. The width of the lateral fields in the region of metacorpus is 4-5 μ, at nerve ring 5-6 μ and near the middle of the body about 8 μ (6-10 μ) or 1/3 rd the corresponding body-width. The width of lateral fields is maximum at vulva. In the post-vulval region the lateral fields begin to narrow, in tail region the incisures run close to each other and start converging,

In males, the width of the lateral fields is almost uniform from the oesophago-intestinal junction to the beginning of the caudal alae, about 15-20 μ above the cloaca.

The termination of lateral fields is quite different in the two sexes. In females (Text-fig. 3, F), the incisures terminate 15-20 μ above the tip of tail. The width of lateral fields decreases due to convergence of incisures in the postvulval region, but the narrowing becomes conspicuous only in the anal region. Usually, the inner two (median incisures) disappear at about 10-20 μ above the tip of the tail followed by the ventral and dorsal ones which terminate at about 5-15 μ above the tip of tail. In males (Text-fig. 3, H), posterior to the origin of caudal alae, the ventral and the inner two incisures run a short distance on the two wings of the alae where they expand and gradually disappear, usually above or occasionally slightly below the cloacal aperture. The ventral incisure disappears first, followed by the inners. The dorsal incisures runs along the dorsal margin of the caudal alae and finishes at 5-15 μ above the tip of the tail.

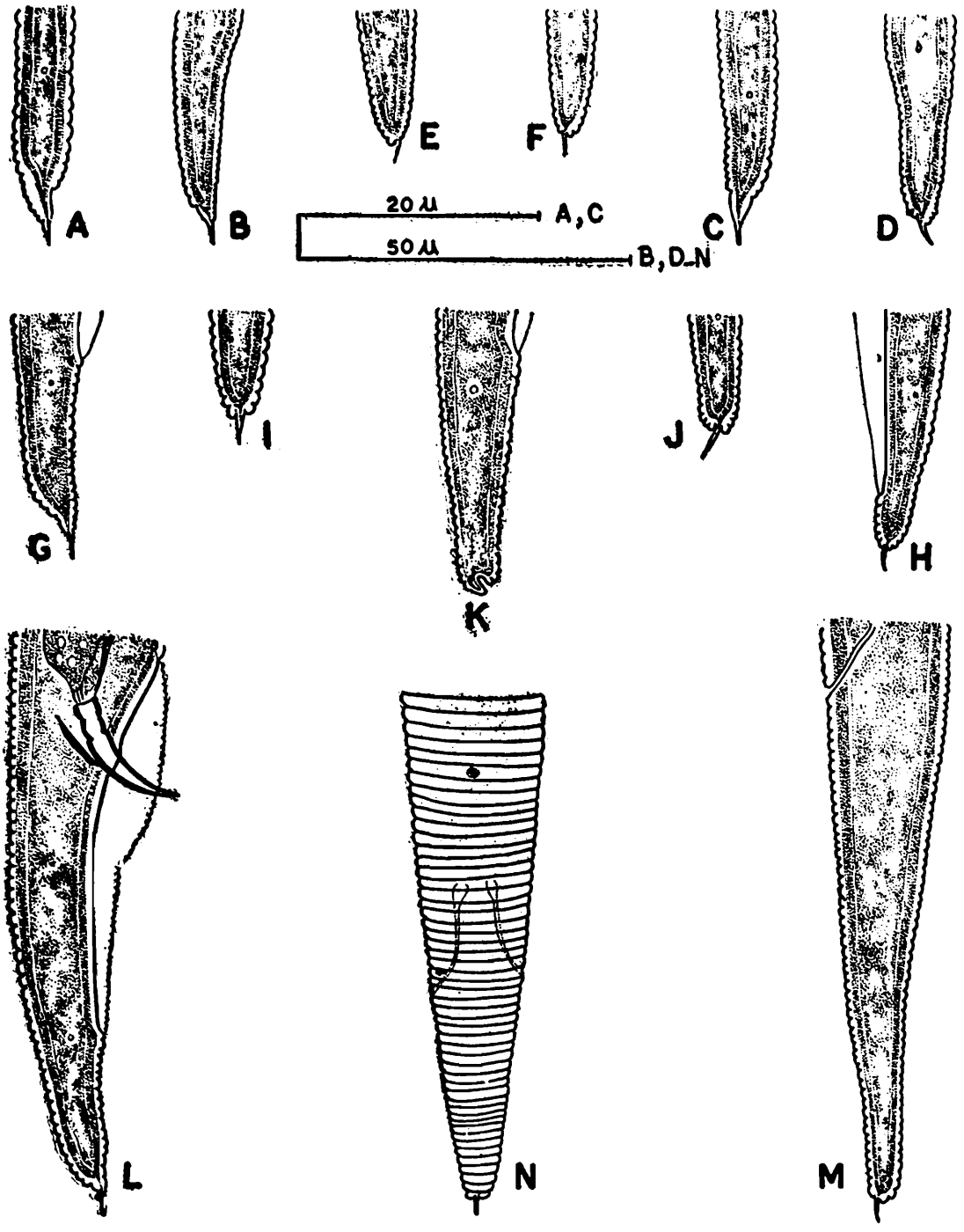
Areolation (Text-fig. 3 ,D-F).—The lateral fields are usually incompletely areolated. The areolation is caused by intrusion of transverse striae into the lateral fields. In the anterior half of the body, the areolation usually does not extend through the entire width of the lateral fields (incomplete areolation), but sometimes it may be almost complete. However, irregularities are very common in the occurrence of complete and incomplete areolation. It has been observed that in the pre-vulval region, the areolation is mostly confined to the outer bands of the lateral fields, the middle band is rarely crossed. However, in vulval and post-vulval regions, the outer as well as median bands of incisures are all interrupted by the transverse striae which may make complete rings around the body of the worm. In a number of specimens the areolation was complete throughout the length of the lateral fields. The areolation in the pre-vulval region is generally incomplete, but in post-vulval region it is mostly complete.

Anastomosis (Text-fig. 3, C and G).—The phenomenon of anastomosis is rarely observed in nematodes, except in Criconematids. However, here it was observed in several specimens. Striations from head to tail are adversely affected in some of the specimens studied. The anastomosis may cause difficulties in the study of lateral fields because both the transverse and longitudinal striae show anamolies in their normal pattern. In specimens showing anastomosis, the lateral fields follow a random course, they do not run parellel to each other and show interruption at several places with the result that they do not look as incisures but as uneven lines made up of several small units. The overlapping of transverse striae was seen on any region of the body and they also do not form complete rings.



Text-fig. 3. Cuticular modifications of *H. oryzae*, A. Head end of female showing Amphids and Cephalids, B. Anterior region of female showing origin of lateral fields, C. Anterior region of female showing anastomosis, D. Lateral fields with incomplete areolation in the pre-vulval region, E. Lateral fields with complete areolation in the post-vulval region, F. Lateral fields with complete areolation in tail region, G. Lateral fields on tail showing anastomosis, H. Lateral fields on male tail, I. & J. Male tails showing origin and termination of caudal alae, K. Male tail in dorso-ventral position.

Caudal alae (Text-fig. 3, I--K).—The males of the species have a pair of wing-like structure known as caudal alae which are sometimes referred to as 'bursa' The term bursa is incorrect as this structure is neither supported by rays nor by ribs as in the strongylid nematodes.



Text-fig. 4. Tail shapes of *H. oryzae*. A-D. Leaf-shaped tails of females, E & F. Tail tips with additional striae, G & H. Posterior halves of male tails, I. Female tail tip dorso-ventral, J. Female tail tip with curved mucro, K. Male tail notched, L. Male tail lateral, M. Female tail lateral, N. Female tail dorso-ventral.

