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Scanning Electron Microscopy of the Mosquito Parasite, *Reesimermis nielseni* (Nematoda: Mermithidae)

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Reprinted from Proceedings of The Helminthological Society of Washington
Vol. 41, No. 2, July 1974
p. 173–177
Made in United States of America

Scanning Electron Microscopy of the Mosquito Parasite, *Reesimermis nielseni* (Nematoda: Mermithidae)[1]

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ABSTRACT: The external anatomy of parts of the male and infective stage of the nematode parasite of mosquitoes, *Reesimermis nielseni*, is described using the scanning electron microscope. Noteworthy findings include: a fine annulation of the cuticle, the large earlike amphid openings in the male, three volcanolike pits probably containing the nerve endings in each cephalic papilla, bifurcation and structure of caudal papillae, the presence of two spicules in the male, and a tooth and a large amphid opening in the preparasitic or infective-stage juvenile.

Reesimermis nielseni Tsai and Grundmann is an important mermithid nematode parasite which causes the death of over 30 species of mosquitoes (Petersen and Willis, 1971). Field tests are currently under way in many parts of the world utilizing this parasite as a biological control agent of pest mosquitoes. Tsai and Grundmann (1969) and Nickle (1972) have studied this nematode with the light microscope. The current authors have studied the male and preparasitic infective stage of this nematode using the scanning electron microscope (SEM). A review of the literature shows that no mermithids have been viewed using SEM.

[1] The authors express their sincere appreciation to W. J. Humphries and Ben Spurlock (University of Georgia Electron Microscope Laboratory) for their suggestions, and the use of equipment and facilities.

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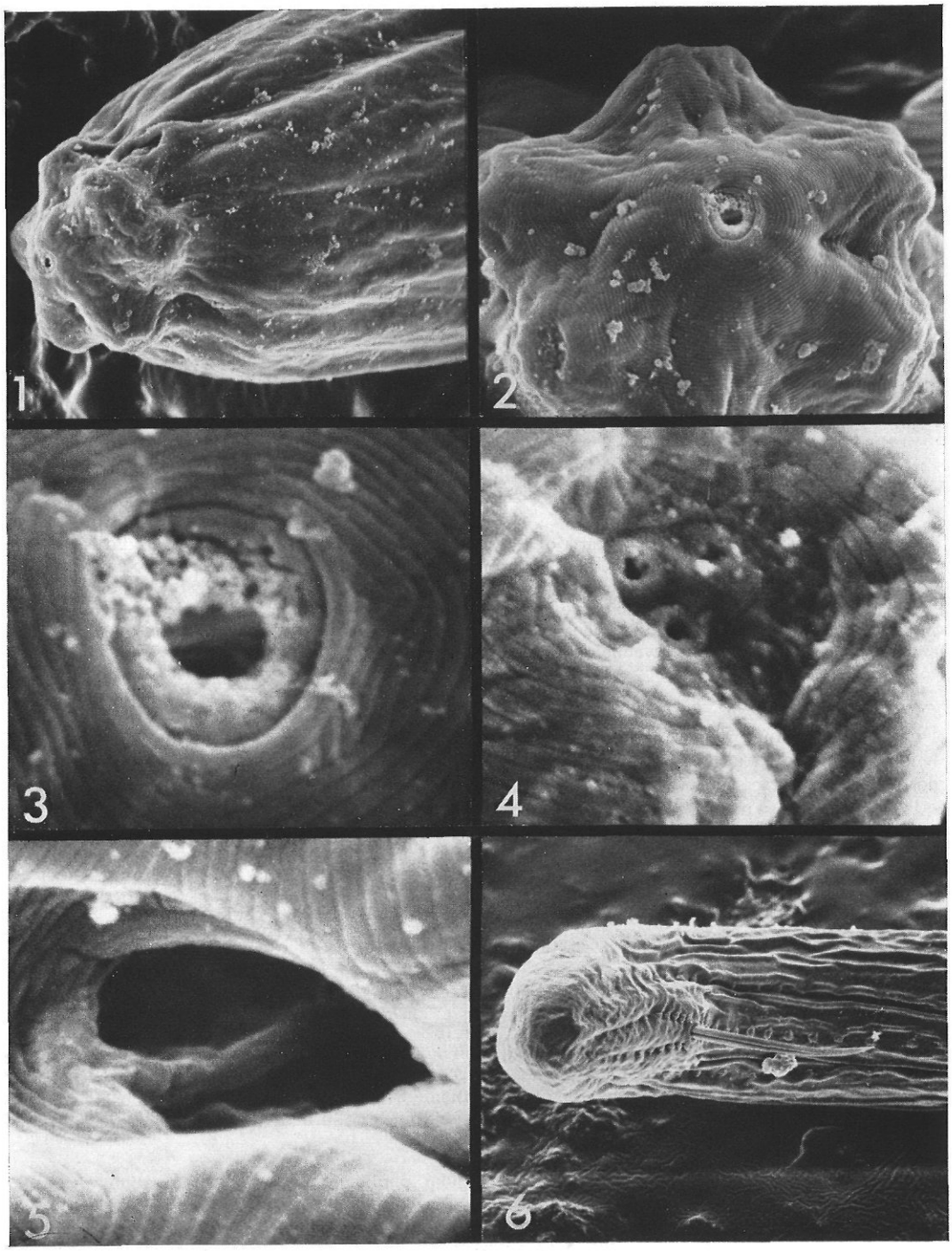
Figures 1–6. 1. Anterior end of male *Reesimermis nielseni* (× 1,650). 2. Face view showing oral opening, cuticular pattern, six lips and cephalic papillae (× 3,900). 3. Close-up of the symmetrically circular oral opening of the male (× 18,400). 4. Cephalic papilla in male with three volcanolike pits, probably containing the nerve endings (× 18,000). 5. Large earlike amphid opening in male (× 1,800). 6. Male tail showing two spicules (× 450).

