قسم الطفيليـــــات
كلية الطب ـ جامعة أسيوط
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بايلز اسكارس بروسيونز (١) دراسة التطور الجنينى والشكل الظاهري للطور اليـــرقى الثانـــى

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نظرا للاهمية القصوي لديدان البايلز اسكارس بروسيونز والتى تصيب حيوان الراكـون الذي ينتشر فى المناطق الشمالية للولايات المتحدة الامريكية ، والتى قد تصيب يرقات هـذه الديدان الانسان وتتجول فى جهازه العصبى والعينين وبعض الاعضاء الداخلية مسببة أضـرار جسيمة تؤدي الى الوفاة السريعة ٠ لذلك قام الباحثون لاول مرة بدراسة التطور الجنيـــــنى لبويضات هذه الديدان ومعرفة الوقت اللازم لكى تصبح معدية وهى فترة ١١ ـ ١٤ يوما ٠ كمـا قام الباحثون بدراسة تفصيلية على يرقات الطور الثانى وذلك باستخدام الميكروسكوب الضوئـى والميكروسكوب الالكترونى للسطح الخارجى حتى يمكن التفرقة بين هذه اليرقات ومثيلاتهـا مـــن مجموعة ديدان الاسكارس الاخـــري ٠

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BAYLISASCARIS PROCYONIS (STEFANSKI AND ZARNOWSKI, 1951) ASCARIDIDAE : NEMATODA I. EMBRYONIC DEVELOPMENT AND MORPHOGENESIS OF SECOND STAGE LARVAE

(With 24 Figs.)

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SUMMARY

Baylisascaris procyonis, the intestinal ascarid parasite of raccons (Procyon lotor), has been implicated as an etiologic agent of human visceral larva migrans (VLM), central nervous system (CNS) disease, and ocular larva migrans (OLM). We have studied the early embryonic development as well as the second larval stage (L2) of B.procyonis in order to identify morphological features which will allow differentiation of this parasite from other nematode larvae.

The eggs of B.procyonis measured 72-81 by 60-66 microns and developed to infective stage in 11 to 14 days at 25°C. L_2 prepared by artificial hatching in vitro measured 14-18 microns in diameter by 275-290 microns in length. B.procyonis L_2 had a characteristic pronounced oral protuberance, lateral alae, a funnel-shaped buccal vestibule, and the tail terminated in a button- shaped knob. Microtopography of B.procyonis second stage larvae was studied in detail by scanning electron microscopy. The anterior end was tapered to form a longitudinally striated cephalic cone. The tip of the cephalic cone was protruded, truncated, and consisted of three dorsal and two subventral lip-like oesophageal protrustions. The dorsal lip-like oecophageal protrusion was separated from the fused subventrals. A pair of amphidial pores were located laterally and slightly dorsal to the subventral oesophageal protrusions. A cuticular cap from the first moult sheath sometimes appeared on the ocsophageal protrusion. Prominent lateral alae extended anteriorly from the base of the cephalic cone to the tail posteriorly. The excretory pore was surrounded by a prominent rim, and an oval cuticular bleb or thickening was found anterior to the anal orifice.

INTRODUCTION

Baylisascaris (SPRENT, 1968) is a genus of ascarids created by SPRENT (1968) to include several species. Bsylisascaris procyonis (STEFANSKI and ZARNOWSKI, 1951), the common intestinal

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ascarid round worm of the raccoon, Procyon lotor, is recognized as an etiologic agent of visceral larva migrans (VLM), central nervous system (CNS) disease, and ocular larva migrans (OLM) in a variety of birds (RICHARDSON, et al. 1980 and REED, et al. 1981), mammals (KAZACOS, et al. 1981 a; KAZACOS, et al. 1983 and KAZACOS, 1983), subbuman primates (KAZACOS, et al. 1981 b; KAZACOS, et al. 1982 and KAZACOS, et al. 1984) and in humans (HUFF, et al. 1984 and FOX, et al. 1985). Recently, considerable attention has been directed to host-foreign nematodes as agents capable of producing disease in man. The etiology in the majority of instances still remains a matter of conjecture. We report here a detailed description of the embryonic development as well as morphologic description of the second stage larvae of B.procyonis using both light and scanning electron microscopy.

MATERIAL and METHODS

Baylisascaris procyonis eggs were obtained from the faeces of naturally infected raccons. A combined sedimentation and flotation technique was employed to concentrate the eggs. B. procyonis gravid adult worms were obtained from a naturally infected raccoon. The terminal uterus was dissected and teased to release the eggs in 0.85% saline solution. Eggs were incubated in a large petri dish containing 2 mm. depth of either 2.5% potassium dichromate solution or 1% hydrochloric acid at room temperature (22-25°C). Incubated eggs were examined at intervals for embryonic development.

Second stage larvae (L2) were obtained by artificial hatching of the embryonated infective eggs in vitro. The eggs were suspended in Medium 199 at 37°C, and rotated with glass beads for 3 hours. Live L2 were separated from egg shells and other debris by the Baermann technique, using an 8 mm-thick cotton pad, through which the larvae migrated. Larvae were studied in the fresh state while immobilized by moist heat. Measurements in microns were made by using a Zeiss Universal microscope equipped with an ocular micrometer, and drawings were made with the aid of a camera lucida.

