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Research Article

A Scanning Electron Microscope Examination of *Heligmosomum costellatum*

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Abstract: The morphology of *Heligmosomum costellatum*, a nematode of field mice (*Microtus epiraticus*), was described by scanning electron microscope. The scanning electron microscopic view of this nematode revealed that the anterior end was surrounded by 2 cephalic vesicles. The 2 copulatory spicules of the male were enveloped in membrane and the male bursa was large. The female posterior end was characterized by a caudal spine. The body of this parasite had transverse ridges.

Key Words: *Heligmosomum costellatum*, scanning electron microscope, mice

Heligmosomum costellatum'un Scanning Elektron Mikroskobik İncelenmesi

Özet: Bu çalışmada tarla farelerinin nematodlarından biri olan *Heligmosomum costellatum* scanning elektron mikroskobu ile incelenmiştir. Bu nematodun ön ucunun iki sefalik vezikül tarafından çevrelendiği gözlenmiştir. Büyük bir bursaya sahip olan erkek parazitin spikülümlerinin membran ile çevrili olduğu izlenmiştir. Dişi parazitin arka ucundaki spin karakteristiktir. Ayrıca parazitin gövdesinde transversal çizgiler bulunmaktadır.

Anahtar Sözcükler: *Heligmosomum costelletum*, scanning elektron mikroskobu, fare

Introduction

Heligmosomum costellatum, a nematode belonging to the family Heligmosomatidae (Trichostrongyloidea), is a parasite occurring in the intestines of holarctic and neotropic rodents (1-3). The characteristics of this genus are based on light microscopy; the body of this worm parasite has complex cuticular ridges (4,5) known as the synlophe (1). The axis of the orientation of the ridges is subfrontal (1). The male parasite has a large bursa and long filiform spicules surrounded by membrane distally. The vulva of the females is next to the tail. A caudal spine is present on the female tail (1,4,5).

There has been no research on the electron microscopic examination of *H. costellatum*. The purpose of this study was to observe various structures of this parasite by scanning electron microscope (SEM).

Materials and Methods

Specimens of *H. costellatum* were collected from the intestines of field mice (*Microtus epiraticus*). 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 (6,7). Specimens were mounted onto stubs by conductive double-sided adhesive tape, sputter-coated with a thin layer of gold by Polaron SC-500 and viewed by SEM (JSM 5600 JEOL SEM).

For comparison with SEM views, *H. costellatum* specimes were put on slides and investigated by binocular light microscope.

Results

The mouth of male parasites located in the anterior end was surrounded by 2 cephalic vesicles behind which transversal cuticular ridges began (Figure 1). The body had transverse ridges (Figure 2). These ridges under higher magnification revealed deep grooves (Figure 3). A large bursa was seen in the male parasites (Figure 4). The spicules of the male were clearly protruding in some cases and were enveloped in membrane distally.

Figure 5 shows the structure of a ray in the bursa. Belt-like structures on these rays were observed under higher magnifications (Figure 6).

In the SEM, the cephalic structures of female worms did not differ from those of males. The anus, situated on the point of the posterior end, formed a "Y" shaped fissure, which was surrounded by 3 papillae (Figure 7). Figure 8 shows the groove shaped structure of these papillae. The cuticular ridges were circular on the female caudal end, and longitudinal in the anterior half of the parasite. A spine was present on the tail of female parasites (Figure 8).

PLATE 1

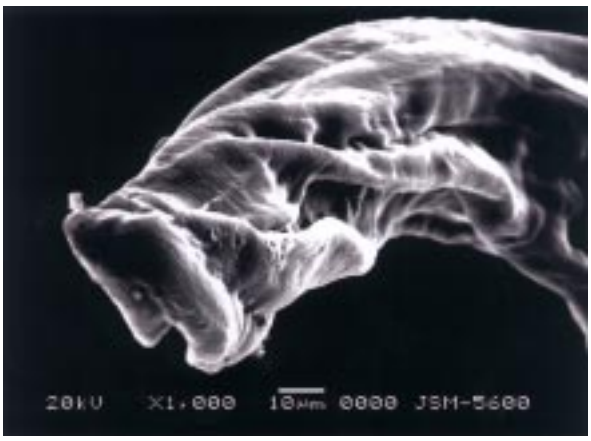

Figure 1. Anterior end of the parasite with 2 cephalic vesicles.

Figure 2. Body of the parasite with cuticular ridges.

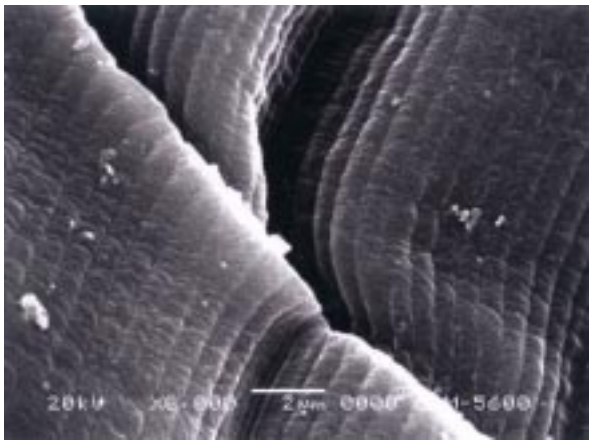

Figure 3. Cuticular ridges under higher magnification.