The caudal alae are formed due to the lateral extentions of the body cuticle. They originate about 15-20 μ above the cloacal aperture on the lateral sides of the body and terminate before the tip of the

tail and thus do not envelop the tail completely. The alae in the anterior half of their length are distinctly convex, but gradually narrowing on the rest. The outer edges of the alae are crenate due to the continuation of the transverse striae of the body. The alae normally neither originate at the same level on the body nor do they terminate.

The relation of caudal alae with the phasmids is variable. In most of the specimens, it was found that while one ala reaches beyond phasmids, the other one stops slightly short; in few specimens, both the alae reach beyond the level of phasmids. However, it was never found to end before the phasmidial level.

Amphids (Text-fig. 6, A).—The amphids are situated on the lateral sides of the body in the head region. The amphidial slits 2-3 μ wide are crescentric in shape, situated on the lateral lips. The amphidial ducts have ellipsoidal pouch-like structure which are known as sensillar pouches, the latter being innervated by minute fibres from the cephalic sensory nerves (Text-fig. 9, A).

Cephalids (Text-fig. 3, A).—The cephalids are present in the stomal region. The first or the anterior cephalid is situated 2-4 striae behind the basal plate. The second cephalid can be seen at about the level of spear knobs. The cephalids form complete rings around the body of the worm.

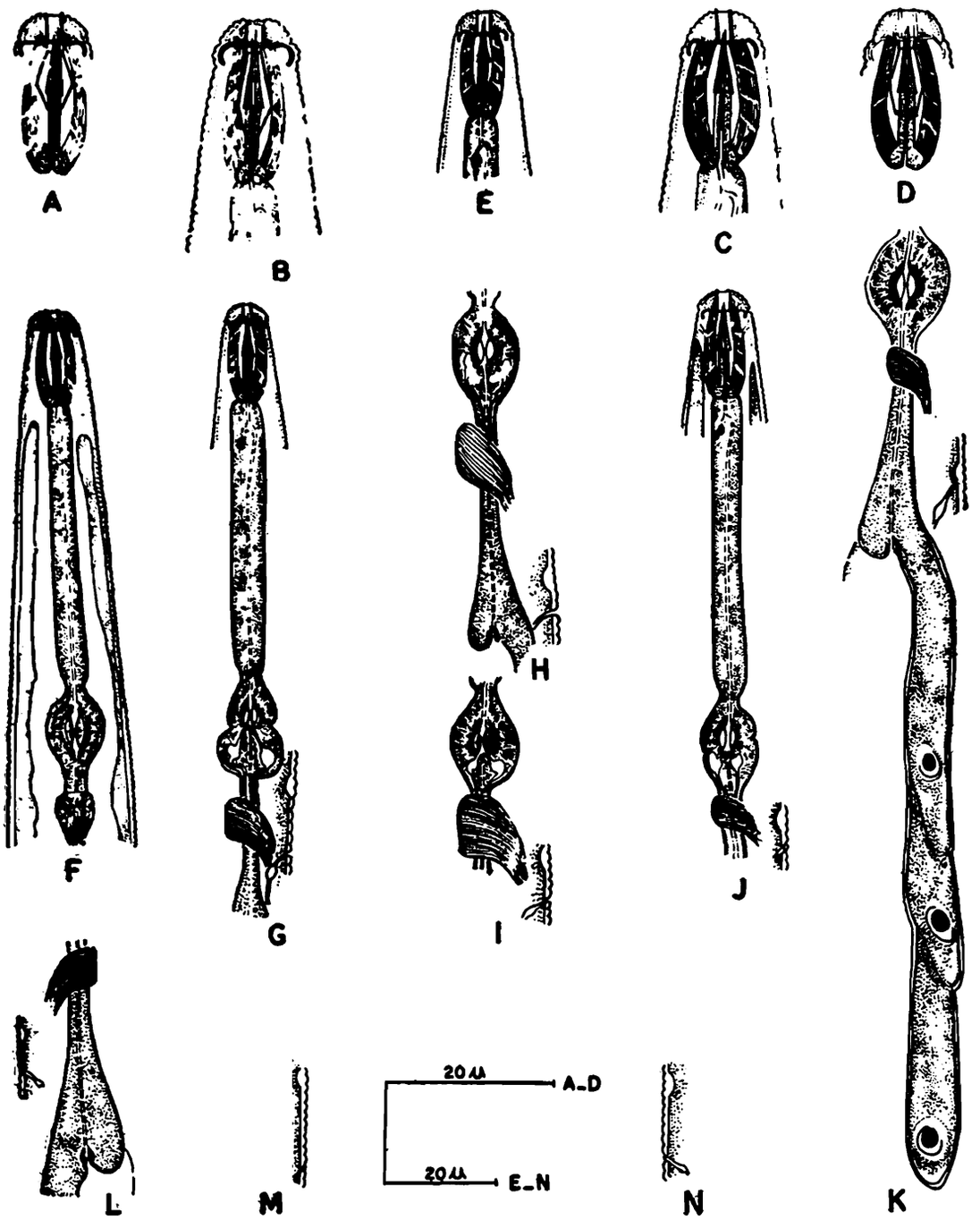
Hemizonid.—The hemizonid appears laterally as a cup-shaped structure, highly refractive in nature. It is situated in the vicinity of the excretory pore, usually 2-8 body striae above the former, 2-3 body striae long. The hemizonid is supplied with prominent nerve fibres arising from the nerve ring (Text-fig. 9, A).

Phasmids (Text-fig. 3, F-K).—The phasmids are located on the posterior half of the tail on the lateral sides as refractive pore-like structures. The position of the phasmids is variable and in the same specimens they are not situated at one level. The phasmids are situated in between the median incisures. Sometimes abnormally they are situated below the termination of the incisures but in the centre of the lateral fields.

Hypodermis

(Text-fig. 5, F)

The hypodermis is a rather thin, syncytial layer which is thickened at four points to form the longitudinal chords. These thickenings are dorsal, ventral and lateral in position. Towards the anterior



Text-fig. 5. Head ends and oesophageal regions of *H. oryzae*. A. Head end of female, B & C. Head ends of male, D. Head end of female showing head skeleton, E. Head end of female showing opening of dorsal-oesophageal gland in the lumen of procorpus, F. Anterior region of female in dorsoventral view showing lateral hypodermal chords, G-K. Different regions of oesophagus showing oesophageal lumen, valvular apparatus, oesophageal gland lobes, their ampullae and orifices. H-N. Relative positions of Hemizonid and Excretory pore.

and posterior ends of the body these chords are rather poorly defined. Anteriorly they originate just behind the spear base, becoming prominent in the region of median bulb and continue as such up to the

level of anus where these chords become less prominent and finally disappear at the hinder end of the tail. In cross sections of the body (Text-fig. 6, D-L) at different levels, these chords can be clearly seen bulging out in the body cavity (pseudocoel) in the dorsal, ventral and lateral positions of the body. However, the dorsal and ventral chords appear to be slightly different in shape and comparatively more prominent than the laterals. The lateral hypodermal chords posterior to level of excretory pore enclose the lateral excretory canals. These four hypodermal chords divide the visceral cavity though incompletely into four quadrants in which sets of somatic muscles are arranged.