Scanning electron microscopy: Suspension containing early second stage larvae obtained by artificial hatching of the embryonated infected eggs in vitro as well as suspensions of the embryonated eggs were fixed in 2.5% glutaraldehyde - 4 mM $CaCl_2$ - 64 mM Na cacodylate buffer, pH 7.4 at 4°C for 14-16 hours. Specimens were washed three times and resuspended in fresh Na cacodylate buffer containing 131 mM sucrose. Specimens were dehydrated through ascending grades of ethanol, washed three times in amyl acetate, placed on a glass coverslip, dried at room temperature over silica gel, sputter coated with 100 A gold and examined with an AMRAY 1400 scanning electron microscope.

RESULTS

Baylisascaris procyonis eggs are rounded, oval or elliptical, golden to deep brown in color, and measure 72-81 u in total length and 60-66 u in width. The egg shell is thick and three layered and has a finely granulated coat, measuring from 3 to 5.5 u in thickness. A few decorticated eggs with 2 u thick shells are observed. The females produce large numbers of unsegmented eggs which reach 75,000 eggs per gram of faeces in heavily infected racoons.

Scanning electron microscopy of the B.procyonis egg reveals the granulation or particulate appearance of the surface (Fig. 1). On high magnification SEM, a fibrillar network with irregular

meshes is readily apparent underneath the granular surface (Fig. 1). No operculum or sutures can be seen on the egg surface. The larvae are released from the eggs during hatching through a crack at one pole (Fig. 2).

The cleavage of B.procyonis eggs was followed in vitro under laboratory condition (22-25°C and 100% humidity). The eggs when freshly laid by the oviparous females contain embryos in an early stage of development, usually unsegmented containing a single cell. Within the first 1-4 hours, the first cleavage divides the embryo transversely into two blastomeres (Fig. 3). The two blastomeres divided at different rates.

The second cleavage, T stage or 4 cell stage (Fig. 4), in which the somatic blastomere divides longitudinally and the germinal cell divides tranversely, occurs within the next 4 to 8 hours. After cleavage, these four cells are rearranged to give a rhomboid shape (Fig. 5). Cleavage proceeds and the embryonic development passes through the morula stages within 8-36 hours (Fig. 6). Within 36-96 hours the embryo passes through a blastula stage (Fig. 7). A gastrula-stage is reacehed within 5-6 days (Fig. 8). On the seventh day, a tadople stage is reached in which the embryo develops an elongated vermiform shape (Fig. 9). Organogenesis and rearrangement continue and the larvae become visible inside the eggs after 8-10 days (Fig. 10). After eleven to fourteen days of incubation, the larvae molt for the first time within the eggs and become the infective stage. The development of the thick-shelled corticated eggs was observed to be slower than the decorticated eggs under laboratory conditions. The second stage larvae remain within the infective egg ensheathed in the cast skin of the first molt (Fig. 11).

Artifical hatching of the infective eggs released sluggishly motile unsheathed larvae, leaving a smooth sheath of the first molt inside or outside the eggs. A few larvae were observed to remain ensheathed when released from infective eggs by pressing the eggs between a coverslip and the slide.

Morphology of the Second-Stage Larvae by Light Microscopy:

The second stage larvae are cylindrical with almost parallel margins (Fig. 12 and 13). under low magnification, the larvae can be divided into two portions, a clear anterior oesophageal region occupying about one-third of the total length, and a posterior opaque intestinal region occupying about two-thirds of the total length and terminating anterior to the tip of the tail. Cuticular lateral alae are seen along the sides of the larvae and extend to about 15 microns from the anterior and posterior ends. The total length of 50 larvae varied from 275 to 290u (average 281±7 SD u), and the width varied from 14 to 18 u (average 16±2 SD u). The head of the larva is constricted from the body proper forming a characteristic conspicuous oral protuberance (Fig. 12, 13 and 15). The buccal cavity forms a funnel-shaped vestibule at the anterior end of the oesophagus. The rhabditiform oesophagus appears as a uniform tube, gradually widening at the nerve ring, and terminates in a pyriform bulb (Fig. 14). The oesophagus measures 87 to 95 u in length, or approximately one third of the total length of the larva. The nuclei of the oesphageal muscles appear in the dorsal sector of the oesophageal wall arranged in two rows. Ganglionic nuclei occur in the body cavity at either side of the oesophagus.

The excretory columns are largely obscured bythe gut, and little can be seen except in the left ventro-lateral margin. The single nucleus of the excretory cell lies anterior to the oesophagointestinal junction. The excretory pore is found on the ventral line nearly at the mid-oesophageal levels, about 43 to 47 u from the anterior end, and leads to a thin excretory tube (Fig. 15).

The nerve ring appears as a homogeneous structure in the mid-oesophageal region. The intestine is a sac-like uniform tube without lumen and the intestinal cells are packed with yellow-

ish refractile fat globules. The intestine terminates 10 to 15 u anterior to the anal pore to which it is connected by a thin tube. The anus is located on the ventral line, about 26 to 29 u from the tail end. The anal orifice protrudes slightly when viewed laterally (Fig. 16).

The genital primordium is a spherical mass between the intestine and the ventral body wall slightly posterior to the mid-intestine (Fig. 12). The tail is differentiated and characterised by having a rounded terminal button knob and is slightly curved dorsally (Fig. 16).

