

# A Scanning Electron Microscopic Study of Eggshell Surface Topography of *Leidynema portentosae* and *L. appendiculatum* (Nematoda: Oxyuroidea)<sup>1</sup>

XIONG YU<sup>2</sup> and JOHN L. CRITES, Department of Zoology, The Ohio State University, Columbus, OH 43210

**ABSTRACT.** The eggs of the nematodes *Leidynema appendiculatum* and *L. portentosae* were studied with scanning electron microscopy (SEM). The opercular grooves on the eggshells of both species are oblique, incomplete, and terminal. They deepen as the embryo develops. Numerous pores and pits are distributed on the eggshell surface in both species. The pits on the eggshell of *L. portentosae* are sharply delineated; those on the eggshell of *L. appendiculatum* are less sharply defined. The surface texture of the eggshell of *L. portentosae* is rougher than that of the eggshell of *L. appendiculatum* and sandy in appearance. The entire eggshell of both species is undulated irregularly to a very small degree.

OHIO J. SCI. 88 (5): 195-198, 1988

## INTRODUCTION

As biological units, nematode eggs are extremely important for the survival of the species. They provide a partially controlled environment for development of the embryo; they offer resistance to external environmental factors; and in the case of parasitic species, they act as the means of transfer from host to host. In order to fulfill these functions, the eggshell probably plays the most critical role. However, many nematode eggs, such as in the genus *Leidynema*, are morphologically very similar when examined with light microscopy.

The nematodes *Leidynema portentosae* and *L. appendiculatum* belong to the family Thelastomatidae. When using light microscopy, the eggshells of *L. portentosae* and *L. appendiculatum* appear very similar to one another and to the eggshell of *Hammerschmidtella diesingi*, a nematode of the same family. Yu and Crites (1985) studied the eggshell of *H. diesingi* with scanning electron microscopy (SEM). Their investigation revealed the structure of pits and the opercular groove in addition to the pores. The present study was undertaken to determine if there are differences in the eggshell surface topography between these two species, which can be ascertained with SEM. We also looked for changes in eggshell surface characteristics as the embryo developed to an infective larva inside the eggshell. These changes might be related to hatching of the larva.

## MATERIALS AND METHODS

Gravid females of *L. portentosae* were collected from the hindgut of infected Malagasy cockroaches, *Gromphadorhina portentosa*, which were supplied from the Insect Culturing Laboratory of The Ohio State University. Gravid females of *L. appendiculatum* were collected from the hindgut of infected American cockroaches, *Periplaneta americana*. These females were washed and kept in Ringer's invertebrate solution at room temperature until they laid most of their eggs. Eggs were kept in Ringer's invertebrate solution at 25°C as development of the eggs continued. Based on developmental time and embryo morphology (Yu and Crites 1989), eggs at early, middle, and final developmental stages, from the zygote through the second-stage larva, were used for study. The eggs were rinsed very quickly in acetic acid, fixed in 2% osmium tetroxide (OsO<sub>4</sub>) for 2 h, washed in 50% ethyl alcohol, and dehydrated in a 60 to 100% ascending series of ethyl alcohol solutions. The dehydrated specimens were critical point-dried in absolute ethyl alcohol with CO<sub>2</sub> in

a critical point dryer (Autosamdri-810). Finally, the dried eggs were mounted on double sticky tape, coated with gold, and viewed with a scanning electron microscope (Hitachi S-500) at an accelerating voltage of 20 KV. About 20 eggs of each species were examined.

## RESULTS

The egg of *L. portentosae* was spherical and elongated with one side slightly flattened (Fig. 1). The eggshell surface was relatively rough and sand-like in appearance. The entire eggshell was irregularly and very finely undulated (Fig. 2). There were many pores and pits distributed over the entire eggshell surface. The size of the pores and the pits on the eggshells were small; the diameter and depth varied randomly from place to place. The pits were sharply defined and usually larger in diameter than the pores, which were much deeper.

An opercular groove at one end of the eggshell was oblique and incomplete. The opercular groove of the eggshell of *L. portentosae* lacks salient zones surrounding it. The groove was shallow at the beginning of embryonic development, but it deepened and two sharp edges formed as embryonic development proceeded (Figs. 3-6).

