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مشاهدات میکروسکوپیک الکترونی اسکنینگ همانکوس کونتورتوس بالغ (نماتود)

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مورفولوژی کرم بالغ همانکوس کنتورتوس با استفاده از میکروسکوپ الکترونی اسکنینگ مورد مطالعه قرار گرفت. یک محوطه دهانی کوچک با تیغهٔ پشتی، ۶ عدد پاپیلی لبی داخلی، ۶ عدد پاپیلی خارجی، ۴ عدد پاپیلی سوماتیک و دو عدد فرورفتگی جانبی مربوط به آمفید در قسمت قدام انگل مشاهده گردیدند. پاپیلی‌های گردنی مشخص و خار مانند بوده و منفذ ترشحی نیز مشاهده گردید. کیسهٔ جفتگیری کرم نر وسیع بوده و لُب‌های جانبی آن شامل شعاع‌های بلند و باریک می‌شدند. لُب پشتی، کوچک و به‌طور غیر قرینه در مقابل لُب جانبی سمت چپ قرار گرفته و شامل شعاع پشتی به شکل Y بود. در قسمت شکمی مخروط تناسلی، ساختمان "O" یا پاپیلی شکمی مشاهده گردید. اسپیکول‌ها بلند بوده و هر یک در نزدیک به انتها دارای خار و منفذ بودند. وولوا در کرم ماده دارای یک پوشش به نام فلپ بوده و نیز منفذ مقعدی شامل یک لبهٔ پشتی ساده و یک لبهٔ شکمی عضلانی می‌شد. همچنین در دو طرف کناری دم ماده، سوراخ‌های فاسمید مشاهده گردیدند.

واژه‌های کلیدی: نماتود، تریکوسترونجیلیده، همانکوس کونتورتوس، مورفولوژی، میکروسکوپ الکترونی اسکنینگ.

rostochiensis two small pores near the tip of each spicule, and it is clear from TEM observations that the dendritic processes end just beneath these pore openings (Clark et al.1973). These authors have concluded, therefore, that the spicules of these nematodes may be regarded as sensitive organs.

The vulva was located at the beginning of the postreior third of the body of the female thus permitting to differentiate H. contortus from Mecistocirrus, the only genus with which a confusion is likely to occur. In Mecistocirrus the vulva is near the tip of the tail (Dunn, 1978). Chitwood (1957) grouped the females of Haemonchus depending on the presence or absence, or shape of the vulvar flaps, in to three basic types: linguiform: knobbed and smooth without flaps. Variations in vulvar region were also noticed in specimens from different hosts and at differenrt seasons (Roberts, 1941). Das and Whitlock (1960) have studied the comparative morphology of H. contortus spp. from various geographical regions. Studies of the vulva flap led these authors believe that it represents the character in Haemonchus. The present observation showed that the vulva was covered with a flap and supported by two lateral knobs. Daskalov (1972) suggested that the increase in the presence of the additional knob on linguiform types is correlated with the reproductive activity of the mature worm, Daskalov therefore considered that the vulvar flaps have no taxonomic importance and are age related. Also, this study revealed the details of the anal pore. Observations of Lichtenfels et al. (1990) showed the anus in infective larva and parasitic third-stage larva of H. contortus to be semilunar in shape with lateral corners turned down and a slightly protruding upper lip. New information on female H. contortus provided in the present study includes descriptions of phasmidal apertures.They were flat and botton-like. The fact that the phasmids and amphids are located at the extremities of the nematode suggests that they may be involved in the detections of intensity of a given stimulus and thus helping to maintain the worm in a favourable environment (McLaren, 1976). Previous work on immature forms of H.contortus (Lichtenfels et al. 1990) Showed that phasmidal ducts opening on the body wall halfway between the anus and the distal ends of the lateral alae and slightly ventral to the alae.

The present study indicates the presence of differences in the cuticle of adults H. contortus in different parts of their bodies. The nematode cuticle has important basic functions both as a structural framework and acting to protect the organisms from dehydration, abration ,predation (in free-living species) and immune attack (in parasites). Finally,the negatively charged surface coat is a feature shared both by free-living species and by parasitic nematodes, in which it may act as a disposable carrier of antigenic products and thereby be a major element of successful immune evasion (Maizels et al . 1993).