Scanning Electron Microscopy of the Second Stage Larvae:

The second stage larvae in general are cylindrical and fusiform. The parallel margins taper gradually at the posterior end to form the tail and are abruptly tapered anteriorly commencing half-way between the nerve ring and the anterior end to form a slender attenuated cephalic cone (Fig. 17). The most anterior tip of the cephalic cone is conically protruded with a truncate blunt tapering termination (Fig. 18). In the enface view, a wide triangular oesophageal opening leading directly into the oesophagus is seen (Fig. 19 and 20). A dorsal and two subventral lip-like thick and blunt prominent fleshy structures form the anterior tip of the oesophagus (Fig. 18, 19 and 20). A deep triangular vestibule separates the dorsal lip-like oesophageal protrusion from the subventral ones dorso-laterally (Fig. 18). The subventral oesophageal protrusions are fused together except for a shallow triangular fossa marking the separation borders antero-ventrally (Fig. 20). The cuticle of the body wall proper extends from the tips of the lip-like oseophageal protrusions externally to the end of the cephalic cone, and has marked longitudinal striations ro streaks. A pronounced external indentation appears in the form of two cylindrical thickened cuticular rods on the outer surface of each oesophageal protrusions (Fig. 20 and 21). The blunt ends of the lip-like structures are sole-shaped (Fig. 20 and 21).

A pair of amphidial pores are seen laterally and slightly dorsal to the subventral oesophageal protrusions, located about one-third of the cephalic cone length from the anterior end (Fig. 19 and 20). Based on the enface and lateral views, the cephalic sensory organs including the inner and outer circle of labial papillae are not evident.

In some newly hatched larvae, a cuticular cap appears adherent to the lip-like oesophageal protrusions and continuous with the oesophageal lining (Fig. 22). The annular cuticular striations of the body surface are clearly seen. Two prominent longitudinal lateral alae commence anteriorly at the base of the cephalic cone and extend to the tail posteriorly along the sides of the body (Fig. 19 and 20). On each side of the lateral alae is a small cuticular ridge (Fig. 17). The excretory pore is situated in the ventral mid-line anteriorly and is surrounded by a prominent rim (Fig. 21). The anal pre is circular, situated mid-ventrally and posteriorly. An oval cuticular bleb or thickening is evident anterior to the anus (Fig. 23). The tail is relatively long and straight, and ends with a knob-like tip which is longitudinally striated (Fig. 24).

DISCUSSION

Development and morphogenesis of different ascarid nematodes in both normal and abnormal hosts has been extensively studied by many investigators. However, the embryonic development and morphogenesis of B.procyonis second-stage larvae have not been described. Several aspects of the early immature stages of B.procyonis previously unreported are revealed using light and scanning electron microscopic methods.

The cleavage of nematode eggs has been followed by a number of investigators (LEVINE, 1980). The descriptionof the cleavage of B.procyonis eggs in the present study agrees in general

with the embryogenesis of other ascarid worms. Based on the time required for egg incubation, KAZACOS, et al. (1981 b) stated that eggs were embryonated in 2% formalin-saline in 30 days at 28°C. However, on the basis of our experimental studies, B.procyonis eggs became fully infective following 11-14 days incubation at 22-25°C, a substantially shorter period. SPRENT (1953) reported that the eggs of Baylisascaris devosi, the parasite of the fisher and marten were infective for mice at 12 days after culturing in moist charcoal at 68-80°F. Moreover, SPRENT (1970) was able to infect laboratory mice with B.tasmaniensis eggs, the parasite of the marsupial carnivores, after culturing the eggs for 3 weeks at 72°F.

The measurement of the eggs in the presnet study felt in the measurement range presented by SPRENT (1968). The measurement given by KAZACOS (1981) do not match the size range of the B.procyonis eggs given by SPRENT (1968) or in this report. However, the description of the eggs by SEM given by KAZACOS and TUREK (1983) are essentially identical to ours.

The origins of the oral protuberance of B.procyonis may differ from those previously reported for other ascarids. From our SEM study of B.procyonis L2, we concluded that the oral protuberance was formed by three lip-like esophageal protrusions and not by true labia, a new finding concerning second stage larvae of ascaridoid nematodes. Of the previous investigators, ROBERTS (1934) described the second-stage larvae of Ascaris suum as having three clearly defined lips, one broad dorsal and two ventral. SPRENT (1953) described that the second-stage larvae of B.devosi develops a conspicuous tooth-like projection at the anterior end. NICHOLS (1956 a) stated that the anterior quarter of the body of the second-stage larvae of Toxocara spp. tapers evenly to a three-lipped, subterminal, dorsally inclined mouth. Moreover, he noted that the second-stage larvae of Ascaris lumbricoides are specifically characterized by three lips which form a defined oral protuberance (1956 b). DOUVERS, et al. (1969) reported that Ascaris suum larvae have a head comprised of three cuticular lips, one dorsal and two subventral, in all phases of development. They added that the cuticular lips of the second stage appear as a transparent cap on the opaque cephalic tissues, and are not constricted from the body proper. SPRENT, et al. (1973) mentioned that the anterior end of B.tasmaniensis second stage larvae was rounded with a triradiate oral opening on small lips with four minute submedian papillae. In studies of B.procyonis L3 (in preparation) we have observed the formation of primary labia and labial papillae around the protuberant esophageal tip in the developing third stage larvae.

The presence of the cuticular caps adherent to the lip-like oesophageal protrusions of some newly hatched larvae may explain the interpretation of the cephalic structure of some ascarid second-stage larve by previous workers. This cuticular cap may be the remnant of the first moult sheath. SEM microtopography of B.procyonis second-stage larvae may prove useful in species differentiation within this genus and for defining the relationships of these groups of parasites of other ascarids.

The B.procyonis second-stage larvae described in this report reveal some useful morphologic features for distinguishing them from other species of ascarids. B.procyonis second-stage larvae have a characteristic prominent oral protuberance seen by L.M. The tip of the tail fo the B. procyonis L2 terminates in a distinct knob-shaped appendage. The lateral alae of the L2 are thin and membranous in appearance. We also have prepared an antiserum to B.procyonis adult extract which stains both B.procyonis larvae and larval antigens deposited in granulomata (unpublished observations). Thus combined microtopographic studies of recovered larvae, and immunohistologic studies of host tissues, may provide for definitive identification of tissue stages of B. procyonis larvae.

