Microscopic Study of Mechanoreceptors and Chemoreceptors of Anterior and Posterior Ends of *Toxocara Canis* Using Scanning Electron Microscopy (SEM) and Light Microscope (LM)

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**ABSTRACT**

In this study, fine structure of amphids and phasmids of *Toxocara canis* and cuticle, muscles and digestive tracts in this parasite were investigated using optical and electron microscopy and hematoxylin-eosin (H&E) staining as well as other specific stains. Thirty-eight adult *T. canis* worms were used in this study. These worms were collected from the small intestines of Urmia’s canines and fixated in AFA and 10% formalin solutions. The anterior and posterior part of *T. canis* male and female worms were prepared according to the conventional method in the histological laboratory and cut at 4-5 μm thickness. The samples were then stained using H&E and specific PAS, Masson's trichrome and Orcein staining and the structure of amphid (anterior), phasmid (posterior), cuticle, muscles and digestive tracts of male and female worms were studied under light microscopy. Basal, intermediate, cortex and cuticle surface coating of the parasite were visible. Alae are also seen as the thickenings in the cuticle. The muscle layer structure consists of non-branchcd cylindrical cells. The intestinal tract is composed of cuticular cogs, the esophagus is of filamentous-muscular structure, and the intestine is made of columnar epithelial tissue with microvilli and glycocalyx. The amphid structure consisted of cuticular protrusions with penetrations of cephalic framework to their inner layers. Phasmid structure also includes protrusions in the cuticle and invagination of sensory neurons. It was concluded that for the most part, the histological structure of the cuticle can be studied using optical microscopy. The muscle structure in this parasite is very similar to the skeletal muscle in mammals. Also, the epithelial structure of the intestine in this parasite was largely similar to the intestinal epithelium in mammals. Finally, in relation to the amphid and phasmid structure, it was observed that they were protrusions covered by cuticles where neural, filamentous and muscular structures were the core of these protrusions.

**Keywords:** *Toxocara canis*, Receptors, Papillae, Histology, Light Microscope (LM)

**INTRODUCTION**

Nematodes are considered as a threat to human and animal life due to their high diversity and high prevalence. On the other hand, the coexistence of humans and dogs has been a cause of the common diseases among them (Baker, 2002). The role of dogs as a definitive host for a number of parasites common between humans and animals has been extensively studied and has been a worldwide public health issue for many years (Shalaby et al., 2010). Humans also exacerbate the risk of transmitting the disease to or from semi-domestic dogs in rural areas, or in societies with poor socio-economic conditions with lacking in veterinary attention and awareness for common diseases (Zhou et al., 2008).
Worms nourish by sucking and feeding on blood (*Ancylostoma*), ingestion of degraded tissues and blood by worms immersed in the mucus (*Trichuris*), feeding on the contents of the intestine (*Ascaris*) or ingesting bodily fluids (*fillers*). Cuticle, which is the outer covering of the nematode, is resistant to digestive secretion. In nematodes, there are usually four papillae in different sizes and shapes with sensory function. There is also a pair of cervical papillae which, depending on the genus and species of nematode are located at a certain distance from the anterior end of the parasite. Some cervical papillae are horn-like, some are minuscule and, in some cases, nonexistent. Some throat-like papillae, some are very small and not seen in some nematodes. Larger papillae can be seen in bumps in size from a needle-like appendage to a complex structure with posterior toothed portions. Larger papillae can be seen as needle-like projections or complicated structures with dentigerous posterior. The location, shape and size of these papillae are used in the study of taxonomic characteristics. These organs act as mechanical receptors and help nematodes to cross small pore spaces (McLaren, 1976; Naem, 2007). Amphids are located in the anterior part of the nematodes and can be seen in various shapes and sizes. Some amphids act completely as chemical receptors while some act as optical receptors in coordination with a gland. Phasmids are found in the posterior part of the male and female nematodes. They are involved in evaluating the received stimuli and help the worm to establish and relocate to a favorable environment (McLaren, 1976). A microscopic investigation of adult worms revealed that the worm is coated by a noncellular cuticle with various and usually thick layers. The cuticle is produced by an underlying layer that is predominantly a smooth hypodermis (epidermis). Nuclei of the hypodermis are found in the four cords that are formed by hypodermal thickening. Dorsal and ventral cords are encapsulated by longitudinal axons while the lateral cord runs along the secretory canal and is covered by a series of apparently branched tubes. Somatic cells have contractile swellings and within the cytoplasmic region of cells there is a translucent nucleus with nucleoli (Shalaby et al., 2009). Toxocarasis is caused by *T. canis* in canids and *Toxocara cait* in the felids. Nematodes are pervasive parasites with a complex life cycle. The apparent differences between the species has been investigated using optical and electron microscopy. Species of this parasite can be detected by light microscopy mostly based on the size and shape of the lips. The difference between the visual aspects of the lips and the shape of the dentigerous ridges are also examined by scanning electron microscopy (Minciullo et al., 2018). The anterior end of the *T. canis* is bent in the ventral direction and has a large monotone caudal ala. Scanning electron microscopy revealed worms with three well defined lips, each with dentigerous ridges and two small holes or invaginations bordering the outer margin of the lips. Dents are triangular in lateral view but blunt and wide in when viewed in other directions. A report has shown that the dorsal lip has two large papillae while each ventral and lateral lips have a large papillae and a small papilla. Moreover, a large amphid pair is seen near the dorsal lip while there are a large amphid and a small one on the lateral and ventral lips (Shalaby et al., 2009; Shalaby, 2018). The anterior is lance-shaped and has three swollen lips. The inner and outer rings as well as the papillae are easily visible. The male length is 4-10 cm. It has a caudal ala and a digitiform appendage can be seen at the posterior end of the male worm. *T. canis* adult worms have been seen in the small intestine of dogs, red foxes, gray and Egyptian-Mongolian wolves and jackals from all over the world. The occurrence of disease is more common among younger animals. Adult worms feed on nutrients in the shedding of epithelial cell. In ascariasis induced by adult worms the clinical signs are only seen in abnormal locations such as bile duct. Affected animals show signs of depression and anorexia while anemia is seen in chronic cases. In the present study, we have been trying to study the structure of chemical receptors of the *T. canis* parasite (amphid and phasmid) and some of its internal organs using hematoxylin-eosin staining and specific staining for mammalian and avian tissues.
MATERIALS AND METHODS

