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Jea Kim Yi  
*Brigham Young University*

Richard A. Heckmann  
*Brigham Young University*

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MORPHOLOGICAL CHARACTERISTICS OF DENTOSTOMELLA TRANSLUCIDA, A NEMATODE (OXYUROIDEA) FOUND IN MONGOLIAN GERBILS

Jea Kim Yi¹ and Richard A. Heckmann¹

ABSTRACT—Dentostomella translucida Schulz & Krepkorgorskaja (1932) was found in the small intestine of the Mongolian gerbil, Meriones unguiculatus Milne-Edwards, housed at the small animal center at Brigham Young University, Provo, Utah. Pertinent taxonomical characteristics were studied to differentiate D. translucida from D. kuntzi (Myers 1961), D. grandmanni (Chitwood 1963), D. legerae (Quentin 1975), and D. karachiensis (Bilqees 1978). Dentostomella translucida is distinguished by a large, evenly proportioned body, the presence of five unequal teeth per esophageal sector, and a spicule tip bifid in ventral view in males. This project included the analysis of the structure and histochemistry of the adult nematode cuticle layers and egg-shell layers through the use of light and electron microscopy. Embryonation of D. translucida eggs was attempted to recover various larval stages. Additional information on D. translucida includes the presence of six cuticle layers, one exogenous with three endogenous egg-shell layers, and an egg operculum similar to that of D. kuntzi.

Dentostomella translucida Schulz & Krepkorgorskaja (1932) is a parasitic nematode found in the intestine of wild rodents including Meriones unguiculatus Milne-Edwards (Wightman et al. 1978, Pilitt and Wightman 1979), Meriones meridianus (Danzan 1978), Rhombomys opimus (Schulz and Krepkorgorskaja 1932, Shleikher and Samsonova 1954, Danzan 1978), Dipus sagitta (Danzan 1978), Mastomys fumatus (Chitwood 1963), and Mesocricetus auratus (Greve 1985). Even though the genus Dentostomella has not been shown to be detrimental to the hosts, it is highly infectious and continues to appear in parasite surveys for rodents. Dentostomella was established by Schulz and Krepkorgorskaja in 1932 while describing D. translucida in Rhombomys opimus Lichtenstein in Kazakhstan, USSR. The genus was later placed in the suborder Oxyurata due to the presence of male caudal alae, genital papillae surrounding the cloaca, spicule weakly chitinized, absence of gubernaculum, and the position of vulva in the anterior half of female worm. Skrjabin et al. (1960) established the new family Heteroxynematidae (superfamily Oxyuroidea), and Petter and Quentin (1976) established the subfamily Heteroxynematinae, which included Dentostomella as one of the five known genera. The characteristics of Heteroxynematinae include: the absence of a cuticular shield at the cephalic end, and a cuticular ornamentation before the male cloaca consisting of curry combs and plates with suckerlike membranes. There are four other known species in the genus, D. kuntzi, D. grandmanni, D. legerae, and D. karachiensis described by Myers (1961), Chitwood (1963), Quentin (1975), and Bilqees (1978), respectively. Compared to other pinworms in the same genus, D. translucida is distinguished by a large, evenly proportioned body, the presence of five teeth per esophageal sector, and a spicule tip bifid in ventral view for the male.

Mongolian gerbils, Meriones unguiculatus, housed at the small animal center at Brigham Young University, were hosts for D. translucida. The source or route of infection to the host is probably via infective eggs in the bedding and food. The probable life cycle of the worm would be similar to that of other pinworms, namely: eggs are released in large numbers from a mature, gravid female nematode at death and subsequently appear sporadically in the fecal mass of the host. The eggs embryonate within 1–4 days into an infective stage and, following ingestion, develop into larvae in the stomach of the host because of the favorable low pH and digestive enzymes.

The objective of this study is to incorporate different levels of microscopy (from light to electron optics) and histochemistry to provide

¹Department of Zoology, Brigham Young University, Provo, Utah 84602.
pertinent morphological and anatomical features whereby *D. translucida* can be further described and taxonomically delineated from other species of the same genus. These techniques provide the first information on the structure of the egg-shell layers and the cuticle layers of the adult *D. translucida*.

**Materials and Methods**

Live *Dentostomella translucida* were obtained from the small intestine of 50 necropsied *Meriones unguiculatus*. The nematodes were washed in tap water and were further prepared for various microscopic analyses as follows.