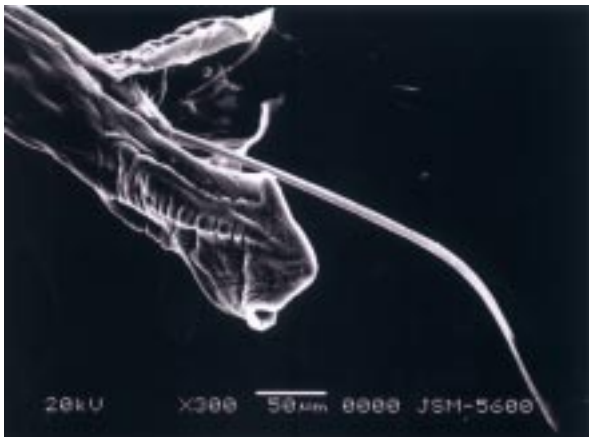

Figure 4. Bursa of male parasite.

PLATE 2

Figure 5. The structure of a ray in the bursa.

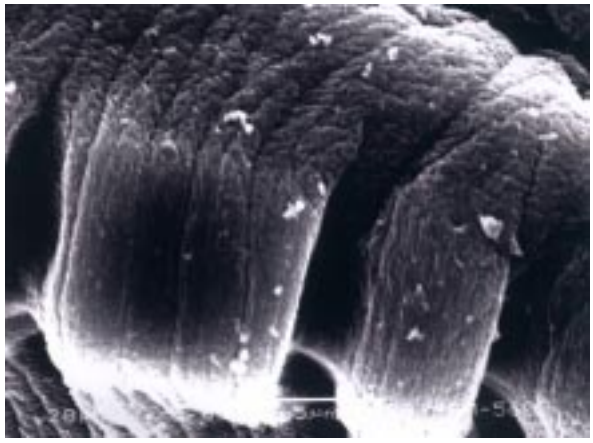

Figure 6. Belt-like structures on the bursal rays.

Figure 7. The anus surrounded by 3 papillae.

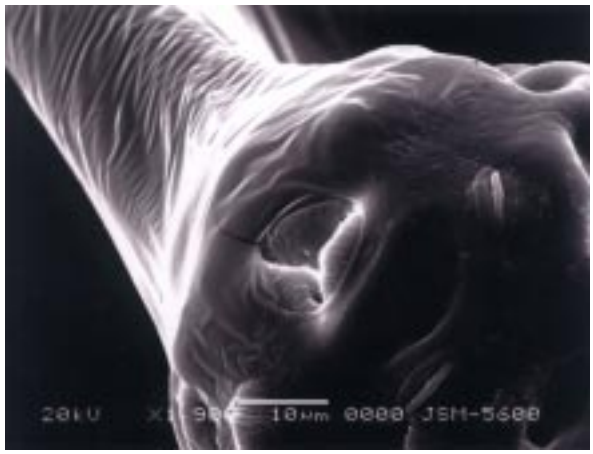

Figure 8. Posterior end of female parasite.

In the light microscopic views, the anterior end of the parasite was surrounded by cephalic vesicles (Figure 9). The male bursa had a small incisure between the lobes. Externodorsal rays were elongated with thin structures. The dorsal ray was divided into 4 branches (Figure 10). A characteristic caudal spine was observed on the posterior end of females (Figure 11).

Discussion

In Turkey, *H. costellatum* was detected in field mice for the first time in our study. The present study provides a detailed morphological redescription of *H. costellatum* by means of SEM. In the light microscopic views, the present material agrees with the description in previous studies (2,3). The electron microscopic observations confirmed and extended the light microscopic views of *H. costellatum*.

Electron microscopic views of the body surface showed that it consisted of ridges with deep grooves. A spine was present on the tail of the female worm. A large bursa and its thick lateral ray were observed on male parasites. The long spicules were protruded in the bursa. The anus shape and its surrounding papillae, and circular cuticular ridges in the female parasites were interesting

