

FIRST SCANNING ELECTRON MICROSCOPE OBSERVATION ON ADULT  
*OESOPHAGOSTOMUM VENULOSUM* (RUDOLPHI, 1809)  
(NEMATODA: *STRONGYLIDA*, *CHABERTIIDAE*)

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**Abstract.** *Oesophagostomum* sp. belongs to the family Chabertiidae (Nematoda: Strongylida). Members of this genus have a cylindrical buccal capsule, usually narrow. The mouth of male parasites located in the anterior end is surrounded by internal and external leaf crown. The buccal capsule is shallow. There are no lateral cervical alae. The cervical papillae are present and situated behind the level of the esophagus. The external and the internal leaf-crown consist of 18 and of 36 elements, respectively. The male parasite has a large bursa and spicules long, tubular, slender and with an accessory piece present. A curved sword-shaped structure of these spicules is observed under higher magnifications. The tail of the female parasite is finely pointed. In the female parasite, the anus was seen as a fissure in shape. The vulva of the females is next to the tail and sexual cement is observed. The anus is situated on the point of the posterior end. Analysis by SEM, revealed no difference between anterior ends of male and female parasites.

**Keywords:** *Oesophagostomum venulosum*, Scanning Electron Microscopy (SEM), parasite, sheep, intestine.

*OESOPHAGOSTOMUM VENULOSUM* (RUDOLPHI, 1809) (NEMATODAI *STRONGYLIDA*,  
*CHABERTIIDAE*) ANALIZĖ NAUDOJANT SKENUOJAMĄJĮ ELEKTRONINĮ  
MIKROSKOPĄ

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**Santrauka.** *Oesophagostomum* sp. priklauso *Chabertiidae* (nematodai *Strongylida*) šeimai. Šios genties atstovams būdinga siaura cilindro formos bukalinė kapsulė. Parazitų patinų burna yra priekiniame kūno gale ir turi vidinį bei išorinį praplatėjimą. Bukalinė kapsulė negili, nėra išilginių kaklo sparnų. Yra kaklo ataugos, išsidėsčiusios už stemplės. Vidinis ir išorinis praplatėjimai susideda atitinkamai iš 18 ir 36 elementų. Patinų bursa gerai išsivysčiusi (didelė). Taip pat yra dvi vienodos vamzdelio formos ilgos ir plonos spikulos su ataugėlėmis. Labiau padidinus matyti, kad spikulos yra kardo formos. Patelių uodega šiek tiek smailėja, plyšio formos analinė anga pačiame kūno gale. Patelių vulva yra šalia uodegos; matyti lytinės išskyros. Tiriant skenuojamuoju elektroniniu mikroskopu nepastebėta patelių nei patinų galvos ir priekinės kūno dalies skirtumų.

**Raktažodžiai:** *Oesophagostomum venulosum*, skenuojamasis elektroninis mikroskopas (SEM), parazitai, avys, žarnynas.

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**Introduction.** Parasitic gastroenteritis is one of the major causes of productivity loss in sheep and goats. *Oesophagostomum* sp. belongs to the family Strongylidae (Nematoda: Strongylida). *O. venulosum* is mainly a parasite of sheep and goats but has been found in deer, bighorn sheep, chamois and other ruminants throughout the world; occasionally humans are involved. In the past decade, it became clear that, in some parts of Africa, humans are satisfactory final hosts. In those areas, prevalence of infection is high and morbidity is significant. (Polderman et al., 1995, Soulsby, 1969). Life

cycle is similar to that of *O. columbianum* (Goldberg, 1952, Soulsby, 1969). Eggs passed in faeces of the host were usually in the 16 to 32 cell stage. In culture (charcoal-faeces), the first-stage larva hatches from the one end of the egg in about 24 h. The first moult occurs in about 24 h after hatching. At room temperature, the third stage larvae develop 3 to 5 days after hatching. Goldberg (1951) gave infective larvae to ten lambs and a goat and examined them at various developmental stages thereafter (Goldberg, 1952). Three days post infection, third stage larvae were found coiled and encapsulated in the mucosa