**Fecal Examination and Embryonation**

Fecal samples were collected and thoroughly mixed with Sheather's solution, strained through a wire sieve to remove coarse elements, transferred to test tubes, and allowed to stand undisturbed for 1 hr. The tubes were filled so that a clean slide placed over the mouth closed the tube without trapping air between the suspension and the slide. Each slide was then examined with bright field, as well as Nomarski interference, light microscopy to determine the presence of helminth eggs. Gravid female nematodes, teased from the small intestine, were put in petri dishes with warm water and cut into pieces to expel the eggs. The petri dishes were kept in the dark at 37°C for 4–7 days and observed daily; digestive enzymes were added to attempt in vitro hatching following embryonation.

**Histochemistry**

Specimens of *D. translucida* were placed in 10% buffered formalin and Bouin's fixatives. After dehydration with ethanol and embedding in paraffin, the specimens were sectioned at 4–6 μm with a rotary microtome. Sections were mounted on slides and stained with haematoxylin and eosin (H & E), Masson trichrome, pentachrome, orcein, Sudan IV, periodic acid schiff (PAS), and azure with toluidine-blue stains in order to identify the presence and the locations of various types of tissues.

**Scanning Electron Microscopy (SEM)**

Male and female specimens of *D. translucida* were fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.3). They were rinsed in the buffer and dehydrated through a graded series of ethanol, followed by changes in absolute acetone, and critical-point dried with liquid CO₂ as the transitional fluid. The specimens were mounted on aluminum stubs, coated with gold utilizing a Polaron sputter coater, and examined in an Amray 1000A at 20 KV.

**Transmission Electron Microscopy (TEM)**

Specimens of *D. translucida* were rapidly removed from the gerbils. Whole nematodes were cut into 3–4 parts to allow fixation of internal viscera. Sections were fixed for 2 hr in 2% glutaraldehyde–3% acrolein in 0.1 M sodium cacodylate buffer (pH 7.3), then rinsed with several changes in 0.1 M sodium cacodylate washing buffer. The specimens were post-fixed in 1% osmium tetroxide for 2 hr, washed several times in 0.1 M sodium cacodylate buffer, and left in 0.5% uranyl acetate overnight. Following dehydration with a graded series of ethanol, the specimens were embedded in 100% Spurr resin (Spurr 1969). The tissue was sectioned with glass knives on a Sorvall MT-2 microtome and placed on formvar-coated grids (200 mesh). Prior to viewing, sections were stained with lead citrate and 1% uranyl acetate. The grids were examined in a Philips high-resolution EM 400 transmission electron microscope.

**Results**

**Description**

The following features, determined with light and electron optics, characterize *D. translucida* specimens.