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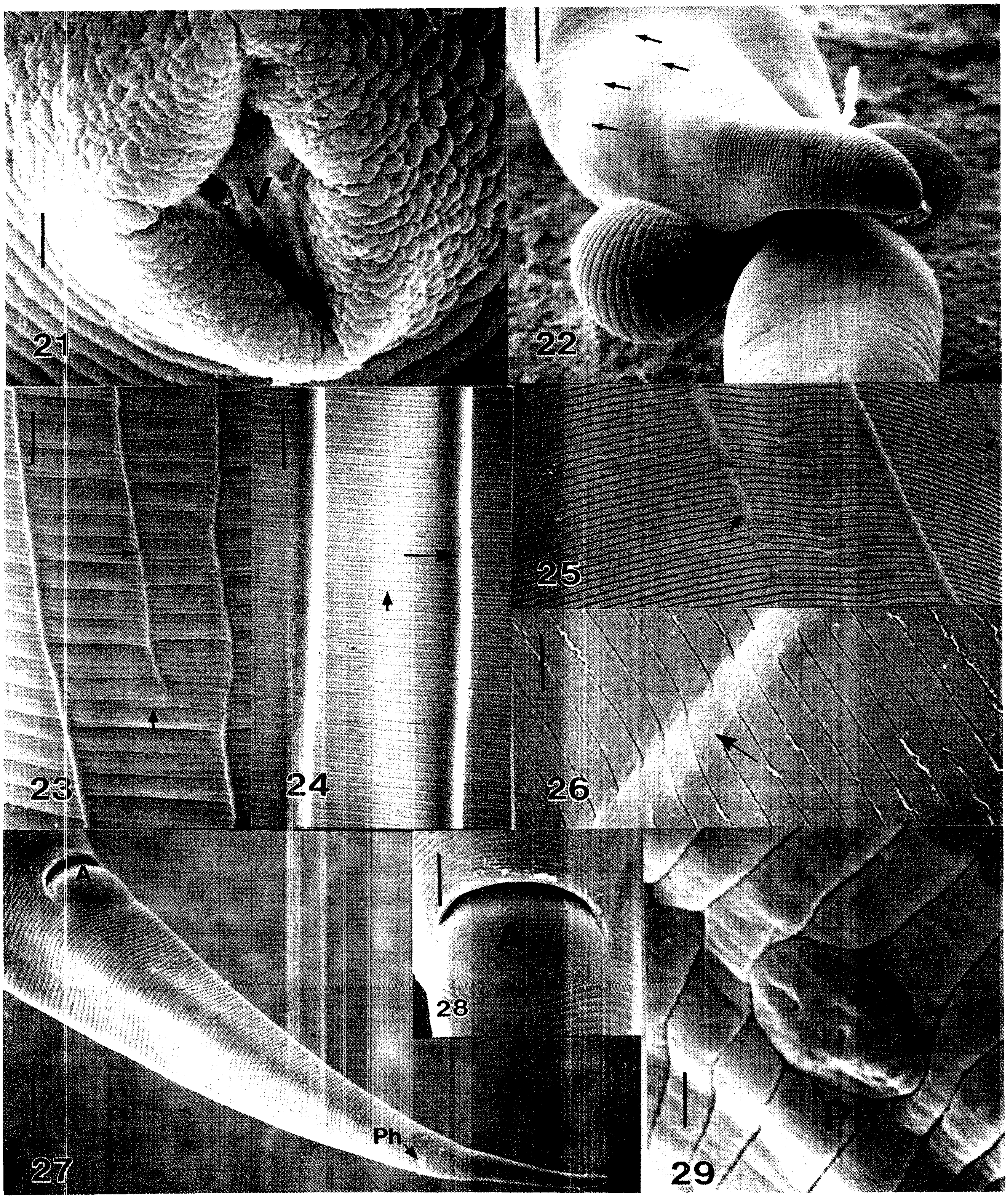