of the small intestine, the area forming the provisional buccal capsule was clear. Larvae were quiescent. On the 4th day after infection, most larvae were found in the lumen of the intestine near the mucosa. Nearly all larvae (96%) had the well developed provisional buccal capsule of the fourth stage and some were exsheathing and the data showed that during the development from the third to the Super family Strongyloidea the fourth stage occurred while larvae were encapsulated in the intestinal wall (Anderson, 2000). In the fourth stage, worms emerged into the lumen of the gut and shed the third-stage cuticle. By the 5th day after the infection, most larvae (95%) had migrated to the caecum and the first part of the colon. Approximately 98% of the larvae were in the fourth stage of development (Anderson, 2000). The fourth and final moult usually occurred 13–16 days post infection, when worms were little more than a third the length of mature adults. Most worms were mature by 31 days post infection and the average pre patent period was 28 days (Anderson, 1926, Anderson, 2000). The diagnosis is based on the examination of adult nematodes. Important characters include the structure of the leaf crowns, relative degree of development of the cephalic vesicle, placement of the cervical papillae, and specific attributes of the copulatory bursa and spicules (Goldberg, 1952, Levine, 1980). Oesophagostomiosis in domestic hosts, however, is associated with inflammation and the development of characteristic nodules in the intestinal wall (Levine, 1980). Though, pathogenesis of *O. venulosum* is very similar to *O. columbianum* and occurs in the same site in host, the pathogenic effects are quite different. *O. venulosum* is generally considered to not cause a problem for sheep. Some experimental infections have resulted in scours and ill thrift (Goldberg, 1952, Levine, 1980). *O. venulosum* is relatively harmless and does not form nodules or any specific lesions in the intestines, but worms are clearly visible in the caecum of infected sheep. Even in heavy experimental infections the clinical effect are of a low order (Anderson, 2000, Goldberg, 1952). Identification of the species of the genus *Oesophagostomum* is based on the examination of adult nematodes (Levine, 1980). Important characters include the structure of the leaf crowns, relative degree of development of the cephalic vesicle, placement of the cervical papillae, and specific attributes of the copulatory bursa and spicules (Levine, 1980). The characteristics of this genus is based on light microscopy, the body of this worm is slender, and white or grey. The oral opening of *O. venulosum* is small and narrow with leaf-crowns. There has been no research on the electron microscopic examination of *O. venulosum*. The purpose of this study was to observe various structures of this parasite by scanning electron microscope (SEM).

**Material and methods.** Specimens of *O. venulosum* were collected from the intestines of sheep. For SEM study, the parasites were preserved in a 4% phosphate buffered formalin solution. The worms were rinsed in distilled water, and then in sodium phosphate buffer. Samples were fixed in 3% glutaraldehyde buffered with sodium phosphate (PH 7.2) for 2 h at +4°C, rinsed in

sodium phosphate buffer 3 times and fixed again in 1% Osmiumtetroxide in sodium phosphate buffer for 2 h. After fixation, the samples were washed in sodium phosphate buffer overnight, then dehydrated in ascending ethanol solutions gradually (50%, 60%, 70%, 80%, 90%, 95% and 99%), and air-dried. Specimens were mounted on to stubs by conductive double-sided adhesive tape, sputter-coated with a thin layer of gold by Polaron SC-500 and viewed by SEM (JEOL JEM-2000FX). (Bozzola and Russel, 1992, Hayat, 1981, Naem, 2004, Yıldız et al., 2003, Yıldız and Çavuşoğlu, 2004).

**Results.** Members of this genus have a cylindrical or large and sub globular buccal capsule, usually narrow. The mouth of male parasites located in the anterior end was surrounded by internal and external leaf crowns (Fig. 1). The internal leaf crown either present or absent. The external leaf crown consists of 18 and the internal leaf crown of 36 elements (Fig. 4). The buccal capsule is shallow. A transverse ventral cervical groove presents (Fig. 5) the anterior end usually with a cuticular inflation or vesicle limited behind, about the level of the excretory pore, by a transverse ventral groove (Fig. 3). There is a ventral cervical groove near the anterior to which the cuticle is dilated to form a cephalic vesicle. This groove extends for a varying distance on the lateral surfaces of the worm. The cuticle of the anterior end may be dilated to form a cephalic vesicle, limited ventrally by the cervical groove (Fig. 3). There are no lateral cervical alae. The cervical papillae are situated behind the level of the esophagus (Fig. 7). The male bursa is well developed and there are two equal, alate spicules, 1.1–1.5 mm. Medio lateral and posterior lateral rays of bursa closely applied to each other and are somewhat divergent from the anterior lateral ray. The external dorsal arising high up on the main stem of the dorsal ray, bifurcated in its posterior two-fifths (Fig. 12). A much reduced accessory branch arises from each of the main branches. The second pair of accessory branches is occasionally represented by a minute, slender branch on each side posterior to this. The tail of the female tapers to fine point (Fig. 9). The vulva is situated about 0.6 mm anterior to the anus. A large bursa was seen in the male parasites. In the female parasite, the anus was seen as a fissure in shape (Fig. 9). The male parasite has a large bursa and spicules long, tubular, slender an accessory piece present (Fig. 12). Any difference between anterior ends of male and female parasites was not observed in the present study. The anus is situated on the point of the posterior end. The vulva of the females is next to the tail and coupling cement was observed (Fig. 9). The vagina is very short, transverse and kidney shaped and coupling cement was seen. In the female parasite, the anus was seen as a fissure in shape. Fine tail is present on the female worm. Curved sword structures on these spicules were observed under higher magnifications (Fig. 10). In the SEM, the cephalic structures of female worms did not differ from those of males and any difference between anterior ends of male and female parasites was not observed in the present study.