**Adult.**—The body is cylindrical and elongated with a transparent cuticle possessing transverse striations. There are ventral and dorsal annulations interrupted by two lateral longitudinal ridges extending the full length of the body (Fig. 1). The cuticle is divisible into the following six rudimentary layers (Figs. 2–5): (1) layer one is 30 nm thick and consists of a highly electron-dense, double membranelike structure; (2) layer two is 950 nm thick, separated by a fine granular band from layer one, and consists of an electron-lucent matrix with granular patches; (3) layer three is 1.3 μm thick and is composed of two
Figs. 1–5. *Dentostomella translucida*. 1. Scanning electron micrograph showing the cylindrical body with annulations and longitudinal ridge (bar = 100 μm). Inset showing the interruption of annulations by the ridge (bar = 5 μm). 2. Azure with toluidine-blue stained photomicrograph showing a rugose pattern of the cuticle layers (Cu), hypodermis layer (Hd), and muscle band (Mh) (bar = 50 μm). Inset showing six distinct cuticle layers (TEM) (bar = 4 μm). 3–5. Transition electron micrographs of six cuticle layers and hypodermocyte. 3. Layer 1, electron-dense double bands; layer 2, electron-lucent granular patches; layer 3, electron-dense and electron-median globules (bar = 670 nm). 4, 5. Layers 4–6, homogenous matrix with radial channels (RC), and the layers separated by electron-dense bands (arrows). Note hypodermocyte (Hd) bounded with microtubules (Mt) and mitochondria (Mc) (bars = 670 nm).
Figs. 6–11. Cephalic end of *D. translucida*. 6. Nomarski interference micrograph showing small cephalic inflation (CI), esophageal corpus (Co), bulb (Bu), and enlarged intestine (It) (bar = 180 μm). 7. Presence of four cephalic papillae (Pa) and two amphids (Am) on the outer circle (bar = 18 μm) (SEM). 8. Mouth opening showing three esophageal sectors, each sector with one median tooth (Md), two perimeter teeth (Pr), and two small teeth (Sm) (bar 2.8 μm) (SEM). 9. Toluidine-blue with azure-stained photomicrograph showing neurons that form a nerve ring (NR) (bar = 140 μm). 10, 11. Male *D. translucida* caudal end. 10. Male spicule tip bifid in ventral view (arrow) (bar = 10 μm). 11. Male caudal papillae on the bursa; one adanal pair (AD), one unpaired (UP), one lateral pair (La), and one postanal (Po) pair (bar = 50 μm).
types of globular patches—an electron-dense and an electron-median; and (4) layers four, five, and six are composed of a similar homogenous matrix and measure 2.1, 1.0, and 0.4 μm, respectively. The latter three layers are separated by electron-dense granular bands. Electron-dense radial channels running perpendicular to the surface extend through layers four, five, and six. With staining for LM, the cuticle layers had an affinity for orcein, aniline blue, and pentachrome (yellow coloration), but no affinity for PAS and acid fuchsin. Toluidine-blue showed a strong affinity with layers two and three, but weaker affinity with four and five. The clarity of pattern and thickness of the cuticle layers varied among specimens and probably depends on the maturity of *D. translucida*. Due to the constriction at the annulation of the cuticle, a rugose pattern is expressed for this cuticular region (Fig. 2). The measurements for the six layers are taken at the thickest portions of the annulae. Below the cuticle layers, hypodermal cells are bound with numerous infolding microtubules (Fig. 5). A slight lateral cervical inflation (Fig. 6) is present, while the pharyngeal cavity, cephalic vesicle, lips, and alae are absent. One pair of subventral papillae, one pair of subdorsal papillae, and two lateral amphids are present at the external circle of the cephalic end (Fig. 7). The buccal cavity is characterized by a row of teeth arranged symmetrically on the margin of the triradiating esophagus sectors, one dorsal and two subventral. One sector of the esophagus includes a conical-shaped median tooth, which is the largest, and projects outward. Two perimeter teeth are situated on either side of the median tooth. The two smallest teeth connect to the perimeter teeth at the base located at the outer edge of the buccal cavity (Fig. 8). The esophagus is short (1/45 of body) and thick, with a constriction at the posterior end at the position of the esophago-sympathetic nerve ring that divides the spherical bulbous part from the cylindrical part (Fig. 9). The triradiating lumen of the esophagus is continuous with a fibrous esophago-intestinal valve. The esophagus is covered externally by a semicircular membrane but without internal chitinized armament.

**MALES.**—The average length of the male nematode is 10.25 mm; the width at the level of the esophagus bulb, midbody, and anus measures up to 0.24, 0.47, and 0.23 mm, respectively. The esophagus is 0.29 mm long, 0.07 mm wide at the level of the esophagus bulb, and the cylindrical esophageal corpus is 0.21 mm long. The nerve ring, which measures 0.07 mm wide, encircles the base of the corpus (Fig. 9). There is an excretory pore 2.29 mm from the cephalic end. A cuticular swelling (bursa) at the ventral surface of the tail forms caudal cords on both sides, which range from 0.5 mm to 0.95 mm (situated 0.11 mm from the tail tip). The bursa is without supporting rays but has transverse annulation with cuticular platelets situated on the anterior surface of the cloaca. A single spineulc, weakly chitinized, is 0.35 mm long, cylindrical, with a blunt distal end rounded in lateral view and bifid in ventral view (Fig. 10). The ventral caudal papillae on the fleshy bursa are present (Fig. 11). There is a pair of large adanal papillae 0.32 mm from the tail tip, an unpaired papilla between the adanal pair, and a pair of small lateral papillae slightly posterior to the protuberance. One pair of asymmetrically arranged postanal papillae is found 0.174 mm from the tail tip.