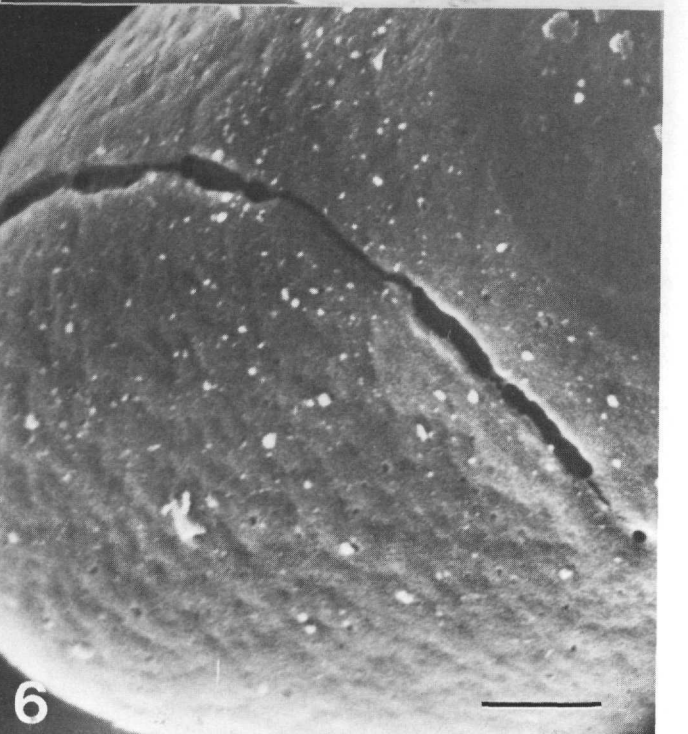
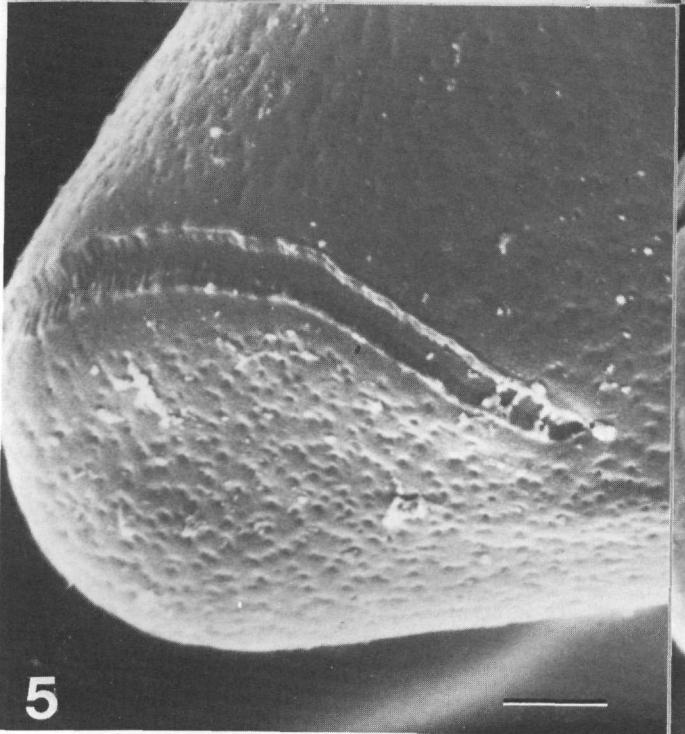
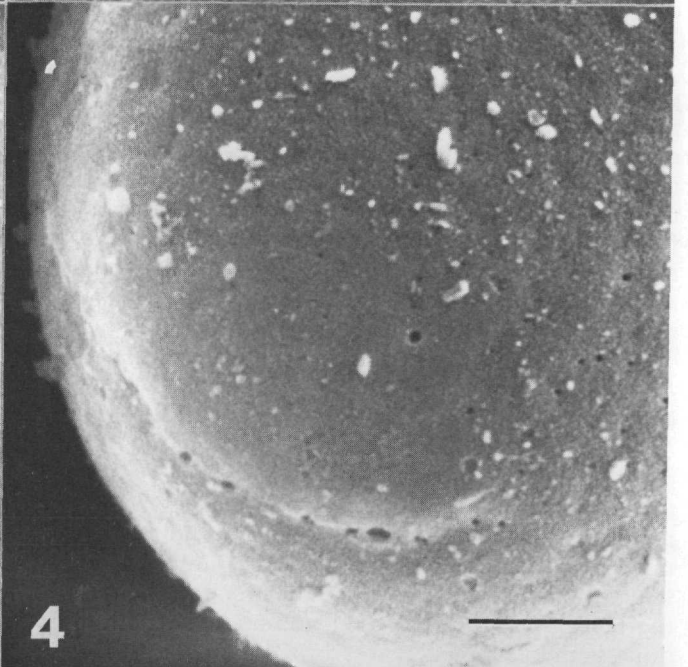
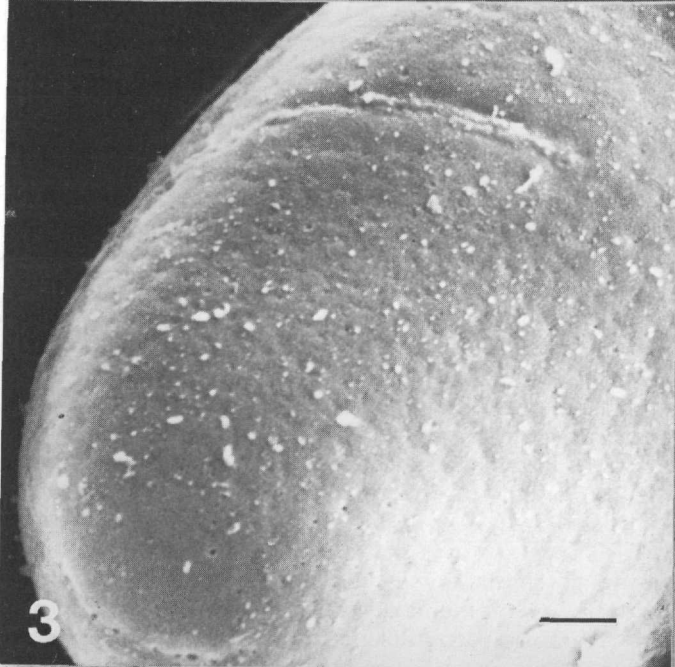
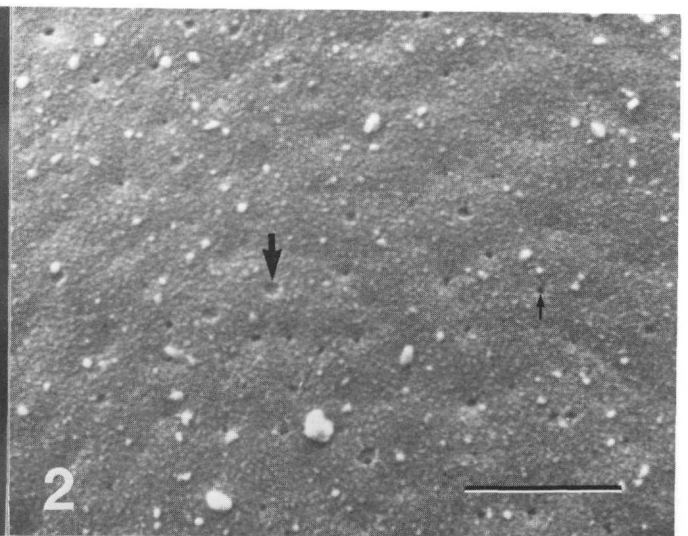
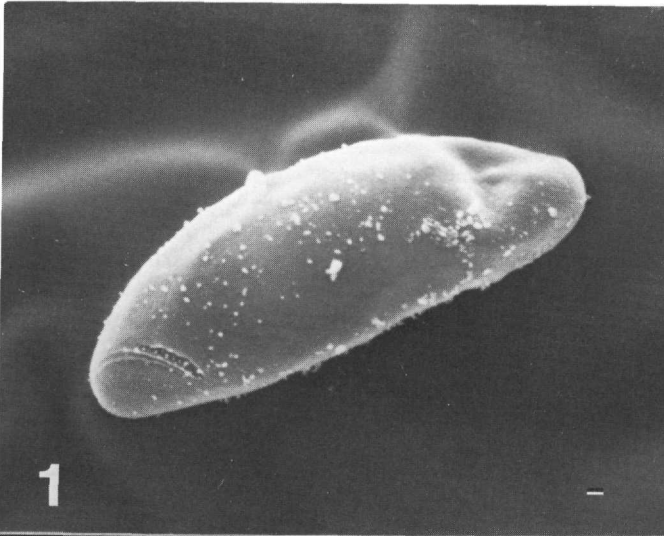
The basic pattern of the eggshell of *L. appendiculatum* was almost the same as that of *L. portentosae* with only minor differences. The opercular groove was oblique, incomplete, and terminal (Fig. 7). The pits on the eggshell were numerous, but not as sharply defined as in *L. portentosae* (Fig. 8). The operculum groove was surrounded by two narrow but slightly salient zones. As in *L. portentosae*, the eggshell also was undulated slightly and had many randomly situated pores and pits (Fig. 9).