Musculature

(Text-fig. 8, A-G)

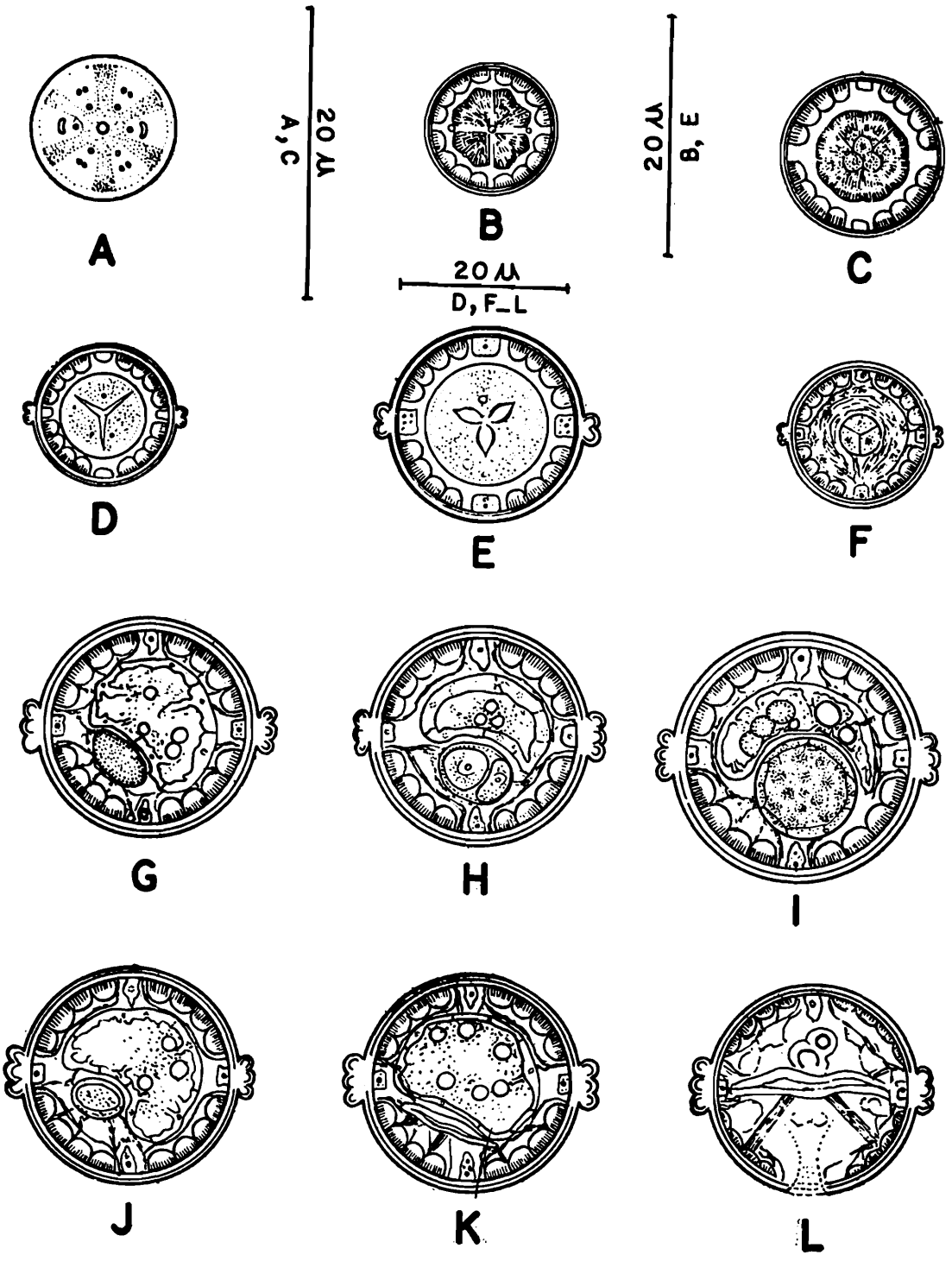
The muscles in the body of the nematodes were studied under two headings : 'Somatic or unspecialised musculature' and 'Specialised musculature'

Somatic or unspecialised musculature (Text-fig. 8, A-C).—Each quadrant of the hypodermis is provided with 4 cells which are flat at the point of their attachment with the hypodermis. Thus the nematodes are typical meromyarian with platymyarian type of somatic musculature. The number of muscle cell was found to be always constant, i.e. 4 cells per quadrant from the region of spear to anus, but normally they are more prominent towards the middle of the body and less towards extremities depending upon the width of the body. The 'thorneian cells' of Sher (1968) could not be detected in this species.

Specialised musculature.—In addition to the somatic muscles there are 'specialised muscles' associated with the various organs of the body to help them in their activities. The various body parts such as spear, oesophagus, intestine spicules, gubernaculum, etc., each having muscles attached to them. In addition, all the openings to the exterior are guarded by specialized type of muscles known as sphincters.

(*i*) *Spear muscles* (Text-fig. 5, A-D and 6, B and C).—There are four primary sets of muscle bands associated with the spear, but each set is ultimately subdivided. Thus there appear to be 8 muscle bands. They are attached to the base of spear knobs at one end and to the basal plate of the lip region at the other. These muscles serve for the forward and backward movement of the spear. When the nematodes are relaxed by gentle heat these muscles keep the spear in its natural position.

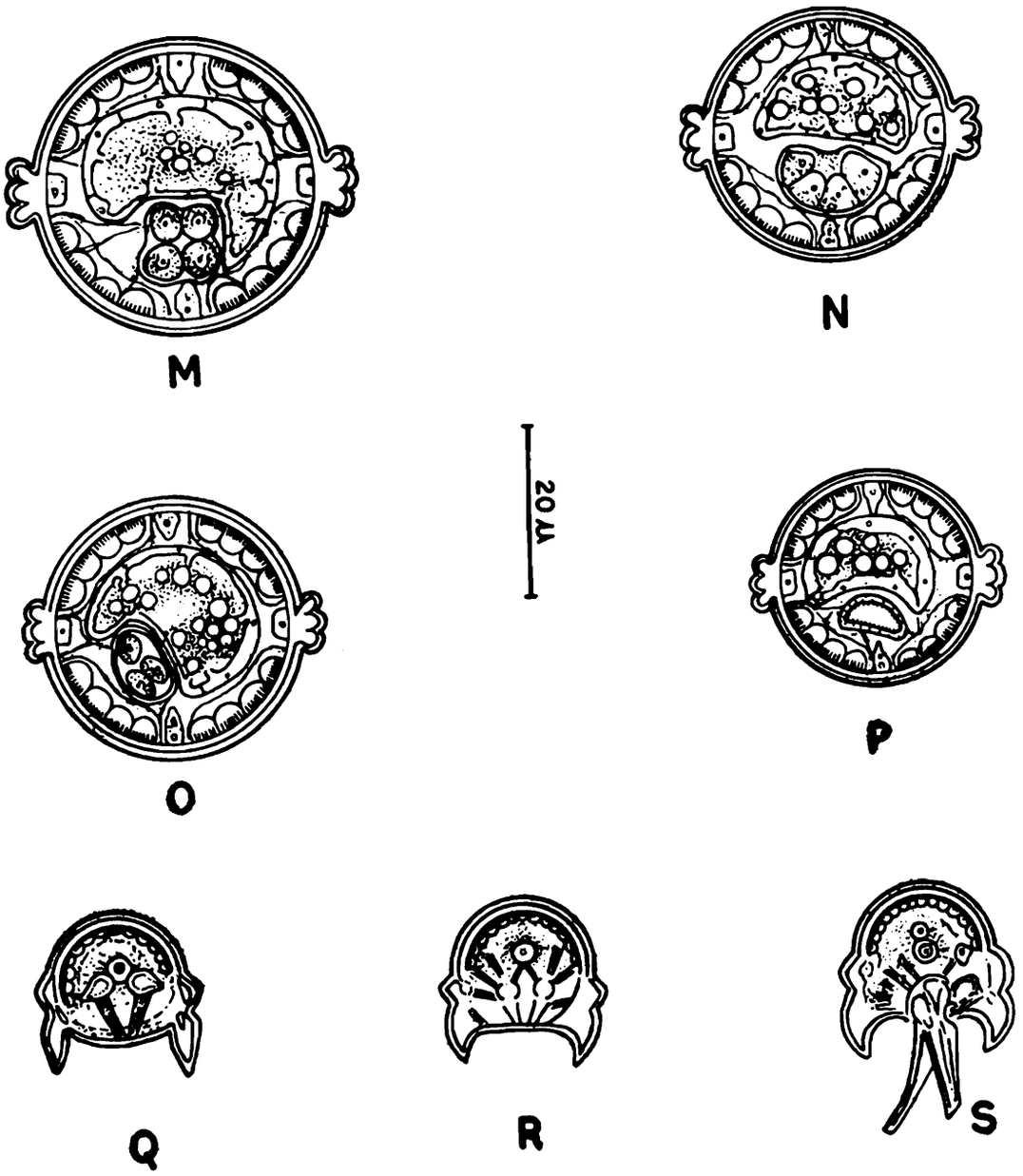
(*ii*) *Somato-esophageal muscles.*—Associated with oesophagus. Not very prominent.