**FEMALES.**—The average length of the female nematode is 29 mm, and the width at the level of the esophagus bulb, midbody, and anus is 0.52 mm, 1.15 mm, and 0.35 mm, respectively. The esophagus is 0.4 mm long and 0.2 mm wide at the bulb, with a cylindrical corpus part 0.25 mm long terminating at the encircling nerve ring. An excretory pore situated behind the esophagus is 3.9 mm from the cephalic end. Two bulbous seminal receptacles are present, one above and one below the vulva. The vulva is characterized by a transverse slit 12 mm from the cephalic end. The vagina is 1.1 mm long by 0.23 mm thick, directed cephalad and connected to a muscular, walled vagina vera. The vagina vera is directed caudad after a reflex to an unpaired uterine tube and widens into a common egg chamber. The egg chamber, 1.4 mm long, divides into two uteri situated posterior to the vulva and terminates at 0.64–1.4 mm from the tail (Fig. 15) tip.

**OVA.**—*Dentostomella translucida* eggs are elongated, asymmetrical, flattened on one side, and measure 130 μm long by 44 μm wide (Fig. 12). The thickness of the shell is uneven, and the egg-shell consists of: (1) an exogenous uterine layer, (2) a vitelline layer, (3) a
Figs. 12–17. Dentostomella translucida eggs. 12. Azure with toluidine-blue-stained photomicrograph. Note the position of endogenous egg-shell layers (small arrows) on the flattened side of the egg. Large arrows show dense granules secreted by uterine cells (bar = 30 μm). 13. Four egg-shell layers: exogenous (Ex), vitelline (Vt), chitinous (Ch), and lipid (Lp) layers (bar = 530 μm). 14, 15. Exogenous egg-shell layers showing electron-dense plugs and columnous materials. Note the fringe materials on the surface (arrows) (bar = 300 nm). 16. Four egg-shell layers (abbreviations same as 13) showing the endogenous layers being separated from the exogenous layer (bar = 640 nm). 17. Flattened side of the egg showing the embryo (Em) adjacent to the endogenous layers (Ed) (bar = 4 μm).

chitinous layer, and (4) a lipid layer (Figs. 13–17). This nomenclature for the egg is based on Wharton's description of oxyurid eggs (Wharton 1979a, 1979b, 1979c).

exogenous uterine layer is 560 nm thick, continuous, and highly variable in morphology. This layer consists of electron-dense plugs or columnous materials embedded between coarse fibrils that are perpendicular to the outer surface. Histochemically, the exogenous uterine layer shows an intense red coloration with pentachrome, Masson trichrome, and Sudan IV. The exogenous uterine layer has an affinity for azure with toluidine-blue, but has no affinity for orcein and PAS. At the outer surface of the egg-shell, there are irregular fringes of electron-dense materials that are tightly adhered (Figs. 14, 15). The vitelline layer is membranelike, 30 nm thick, and situated adjacent to the curved side of the egg, but this layer is separated from the exogenous layer on the flattened side of
TABLE 1. Comparative measurements (in mm) and structures of five Dentostomella spp.

<table>
<thead>
<tr>
<th>Species:</th>
<th>D. grandmanni</th>
<th>D. legerae</th>
<th>D. karachiensis</th>
<th>D. kuntzi</th>
<th>D. translucida</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length</td>
<td>4.8</td>
<td>7.5</td>
<td>9.1</td>
<td>13.1</td>
<td>13.2</td>
</tr>
<tr>
<td>width</td>
<td>15.6</td>
<td>21.0</td>
<td>19.9</td>
<td>21.8</td>
<td>29.0</td>
</tr>
<tr>
<td><strong>Esophagus:</strong></td>
<td>0.26</td>
<td>0.27</td>
<td>1.17</td>
<td>0.29</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Excretory pore:</strong></td>
<td>3.9*</td>
<td>2.32</td>
<td>6.40</td>
<td>2.64</td>
<td>2.88</td>
</tr>
<tr>
<td><strong>Spicule:</strong></td>
<td>0.26</td>
<td>0.24</td>
<td>0.2</td>
<td>0.16</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>distal end:</strong></td>
<td>bidentate</td>
<td>rounded</td>
<td>brushlike</td>
<td>rounded</td>
<td>bifid</td>
</tr>
<tr>
<td><strong>Valva from anterior end:</strong></td>
<td>8.3</td>
<td>10.0</td>
<td>7.43</td>
<td>13.0</td>
<td>12.0</td>
</tr>
<tr>
<td><strong>egg:</strong></td>
<td>0.13</td>
<td>0.15</td>
<td>0.13</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>width:</strong></td>
<td>0.04</td>
<td>0.04</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Host:</strong></td>
<td>Entamias quadrivittatus</td>
<td>Gerbillus campstres</td>
<td>Acrinus calirinus</td>
<td>Acrinus mugiculus</td>
<td></td>
</tr>
<tr>
<td>Cephalic inflation</td>
<td>small</td>
<td>narrow, long</td>
<td>large, long</td>
<td>small</td>
<td></td>
</tr>
<tr>
<td>Cephalic papillae</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>No. of teeth</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Caudal papillae</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>adanal</td>
<td>1 pair</td>
<td>0</td>
<td>1 pair</td>
<td>1 pair</td>
<td></td>
</tr>
<tr>
<td>postanal</td>
<td>3 pairs</td>
<td>3 pairs</td>
<td>2/3 pairs</td>
<td>2/3 pairs</td>
<td></td>
</tr>
</tbody>
</table>