Figs. 21-29 Scanning electron micrographs of middle and posterior parts of the body of female Haemonchus contortus. **Fig. 21** vulva (V) was covered with a flap (F) and supported by two cuticular knobs (CK), at the begining of vulva region, continuation of longitudinal cuticular ridges in oblique position (arrows). Bar=55.55 um. **Fig. 23** middle part of the body cuticle with transversal striae (short arrow) and longitudinal ridges (long arrow) Bar=7.69 um. **Fig. 24** high magnification of middle part of the body cuticle, transversal striae (short arrow), Bar=7.14 um. **Fig.25** near to the vulvar region, cuticular ridges were disappeared (arrows). Bar=7.14 um. **Fig. 26** pattern of cuticular ridges in oblique position (arrow) at the begining of vulvar region. Bar=3.7 um. **Fig. 27** posterior third of the body, ventral side, anus (A), pasmid (Ph) on right lateral side of the tail. Bar=25 um. **Fig. 28** anus (A) with thin upper rim and strong, smooth lower rim. Bar=13.33 um. **Fig. 29** botton-like phasmidal aperture (Ph) on lateral side of female's tail. Bar=1.66 um.

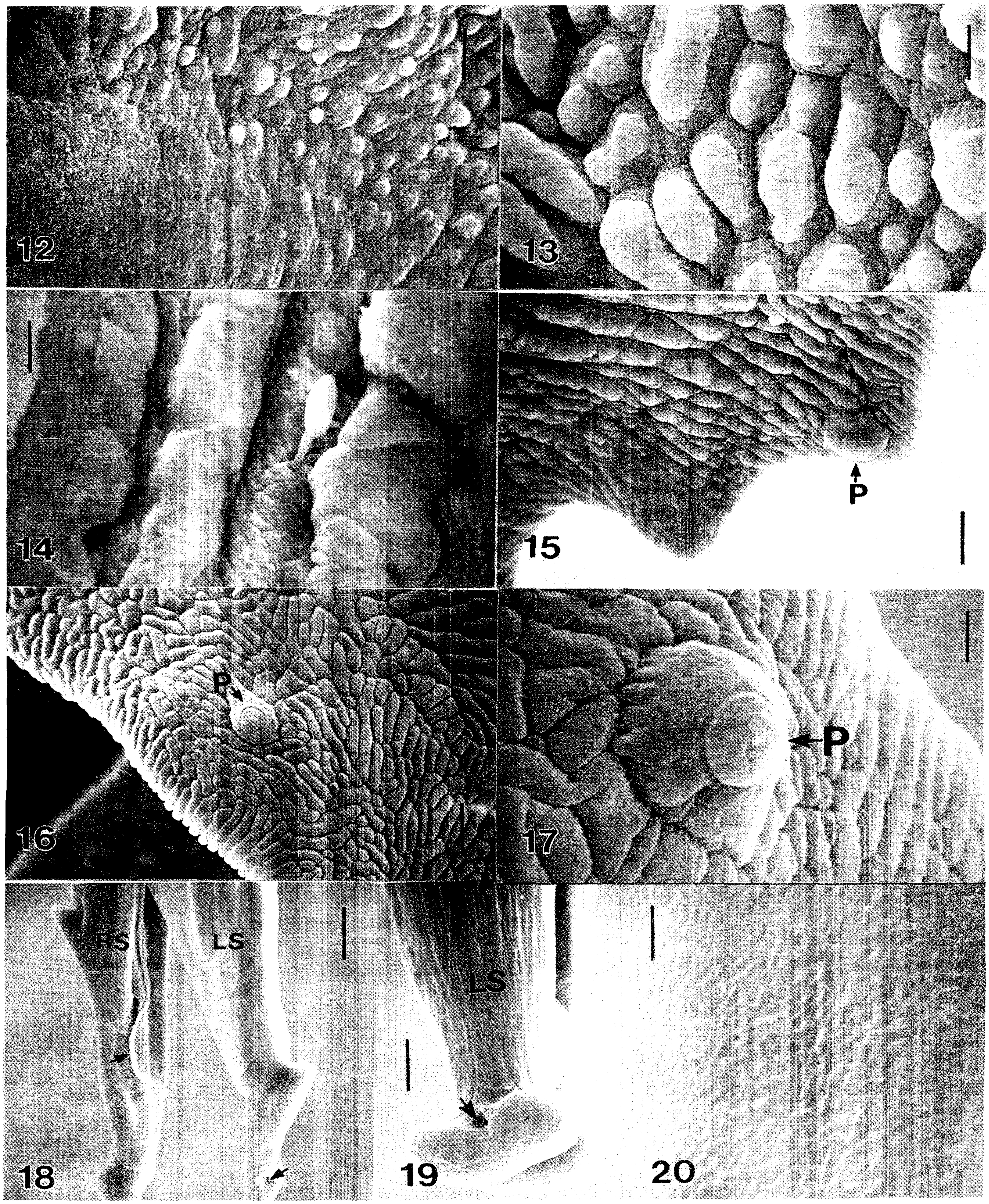
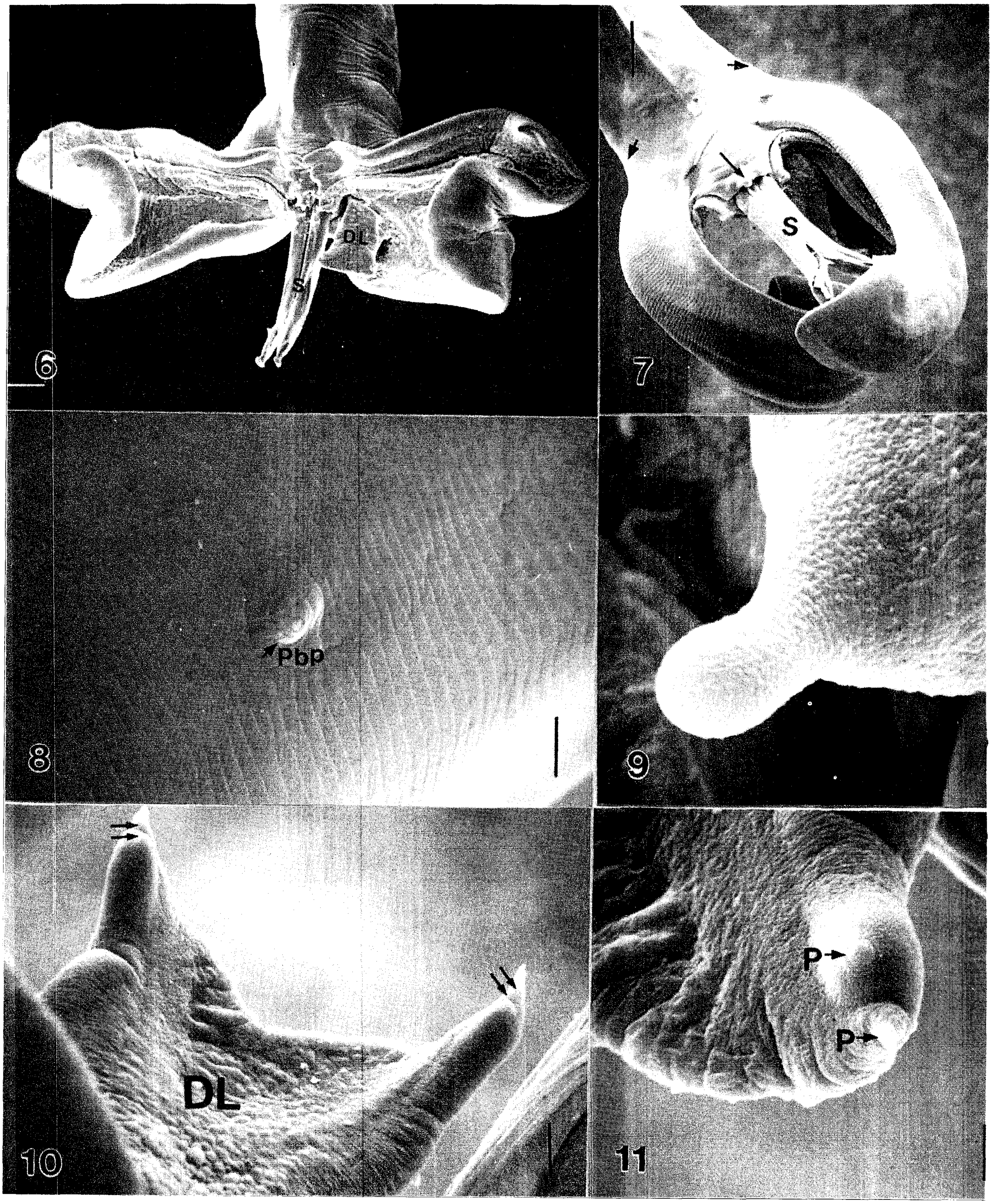
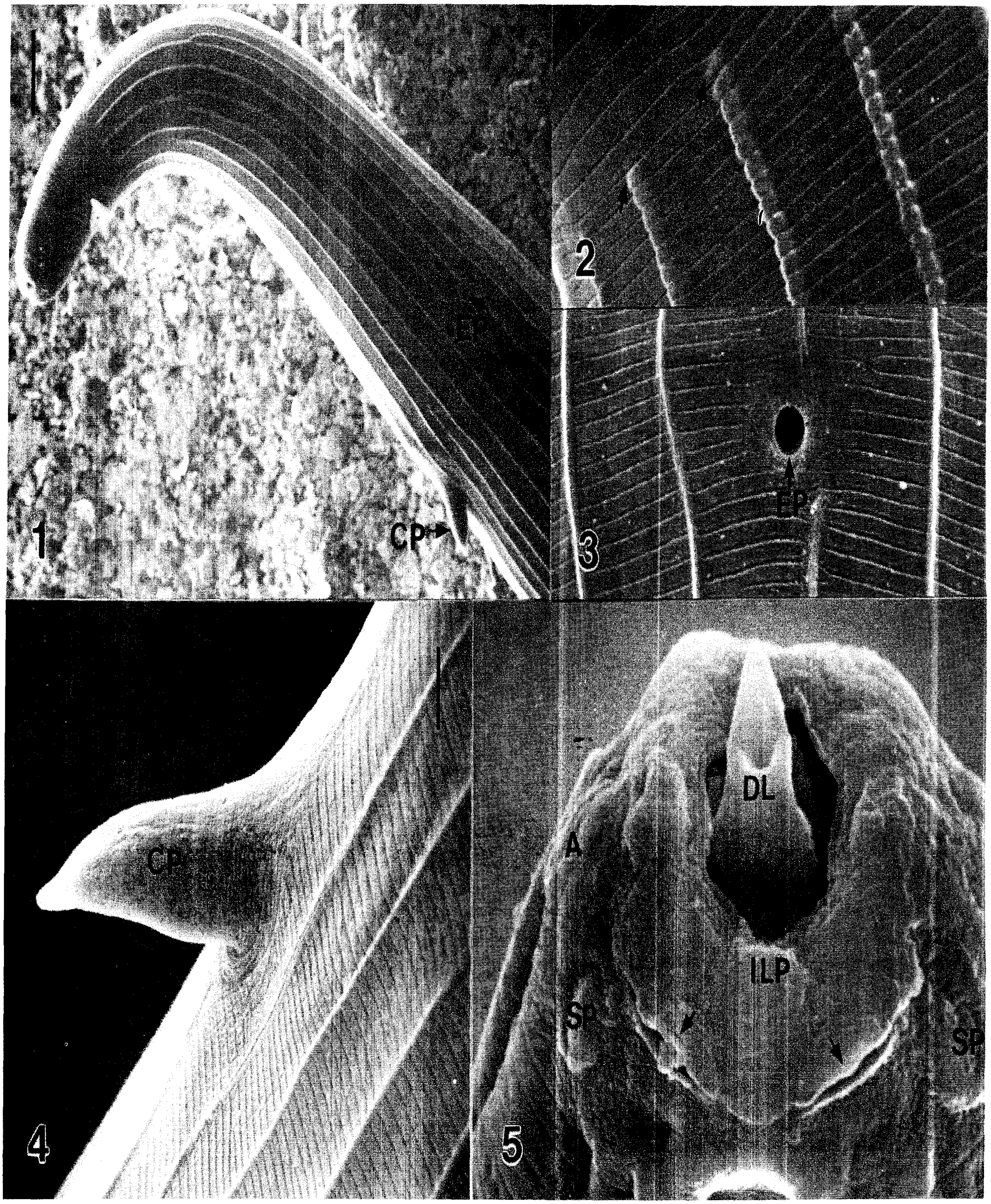


