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Caudal Papillae in *Romanomermis culicivorax*[1]

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Abstract: Distribution of caudal papillae in adult *Romanomermis culicivorax* was determined by scanning electron microscopy. Ninety eight caudal papillae, each containing one pore, were present in males but absent in females. Papillae were arranged in three longitudinal rows, one ventral, two ventrolateral; the middle ventral row bifurcated anterior to the spicule. The appearance of the papillae was different anterior and posterior to the spicule. The role of the caudal papillae in mediating copulatory behavior was discussed.

Key words: caudal papillae, nematode, *Romanomermis culicivorax*, scanning electron microscopy.

The nervous system of the mermithid nematode, *Romanomermis culicivorax*, contains two systems of neurons, one of which secretes catecholamine(s) (4), the other a peptide with the immunocytochemical properties of the molluscan neurohormone FMRF-amide (5). Copulatory behavior may be regulated by catecholaminergic neurosecretions (6).

In order to mediate copulatory behavior, particularly attraction between the sexes, the nematode must possess a well-defined system of sensory organs, capable of responding to external stimuli. The most likely location of such sense organs, in addition to the head tip, a site of sensory activity in other nematodes (1), would be in the tail region, where sex-related differences in the distribution of neurosecretory sites has been observed. In contrast to the male, the tail of the adult female contains clusters of peptidergic ganglia (5). However, the tail of the adult male contains a much larger number of catecholaminergic ganglia than does that of the female (4).

The nature of the sensory organs in *R. culicivorax* requires elucidation. Wright and Richter (12) described the arrangement of anterior sense organs in this nematode, but no information is available on the distribution of caudal sense organs. In this study, we provide a description of the distribution pattern of the caudal sense organs in both sexes of the adult nematode.

MATERIALS AND METHODS

Source of nematodes: Romanomermis culicivorax was reared in vivo following the technique of Platzer and Stirling (10), but by propagating it through the larval mosquito *Aedes aegypti* rather than *Culex pipiens*. Immediately after emergence from the host, free-living post-parasitic nematodes were collected in a petri dish (9.0 cm d) containing sterilized water and kept in an incubator at 27 C. Under these conditions, molting to the adult stage occurred about 5 days after emergence from the host.

Scanning electron microscopy: Immediately after molting, 20 adult males and 20 adult females of *R. culicivorax* were killed by transferring them to a small beaker containing distilled water, which was surrounded by another beaker filled with water (100 C). Nematodes were heat treated for 2–3 minutes, or until the specimens assumed the almost straight form characteristic of heat-death (3), then transferred into small vials containing Karnovsky's fixative (7) in 0.2M Plumel's cacodylate buffer (PCB; 2) and fixed for 1 hour at room temperature (24–27 C). After several rinses in PCB, specimens were post-fixed in 1% (v/v) osmium tetroxide (J. B. EM. Services, Inc. Dorval, Quebec, Canada) in 0.2M PCB for 2 hours at room temperature. After several rinses in PCB, specimens were dehydrated through a

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graded series of ethanol (35, 50, 70, 80, 90%), spending 5 minutes in each, then in 100% ethanol for 1 hour. Dehydrated specimens were critical-point dried using liquid carbon dioxide in a Polaron E 3000 critical-point drying apparatus (J. B. EM. Services, Inc.). Nematodes were attached to two separate aluminum stubs (J. B. EM