*Sex not available.

the egg (Figs. 16, 17). The chitinous layer is 650 nm thick, electron-lucent, and demarcated on its inner face by the innermost lipid layer, which is 16 nm thick. Endogenous layers, which include the vitelline, chitin, and lipid layers, were not found in the histological sections presumably because of their fragile nature. However, research has confirmed that the chitinous layer contains chitin secreted by the embryo (Bird and McClure 1976, Pearse 1968). The lipid layer is characterized by positive reactions with lipid and phospholipid stains (Wharton 1979a). For the scanning electron micrographs, the surface of the shell was covered with numerous bumps, but no pores or ridges were present (Fig. 18). Although we were unable to detect a marked opercular spot using LM and TEM, SEM showed sutures on an operculum near one pole of the curved side of the egg (Figs. 18, 19). This observation is consistent with another report for operculated D. kuntzi ova (Ashour and Lewis 1982).

Embryonation

Most D. translucida eggs recovered from the host’s feces contain a single-celled embryo (Fig. 20). The eggs from the feces or directly from the uterus of a fertilized female worm can develop into a coiled (a ring and a half) larva within a few hours (Fig. 21). The next stage is a vermiform larva, which appears within 48 hours when incubated in water at 37 °C (Fig. 22). The vermiform larva is stout, 130 μm long, and possesses a well-formed anterior portion of the digestive system, but the sex cannot be identified at this stage of development. Chitwood and Chitwood (1950) indicated that oxynurid larvae may have an inactive stage between the first and second vermiform stages, but D. translucida larvae did not exemplify these stages. However, the active larvae seemed to enter an inactive phase and seldom hatched out of the egg-shell. After 6 days of incubation, some larvae emerged from the egg via the operculum, and one larva without the egg-shell was recovered on another occasion (Fig. 23). Further attempts to incubate and hatch the larvae beyond 6 days were not successful.

**DISCUSSION**

Comparisons of Dentostomella Species

Dentostomella translucida is distinguished from the other four Dentostomella species primarily by a large body size with a minute cephalic inflation, the presence of five teeth per esophageal sector, and, in the male, the presence of seven caudal papillae and a bifid spicule tip. Table 1 shows the body measurements and other pertinent characteristics of
D. translucida, D. grandmanni (Chitwood 1963), D. kuntzi (Ashour and Lewis 1982), D. legerae (Quentin 1975), and D. karachiensis (Bilqees 1978). Measurements for five Dentostomella species given in Table 1 were obtained from both full-grown and juvenile nematodes. The juvenile nematodes tend to have different body proportions than adult nematodes. Thus, some of the measurements, such as the length of the esophagus, the position of the excretory pore and vulva from the anterior end, and the size of the spicule in the male, are unreliable sources for differentiating species based on current data. Pertinent characteristics listed in Table 1 will be emphasized in this discussion.

