# Teratological Development in the Cephalic Anatomy of the Nematode Romanomermis culicivorax[1]

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*Abstract:* Adults of *Romanomermis culicivorax* obtained from mass cultures were examined by scanning and transmission electron microscopy to determine the organization of their anterior sense organs. The normal pattern apparently consists of two lateral amphids plus six cephalic papillae. Lateral cephalic papillae contain two sense organs, each with a cuticular pore, while subdorsal and subventral papillae have three sense organs, each with a cuticular pore. About 30% of females and 80% of males examined showed aberrant developments in these sense organs. Both cuticle and underlying cells (hypodermis and neurons) are affected; some sense organs are absent while others are incompletely formed. Few aberrant worms were found in a smaller collection of worms reared in lower population densities. Perhaps aberrant forms are examples of teratological development resulting from, or promoted by, conditions used for mass rearing of biological control agents. *Key words:* teratology, sense organs, electron microscopy, *Romanomermis culicivorax*.

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Nematode development is generally considered to be strictly genetically controlled. Indeed, head structures in various groups are therefore of considerable taxonomic value. Yet changes in differentiation of the reproductive systems of mermithids and *Meloidogyne* spp. can result from adverse environmental conditions (6). Such changes can be considered as teratogenic. Johnson and Viglierchio (2) recorded a teratogenic larva of *Heterodera schachtii* resulting from treatment with a juvenile hormone mimic, and Ellington and Golden (1) recorded abnormal numbers and locations of phasmids on 15–18% of a collection of *Hoplolaimus concaudajuvencus*.

During an electron microscope study of cephalic sense organs of the mermithid nematode, *Romanomermis culicivorax* Ross and Smith 1976, a significant number of specimens were found showing varying degrees of abnormal development of head tissues. A comparison of specimens from two culture situations suggests that abnormal development may have arisen or occurred with greater frequency as a result of crowded growth conditions.

## MATERIALS AND METHODS

Sand cultures of *R. culicivorax* were obtained from the Gulf Coast Mosquito Research Laboratory of the United States Department of Agriculture, Lake Charles, Louisiana, in 1978 and 1979. Upon arrival in Toronto, they were immediately refrigerated at 5 C. When worms were to be recovered, samples were taken from the cold so that the bulk of the culture remained refrigerated.

Adult worms were rinsed in fresh dechlorinated tap water prior to fixation. Only actively coiling nematodes were selected. Males and females were fixed separately in about 1.5% glutaraldehyde in cacodylate buffer (osmotic value adjusted to 150 milliosmols). The tips of the worms' heads were cut off in fixative. Fixation was carried out at room temperature for up to 24 h. Tissues were then repeatedly washed in cacodylate buffer (150 milliosmols) and post-fixed for 1–2 h at room temperature in 1% osmium tetroxide in cacodylate buffer. Following ethanol dehydration, worms were processed for scanning or transmission electron microscopy. For scanning microscopy, specimens were transferred gradually to Freon TF and critical point dried from liquid $CO_2$. Specimens were metal coated (gold-palladium) by either evaporative or sputter methods. For transmission microscopy, tissues were embedded in Spurr's medium. Sections were stained sequentially with uranyl acetate and lead citrate.

## RESULTS

It was soon evident by scanning electron microscopy that considerable care was needed to prevent specimens from col-