Fig. 12 the surface of dorsal lobe. Bar=4 um. **Fig. 13** inner surfcae of copulatory bursa. Bar=4 um. **Fig. 14** hyperparasite (arrow). Bar=1.66 um. **Fig. 15** one of two pairs of papillae on each lateral lobe (P). Bar=7.69 um. **Fig. 16** outer surface of left lateral lobe with one of two domed-shaped papillae (P). Bar=7.14 um. **Fig. 17** high magnification of domed-shaped papilla on the outer surface of left lateral lobe (P). Bar=1. 66 um. **Fig. 18** barbed-spicules; left spicule (LS) with pore (arrow), night spicule (RS) with a semi-spicular annulation (arrow). Bar=7.69 um. **Fog. 19** extremity of left spicule (LS) with pore (arrow). Bar=4 um. **Fig. 20** smooth surface of each spicule. Bar=1.82 um.



Figs. 6-20 Scanning electron micrographs of posterior end of male Haemonchus contortus. **Fig.6** copulatory bursa of male, dorsal lobe (DL), spicules (S). Bar=58.82 um. **Fig. 7** prebursal papillae (short arrows), the "O" or the ventral papilla (long arrow), spicules (S). Bar=58.82 um. **Fig. 8** pre-bursal papilla (Pbp). Bar=3.92 um. **Fig. 9** the ventral papilla. Bar=1.96 um. **Fig. 10** dorsal lobe (DL) and Y-shaped dorsal ray with one pair of papillae on each extremity (double aarrows). Bar=7.69 um. **Fig. 11** 1 of 2 extremities of Y-shaped dorsal ray with two papillae (P). Bar=4.25 um



Figs. 1-5 Scanning electron micrographs of anterior end of adults Haemonchus Contortus. **Fig. 1** begining of neck area (without cuticular ridges), excretory pore (EP), one of two cervical papilae (CP). Bar=25 um. **Fig. 2** begining of cuticular ridges (arrows). Bar=3.92 um. **Fig. 3** ventral side of anterior end, excretory pore (EP). Bar=3.92 um. **Fig. 4** lateral side of anterior end, cervical papilla (CP). Bar=3.92 um. **Fig. 5** lateral view showing mouth, dorsal lancet (DL), internal labial papilla (ILP), 2 of 6 external labial papilla (arrows), 2 of 4 somatic papilla (SP), amphidal aperture (A). Bar=1.66 um.

rectangular lappest in a star- shaped arrangement , 6 external labial papillae, 4 somatic papillae, and small amphidal apertures were also present (Fig.5). SEM observation showed that the mouth was simple and carried a bent dorsal lancet with an orifice of the dorsal esophageal gland (Fig.5).

The bursa of the male had elongate lobes supported by long rays, while the dorsal lobe was asymmetrically, situated on the left-hand side and supported by a Y- shaped dorsal ray. Other important diagnostic features were the barbed spicules (Fig.6). At the beginning of the copulatory bursa, on each lateral side, one single prebusal papilla was observed (Figs.7,8), cuticular ridges were absent in this area (Fig 8). on the ventoral aspect of the genital cone, the "O" or ventral Papilla was observed (figs. 7,9). The Y-shaped dorsal ray had one pair of papillae on each extremity (Figs.10,11). The surface of the dorsal lobe was covered by bursal bosses at the beginning and was smooth at its extremity (Fig. 12). Some SEM differences were seen between the inner and outer cuticular surface of the bursa (Figs.13,16). In one case a structure were likely to be a hyperparasite was observed on the inner surface of the right lateral lobe, while absent in other worms (Fig. 14). The SEM observations also showed two pairs of papillae on the inner surface of each lateral lobe (Fig.15) and one pair of papillae on the outer surface of each lateral lobe (Figs. 16,17). These papillae of the bursa were similar and domed-shaped (Figs. 15,17). The spicules of the male measured 460 to 510 um in lenght. The distal tips of the spicules each had a single barb at unequal distances, the barb on the left spicule was 200 um distant from the tip and the right spicule 400 um (Fig.18). There was a single small pore at the end of each spicule (Fig.19). The surface of the spicules were smooth (Fig.20).