In this study, 38 adult *T. canis* worms were collected adult *T. canis* were obtained from animal shelter of Urmia, West Azerbaijan, Iran. For this propose a newly received pups were treated by mebendazole 1g 50 kg\(^{-1}\) for five days (Tolide Darouhai Dami Iran Co. Tehran, Iran), followed by single administration of praziquantel 50 mg kg\(^{-1}\) (Tolide Darouhai Dami Iran). and separated before sending for specimen preparation.

Worms collected were washed in water and drained with filter paper before fixation in 10% buffered formalin and AFA. Due to the small size of the chemoreceptors (amphid and phasmids) in the *T. canis* parasite, scanning electron microscopy (SEM) was first used to observe the exact structure and exact location of these receptors. For optical microscopy study of the amphids and phasmids in male and female *T. canis* worms, the worms collected from the feces of dogs were fixed in 10% formalin after being washed in water and draining. The anterior and posterior part of *T. canis* male and female worms were then prepared according to the conventional method in the histological laboratory and incised into 4-5 \(\mu m\) sizes. These sections were then stained with H&E and the specific stains periodic acid-Schiff (PAS), Masson's trichrome and orcein. The structure of amphid (anterior end) and phasmid (posterior end), cuticle, muscles and intestinal tract of male and female worms were studied under light microscope.

PAS staining was used to examine carbohydrate aggregation and intensity, orcein specific staining was used to investigate the elastic structure and Masson's trichrome staining was used to identify collagen strands in collagenous tissues. The stains used in this study were all the same stains used for mammalian and avian tissues. The reason for this was to determine the similarity or differences in the structures of this parasite with those of mammals and birds (Hesari et al., 2015).

RESULTS

Cuticle’s histology

Primary examination of the body covering in *T. canis* parasites using PAS (Periodic Acid Schiff) staining and light microscopy revealed that the outerCovering of the worm consists of a cuticle supported by somatic tissue. This cuticle reacted positively to PAS and was highly stainable. Hematoxylin-eosin (H&E) staining of the longitudinal slices of *T. canis* parasite showed that the cuticle structure was almost similar throughout the body. The basal layer, as the deepest layer of the cuticle, the median layer which is relatively thick, a thin acidophilic layer above the median which was recognized as the cortical layer and a thick surface coating as the outermost layer were all visible and distinguishable. Outer first shed cuticle and the inner second shed cuticle were not distinguishable from other layers. The wrinkles on cuticle surface of the worm are due to infolding of different layers of the cuticle and they do not form a special structure detectable by light microscopy. At the infolding sites of the cuticle, the thickness of the cortical layer and the surface coating layer are reduced (Figure 1, parts A and B).