Scanning electron micrographs of D. kuntzi published by Ashour and Lewis (1982) show structures similar to D. translucida, such as four submedian papillae and two amphids on the external circle (Fig. 7), and the number and arrangement of caudal papillae for males; they also show differences in the number of teeth when the two species are compared. Dentostomella kuntzi has a more conspicuous cephalic inner circle than D. translucida, which may be why Myers' (1961) LM studies indicated that D. kuntzi has six papillae in the inner circle. Chitwood (1963) indicated that D. grandmanni has four cephalic papillae on the outer circle and six more papillae on the inner circle that are not found in other Dentostomella species. With additional SEM work, the presence of papillae on the inner circle can be confirmed. Dentostomella legerae does not possess structures that are strikingly diverse from or similar to D. translucida. However, D. legerae shows greater similarities with D. Kuntzi in body measurements and conformation for female nematodes. Chitwood (1963) previously distinguished D. grandmanni, D. translucida, and D. kuntzi by the body conformation of adult females as follows: Dentostomella grandmanni is very stout, especially in the post-vulvar region, D. translucida is long and evenly proportioned, and D. kuntzi is very slender. Dentostomella karachiensis appears most different from the other species in morphology: the male caudal papillae do not exist, and eight teeth are present on three esophageal sectors that result in an uneven distribution of teeth. Also, the presence of modified cuticle squares near the cloaca and a brushlike spicule tip are distinct characteristics of D. karachiensis. Characters of the female D. karachiensis cannot be reviewed since it has not been recovered from host animals.

The cuticle of D. translucida has morphological characters that may be used to differentiate it from other oxyurids. The TEM study indicated that cuticle layers are highly complex, with many layers of different chemical composition that aid in worm survival when subjected to digestive enzymes from the host. The histochemical study shows the presence of reticular, elastin, and collagenous-type proteins that maintain the texture and structure of the nematode.

Dentostomella translucida Ova

The egg-shell of D. translucida exemplifies the basic pattern for most oxyurids as described by Wharton (1979a, 1979b, 1979c) for Aspiculuris tetraptera, Sypuctia obvelata, and Hammerschmidtia diecingi. However, the exogenous uterine layer is considerably different in morphology for D. translucida as compared to the other three oxyurids listed above. The structures of the vitelline, chitin, and lipid layers are similar. Wharton (1979a, 1979c) indicated that exogenous layers are formed by secretions from the uterine cells; thus, such differences between the oxyurids he studied and D. translucida may reflect the structural and physiological differences of the female reproductive tract, wherein the variability of egg-shell structure for different species can be a pertinent taxonomic feature. The exogenous layer, being nonporous, indicates that the embryo and the endogenous layers are not under direct influence from the uterine cells. The histochemical reactions of the exogenous layers show the presence of lipids and fibrinoid and reticular proteins. The dense granules (Fig. 12) secreted by the uterine cells that surround the egg have a similar histochemical reaction to that of the exogenous layers, thus indicating a common origin for these layers. Figures 14 and 15 show the egg surface being laced by electron-dense fringe materials that seem to provide stickiness to the oxyurid ovum. The D. translucida egg has an adhesive nature, as do most other oxyurids, which aids in spreading infections to other hosts. The endogenous layers, the vitelline, chitin, and lipid layers, run parallel
to the exogenous layer on the curved side of the egg, but peel at a 90-degree angle on the flattened side of the egg (Fig. 16). It appears that the exogenous layer is not directly associated with nor bonded to any of the three endogenous layers. Although Wharton (1979a), in TEM studies, found that the composition of the operculum is different from the remainder of the egg-shell, only SEM showed the existence of an operculum for *D. translucida* eggs. The presence of an operculum is in contrast to previous reports that the eggs of Heteronematidae do not have an operculum (Petter and Quentin 1976).

A limited number of larval stages were recovered from our attempts to cause embryonation. As indicated by the experimental passages of Wightman et al. (1978), the eggs released with the fecal mass of the host are infectious within 1–4 days. Thus, according to the embryonation studies, eggs with a coiled larva and a vermiform larva are the infective stages. It should be noticed that the size of the larva hatched from the egg is approximately the same as the larva inside the egg. Also, the presence of a rather stout and large esophagus for the larva indicates the abilities to receive a better type of nourishment and to attach to the mucous membranes of gastrointestinal tissues.

**Acknowledgments**

This work was supported by research grants from the Department of Zoology and from the Association of Students at Brigham Young University. The technical assistance from the staff members at the Brigham Young University Center for Electron Optics was greatly appreciated.

**Literature Cited**