PLATE 3

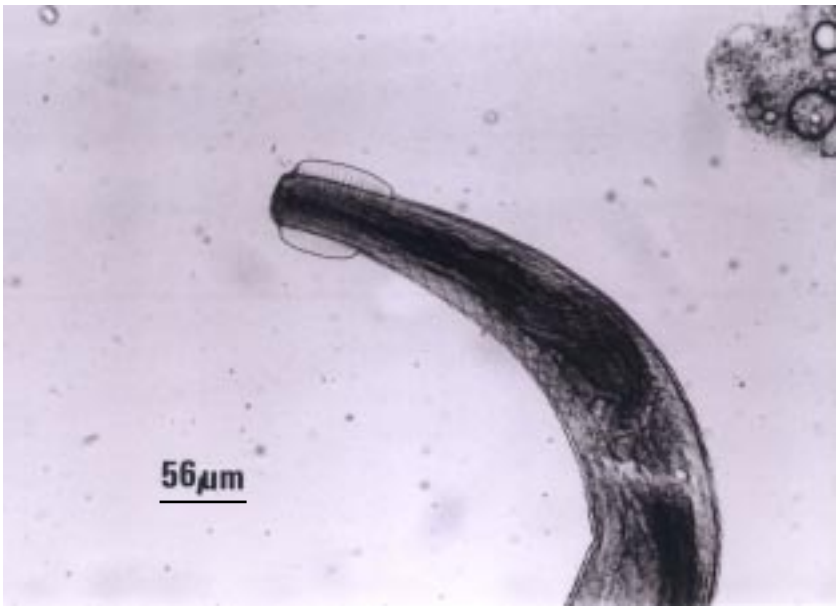

Figure 9. Anterior end of the parasite (under light microscope).

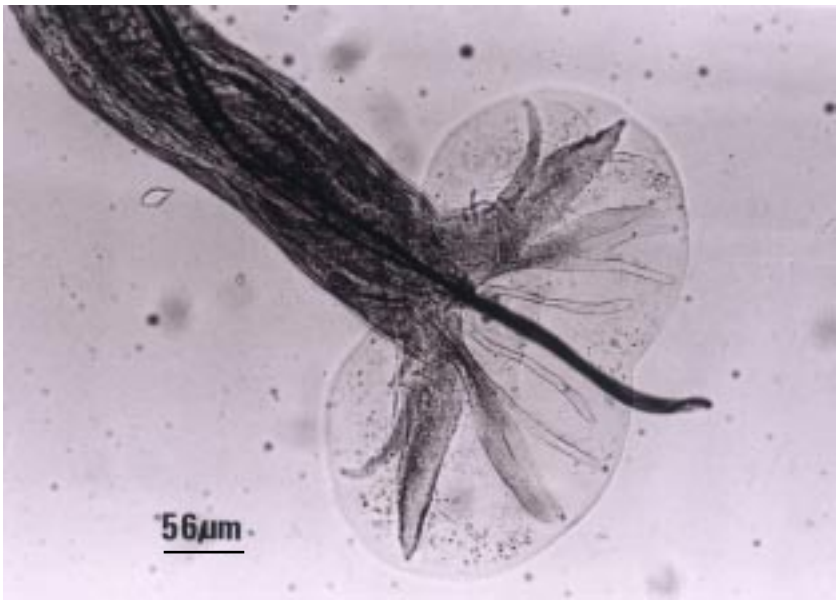

Figure 10. Male bursa (under light microscope).

findings in the present study. The vulva was not observed on examined *H. costellatum* in this study due to the positions of the parasites on the stubs.

Another interesting finding is that belt-like structures were seen on the bursal rays. They were probably related to bursal movement.

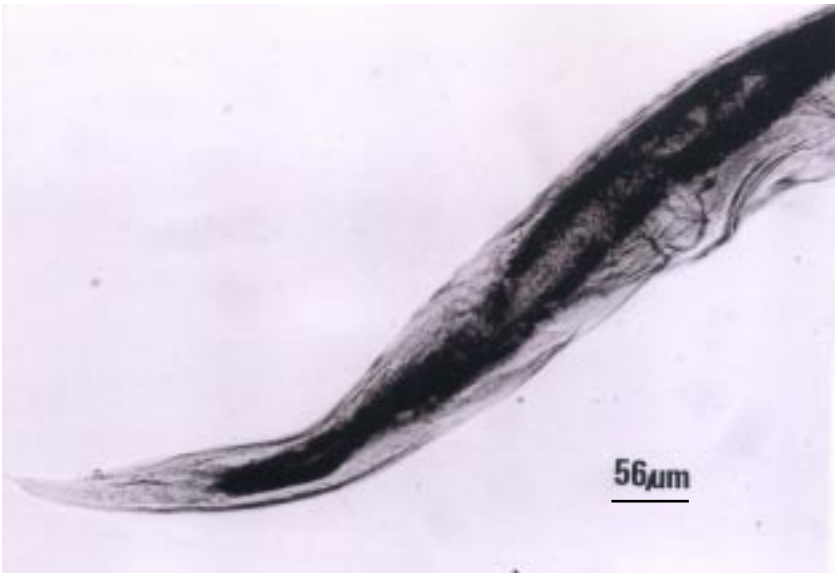

Figure 11. Female posterior end with caudal spine (under light microscope).

The various components of the bursa copulatrix have been discussed by various authors (8-10). Stone et al. (8) investigated the use of the bursa as a taxonomic character in the family Trichostrongylidae and concluded that it has some value as a generic character and, in some cases, can be used as a supportive character for specific identification. The species of the genus Heligmosomum have a smaller incisure between the lobes, the lateral rays have a common trunk, and diverge sharply from neighboring groups and long filiform spicules in the membrane are enveloped distally. In the present study, the genital cone structure and complex cuticular ridges can also be seen to support this character in specific identification.

Acknowledgment

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