Text-fig. 6. Cross-sections of *H. oryzae* at different levels, A. *En face* view, B. Below the basal plate showing spear muscles, C. At level of spear knobs, D. At level of the anterior region of median bulb, E. At level of middle of median bulb. F. Through the isthmus, G. Through dorsal-oesophageal gland lobe, H. Through the ovary, I. Through spermatheca, J. Through the proximal part of the uterus, K. Through the distal part of the uterus, L. At level of anus.

(*iii*) *Somato-intestinal muscles.*—Associated with intestine. Not very prominent.

(*iv*) *Vulval muscles.*—There are several sets of muscles associated with the vulva that help in the dilation and contraction of the vulval opening. These are primarily of two types :



Text-fig. 7. Cross-sections of *H. oryzae*, ♂ ♂ at different levels M-O. Through testis, P. Through vas deferens, Q. Through the head of spicules, R. & S. Through the middle of spicules.

(*a*) *Dilator vulvae* (Text-fig. 8, D-G : 2).—They consist of eight muscle bands, 4 anterior to vulva and 4 posterior. These sets are arranged in 4 pairs — 2 anterior and 2 posterior to vulva. They originate from vulva and run ventro-laterally. These vulval muscles in association with the sphincter muscles control the dilation of vulva.

(*b*) *Constrictor vulvae* (Text-fig. 8, D-G : 1).—The *constrictor vulvae* consist of four muscle bands. They are attached to the base

of the vagina at one end and to the latero-ventral body wall at the other end.

(*v*) *Anal muscles* (Text-fig. 8, H : 3).—A single muscle band in the anal region of female extends from the lower margin of the anus to the subdorsal wall of the body. This muscle band is also present in males, but is inconspicuous.

(*vi*) *Spicular muscles* (Text-fig. 8, I).—There are several sets of muscle bands associated with the spicules of the males to bring about its movements. These muscles are *retractor spiculi* and *protractor spiculi.*

Retractor spiculi ;

(*a*) *Latero-ventral retractor spiculi* (Text-fig. 8, I : 4).—A pair of muscle bands on each side extends from the head of spicules to the body wall in between the lateroventral somatic muscles.

(*b*) *Latero-dorsal retractor spiculi* (Text-fig. 8, I : 5).—A pair of muscle bands on each side extends from the head of the spicules to the body wall in between the laterodorsal somatic muscles.

Protractor spiculi ;

(*a*) *Right and left external protractor spiculi* (Text-fig. 8, I : 6-7) —They extend from the lower margin of the head of spicules, and are inserted in the anterior region on the latero-ventral wall of the tail region.

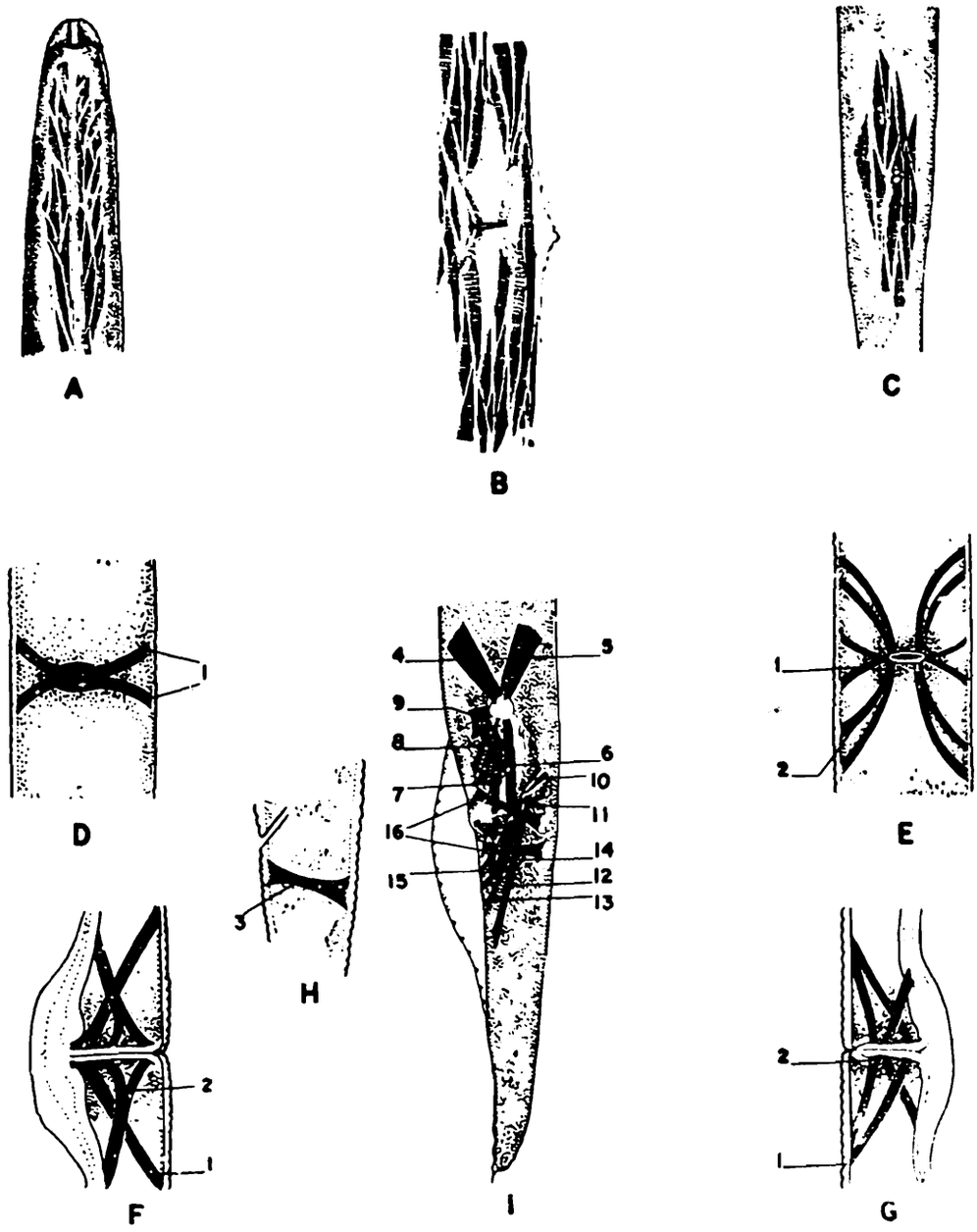
(*b*) *Internal protractor spiculi* (Text-fig. 8, I : 8).—They are less prominent, extending from the lower part of the head of spicules to ventro-lateral wall of the body.