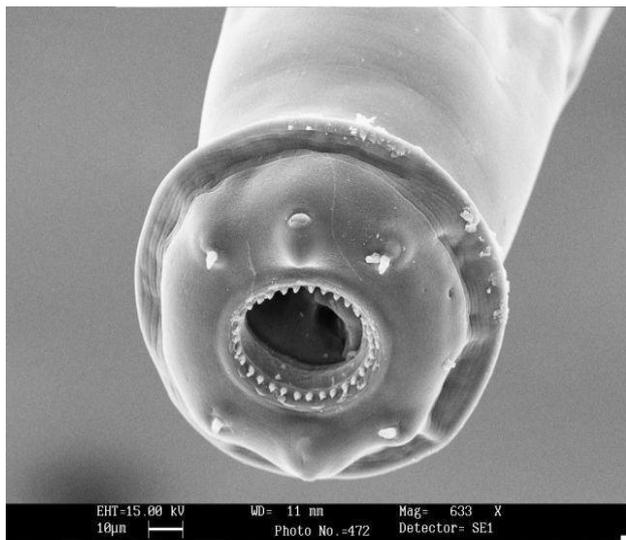


Fig. 1. Anterior end with internal and external leaf crowns

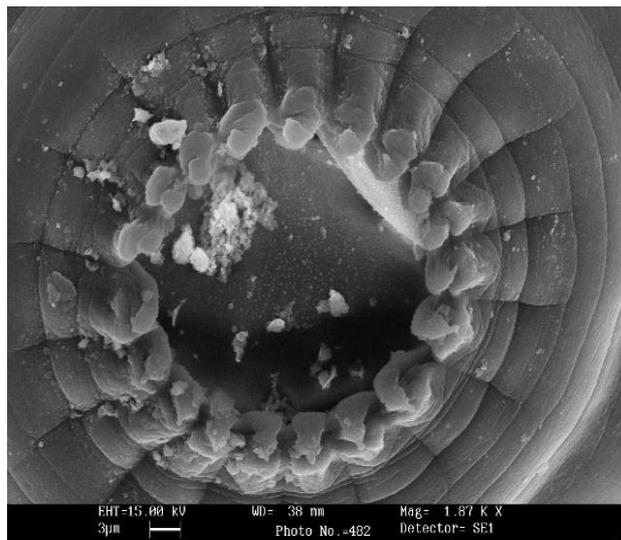


Fig. 4. External leaf-crown under higher magnifications

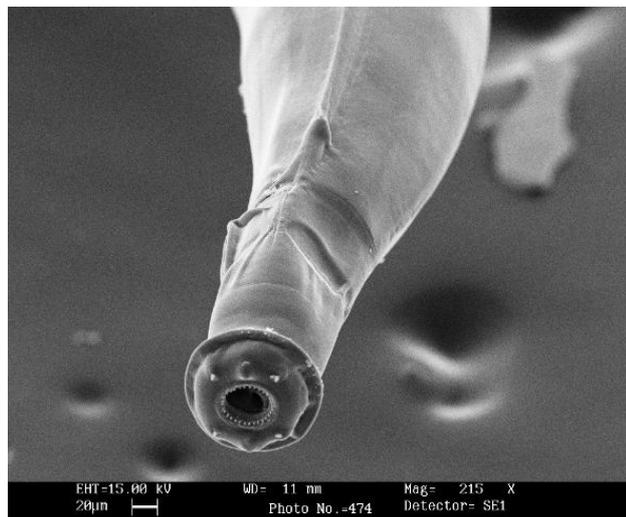


Fig. 2. Body of the parasite with cuticular ridges

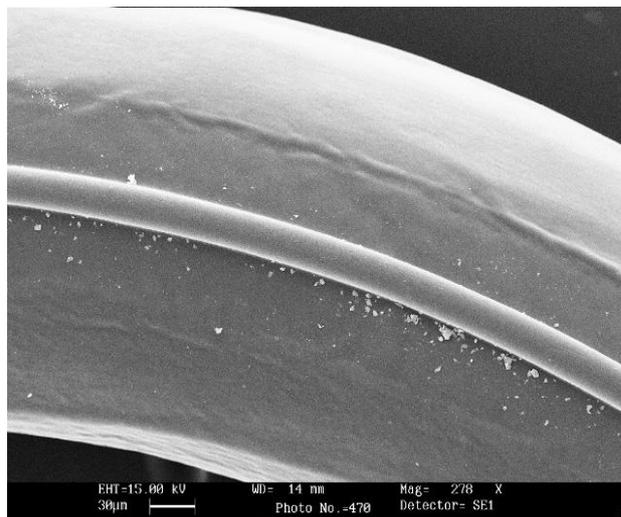


Fig. 5. A transverse ventral cervical groove is present

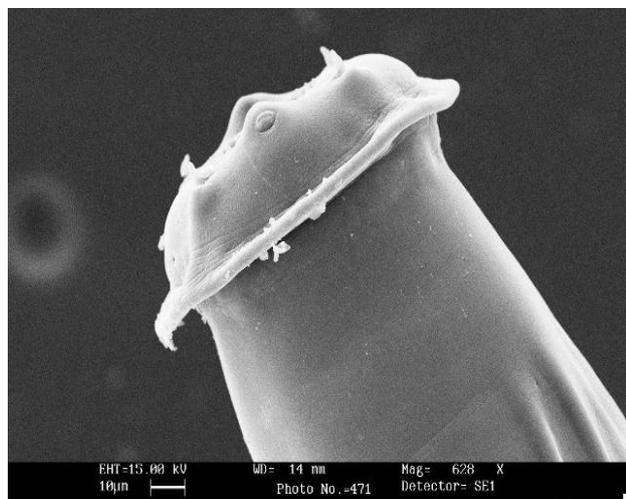


Fig. 3. Cephalic vesicle in head

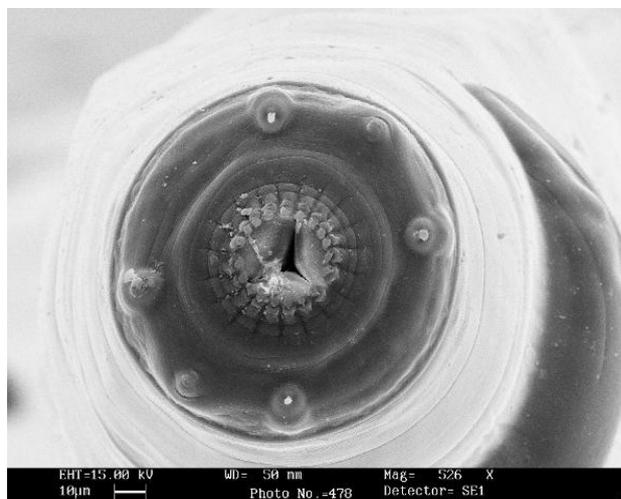


Fig. 6. External leaf-crown

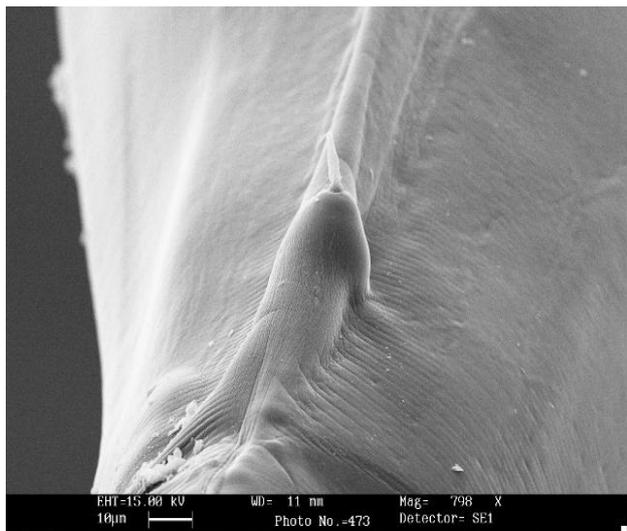


Fig. 7. Cervical papillae situated behind of the esophagus

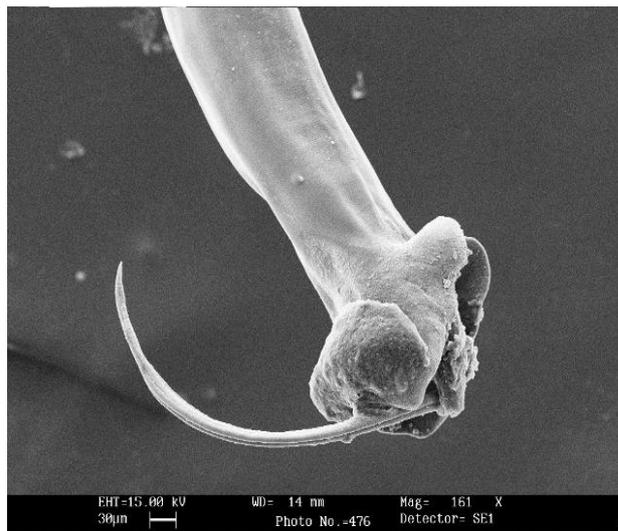


Fig. 10. Curved sword spicules under higher magnifications

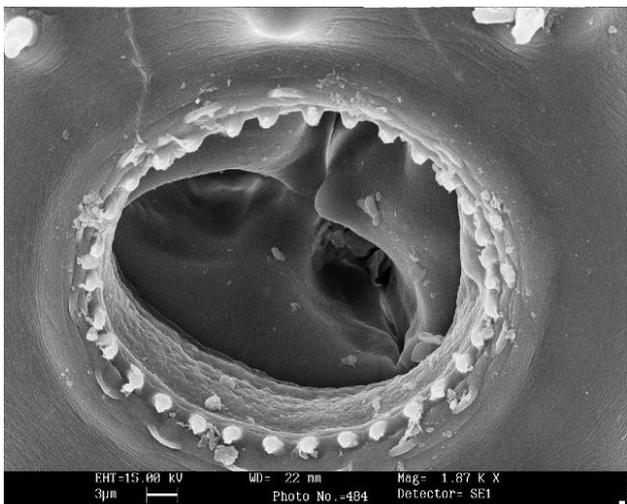


Fig. 8. Leaf crown under higher magnifications

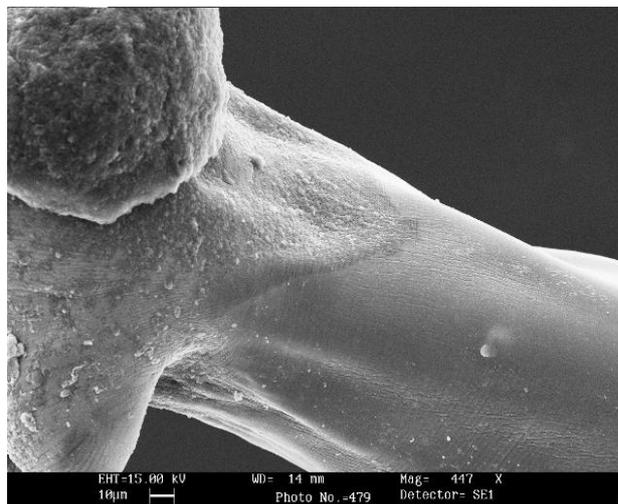


Fig. 11. Posterior end of male parasite

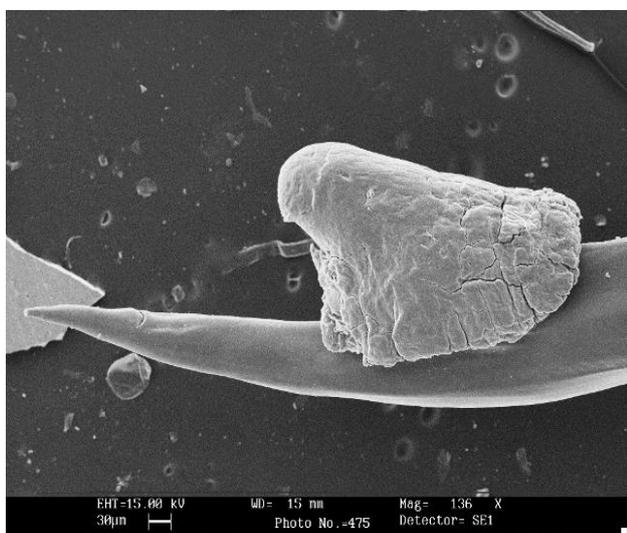


Fig. 9. The vulva surrounded by coupling cement

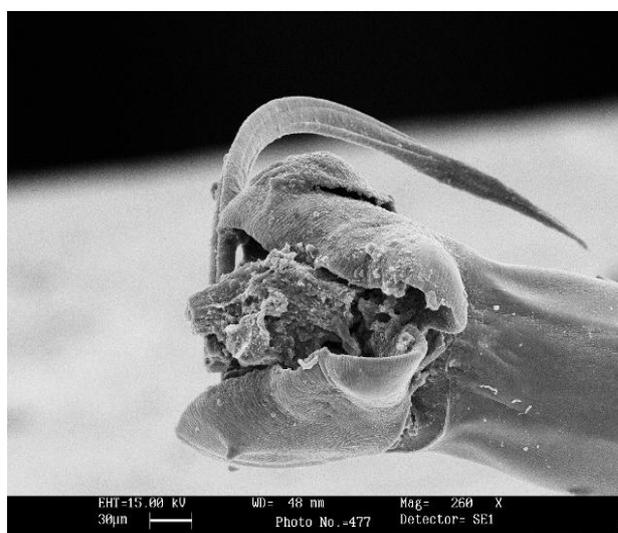


Fig. 12. Bursa of male parasite