Figures 7–12. 7. Male tail showing bifurcation of caudal papillae (× 430). 8. Bifurcation of caudal papillae (× 1,800). 9. Caudal papillae (× 9,000). 10. A single caudal papilla (× 18,000). 11. Anterior end of preparasitic infective-stage juvenile showing protruding tooth and amphid opening (× 8,700). 12. Dorsal/ventral view of preparasitic infective-stage juvenile showing tooth (× 8,700). [This specimen was dehydrated in acetone, air-dried, and coated with gold (Stone and Green, 1971)].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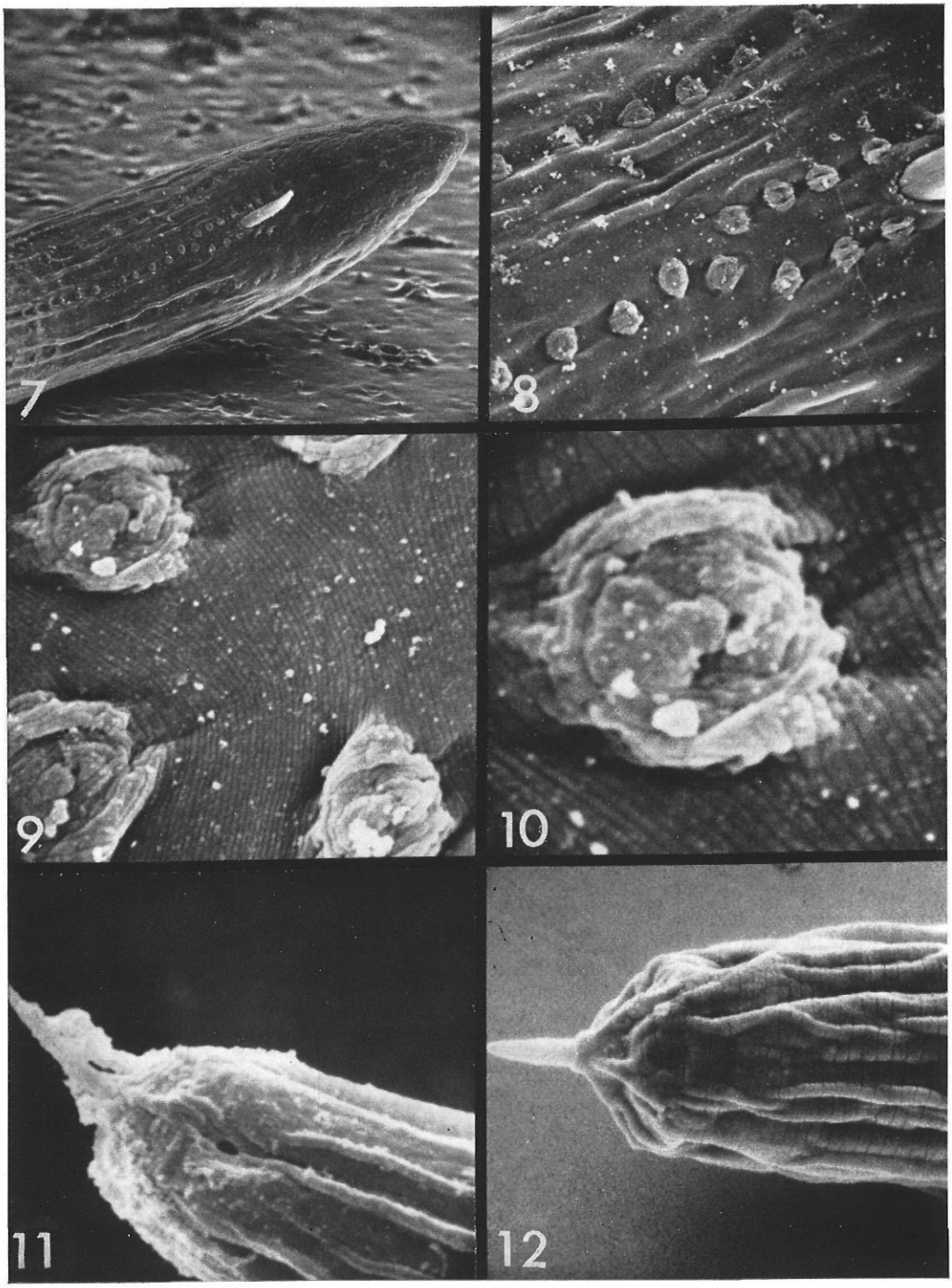
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Materials and Methods