(*c*) *Anterior protractor spiculi* (Text-fig. 8, I : 9).—They extend from the posterior side of the head of spicules, and are inserted straight on the lateroventral wall.

(*vii*) *Gubernacular muscles.*—The muscles associated with the gubernaculum are of following three types :

Retractor gubernaculi (Text-fig. 8, I : 10 and 11).—A set of muscles extending from the distal part of the gubernaculum to the latero-dorsal wall of the body.

Protractor gubernaculi (Text-fig. 8, I : 12 and 13).—They are strongly developed as compared to other muscles associated with the gubernaculum. They extend from the proximal end of the



Text-fig. 8. Musculature of *H. oryzae*, Somatic muscles : A. In the anterior part of body, B. In the vulval region, C. In the anal region ; *Specialized muscles :* D-G. Vulval muscles, H. Anal muscles, I. Male copulatory muscles ; 1. *Constrictor vulvae* ; 2. *Dilator vulvae* ; 3. *Anal muscles* ; 4. *Latero-ventral retractor spiculi* ; 5. *Latero-dorsal retractor spiculi* ; 6. *Right external protractor spiculi* ; 7. *Left external protractor spiculi* ; 8. *Internal protractor spiculi* ; 9. *Anterior protractor spiculi* ; 10. *Right retractor gubernaculi* ; 11. *Left retractor gubernaculi* ; 12. *Right protractor gubernaculi* ; 13. *Left protractor gubernaculi* ; 14. *Right seductor gubernaculi* ; 15. *Left seductor gubernaculi* ; 16. *Copulatory muscles.*

gubernaculum to the subventral body wall and are known as right and left protractor gubernaculi depending upon their position in the body.

Seductor gubernaculi (Text-fig. 8, I : 14 and 15).—These muscles are clearly visible only in the ventro dorsal view, laterally they are obscure. They extend from the proximal end of the gubernaculum to the latero-ventral body wall of the nematode and are known as right and left seductor gubernaculi depending upon their position in the body.

(*viii*) *Copulatory muscles* (Text-fig. 8, I : 16).—The paired copulatory muscles described by Coomans (1963) in *Rotylenchus goodey* are not distinct in this nematode However, a band of muscles in the pre-anal region, extending from latero-ventral position of the body wall to laterodorsal body wall is distinct in some of the specimens in addition to post-anal muscle bands. These muscles may be copulatory in function.

Digestive System

The digestive system of the nematode is made up of the following parts :

(*i*) Stoma, (*ii*) Procorpus, (*iii*) Metacorpus, (*iv*) Basal glandular part of oesophagus, (*v*) Intestine, (*vi*) Rectum and (*vii*) Anus.

Stoma.—The region between the oral aperture and the spear base is termed pharynx, oral cavity, buccal cavity or stoma. This part of the digestive system is connected to the oral aperture which is guarded by six lips and is supported by sclerotized ridges along the six radii which form the head framework. The oral aperture leads into a narrow cavity formed by the union of the inner extremities of the six ridges which provide a passage for the forward and backward movement of the spear. The spear measure 15-19 μ (average 17 μ) in length and is divisible into anterior conical part (metenchium) and posterior cylindrical part (telenchium); the latter is provided with three well developed knobs at its base. The movement of spear is brought about by means of the four paired muscle bands discussed earlier.

Oesophagus.—The oesophagus of the nematode is divisible into an anterior procorpus or precorpus, metacorpus or median bulb, the isthmus, and the glandular lobe overlapping the intestine. The procorpus or the proximal part of the oesophagus is a slender tube narrowing at both the extremities. The lumen of the procorpus is uniform throughout its length. In the anterior part of the lumen of procorpus opens the duct from the dorsal oesophageal gland at 2-3 μ behind the base of the spear. The distal end of procorpus is connected to the median bulb through a narrowing. The median bulb is an ovate muscular organ which has well developed crescentic valvular apparatus. It is situated at 56-85 μ from anterior end of body.

In the specimens stained by modified Cajal's method (Sanwal, 1957), the median bulb shapes differently (Text-fig. 5, F-J). In some specimens, it is pyriform, with a slight constriction in the middle. This shape was evident in the lateral as well as in the dorsoventral position. In all such specimens crescentic valvular plates remain in the anterior half while the ampullae of the two subventral glands occupy the available space (1/3rd of the total space) posteriorly. The ampullae of the subventral glands open just at the base of the crescentic valves.

The isthmus connects the median bulb with the glandular lobe. It is a very narrow tube enveloped at its middle by the nerve ring.

The posterior glandular part of the oesophagus which may also be called as end bulb, is narrow and refractive, forms a long ventral overlap over the intestine, measures 210-350 μ (average 295 μ) in length. It is made up of three oesophageal glands one dorsal, and two subventral in positions. All the three glands are enclosed in a bag of epithelial cells as evident from the cross sections of body in the oesophageal region (Text-fig. 6, G). The glands have a prominent nucleus situated at their hinder end or the lobular part of the oesophageal glands. The dorsal gland is situated in anterior part of the lobe followed by the subventral which occupy the middle and the posterior position. The duct from the dorsal oesophageal gland passes through the isthmus and median bulb into the procorpus where it opens at the base of spear. Before opening into the lumen of the procorpus it forms a prominent ampulla. The two subventral oesophageal glands also form distinct and prominent ampullae before opening in the median bulb at the base of valvular apparatus (Text-fig. 5, G-J).

Intestine.—The oesophago-intestinal junction is guarded by small cells which are refractive in nature. These cells were distinctly visible in those specimens which were specially stained for the study of muscles. The intestinal tube is narrow in the region where it is overlapped by the glandular part of the oesophagus but posteriorly it occupies nearly all the available space. The intestine is supported by somatointestinal muscles in the body cavity. It is pushed to dorsal side in mature specimens in order to accommodate the developing genital organs (Text-fig. 1, A). In the posterior region it gradually narrows to join the rectum.

Rectum.—The rectum is a dorso-ventrally bent tube lined by the cuticle, measuring 16 μ (15-18 μ) in females and 22 μ (19-24 μ) in males, slightly shorter than one anal body-width in females and slightly longer than one anal body-width or cloacal-body-width in males. In males, the rectum opens along with the genital duct into cloaca. The cloaca is also lined by cuticle. The latter communicate outside through cloacal aperture.

Anus.—A circular openning (Text-fig. 4, N) in the posterior region of the body, midventral in position. Through this the alimentary canal communicates with the outside.

Excretory System

(Text-fig. 9, H)

The excretory system comprisis the longitudinal excretory ducts, terminal duct and excretory pore. The excretory pore lies on the midventral position of the body at or below the level of nerve ring. The excretory pore is guarded by a sphincter. It leads into a terminal duct, which has a swelling in the anterior region giving it a beak-like appearance. The anterior and posterior extremities of this swelling are also provided with sphincters. The sphincters were seen in the specimens treated with A.F.A. The terminal duct runs posteriorly, after a distance of about 50-60 μ it ends into an ampulla-like structure. The latter also receives the anterior and posterior longitudinal excretory ducts. The presence of a renette cell could not be confirmed with certainty. Sanwal (l.c.) also failed to locate it in *Hirschmanniella gracilis*. The lining of excretory ducts shows refractive spots which may be protoplasmic projections of the epithelial cells lining these ducts.