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FIGURE LEGENDS

Fig. (1): EM of B.procyonis eggs. High magnification showing granular surface with underlying fibrillar network (Bar, 10 um). Insert, low magnification of entire egg (Bar, 10 um).

Fig. (2): Newly hatched B.procyonis L2 emerging from crack at one pole of the egg (Bar, 10 um).

Fig. (3): B.procyonis fertilized egg, first cleavage (1-4 hours).

Fig. (4): Four-cell stage (T stage) of embryo (4-8 hours).

Fig. (5): Advanced cleavage of four-cell stage giving Rhomboidal early embryo.

Fig. (6): Morula stage (8-36 hours).

Fig. (7): Blastula stage (36-69 hours).

Fig. (8): Gastrula stage (5-6 days).

Fig. (9): Tadpole stage (7 days).

Fig. (10): Immature first stage larva (8-10 days).

Fig. (11): Infective fully embryonated egg containing second stage larva (11-14 days).

Fig. (12): B.procyonis, second-stage larva lateral view, camera lucida drawing - A, anus; B, button-shaped tail; EB, esophageal bulb; EC, excretory columns; EN, excretory nucleus; EP, excretory pore; ESMN, esophageal muscle nucleus, ET, excretory tubule; GN, ganglionic nucleus; GP, genital primordium; INT, intestine; NR, nerve ring.

Fig. (13): B.procyonis second stage larva.

Fig. (14): B.procyonis second stage larva showing the oral protuberance and the pyriform bulb-shaped end of the esophagus.

Fig. (15): B.procyonis second stage larva, anterior end showing the excretory pore and the oral protuberance.

Fig. (16): Tail of B.procyonis second stage larva, showing roundd button-shaped terminal knob and the anal opening.

Fig. (17): SEM of second stage larva showing cephalic cone and lateral alae (Bar, 10 um).

Fig. (18): SEM of lateral view of anterior tip of cephalic cone, showing triangular vestibule separating the dorsal and subventral lip-like protrusions (Bar, 1 um).

Fig. (19): Lateral view showing the position of the amphidial pores relative to the subventral lip-like structures (Bar, 10 um).

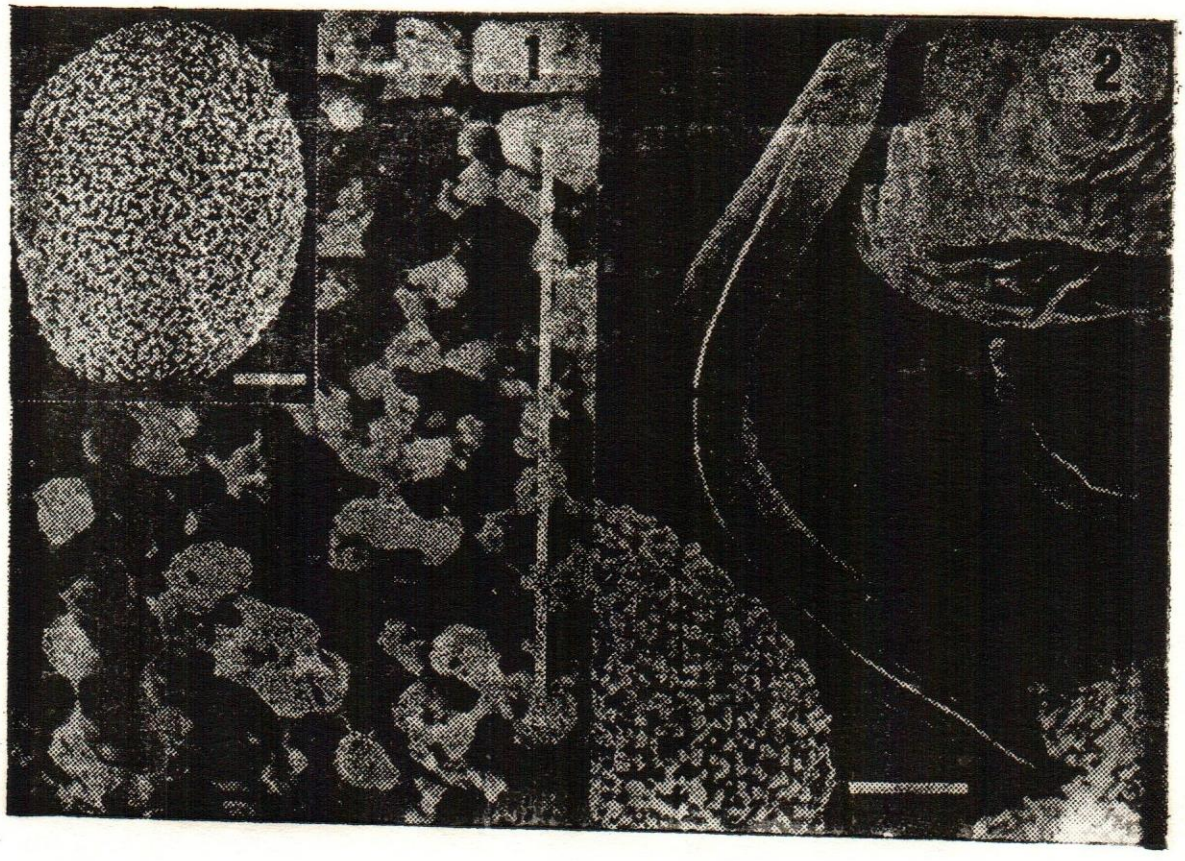
Fig. (20): Enface view showing deep separation between the dorsal and subventral lip-like structures and the shallow groove between the two subventrals. Two cuticular rods support each protrusion giving a blunt sole-shaped end. The lip-like structures completely surround the triradiate esophageal opening (Bar, 1 um).