The vulva was located at the beginning of the posterior third of the female body. Some SEM differences were seen between the cuticle around the vulva and the other parts of the body (Fig.21). The vulva was covered by an anterior flap which was frequently large and prominent and supported by two knobs (Fig. 22). The surface of the vulvar flap and knobs were covered by a cuticle with transversal striae (Fig.22). SEM observations showed longitudinal cuticular ridges were presented only in the first and the second third of the body (Figs.23,24). Near to the vulvar region, the longitudinal cuticular ridges disappeared (Fig.25). In the posterior third of the body these ridges were absent compeletly and only transversal cuticular striae were seen .At the beginning of the posterior third of the body, continuation of longitudinal ridges were seen in oblique position (Figs .22,26).The anal pore was situated on the ventral side of the posterior end of the body (Fig.27).The upper rim of the anal pore was simple and thin and the lower rim was muscular (Fig.28). SEM observations showed one botton-like phasmidal aperture on each lateral side of the femal's tail (Fig.29).

Discussion

Our study on the adult parasitic stage of H. contortus described 6 internal labial papillae, 6 external labial papillae, 4 somatic papillae and lateral amphidal pits around a small mouth . Hope (1965) made one of the first EM studies of nematode amphidal sense organs. Subsequent observations on H. contortus (Ross,1967) confirmed that the sensory terminals within nematode amphids take the from of highly modified cilia whose microtubular numbers and arrangments show extreme variations. The amphidal cilia of H. contortus is said to be enclosed in vascular spaces (Ross, 1967). Because the amphidal sense organs open to the exterior by a pore in the outer cuticle of the worm, they should regarded as chemoreceptors, responsible for detecting changes in the external environment (Mclaren, 1974). There is no evidence that H. contortus amphids have associated glands ,as seen in Ancylostoma caninum (Tharson, 1956). Our present study showed that cervical papillae were 300 um from the anterior end of H. contortus .In parasitic nematodes , these organs are responsible for determining whether or not the nematode can successsfully pass through a restricted space (Mclaren, 1976). Also we showed that the excretory pore in the adults of H. contortus was small, round, unornamented and ventrally located. Lichtenfels et al. (1990) made the same observation on the infective larva and parasitic third-stage larva of H. contortus. The region just posterior to the excretory pore is important in the production of moulting fluid in infective juveniles of H. contortus (Sommerville, 1957; Rogers and sommerville, 1960), and it has been shown that the excretory system of the infective larva of this nematode consists of a tubular H-system with two excretory cells. The excretory cells contain electron-dense granules which may be a source of exsheathing fluid (Wharton and Sommerville, 1984; Wharton, 1991).

In the present study, the number, arrangment and shape of caudal papillae of the male H. contortus were studied. Male strongyloid nematodes terminate in a copulatory bursa, and the caudal papillae are generally situated at the extremities of the bursal rays as also described by Chitwood and chitwood (1950). Our study showed that the papillae of the inner and outer surface of the bursa were domed-shaped. Some authors consider them as sensitive to pressure or lateral deflection (Tulloch et al., 1972; Dick and Wright,1973). Our observations showed some differences among the prebursal papillae, papillae of the inner and outer surface of the copulatory bursa and the papillae at the extremities of the Y-shaped dorsal ray in shape and size .The configuration of the "O" or ventral papilla was compeletly different from the caudal papillae. We postolate that these structures correspond to sensory organs, TEM studies are required to confirm our hypothesis. Also in this study a single small pore was observed at the end of each spicule. Some SEM studies have shown on the male worms of Heterodora

Scanning electron microscopical observations on adult Haemonchus contortus (Nematoda)