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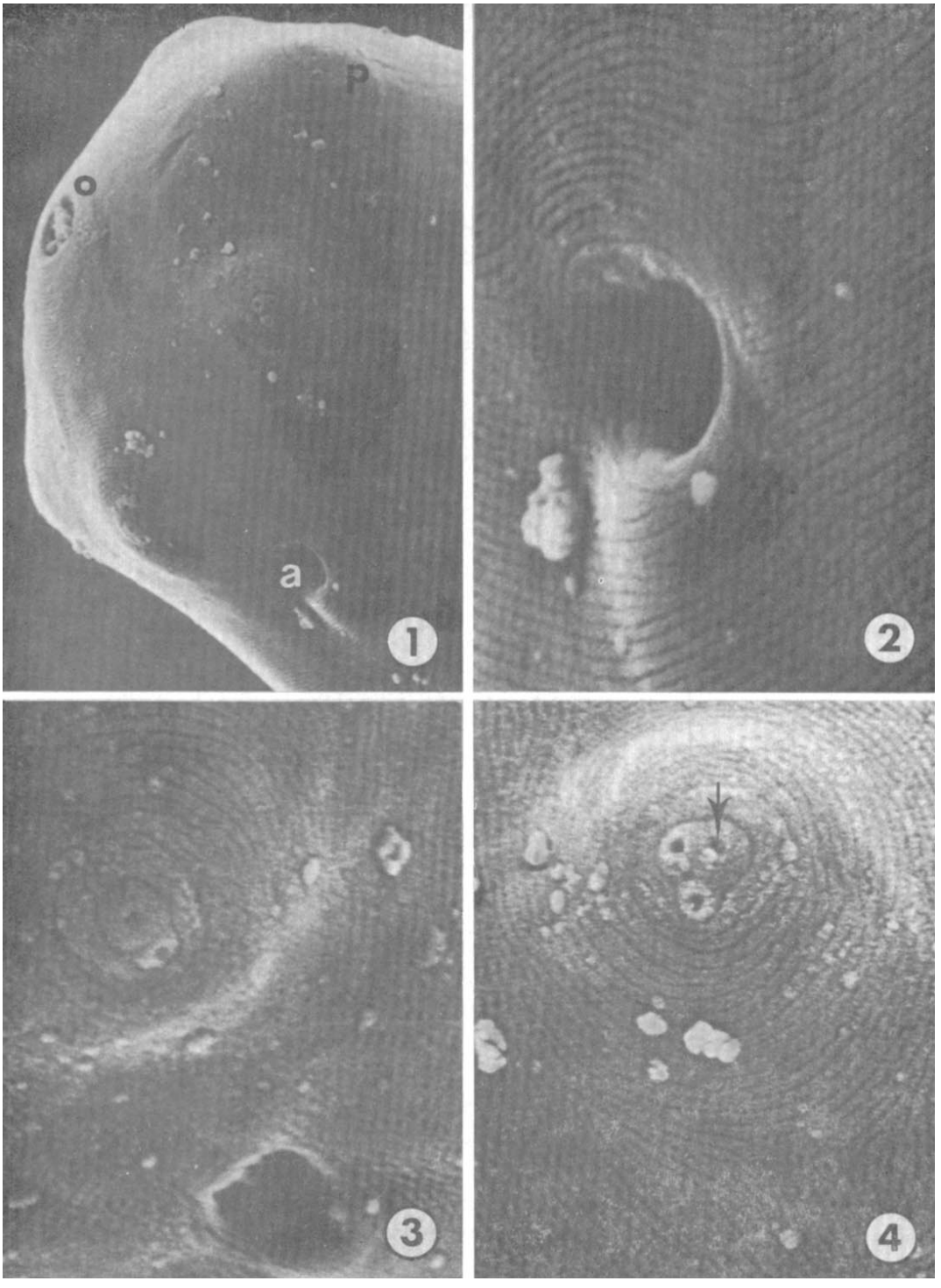
lapsing. Longer fixation in 150 milliosmol glutaraldehyde, gradual ethanol dehydration and transfer through Freon TF, followed by critical point drying usually gave good results. The heads of most females had a centrally located oral opening. Around the outside of the head, six rounded papillae gave the head a hexagonal form (Fig. 1). The large amphidial openings (about 1.6 $\mu$m across) occur just posterior to the cephalic papillae (Fig. 2). Small pores (about 0.2 $\mu$m) (Figs. 3, 4) were located at the apex of each papilla. Each lateral papilla had two pores, one anterior to the other, and the remaining papillae had three pores arranged with one pore more anterior (Figs. 3, 4); presumably these are openings of the cephalic sense organs. Cuticle posterior to the amphid had fine circular annulations (about 0.15–0.2 $\mu$m per annule). Annules around the papillae were arranged concentrically around the pores (Fig. 4). Similar annulations surround the oral opening. This description is assumed to apply to normally developed males and females.