Fig. (21): Enface view showing the beginning of the lateral alae at the base of the cephalic cone, the excretory pore and the amphidial pores (Bar, 10 um).

Fig. (22): Newly hatched second stage larva with cuticular cap adherent to the esophageal protrusion (Bar, 10 um).

Fig. (23): Posterior end, ventral surface showing anal opening with raised oval cuticular bleb (Bar, 10 um).

Fig. (24): Tail, lateral view showing blunt, button-shaped, longitudinally striated terminal knob (Bar, 1 um).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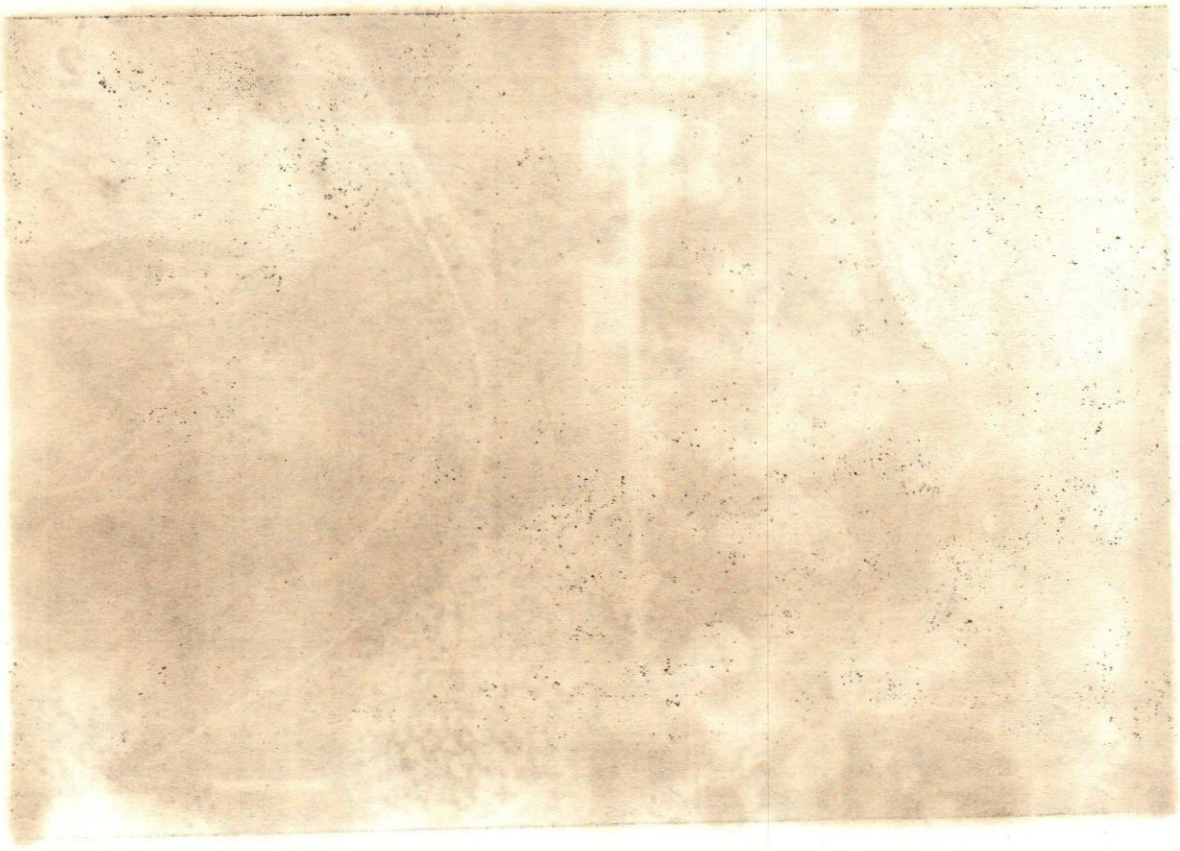