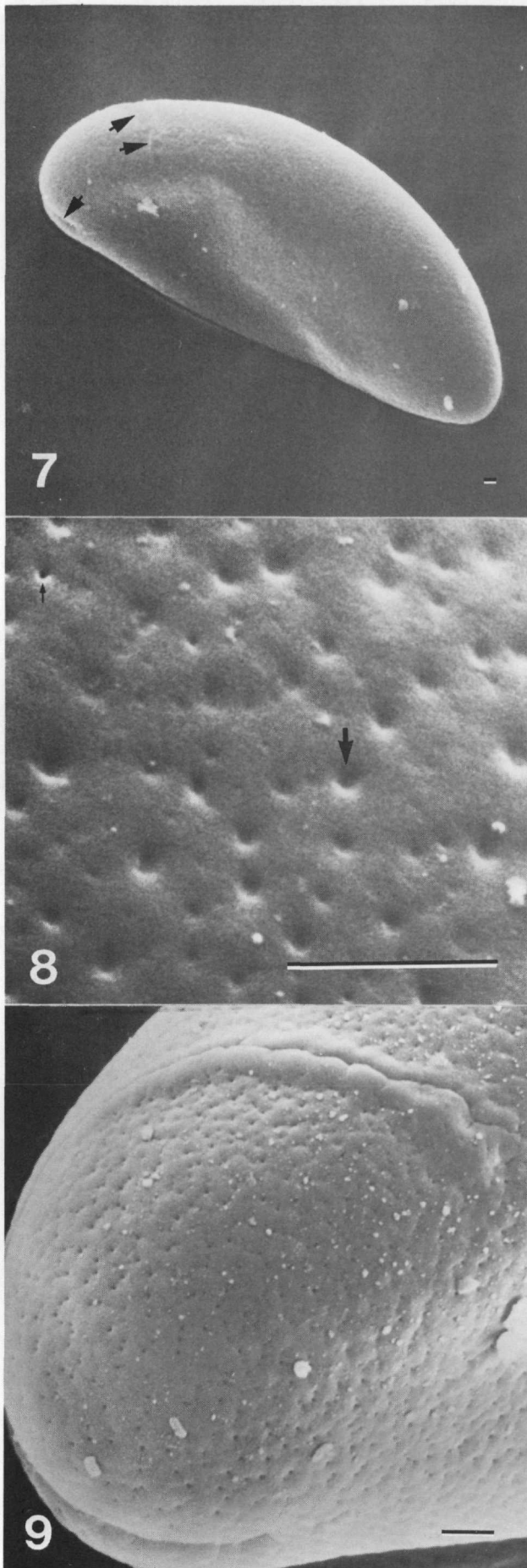
## DISCUSSION

Wharton (1975) noted that the eggshell of *L. appendiculatum* had a structure similar to that of *Hammerschmidtella diesingi*. Both have numerous spaces in the outer layer which open to the exterior via pores. However, he did not give any detailed description and no photographs or drawings were included. *Hammerschmidtella diesingi* is the only species in the family Thelastomatidae in which the eggshell has been studied by SEM (Wharton 1979, Yu and Crites 1985). Wharton (1975, 1979) described the five-layer structure of the eggshell of *H. diesingi*. The internal uterine layer contains discrete spaces which open to the exterior via pores. Yu and Crites (1985) further described the pits and the appearance of the opercular groove on the eggshell of *H. diesingi*.

<sup>1</sup>Manuscript received 4 December 1987 and in revised form 20 September 1988 (#87-58).

<sup>2</sup>Present address: Dept. of Molecular Biophysics and Biochemistry, Yale University, 260 Whitney Ave., P.O. Box 6666, New Haven, CT 06511.