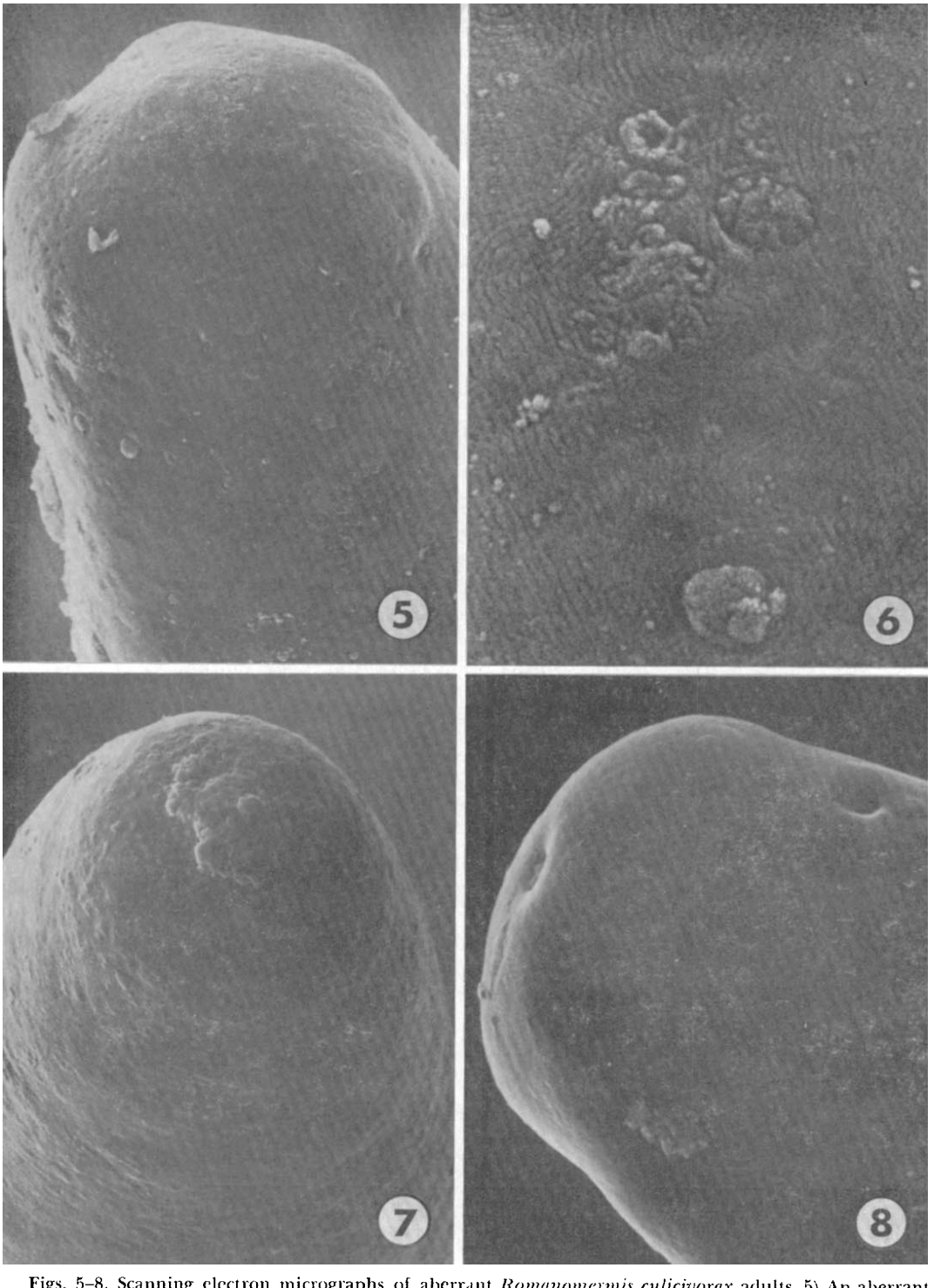
About 30% of the females (7 of 24) and 80% of the males (17 of 21) showed a range of aberrant patterns, although all had been selected as active mature adults. Five males were observed that had no cuticular annules, no amphidial or cephalic sense organ pores, not even an oral opening (Figs. 5, 7). Their heads were variously rounded, lacking definite hexagonal form. Another five males lacked openings of sense organs although in one or two a depression may have marked the site of an amphid. Their oral opening was present, but shifted out of the central position, and although some sculpturing of the cuticle surface was visible, it was variously irregular (Fig. 6). Another male was similar to these but had one amphid and a centrally placed oral opening, while another had one cephalic papilla with two pores and two oddly shaped groups of three pores. Its oral opening was a central but incomplete depression. The last male had two amphidial pores, three cephalic papillae with three pores, one papilla with two pores, a misplaced oral opening, and irregular annulations. Females showed similar aberrations, including lack of cuticular annulation, absence of amphidial pores and/or cephalic sense organ papillae and pores, and displacement of the oral opening (Fig. 8).