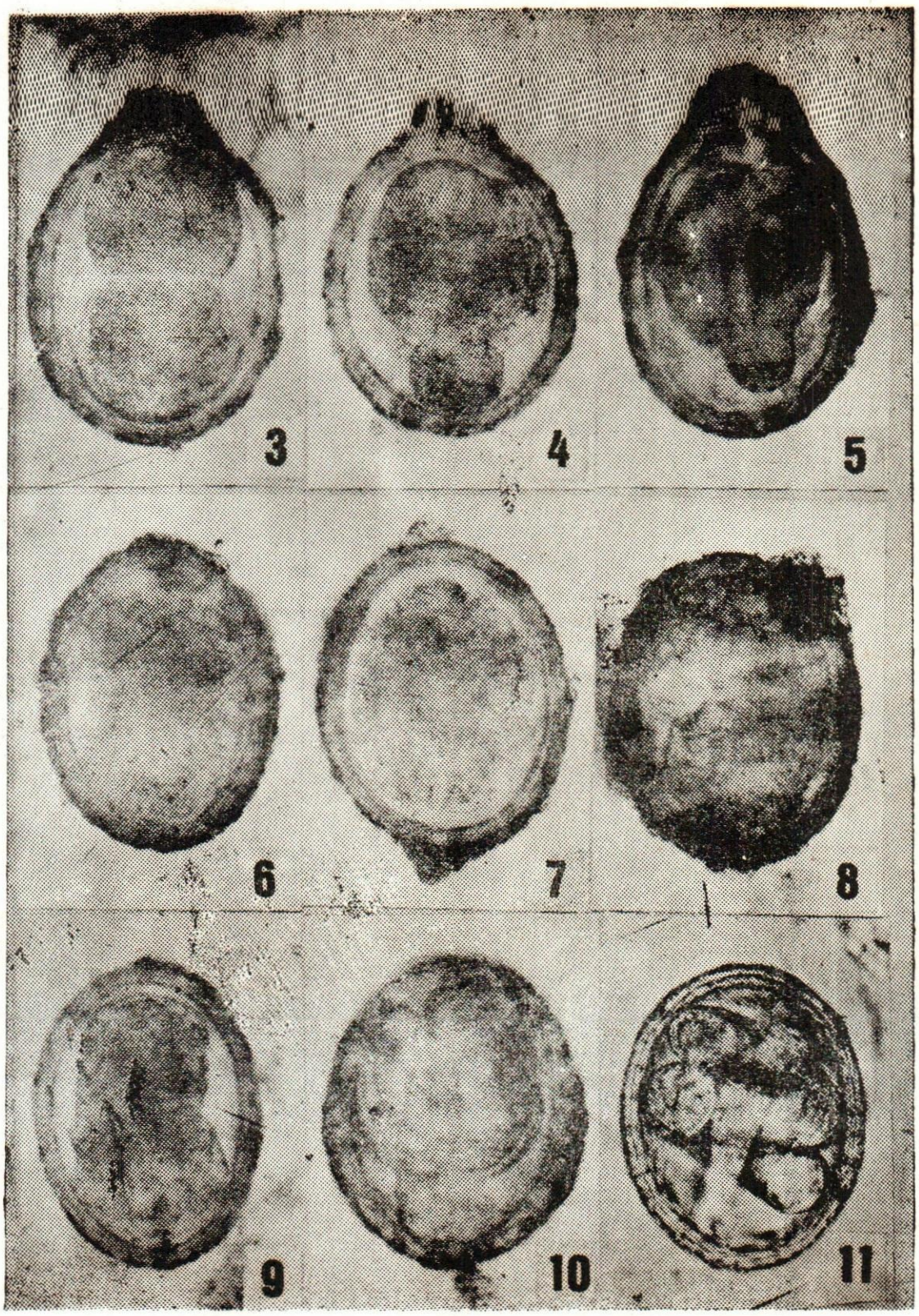
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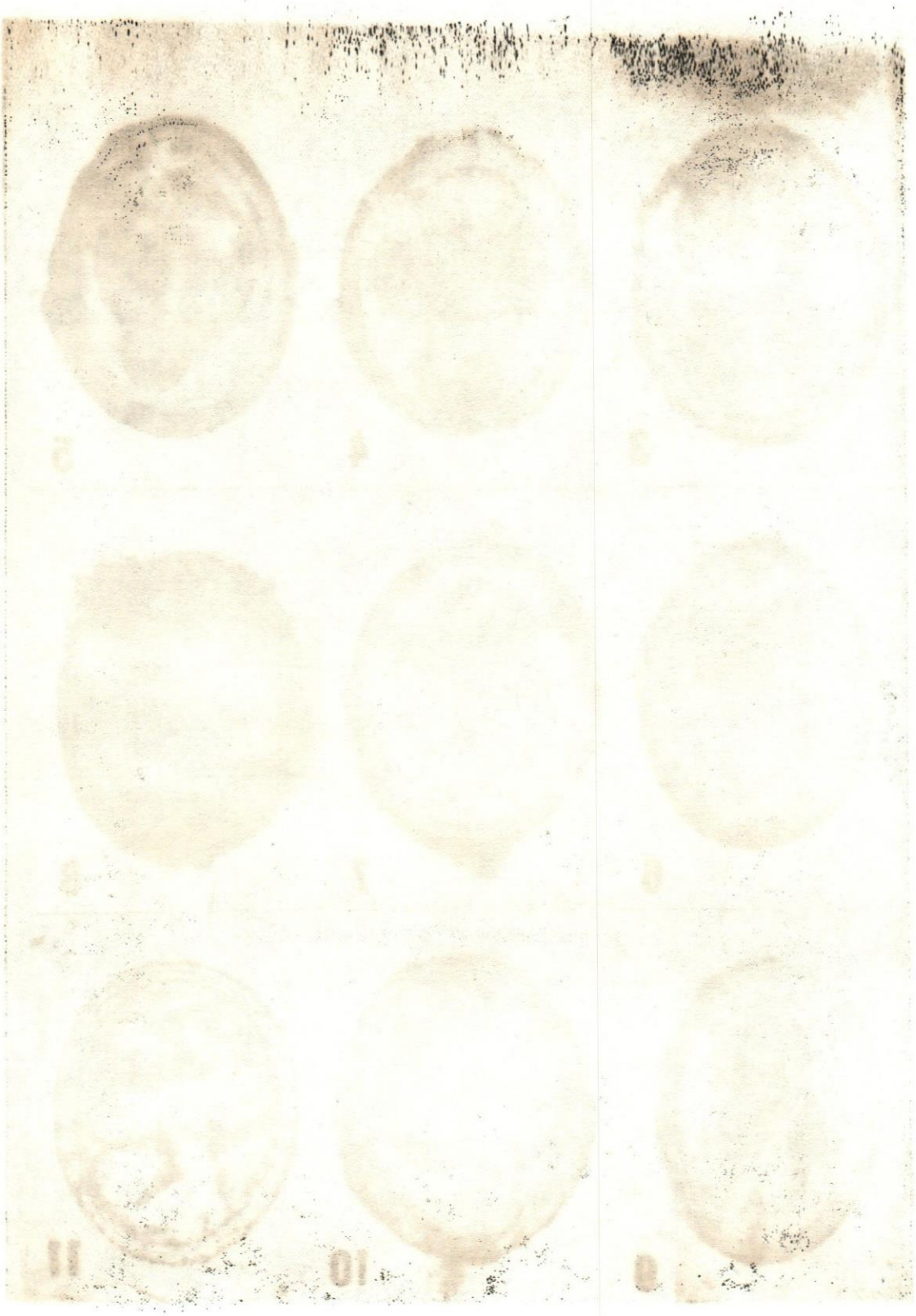