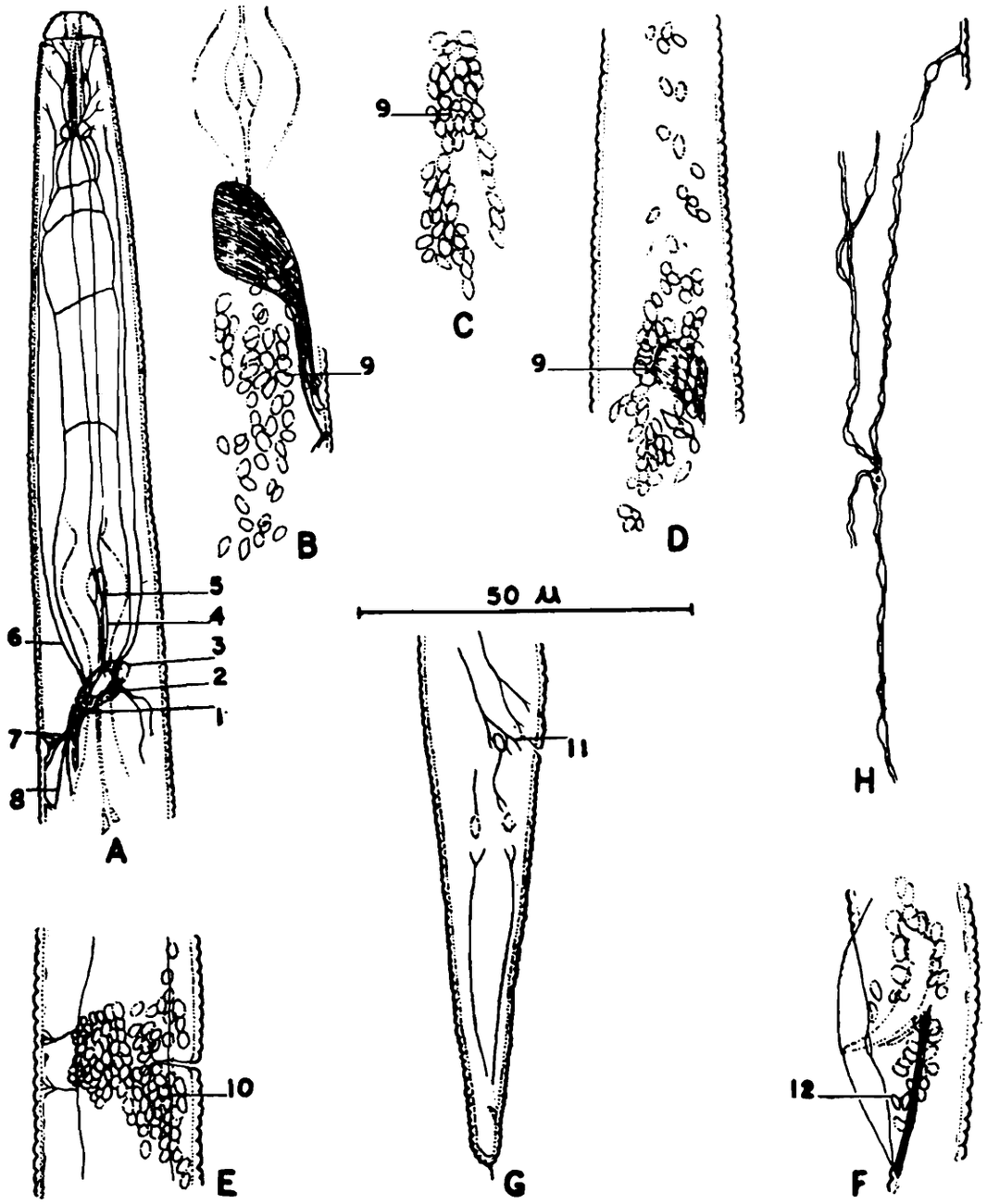
Sanwal (l.c.) was doubtful about the connection of ampulla with the excretory ducts. The present study which is based on a larger number of specimens clearly shows that there is definite connection between the two. The tubular longitudinal excretory canals extend from the ampulla-like structure anteriorly and posteriorly (Text-fig. 9, H). A transverse duct arising out of the ampulla is clearly visible. The anterior canal breaks up into two at about 25-30 μ from the excretory ampulla. These two branches cover up the anterior part of the body running laterally. In some specimens, they could be traced up to the base of spear. On the other hand the posterior duct is visible with distinction only in the pre-vulval region of the freshly stained specimens. The two canals are dilated, at several places throughout their lengths into ampulla-like structures. At these places, they are supposed to receive smaller excretory tubules from different parts of the body. The excretory ducts were also observed in living specimens where they appear smooth.

Nervous System

(Text-fig. 9, A-G)

The nervous system consists of a nerve ring, the ganglia and the nerves. In addition, there are scattered nerve cells which are also associated with this system and are perhaps sensory in function. The nerve ring which is the centre of nervous system is fibrous in

nature. The ganglia (one ventral, one dorsal and two laterals) were visible only in some of the specimens. The nerves arising out of the nerve ring and the associated ganglia are delicate. Two nerves known as cephalic sensory nerves arise out of the dorsal and



Text-fig. 9. Nervous and excretory system of *H. oryzae*, A. Nerve ring complex, B. Nerve ring and associated neuro-sensory cells. C. &D. Nerve ring and associated neuro-sensory cells at different levels, E. Neuro-sensory cells in the vulval region, F. Neuro-sensory cells in the cloacal region, G. Neuro-sensory cells and nerves in the anal region, 1. Nerve ring ; 2. Lateral cephalic ganglion ; 3. Dorsal ganglion ; 4. Lateral cephalic-sensory nerve ; 5. Lateral cephalic-sensory nerve ; 6. Ventral cephalic sensory ; 7. Nerve to hemizonid ; 8. Nerve to excretory pore ; 9-12. Neuro-sensory cells, H. Excretory system.

two ventral ganglia. Two more nerves one to each amphids, originate from the lateral ganglia, the branches of which also supply to the submedian lips. Branches from the nerve ring also supply the hemizonid, and cephalids. The nerve supply to other parts of the body such as excretory pore, digestive and reproductive systems, was observed in some of the specimens, but on the whole they are very faint.

Nerve cells.—A number of nerve cells were found scattered in the body, but they accumulate in the vicinity of nerve ring, vulva and cloaca. According to Sanwal (1957) the number of these cells in *H gracilis* is definite but in the present material their number as well as distribution is variable.