**Discussion and conclusions.** *Oesophagostomum* species are free-living nematodes of the family. *Oesophagostomum* spp. is prevalent worldwide and these nematodes are often referred to as nodular worms, owing to the fact that several species cause nodule formation on the wall of the intestine. Species are parasites in the small intestine and the large intestine (caecum and colon) of cattle, sheep, goats, deer, camel, pig, primates and many other ruminants. Wild cervids and bovids, however, are unlikely to be important in the epizootiology of other species, which circulate primarily among domestic hosts (Goldberg, 1952, Levine, 1980). Wild cervids, particularly elk and deer, are the probable source of *O. venulosum* reported in cattle from the western United States (Baker and Fisk, 1986, Hoberg and Rickard 1988, Soulsby, 1969). In Iran, *Oesophagostomum*, spp. occur in the caecum and colon of cattle, sheep, goat, camel, wild sheep, camel and other wild ruminants (Eslami and Fakhrzadegan, 1972, Eslami et al., 1976, Eslami et al., 1980, Eslami and Nabavi, 1979, Skerman et al., 1967). Members of this genus have a cylindrical buccal capsule, usually narrow. Leaf crowns are present. There is a ventral cervical groove near the anterior and anterior to which the cuticle is dilated to form a cephalic vesicle. The purpose of this study was to observe various structures of this parasite by SEM. In a study carried out by Neuhaus and his colleagues on ultra structure and development of the body cuticle of *O. dentatum*, it was shown that of a structural change in the cuticular morphology between the 3rd and 4th juvenile stage harmonizes with earlier reports about the Strongylida. Such a change occurs at different ontogenetic stages or seems to be missing in other nematodes (Neuhaus et al., 1996). Morphogenetic events such as the formation of the radial striation layer from amorphous precursor material agree with previous observations on strongylids (Neuhaus et al., 1996). Other study carried out by Duggal et al. on the copulatory apparatus of male *O. columbianum* by SEM method showed that genital cone is provided with a ventral lip and a pair of sub dorsal genital appendages (Duggal and Kaur, 2006). The ventral lip is a triangular structure having a single papilla on it and the genital appendages are covered with wrinkled cuticle with a nerve process projecting to the exterior in centre. The bursa is supported by muscular rays, which end as knob like sessile genital papillae. The inner surface of the bursa is porous. Spicules are two, equal, each provided with an ala, which decreases in height distally, and end much prior to the spicular tip (Duggal and Kaur, 2006). The present material agrees with the description in previous studies. Our finding in the electron microscopic views of the body surface showed there is a ventral cervical groove near the anterior and anterior to which the cuticle is dilated to form a cephalic vesicle. A fine point was present on the tail of the female worm. A development bursa and its thick lateral ray were observed on male parasites. The long spicules were salient in the bursa. Spicula and cloacae of male parasites were observed. In the present study the vulva was not observed and was hidden under the coupling cement. Another interesting finding is that

curved sword structures on these spicules were observed under higher magnifications.

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