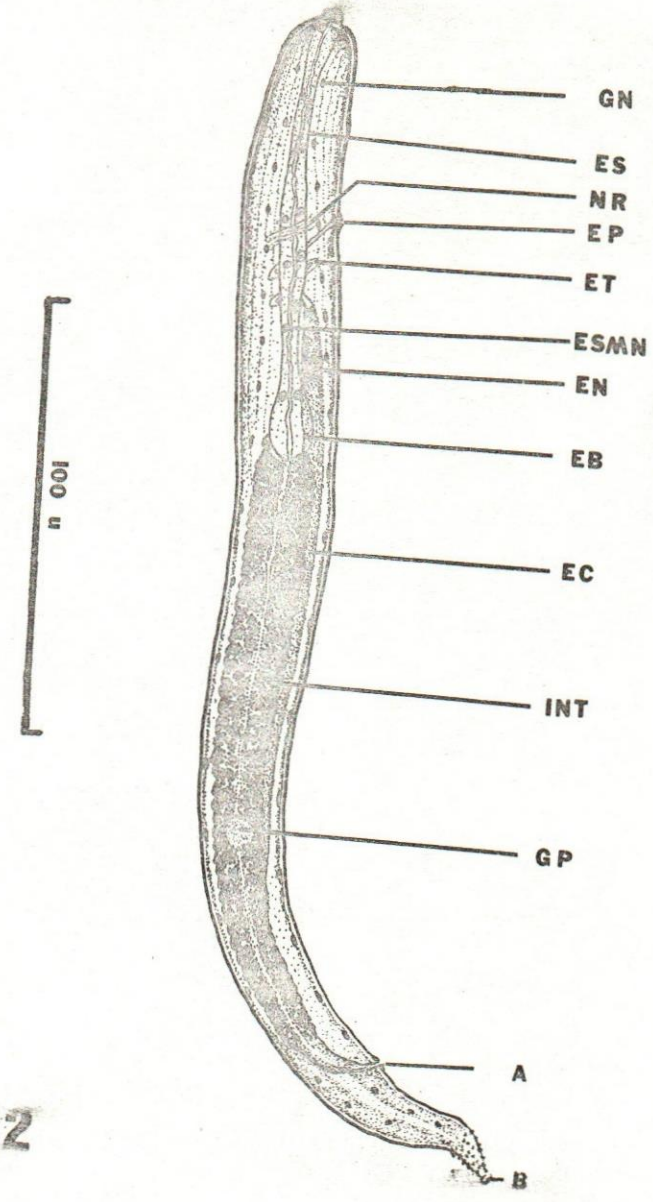

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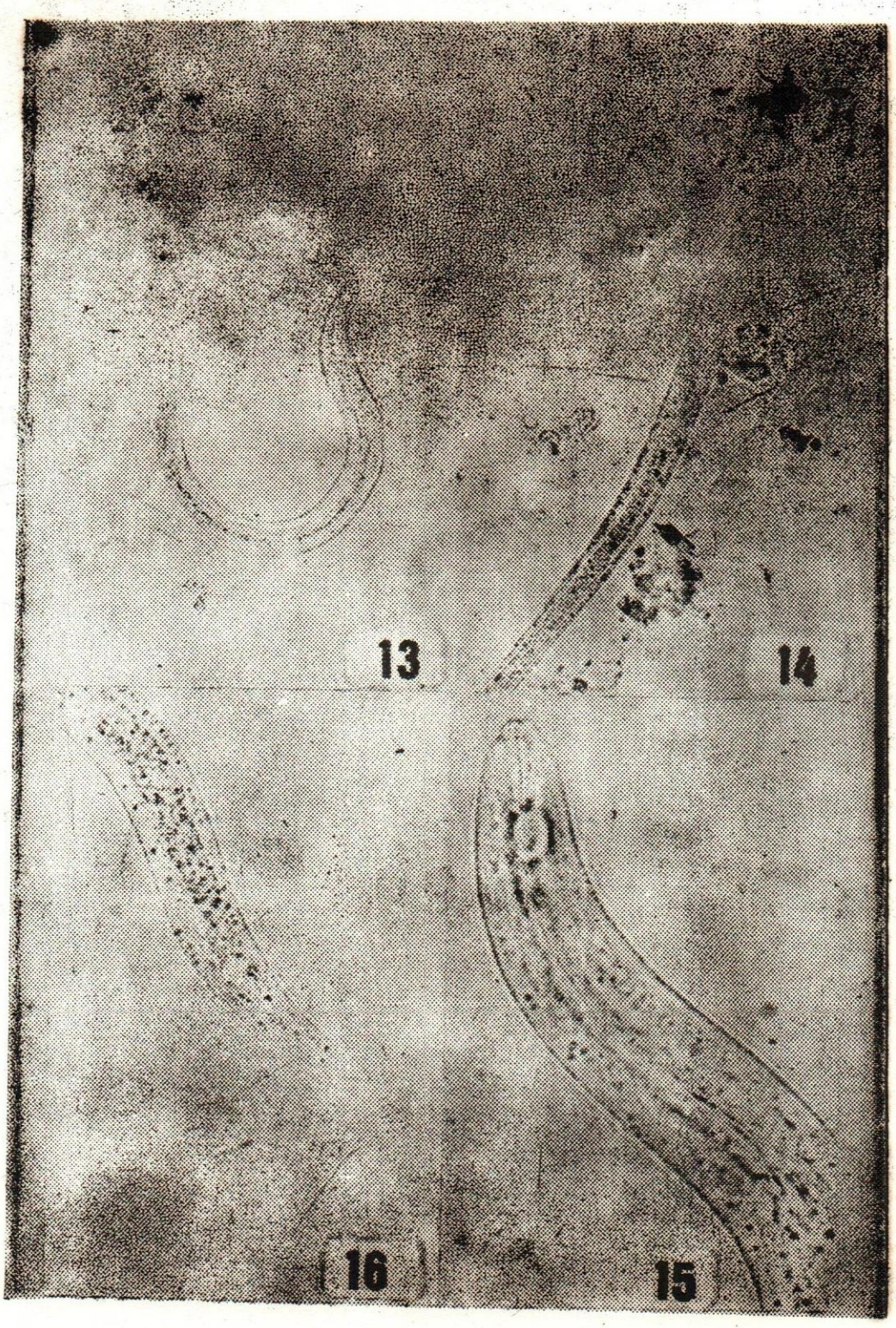