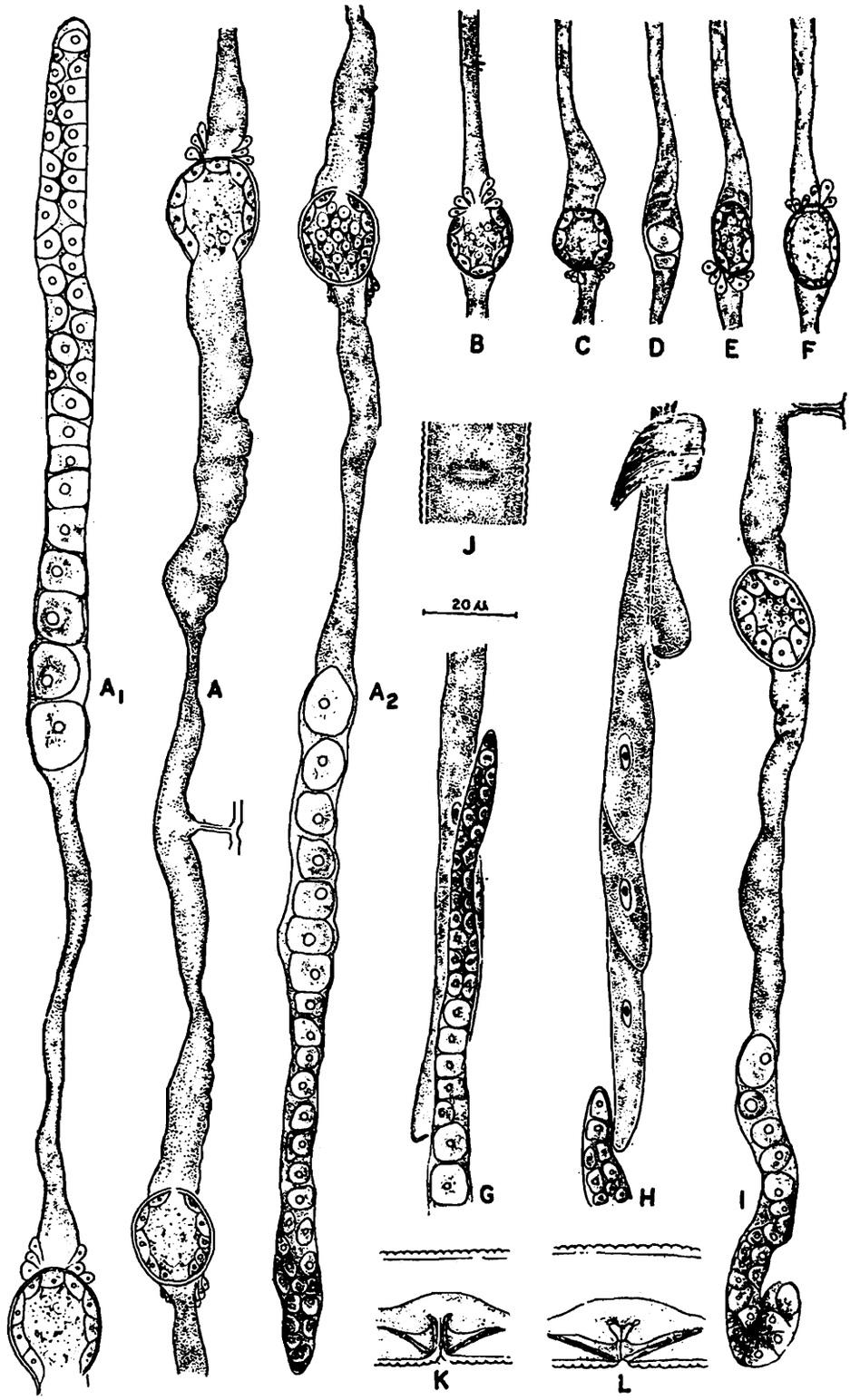
Reproductive System

Sexes are separate, but there is no sexual dimorphism except for the differences in gonads and their associated structures. The males and females are found in ratio of 3 : 7 in the soil as well as in the roots of paddy.

Female reproductive organs.—The female (Text-fig. 10, A-L) gonads are amphidelphic with outstretched ovaries. Each sexual branch consists of an ovary, an oviduct, a spermatheca and a uterus. The total space occupied by the gonads is 46% (40-75%) of the total body length. The anterior and posterior branches measure 285 μ (231-440 μ) and 268μ (234-416μ) or 24% (17-43μ) and 22% (17-41%) respectively of the total body length.

Each sexual branch consists of an ovary which is a long tubular structure lying ventral to the intestine. The öocytes are arranged in a single row, except in the region of the multiplication. In few specimens, the öocytes were found arranged uniformly in a single row. The nuclei of the öocytes are very prominent. The number of öocytes in each ovary varies from 37-43 (average 39). In some older females there were only 17-18 öocytes in one ovary.

The ovary leads into an oviduct which is a narrow muscular tube having almost a uniform width throughout its length. The oviducts of anterior and posterior sexual branches measure 87 μ (28-153 μ) and 91 μ (28-191 μ) respectively. The oviduct terminates into a round spermatheca which measures 14-22 μ× 7-10 μ. At the junction of oviduct and spermatheca, three pairs of unicellular structure are present. They may be glandular in nature. Their function could not be determined. The shape of spermatheca varies from a simple pouch to a more or less spherical structure (Text-fig. 10, B-F). In some females, it assumes somewhat oval or elongate shape. In the younger



Text-fig. 10. Gonads of *H. oryzae*, ♀ ♀ A. Uterine part ; A_1. Anterior sexual branch ; A_2. Posterior sexual branch ; B. Anterior spermatheca ; C. Posterior spermatheca ; D-F. Developing spermatheca ; G. Abnormally developed ovary reaching the glandular part of oesophagus ; H. Ovary touching the tip of the glandular overlap ; I. Posterior gonad showing a part of ovary reflexed ; J. Vulva (surface view) ; K & L. Vulva (lateral) showing structure of the vagina at two different levels.

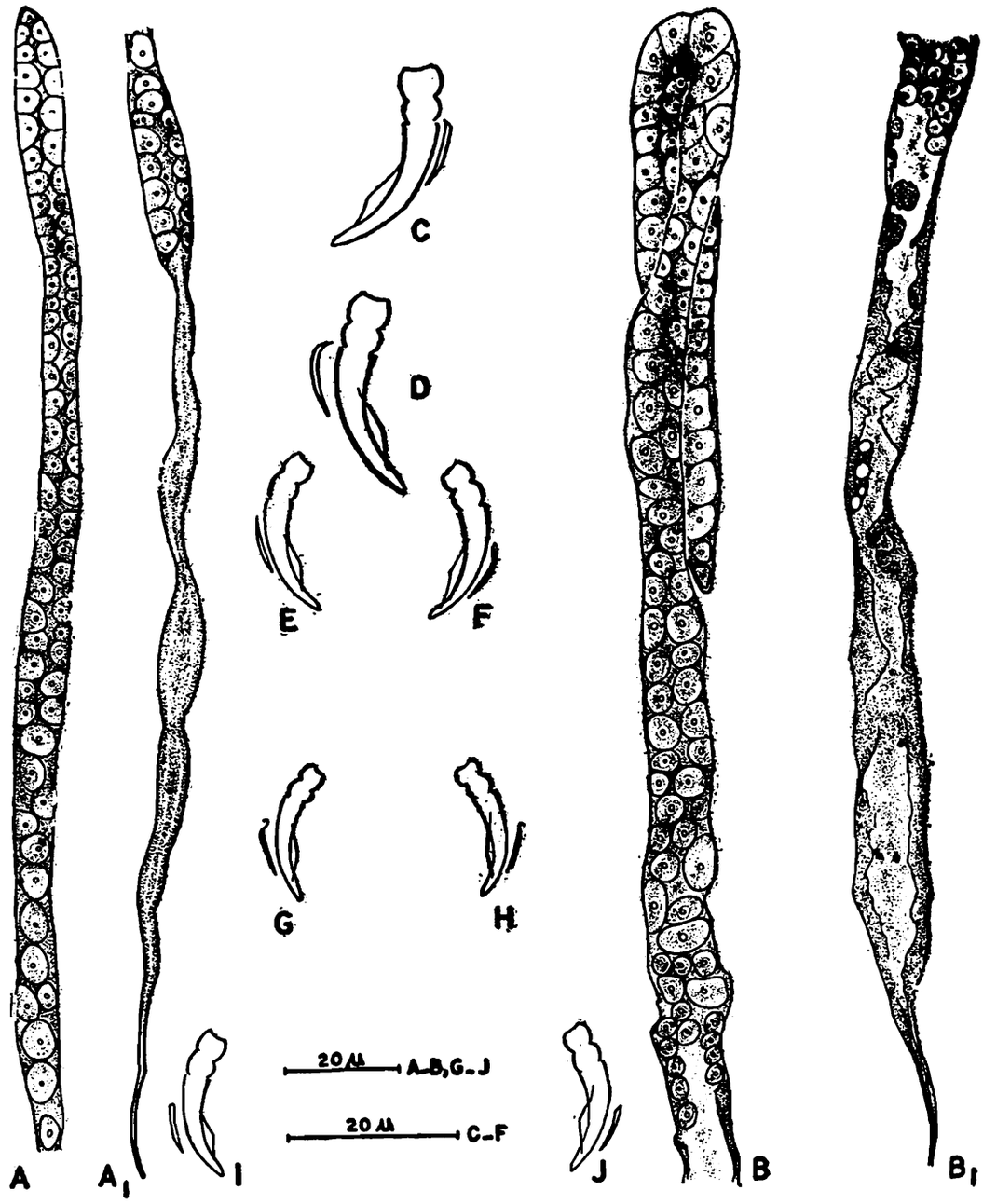
females the spermetheca is inconspicuous in the form of a swelling (Text-fig. 10, D) and assumes a definite shape upon the maturity of the worm. They are usually filled with oval to spherical sperms. The spermatheca lies at the oviduct-uterus junction. The proximal part of the uterus is muscular, while the distal is glandular. The distal part is also known as quadricolumella or 'shell-gland' part. The uterine or proximal part is more conspicuous in appearance than the quadricolumella region due to its having strong muscules which are used for the ejection of the eggs. The total length of the anterior uterus is 90 μ (58-197 μ) and of the posterior uterus is 80 μ (58-105 u).