The actual shape, size, and surface topography of the eggshell of *L. portentosae* and *L. appendiculatum*, especially the latter, are close to those of *H. diesingi*. The pores and pits on the eggshell surface of *L. appendiculatum* are almost the same as those of *H. diesingi* (Yu and Crites 1985). In both, the pits are not sharply delineated and the texture of the eggshell surface is smooth. The morphology of the opercular groove of *L. appendiculatum* is also similar to that of *H. diesingi*. Both have two narrow salient zones surrounding the opercular groove.

It is not surprising that *L. portentosae* and *L. appendiculatum* have the same basic pattern (i.e., incomplete opercular shape, etc.) for the opercular groove, as they are two closely related species. The two species differ in such detailed characteristics as the morphology of the pits, the texture of the eggshell surface, and the width of zones surrounding the opercular groove.

There are two major differences in the eggshells of the three species. First, the opercular groove of the eggshell of *L. portentosae* and *L. appendiculatum* is an incomplete circle rather than a complete one as in *H. diesingi*. Second, the opercular groove in *L. portentosae* and *L. appendiculatum* is inclined at a much greater angle to the central axis of the egg, but not in *H. diesingi*.

For all of the specimens examined, the characteristics of the pores, the pits, the operculum, the surface texture, and so on, are constant within a species. This indicates that detailed eggshell surface morphology can be added into the characteristics of the species.

It is also interesting that the opercular groove deepens gradually during the course of egg development from the embryo to the second-stage larva. In *L. portentosae*, the opercular groove is very shallow just after egg laying and the embryo has only one or a few cells. Later, the outermost layer of the groove gradually disappears as the groove surface sinks in. This results in a deeper groove with two sharp edges. The process of groove deepening continues as the first-stage larva develops to a second-stage larva. In the final stage of larval development inside the eggshell, the groove becomes very deep. Presumably only a very thin layer is left to maintain the operculum on the eggshell. The final opening of the operculum probably needs some signal which may be given by the next host, since the eggs usually do not hatch until they are ingested by another host. Van der Gulden and Van Aspert-van Erp (1976) suggested that the hatching of oxyurid eggs in-

FIGURES 1-6. Scanning electron micrographs of *Leidyndema portentosae* eggs. All scale bars = 2  $\mu\text{m}$ . 1, an entire egg showing the opercular groove; 2, a close view of the eggshell surface showing the pores (small arrow), pits (larger arrow), and the slightly undulated sand-like surface; 3, the operculum area of the egg, showing the incomplete opercular groove which is shallow during the early embryonic development; 4, a close view of the opercular groove at the apex of the eggshell; 5, a deeper opercular groove with two sharp edges in the middle of prehatching development; 6, a further deepened opercular groove at the end of the prehatching development.

FIGURES 7-9. Scanning electron micrographs of *Leidyndema appendiculatum* eggs. All scale bars = 2  $\mu\text{m}$ . 7, an entire egg showing the opercular groove (arrows); 8, a close view of the eggshell surface showing the pores (small arrow), pits (large arrow), and smooth surface; 9, the operculum area of the egg.

volves an increase in the permeability of the eggshell followed by the opening of the operculum. The results of this study have further suggested that changes in the characteristics of the opercular groove during prehatching development could also have a critical role in the hatching of the egg.

ACKNOWLEDGMENT. We thank J. M. McCabe who supplied the host cockroaches for this experiment.

#### LITERATURE CITED

- Van der Gulden, W. J. I. and A. J. M. Van Aspert-van Erp 1976  
*Syphacia muris*: water permeability of eggs and its effect on hatch-  
ing. *Experimental Parasitol.* 39: 40-44.
- Wharton, D. A. 1975 Structure and function in an Oxyurid egg. *Parasitol.* 71, xxii.
- \_\_\_\_\_ 1979 The structure and formation of the egg shell of *Hammerschmidtella diesingi* (Nematoda: Oxyuroidea). *Parasitol.* 79: 1-12.
- Yu, X. and J. L. Crites 1985 Surface topography of *Hammerschmidtella diesingi* (Nematoda: Oxyuroidea) eggs using scanning electron microscopy. *Ohio J. Sci.* 85: 266-267.
- \_\_\_\_\_ and \_\_\_\_\_ 1989 The prehatching development of *Leidynema portentosae* (Nematoda: Oxyuroidea). *Proc. Helminthological Soc. of Washington*; in press.
-