Transmission electron microscopy of adults from the mass cultures showed a surprising range in development of internal anatomy. Some worms had a "normal" body wall with the cuticle underlain by a thin hypodermis and well-developed longitudinal muscles. In these worms, nerves were identified and some aspects of the internal anatomy of the cephalic sense organs were determined. Amphids contained 11 dendritic processes in a cuticle-lined canal. Doublets of microtubules and a distinctive ciliary necklace region occurred near the base of these processes, but no true basal bodies were seen. The number of sensory nerve cells was not determined, but the tips of dendrites showed dense junctional complexes with membranes of the adjacent cell (presumably the sheath cell) indicating the formation of a receptor cavity. The cephalic sense organs contain similar sensory dendrites and sheath cells. Although all details of the sense organs were not fully defined, it appears that each papilla contains either two or three groups of sensory dendrites, each associated with a cuticular pore and receptor cavity surrounded by a sheath cell (Fig. 9). Significant observations could be drawn from 8 of the 12 worms sectioned. Four of the five females appeared to have complete sense organs, while in one, only one amphid, no other sensory elements, and no anterior body musculature was identified. All three males sectioned showed internal abnormalities and it was difficult to identify sensory units or assign them to elements of a "normal" pattern. Although sensory neurons, sheath cells, and receptor cavities were sometimes identifiable, pores through the cuticle seemed absent. In one worm, tissue surrounding the esophagus was broken and contained many irregular vesicles while in another, all tissues were broken and vesiculated.

A selection of male and female worms supplied by Dr. T. Galloway of the University of Manitoba were examined by the same scanning electron microscopy techniques (worms had been previously fixed in a formalin-acetic acid-alcohol fixative but




Figs. 1–4. Scanning electron micrographs of normal *Romanomermis culicivorax* females. 1) The head showing a central oral opening (o), one of the amphids (a), and five of the six papillae (p). × 3,000. 2) An amphidial opening and annulations in the adjacent cuticle. × 11,000. 3) One of the lateral papillae with two pores. × 9,000. 4) A submedian papilla with three pores. Arrow indicates the more anterior, possibly inner labial pore. Note patterns of cuticular annulations. × 8,200.




Figs. 5–8. Scanning electron micrographs of aberrant *Romanomermis culicivorax* adults. 5) An aberrant male. Neither sense organ pores nor patent oral opening were evident. × 2,000. 6) Irregular sculpturing of the head cuticle of the male shown in figure 5. × 7,200. 7) A very aberrant male with encrusting materials around the site of the oral opening. × 1,700. 8) An aberrant female showing smooth cuticle, a central oral opening, one of two amphids, but no papillae. × 2,300.