The collagenous structures of basal membrane of cuticle were easily stained by Masson's Trichrome stain. Positive Masson's Trichrome streaks and bands (blue) were also evident in cuticle’s thickness and length (Figure 1, parts C and D). Orcein staining of elastic fibers clearly showed these streaks in outer layers especially in cortical and surface coating layers (Figure 1, part E). Finally, histological examination of the ala showed thickening in cuticle which was apparent along the whole body of the ala. The muscle structure underneath the ala was changed and it seems that the subcuticle structure at the location of the ala has been differentiated, creating a special structure. Histological sections and H&E staining on the ala shows that ala is mostly created by thickening of the median layer which is bounded by basal layer and surface layers of cuticle. There was no somatic layer in the ala structure (Figure 1, part F).

Histology of muscles
Histological examination of the muscle layer in *T. canis* revealed that this tissue was mainly in the subcuticle region and in direct contact with the cuticle. This layer was not seen in distal parts of coelom cavity. This muscle layer is composed of cylindrical, non-branching, somatic cells with multiple cornered nuclei. The striated from seen in the cytoplasm of mammalian muscle cells is not detectable in this worm. Using specific staining, it was found that the muscle cells in this worm are not grouped, lack muscle support structures such as epimysium, perimysium, and endomysium and form a uniform muscle structure (Figure 2).

**Histology of intestinal organs**

Histological examination of the dentigenous ridges in this parasite, as the apparatus for attachment to the host, revealed that the dents were visible as a sharp cuticular protrusions in a row on both sides of each lip (Figure 3, part A).

Histological study of the structure of the esophagus as the second recipient of nutrient intake revealed that the different esophageal sections had the same histological structure except the variation in the lumen diameter. The esophagus of this parasite is formed as a tube, with two adjacent lips located in the interior of the corpus (the anterior part of the esophagus). These two lips have filamentous-muscular structures and show parasite-specific histological features when when stained with H&E. The difference in the inner diameter of the esophagus has led to various names such as isthmus and bulb for different sections (Figure 3, part B).

Examination of the intestinal structure of *T. canis* revealed that the intestinal wall in this worm has a fairly uniform structure along the intestine. It consists of a basal membrane, a simple elongated columnar epithelium as well as microvilli and glycocalyx on its surface which covers the entire inner surface of the intestine. The nucleus of the epithelial cells is usually round a well-defined and located at the base of the cell. The nucleus of some simple columnar epithelial cells may be in rows above other nuclei (Figure 3.C). Microvilli and glycocalyx were clearly visible in PAS staining of the basal membrane. Also, the PAS staining of the cytoplasm of simple columnar epithelium of intestine shows relatively large fragments of carbohydrate structures (Figure 3, part D). Finally, the intestinal tract is connected to the outside by a conduit at the far end of the worm. Throughout the respiratory tract up to the anal canal, the intestinal epithelial canal is identical covered by simple ciliated columnar epithelium (Figure 3, parts E and F).

**Histology of Physical and Chemical Receptors**

Using SEM imaging, we could identify and prepare serial sections of the lips and the posterior end of *T. canis* to study the chemical and physical receptors in this worm. The lips were covered with cuticle from the sides. Inside each lip there is a framework or secretory network that contains the nerve fibers and filamentous muscles specific to the parasite. At the apex of the dorsal lip there is a pair of large double papillae while at the site of each ventral lip, a large double papillae, a small papillae and an amphid is seen. This amphid is engorged by cuticular tissue and the cephalic framework is visible in the subcuticular region. The location of the amphid was detectable using SEM imaging and with the help of papillae positions. Amphids’ measured sizes were 3-4 µm, large papillae were 7-10 µm and small papillae were 6-8 µm (Figure 4).

Phasmid structure was also investigated using SEM and serial sectioning method. The phasmid location was first verified after observing post cloacal papillary structures and then studied under an optical microscope. As for the histological structure of the phasmds, similar to the amphids, they are surrounded by cuticle which is engorged at the location of phasmid. Muscular and nerve tissues are detectable inside this swelling. Phasmds were measured to be 2-3 µm in size (Figure 5).