The nematode specimens were obtained from the bottom sand in an aquarium about 2 weeks after the postparasitic larval mermithids emerged from the host mosquito, *Culex pipiens quinquefasciatus* Say.

The specimens were prepared for scanning electron microscopy by a modified critical point-drying method (Hayatt and Zirkin, 1973; Högger and Bird, 1974). In this procedure biological specimens are dehydrated in ethanol or acetone; the ethanol or acetone respectively is replaced by a transitional fluid (here liquid CO_2), which upon heating to its critical point (31C for CO_2) transforms without interface from the liquid to the gaseous state leaving the specimens dry with little or no distortion. Nematodes were relaxed by gentle heat and were fixed in 2.5% formaldehyde, 1% glycerine. An incision was made in the large males about in the middle of the body to allow better penetration of dehydration fluids. Specimens were washed by shaking them in a 0.5% Kodak Photo Flo® solution for 1 min. Dehydration was initiated by placing the nematodes in 20% ethanol in a dish standing above absolute ethanol in a sealed container at 42 C overnight, similar to Stone and Green's (1971) acetone technique. On the following day absolute ethanol was added slowly to the solution containing the nematodes. Then two changes of absolute ethanol were made. In preliminary preparations *R. nielseni* males collapsed irreversibly during more rapid changes of ethanol concentrations. Also for this reason amyl acetate was omitted, in contrast to earlier investigations (Högger and Bird, 1972). The specimens, immersed in absolute ethanol in a brass well covered with 25-μm and 1-mm screens, were transferred to a pressure chamber which was subsequently filled with liquid CO_2. After a 10-min equilibration period, the chamber was flushed with liquid CO_2 for 10 mins to remove the ethanol. The chamber was heated to 42 C at 180 atm. Then the CO_2 was released slowly and dry specimens were obtained. These were hand-picked and stuck to an SEM specimen carrier. The glue was made by washing the adhesive from adhesive tape with ethyl acetate, similarly to the Stone and Green (1971) procedure. The thin sticky film on the carrier prevented the specimens from being blown off in subsequent operations. The whole adhesive layer of tape is too thick and nematodes become submerged in it. At the end of the dehydration period the juveniles often stuck irretrievably to the bottom of the small Syracuse watch glass in which they had been processed. Therefore, a small round cover glass was used as a false bottom on which the specimens then settled. This allowed the majority of specimens to be transferred at once to the brass well. After drying, the cover glass was glued to an SEM specimen carrier with electrically conductive silver paint. The specimens on the carrier were coated with gold under vacuum and observed with a Mark II Cambridge scanning electron microscope at 30kv.

Acetone permitted faster dehydration. Collapse resulting from too steep a gradient was reversible in a lower concentration. This was not the case when ethanol was used.

Juvenile specimens dehydrated in acetone and then air-dried according to Stone and Green (1971) shrank more than those which were critical point-dried after acetone or ethanol dehydration. Compare Figures 11 and 12.

The main problem in all techniques was foreign matter, probably from the habitat. Dirt may have in part become attached to the specimens during the routine formalin fixation for light microscopy. Therefore, it appears to be advantegeous to wash the specimens before fixation (Högger and Bird, 1974).

Results and Discussion

Most SEM studies concentrated on the male of *R. nielseni* and some pictures were taken of the much smaller preparasitic juvenile. Figures 1 and 2 show the male front end with the six lips, cuticle finely striated on the surface (not crisscross), the symmetrically circular oral opening Figure 3, a large amphid, and the six cephalic papillae. Figure 5 shows a close-up of the large earlike amphid and Figure 4 under high magnification shows three volcanolike pits probably containing the nerve endings in a single cephalic papilla. The original description of *R. nielseni* stated that there was only one spicule; however, Nickle (1972) emended this description to show the

presence of two spicules as shown in Figure 6. The caudal papillae form a double row around the cloacal opening as shown in Figures 7 and 8. Individual caudal papillae are seen in Figures 9 and 10.

Figures 11 and 12 show the anterior end of the preparasitic juvenile infective stage of *R. nielseni*. This stage is very slender and difficult to view under the light microscope. The tooth used to penetrate the mosquito wriggler and the large amphid opening which has not been reported before, are easily seen. The cuticle is superficially finely annulated as in the adult male (Figs. 9, 10).

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