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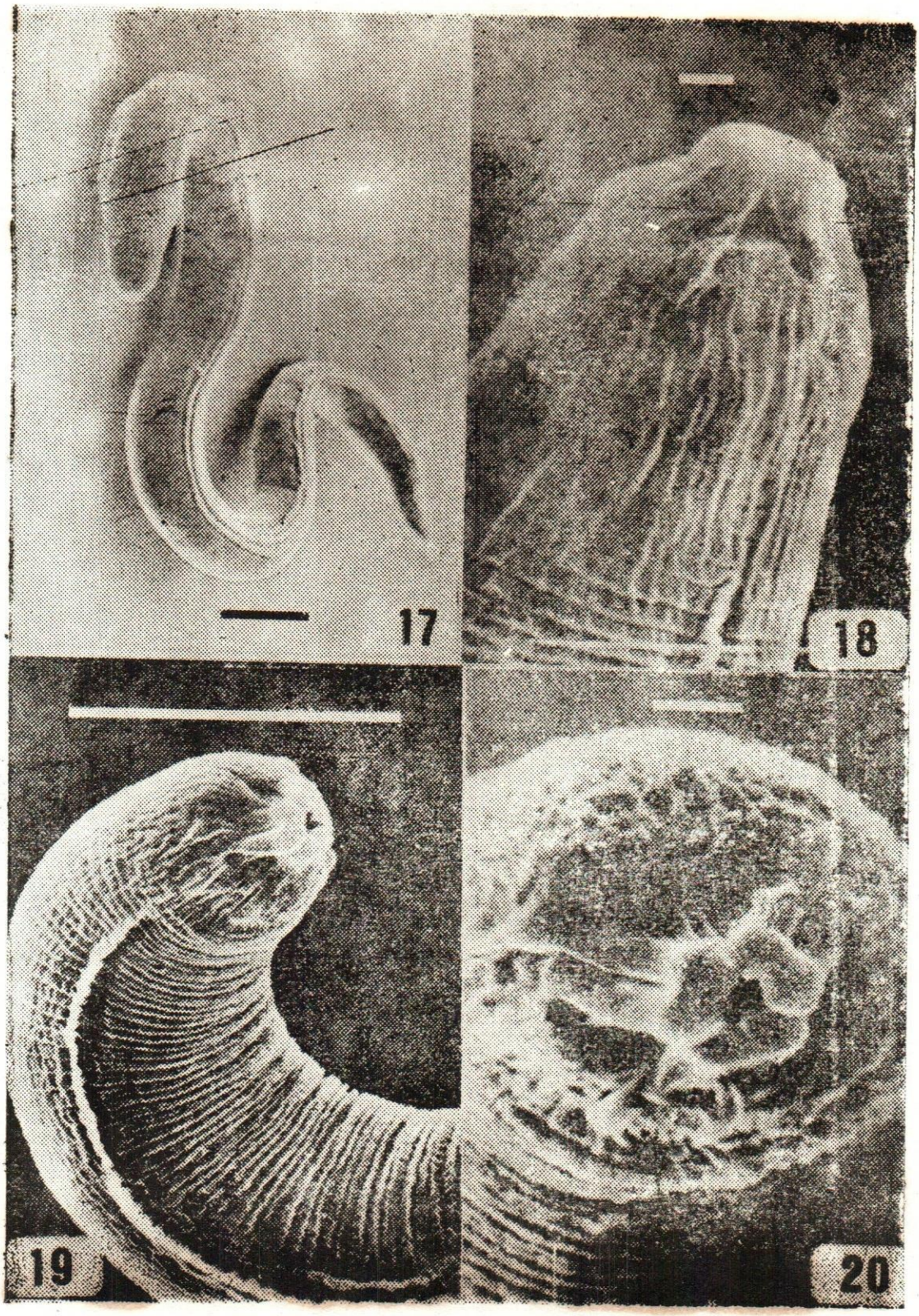
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