The uteri jointly open into the vagina. The vagina is 10 μ (8-12μ), its walls are lined with cuticle. In lateral view, the vaginal walls show two distinct parts which have been regarded as rods by Sanwal (l.c.). The anterior part is smaller than the posterior one. There are some cells conspicuously visible in lateral view which lie at the vaginauteri junction. The vagina opens outside through a depressed transverse slit-like vulva 7 μ (6-8 μ) wide. In some of the specimens, the body wall is slightly raised (protruded) in the region of vulva. The position of the vulva is 53% (46-67) of the body length from the anterior end.

In one female, the anterior sexual branch was normal, but the posterior branch had a reflexed ovary (Text-fig. 10, I). In some females (Text-fig. 10, G and H) whose gonads were parasitized by a species of Sporozoa, the sexual branches were extensively developed. Usually the anterior sexual branch ends well below the ventral oesophageal gland lobe but in these specimens it reaches up to the middle of the subventral gland lobe of oesophagus.

Male reproductive organs.—The male gonad (Text-fig. 11, A-J) consists of a testis, a vas deferens, an ejaculatory duct and the copulatory apparatus, and occupies 33% (30-43) of the total body length confining itself to the posterior half of the body. The testis is outstretched rarely reflexed (Text-fig. 11, Band B1). The germ cells are arranged in a single file except in the zone of multiplication. The number of germs cells in the testis varies from 49-69 (averaging 59). The testis leads to the vas deferens, which is thin and tubular at the anterior end, and thick and glandular towards the posterior end. The vas deferens (Text-fig. 11, A_1) measures 230 μ (156-310 μ) and terminates into a narrow muscular ejaculatory duct which opens to the outside through the cloaca.

The associated copulatory structures are paired spicules, a gubernaculum and the caudal alae. Spicules are identical, each measures 25 μ (22-29 μ) medially and consists of a well developed head, a short neck, and a long concave tapering blade with blunt



Text-fig. 11. Gonads of *H. oryzae*, ♂ ♂ A. Testis ; A_1. Vas deferens and ejaculatory duct ; B. Abnormally reflexed testis ; B_1. Posterior part of the same ; C-J. Spicules and gubernaculum.

tips. There is a distinct membranous structure along the concave margin or the ventral side of the spicules. The gubernaculum is generally trough-shaped, measures 8 μ (7-10 μ) but it shows polymorphism (Text-fig. 11, C-J).

Discussion

The observations on the morphology of *Hirschmanniella oryzae* show many intra-specific variations which are important for the

taxonomic study of the genus *Hirschmanniella* and also have a bearing on the related groups. An evaluation of the diagnostic characters studied in *H oryzae* is given below :—

The body shape upon fixation in the two sexes varies from an open "C" to almost straight. The body size ranges significantly. This variation may be as much as 0.45 mm from the mean. However, worms with considerable slender or stout bodies are of frequent occurrence thereby altering the value of 'a'

The transverse striae may vary in nature and on any particular part of body may be fine or coarse, but extreme variations are rare. Normally the midbody has striae with greatest inter-strial width. They usually form complete rings on the head region before the appearance of the lateral incisures. The lateral fields interrupt the striae. Areolations are also present. The phenomenon of anastamosis of striae was seen in some specimens.

The number of incisures in the lateral fields was found to be constant, variations occur only in the manner of its termination. They may terminate 5-20 μ from the tip of tail, either above or below the level of phasmids. This is correlated with the fact that the position of phasmids is also variable. Therefore, the relation of phasmids with the termination of lateral incisures is no longer a dependable character for taxonomic purposes and should be used with caution after studying a large number of specimens.

The height and the width of the lip region ('head') are constant features and should be given importance. The shape of head is hemispheroid with or without a flat apex. In the specimens not fixed properly the actual shape of head may change to considerable extent. In order to determine the exact shape of head the specimens should be excellently preserved and neatly mounted. The number of head annules varies little, in the present specimens from 5 to 6 and is a good taxonomic character.

The average length of spear is 17 μ ranging from 15 to 19 μ. The deviations from the average length should be noted carefully. As recently pointed out by Taylor (1969), the spear, being a cuticularised structure, shows very little variations. Sometimes, it becomes rather difficult to see the tip of the spear and thus measure it accurately. This may result in error if proper care is not taken. In poorly fixed specimens, the head becomes a bit twisted and this results in faulty observations on the spear. Usually spear is slender in *H. oryzae* but abnormally it may be thicker in some specimens. The spear knobs are always rounded and slightly sloping. There was hardly any variation in the shape of the spear knobs. Spear,

therefore, should be given considerable importance in the separation of species of *Hirschmanniella* or any other Tylenchida.

The orifice of dorsal oesophageal gland is also quite constant. It varies 1 μ anterior or posterior to its usual position which is negligible. Therefore, the distance, of dorsal oesophageal gland opening from spear base stands out as a dependable character.

The median bulb is always located posterior to middle of oesopha gus. The change in position, if considerable, may be important. This could not be found in the present species. The length and width of the median bulb has little value.

The relation of hemizonid with excretory pore is not very significant because the former is found 1-8 striae above the latter. Sometimes, the hemizonid appears double. Therefore, much importance to these two structures cannot be given. The excretory pore is always situated anterior to oesophago-intestinal valve. This relation is very constant and should be given due consideration in the description of the nematode species.

SUMMARY

Morphological studies have been made on *Hirschmanniella oryzae* (Soltwedel). Intra-specific variations, important for the taxonomic study of the genus *Hirschmanniella* and also having a bearing on the related groups, have been observed. Number of incisures, height and width of the lip region, number of head annules, structure and length of spear, distance of the orifice of dorsal oesophageal gland from spear base, situation of the excretory pore in relation to oesophagointestinal valve are considered constant characters whereas the body shape and diameter, transverse striae, termination of incisures from the tip of the tail and in relation to phasmids, length and width of the median bulb and the relationship of hemizonid with excretory pore are of little significance.

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