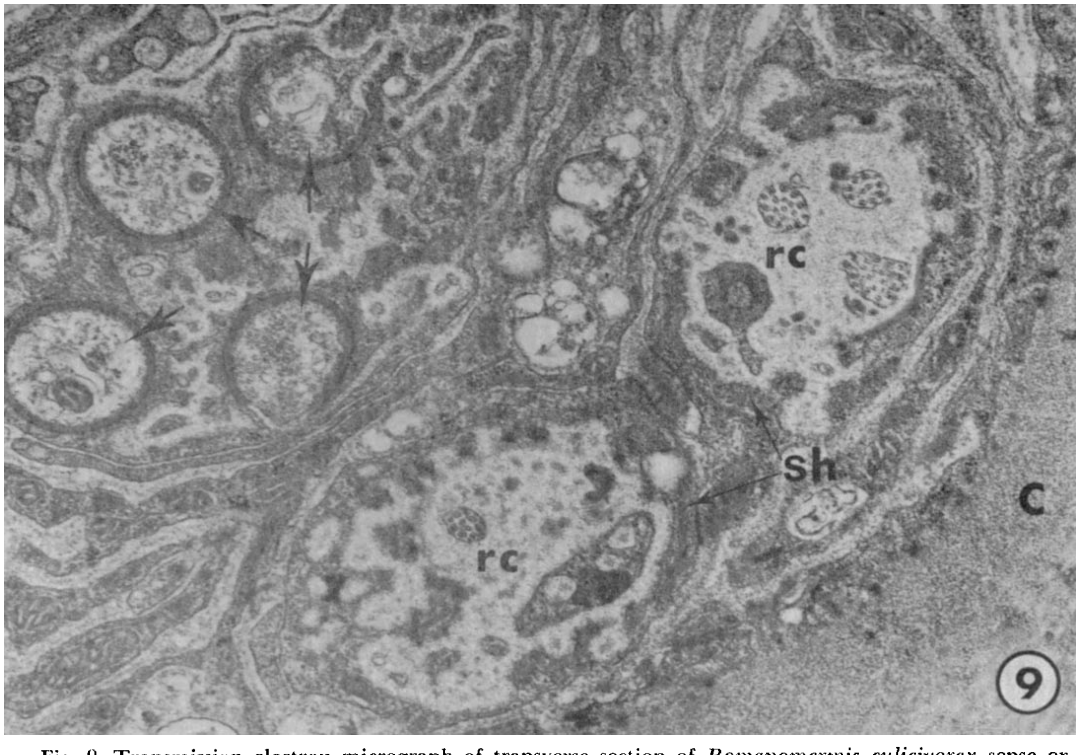



Fig. 9. Transmission electron micrograph of transverse section of *Romanomermis culicivorax* sense organs within a three-pored papilla. Arrows note the tips of four sensory dendrites that make dense junctional complexes with the adjacent sheath cell. Two separate receptor cavities (rc) contain sensory dendritic processes with microtubules and are surrounded by separate sheath cells (sh). C = cuticle. × 30,000.

were then post-fixed in osmium). All of 17 females had the complete complement of sense organ pores, a centrally placed oral opening, and regular cuticular annulations. Only 2 of 11 males showed aberrant patterns. One of these lacked amphidial and cephalic sense organ pores, while the other lacked amphidial pores and had only three groups of three cephalic sense organ pores. Both these males had irregular cuticular sculpturing rather than well-formed annulations.

## DISCUSSION

It is likely that those animals with symmetrical head patterns are normally developed. A previous SEM examination of males of *R. culicivorax* identified cephalic papillae with three pores (3), but the presence of only two pores in the lateral papillae was not noted, although they might have been anticipated from taxonomic studies. It is likely that each cuticular pore is associated with a separate group of sensory neurons, a sheath cell, and probably a socket cell, thus conforming to the basic form of sense organs in other nematodes (7). Possibly the complete complement of cephalic sense organs is present, as the inner or anterior pores may represent inner labial sense organs while the others may represent cephalic and outer labial sense organs. Thus "cephalic papillae" may be complexes of two and three sense organs: amalgamation of outer labial and cephalic sense organs has been noted previously in some animal parasites (7). The fact that all of the cephalic sense organs are associated with cuticular pores further supports the observation that adenophorean nematodes seem to have a higher chemosensory capacity than secernentian nematodes (7).

Anatomical patterns identified here as aberrant involve abnormalities in both cuticle and internal tissues. In some worms, cuticular pores may be absent, although cells of the sense organs may be present but in an unusual internal arrangement. In some specimens, only some of the groups of sensory elements could be identified. Cells comprising the internal tissues exhibited

organelles and cytoplasm, indicating that they were viable. However, two specimens showed tissues that appeared to be damaged or broken. Although these worms were mobile, the heads may have been locally damaged. This could have occurred through shearing action of shifting sand in which they were packed for shipping, similar to the reason suggested by Petersen and Levy (5) for loss of viability of worms or eggs during shipping. It is highly unlikely that the absence of cuticular pores and/or sensory neurons and the subterminal location of the oral opening could arise during the adult stage as a result, for instance, of aging. Cuticular pores are presumably formed only at molt periods. The abnormalities are more likely to be teratological development. Its prevalence is significantly increased in males.

When these findings were recognized in the Louisiana-reared worms, we obtained a small collection of preserved worms from Dr. T. Galloway, for comparison. These worms had been derived from a Louisiana mass culture by growth in a smaller colony of *Culex pipiens quinquifasciatus* at much lower population densities. It is interesting that many fewer abnormal worms (only males) were found. This suggests that teratogenesis may arise or at least be enhanced in crowded conditions, characteristic of mass cultivation systems required for biological control programs. Galloway (personal communication) has observed a variety of gross physical deformities in worms, presumably as a result of overcrowding or major temperature changes. Although large numbers of worms have been grown in laboratories, some problems have been encountered in shipping them and in establishing them commercially (4,5). The induction of teratological development during mass rearing of mermithids for biological control purposes should be considered as a possible factor in determining effectiveness of mass rearing techniques.

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