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Scanning electron microscopy (SEM) was used to examine the surface ultrastructure of adult worms of Haemonchus contortus.They possessed a small bucall cavity with a dorsal lancet,6 internal labial papillae,6 external labial papillae, 4 somatic papillae and two lateral amphidal pits. The cervical papillae were prominent and spine-like. The excretory pore was present. The male bursa had elongated lateral lobes supported by long, slender rays; the small dorsal lobe was asymmetrically situated against the left lateral lobe and supported by a Y-shaped dorsal ray. On the ventral aspect of the genital cone,the "O" or ventral papilla was observed. The spicules were long, each provided with a small barb and pore near its extremity. The vulva of the female was covered by a linguiform process or flap and the anal pore had a simple dorsal rim and a muscular venteral rim. On the lateral sides of the female's tail, phasmidal apertures were observed.

Key words: Nematode,Trichostrongylidae , Haemonchus contortus, Morphology, Scanning electron microscopy.

Haemonchus contortus (Rudolphi, 1803), is a blood-sucking abomasal nematode responsible for extensive losses in sheep and cattle,especially in tropical areas (Urquhart etal.1991). The anatomy and life - history of H. contortus was studied by Veglia in 1915 . Chabaud et al ., specifically numbered cloacal papillae of this nematode in 1970 . Also, revision of the genus Haemonchus was carried out by Gibbons in 1979. In another study, Gibbons in cluded some scanning electron. micrographs of H. Controtus in her SEM guide to the nematode parasites of vertebrates (1986). The pattern of cuticular ridge of H. contortus and H. placei was studied by Lichtenfels et al. (1986), these authors measured the lenghts of the ruminant stomach nematodes H.contortus and H. placei in 1988. SEM data on the sheated infective larvae and parasitic third-stage larva of this nematode has shown details of triradiated mouth, star-shaped arrangement of the 6 laplets, internal, external and somatic papillae ,an excretory pore, amphidal and phasmidal apertures (Lichtenfels et al. 1990). Also, Lichenfels et al., showed new morphological characters for identifying individual specimens of Haemonchus spp. in 1994. Phylogenetic of the subfamilies of the Trichostrongylidae based on 22 morphological transfromation series produced a single cladogram with a consistency index (Herberg and Lichtenfels, 1994). In another study which is carried out by Lichtenfels et al., in 1995, a new hypodermal gland was discovered in female nematodes of the family Trichostrongylidae. A noval silver-staining method was described for elucidation of the synlophe among the Trichostrongyloid nematodes by Khrustalev and Hoberg in 1995.

The aim of the present study is to provide additional infomation on the morphology of the adult male and female of Haemonchus contortus.

Material and methods

Thirty sexually mature adults (15 male and 15 female) of Haemoncus contortus were obtained from the abomasum of naturally infected sheep. The worms were washed and cleaned with 2% sodium cacodylate buffer (PH 7.2). Diagnosis of the species was confirmed on the basis of light microscope (LM) examination and by using the keys (Skrjabin et al.,1954; Soulsby, 1986; Lichtenfels et al., 1986; Lichtenfels, 1994). Specimens were then placed in 4% glutarldehyde . All these stages were made in Iran. The specimens were transfered to the Department of Pathology, EM Facility of the Faculty of Health Sciences, McMaster University, Canada for SEM study. The nematodes were transfered in fresh 4% glutarldehyde for 2h, postfixed in 1% osmium tetraoxide in cacodylate (PH 7.2) for 1h at 4 C and dehydrated through a series of ethanols (50%, 75%, 90% , 100%). They were dried in a liquid CO2 critical point apparatus, Ladd, 28000. The specimens placed on precleaned aluminum stabs, were coated with gold in an ICS, Polaron E5100 and examined with a Philips 501-B scanning electron microscope at an accelerating voltage of 15 KV.

Results

Anterior ends in both sexes had similar cuticular surfaces including cuticular transversal striae and cuticular ridges or synlophe. Cuticular ridges were absent at the beginning of the neck and some of them were bifurcated (Figs. 1,2). On the ventral side of the anterior end , the excretory pore measured 2 um in diameter (Figs. 1,3). The cervical papillae were spine-like and situated on the lateral sides of the anterior end (Figs.1,4). A small mouth was surrounded by 6 internal labial papillae on 6 prominent , bluntly

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