**DISCUSSION**

Although *T. canis* has been the subject of many studies, yet it seems there is no thorough information available for this parasite especially when considering the chemical receptors. In this study, *T. canis* specimens collected in Urmia were first fixed in the appropriate solutions before sending for SEM
imaging to determine the exact location of the chemoreceptors. Subsequently, for the first time the structure of these receptors was evaluated using specific staining and light microscopy. Histological capabilities of optical microscopy in the investigation of cuticular structure, muscular tissue and intestine were evaluated.

Previous reports state morphological similarities between *Ascaris lumbricoides* and *T.canis* in the sense that anterior end of the ala, a large dorsal lip and two ventral lips, ordered denticles are apparent in their inner sections. The external ring of papillae in *A.lumbricoides* was surrounded by four large papillae, of which two are dorsolateral located on dorsal lip and one ventrolateral located on each ventral lip (Shalaby et al.,2010). According to Shalaby (2010) the anterior end of *T. canis* is lance-shaped, has three prominent lips, and the inner and outer rings of papillae and amphids were clearly visible. Denticles were also reported in a single row on the inner labial surface with relatively equal sizes. Ventral and dorsal papillae are different from those of *A.lumbricoides* while cuticular striations in the vicinity of alae were more packed and less distinct (Shalaby et al.,2010). Our results were in agreement with previous reports regarding the morphology of the *Toxocaridae* family of parasites.

Little is known about the sensory organs and their histological structure in nematodes. In general, the sense organ is composed of one or more ciliated dendritic process, a sensory neuron and two supporting cells, which are a socket and a sheath cell (Fagerholm et al.,2004). Amphids in nematodes have different shapes and sizes, some of which are low-performance chemical receptors and some light receptors accompanied by their associated flushing glands (Mclaren et al.,1976). Dendritic processes situated inside the phasmid canal, indicate that phasmsids have a chemosensory function similar to amphids (Fagerholm et al., 2004). In our study, we confirmed the circular shape of amphid and its central canal using optical and electron microscopy.

Investigating dendrites in amphids of *Strongyloides stercoralis* and *Caenorhabditis elegans* showed no signs of striated rootlets in the mechanocilia (Perkins et al., 1986). The reported amphids of *Physaloptera rara* also had a single pore (Naem and Asadi 2013).

The number of amphid pores can also be different among the members of a family in or an order; as in *Spinitectus beaveri*, *Spirocera lupi* and three species of *Habronema* there is a single large pore (Naem, 2007; Gorganiet al., 2013). While in some other species of *Spinitectus* double-pored amphids have been reported (Gorganiet al., 2013). Phasmsids are involved in assessing the intensity of stimuli to the worm and helping them survive in an appropriate environment (Okulewitz et al., 2012).

Fagerholm (2004) investigated the position of phasmsids on the male *Hysterothylacium auctum* and reported a pair of them positioned laterally and equidistant from the rostrocaudal axis of the body and located 20 μm from the tip of the tail. In female worms the phasmsids were located symetrically on the ventral to the lateral line. Phasmid’s papilla in males was cone-shaped with a diameter of 5 μm with a central 1 μm knob (Fagerholm et al., 2004). In the current study, phasmid in *T.canis* was situated at 50-70 μm from the tip of the tail and had a size of 3-5 μm. Phasmsids in male and female worms were situated symmetrically and had a central pore. These findings were verified with both light and electron microscopy.

A previous study hypothesized that phasmid position may be defined genetically and in different groups of nematodes it could mutate during the first larval division stage and blast cell stage (Fitch,1997). Only in some nematode specimens amphids and phasmsids are clearly visible under the light microscope and are reliably discernible from the particles adhering to the cuticle. Optical microscope examination of male *Hemicycliophora* and *Criconematidae* revealed no signs of phasmids or similar structure on the tail while phasmid-like structures were identified in the mid-tail region of *Sphaeronema* under light microscopy, although with much difficulty (Moravec and Justine, 2014).

Amphid structure in *Acrobeles complexus*, a microbial feeding nematode, has been investigated. In these amphids 13 sensory neurons were identified, 12 of which enter a sensory channel formed by a
proximal sheath cell and a distal socket cell. The length of amphid in this parasite is 15 µm, which is measured as the distance between the opening of the sensory channel and where the sensory neurites enter the socket cell. At the entry point of sensory channels, multiple lamellar projections of the sheath cell wrap the sensory dendrite (Bumbarger et al., 2009).