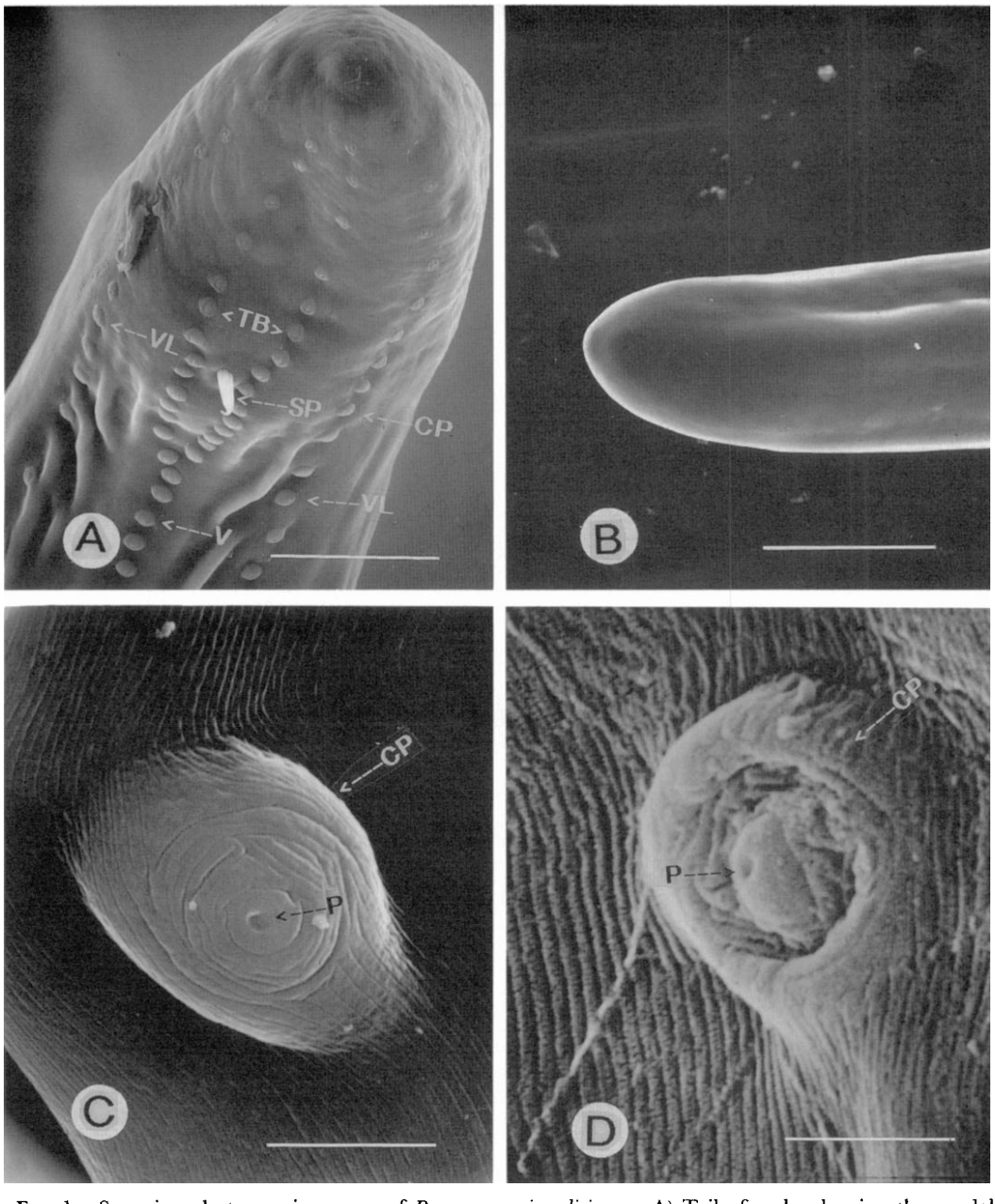

FIG. 1. Scanning electron microscopy of *Romanomermis culicivorax*. A) Tail of male, showing the caudal papillae arranged in three longitudinal rows, one ventral and two ventrolateral; the middle ventral row bifurcated anterior to the spicule. Scale bar = 34 μm. B) Tail of female. Note the absence of caudal papillae. Scale bar = 80 μm. C) Caudal papilla of male, anterior to the spicule showing flattened center with only one pore. Scale bar = 2.40 μm. D) Caudal papilla of male, posterior to the spicule. Note the invaginated center of the papilla. Scale bar = 1.41 μm. CP, caudal papilla; P, pore; SP, spicule; TB, terminal branches of middle ventral row; V, ventral row; VL, ventrolateral row.

Services, Inc.), then gold-coated in an Edwards Model 150A sputter-coater (Edwards High Vacuum, West Oakville, Ontario, Canada). Gold-coated specimens were examined with a Hitachi S570 Scanning Electron Microscope (SEM) operated at an accelerating voltage of 20 kV. Photographs were taken of the tail regions of both sexes.

Results and Discussion

Ninety eight caudal papillae were arranged in longitudinal rows on the surface of the tails of all males examined (Fig. 1A), but were not present on female tails (Fig. 1B). Three rows (two ventrolateral, one ventral) were present until 12.0 ± 0.49 μm (8.0–14.0 μm) anterior to the spicule, at which point the middle ventral row bifurcated. Thus, in the tail tip of the nematode, caudal papillae were arranged in four rows. Each ventrolateral row contained 24 serially arranged papillae, whereas the ventral row contained a total of 50, with 15 pairs of papillae situated in the terminal branches (Fig. 1A). Each caudal papilla had only one pore (Fig. 1C,D). The papillae were dimorphic: those anterior to the spicule had flattened centers (Fig. 1C), whereas the centers of the papillae posterior to the spicule were invaginated (Fig. 1D). The caudal papillae extended from 104.0 ± 2.35 μm (89.0–127.0 μm) anterior to the tail tip almost to the tail tip. The mean distance between each row at the anterior extremities was 8.0 ± 0.28 μm (6.0–10.0 μm) and between the two bifurcated middle rows, measured across the spicule, 5.0 ± 0.21 μm (4.0–7.0 μm).

The distribution and number of catecholaminergic ganglia in the tail region of adult *R. culicivorax* is sex-related. The male possesses 16–20 such ganglia, whereas only two are present in the tail of the female (4). Concentration of catecholamines in these ganglia in males is influenced by the stage of development of the nematode and by the presence of the opposite sex (6). Assuming that the ganglia and papillae in the tail region are functionally interconnected, it seems reasonable to propose that the caudal papillae of the male function as sense organs responsible for regulating copulatory behavior. Such a role was proposed for these structures in other nematode species (8,9,11).

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