Male *Baylisascaris procyonis* have a relatively long and narrowing tail and usually a knob is present at the distal end of the tail. Cephalic papillae are on both sides and irregularly spaced while on outer margin of the anterior section of anus there is a prominent double papilla. There are 5 pairs of papillae in male worm among which the first pair is double in size compared to other pairs. One papillae pair is located near the anus while the other four (caudal) pairs are located near the distal end of the tail. The fourth pair of these papillae is curved and located besides the fourth pair of phasmids which have an open orifice at their center (Snyder and Fitzgerald, 1985). We also viewed the male *T.canis* tail’s narrowing as well as its posterior knob. Six pairs of papillae were placed in irregular distances post-cloaca where the second pair was twice in size compared to others. Phasmids were located adjacent to the fourth and fifth pairs.

Anterior papillae are studied more thoroughly in *T.canis* compared to posterior papillae. There is a pair of amphids at the sub-ventral lip as well as a large and a small papilla. The number as rows of papillae in *A. lumbricoides* and *Ascaris suum* are the same as *B.procynis* except that phasmids in some species of *B.procynis* are located after the third pair of papillae while in other specific are located posterior to the fourth pair. The number and arrangement of papillae in *Parascaris equorum* which is an equine roundworm are similar to *B.procynis* except that in form the phasmids are located on the left side and near the third pair of papillae. Papillae in *Toxascaris leonina* are clearly different from the species of *B.procynis* (Shalaby, 2018; Xue, 2014; Falcone el at., 2001; Snyder and Fitzgerald, 1985). The prominent papillae on the lips of *ascaris* are more likely to function as mechanoreceptors. These cephalic papillae may also have dual functions where the central swelling on the papillae act as mechanical receptor while the lateral welling act as chemical receptors. The cause of the difference in the number of papillae in *Ascaris* is not yet understood (Snyder, 1985).

The scanning electron microscopy of papillae on the lips of *B.procynis* shows similarities in shape and morphology to *T.leonina* and *A.lumbricoides* although it is different to *Toxocara* species (Snyder and Fitzgerald, 1985). The overall number of denticles on three lips In our study we counted 100-128 denticles on each lip of *T.canis* this number of *B.procynis* is reported to be 650, 730 on *A.lumbricoides* and 210 on *T.leonina* (Snyder and Fitzgerald, 1985). The denticles on *T.leonina* are small and their number is reported as 70 on each lip by Snyder and Fitzgerald (1985) while Xue (2015) reported their number as 65-90 on each lip. Difference in hosts, severity of infection and geographical location may be the cause of different reports (Xue et al, 2015).

The presence of papillae at the posterior end of *Ascaris* species has been verified in *A.equorum* using light and electron microscopy (Okulewicz et al., 2012; Owen and Slocombe, 1985). Lim (2008) has reported 25 pairs of pre-cloacal and 5 pairs of post-cloacal papillae in *T.leonina* although these numbers are 23-30 and 4 respectively (Lim, 2008) according to Xue (2015) In the present study we observed two small circular apertures on each lip of *T.canis*. We also verified the presence of pre- and post-cloacal papillae using light and electron microscopy.

A study by Xue (2015) on the morphological characteristics of *T.leonina* showed morphological differences between previous studies and theirs, including differences in the size, width, and length of the cephalic ala and the number of denticles per lip (Xue et al, 2015). There are no reports on the number of denticles in *T.canis* to date for reference purposes.

A 2009 study by Shalaby found that adult *T.canis* worms were coated with an acellular cuticle with several thick layers which produced by a substrate layer that is mostly smooth hypodermis (epidermis). The cuticle is essential for protective, nutritional and shape-preserving purposes as well as being an antagonist for somatic cells. The nuclei were only found in the four cords produced by
hypodermic cells. Dorsal and ventral cords are filled longitudinally with axons while lateral cords are located along the excretory channels and is surrounded by a series of seemingly branching ductules. Muscle cells had contractile and cytoplasmic parts and within their cytoplasmic region a clear nucleus with nucleoli was visible (Shalaby et al., 2009). Our results were in agreement with previous reports regarding the structure of muscle tissue.

Another study that has examined the histological structure of the cuticle has stated that the cuticle in the *T. canis* parasite is composed of six layers, including basal layer, median layer, cortical layer, surface coat layer, the outer first shed cuticle, and finally the inner second shed cuticle (Brusjaska et al., 1995). In the present study, basal, median, cortical and surface coat layers were identified but the outer first shed cuticle and inner second shed cuticle layer were not distinguishable from the adjacent layers.

The thickness of cortical layer and surface coat layer were reduced at the site of cuticle in folding. Histological structure of ala was also attributed to the thickening of a median layer of the cuticle. Using Masson’s trichrome and orcein staining, cuticles’ elastin and collagen was similar to those of mammalian and avian. These results have not been reported in other studies so far.

Regarding the intestinal tract’s structure (teeth, esophagus and intestine), our results confirmed the similarities between the intestinal tract of this parasite and epithelial structure of the intestines in mammals and avian; this tract was composed of a layer of simple columnar cells covered by microvilli and glycocalyx in the absence of goblet cells, the basal layer of epithelium had a collagen-carbohydrate structure and it seems that carbohydrate absorption in the intestine of the parasite is through large portions.

It can be concluded that the basic structure of chemical receptors and their positioning can be studied using light microscopy. Light microscope is also useful in the investigation of other structures and organs of *T. canis* and can be utilized in these studies. This is also true for the study of cuticle, muscle tissue and intestinal tract. The results of the current study regarding the similarities of collagen and elastin structures of the cuticle as well as the epithelium of intestine in this parasite could lead to investigation of new strategies in development of novel drugs and effective anti-parasitic agents which do not damage the host cells.

**REFERENCE**


Figure 1. Histological section of cuticle and ala structures in female *T. canis* parasite

A: Transverse section of *T. canis* with PAS staining, magnification ×100. The body of parasite is wrapped in a cuticle which is supported by a muscle layer. Cuticle has reacted favorably to PAS. 1: muscle layer 2: cuticles.

B: Longitudinal section of *T. canis* with H&E staining, magnification ×400. 1: muscle layer / 2: cuticle / 3: basal layer / 4: median layer / 5: cortical layer / 6: invagination of cuticle.

C: Transverse section of *T. canis* with Masson’s trichrome staining, magnification ×400. 3: basal layer / 8: collagenous strands are present along the whole thickness of cuticle layer

D: Transverse section of *T. canis* with Masson’s trichrome staining, magnification ×400. 3: basal layer / 8: collagenous strands on the upper layers of cuticle which are spread along the cuticle forming an integral stripe.

E: Transverse section of *T. canis* with orcein staining, magnification ×400. 9: elastin fiber strands which are visible on the exterior surface of cuticle.

F: Longitudinal section of ala in *T. canis* with H&E staining, magnification ×100. 10: ala is formed by thickening of median layer of cuticle and the muscle layer does not penetrate it.
Figure 2: Histological section of muscle layer in female *T. canis* parasite

A, B: Longitudinal section of muscle layer stained by H&E, magnification ×400. Somatic cells are completely cylindrical, non-branching with multiple cornered nucleoli.

C: Longitudinal section of muscle layer stained with Masson’s trichrome, magnification ×400. In this staining, no collagenous tissue was found between the muscle cells. The muscle layer in this worm does not form muscle groups and acts as a uniform muscle layer.

D: Longitudinal section of muscle layer stained with Orcein, magnification ×400. The elastic tissue between muscle cells is mostly hardly detectable.
Figure 3: Histological section of different regions of intestinal tract in male and female *T. canis* parasite.
A: transverse section of lips using H&E staining, magnification ×400. 1: positioning and morphology of denticles.
B: transverse section of corpus esophagi using H&E staining, magnification ×100. The esophageal interior of this parasite encompasses a pair of lips with filamentous muscular structure.
C: transverse section of intestinal wall with H&E staining, magnification ×400. 2: simple ciliated columnar epithelial tissue / 3: basal layer / 4: cilia with glycocalyx / 5: nucleoli of some simple columnar cells are located higher relative to other cells.
D: longitudinal section of intestinal wall with PAS staining, magnification ×400. 3: basal layer / 4: cilia with glycocalyx / 6: relatively large carbohydrate bodies absorbed by the absorbing columnar cells.
E: longitudinal section of posterior end of female *T. canis* with orcein staining, magnification ×100. 2: simple ciliated columnar epithelial tissue / 7: anal tube.
F: longitudinal section of posterior end of male *T. canis* with H&E staining, magnification ×100. 2: simple ciliated columnar epithelial tissue / 7: anal tube.
Figure 4: Scanning electron microscopy and light microscopy of lip in male *T. canis* using H&E tissue staining. Magnification: ×400. 1: small papilla on ventral lip / 2: amphid / 3: large double papilla / 4: dentigerous ridges.

Figure 5: Scanning electron microscopy and light microscopy of tail in male *T. canis* using H&E tissue staining. Magnification: ×400. 1: papillae / 2: phasmid / 3: cloaca / 4: intestine.