

Position of Pharyngeal Gland Outlets in Monhysteridae (Nemata)

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Abstract: In this study an attempt was made to determine the position of the outlets and nuclei of the pharyngeal glands in four monhysterid genera. Five *Eumonhystera* spp., seven *Monhystera* spp., and eight *Monhystrella* spp. were studied under the light microscope. Longitudinal sections of an undescribed *Monhystera* sp. and cross sections of *Geomonhystera disjuncta* were also studied under the scanning and transmission electron microscope, respectively. The results of the light microscopic studies were inconclusive about the position of the outlets but showed a number of nuclei in the basal part of the pharynx. The scanning and transmission electron microscopic studies revealed five pharyngeal glands and their outlets; their position was as follows: dorsal gland outlet at the base of buccal tooth, first pair of ventrosublateral gland outlets halfway along the pharynx, and second pair of ventrosublateral gland outlets close to the base of the pharynx. It is concluded that at least three, and possibly five, nuclei are in the basal part of the pharynx. This pattern, in the position of the outlets and nuclei, is similar to that in *Caenorhabditis elegans* (Maupas, 1900) Dougherty, 1953 and may well be the basic plan in the Class Chromadorea (including Secernentia as a subclass).

Key words: *Geomonhystera*, *Monhystera*, Monhysterida, morphology, nematode systematics, pharyngeal gland outlet, pharynx, scanning electron microscopy, transmission electron microscopy, ultrastructure.

The number, shape, and position of pharyngeal glands, and especially the position of their nuclei and outlets, are important features to establish phylogenetic relationships among nematodes at several taxonomic levels (2,4,5,10,13). Typically, nematodes have three or five pharyngeal glands (one dorsal and one or two pairs of ventrosublateral glands). Maggenti (12) considered the five glands to be an ancestral character found in Enoplia and the presence of only three glands in Chromadorea (including Monhysterida) as derived. Three glands in Secernentia was also considered derived. However, he further stated that five glands might be derived from three glands by duplication of the subventrals in some Chromadorea and Secernentia. On the other hand, examples exist in some taxa, e.g., Dorylaimida, where five glands have been reduced to three (Longidoridae) (14).

According to Chitwood and Chitwood (2), Chromadorea, such as Monhysterida, have only the dorsal gland opening at or

near the base of the stoma; the ventrosublateral (= "subventral") glands never open at the anterior end of the pharynx. This arrangement is in contrast to Enoplida, where a pair of ventrosublateral glands often opens through teeth in the stoma or at the anterior end of the pharynx, as the dorsal gland does.

Monhysterida are usually classified as an order in Chromadorea (11,12). Chitwood and Chitwood (2) presented diagrams on the position of pharyngeal nuclei in five monhysterid genera (*Desmolaimus* de Man, 1880, *Siphonolaimus* de Man, 1893, *Terschellingia* de Man, 1880, *Theristus* Bastian, 1865, and *Tripylium* Cobb, 1920). The nuclei of one dorsal and one pair of ventrosublateral glands were shown in the posterior region of the pharynx; the outlets of these glands were depicted at or close to the base of the stoma for the dorsal gland (all five genera), and close to the nerve ring for the ventrosublateral glands (in *Terschellingia*, *Theristus*, and *Tripylium*). This pattern agrees with that of Chromadorea in general.

Riemann (13) described the pharyngeal glands of Linhomoeidae, a family of Monhysterida. In *Paralinhomoeus* de Man, 1907 and *Anticyclus* Cobb, 1920, the orifices of one dorsal and two ventrosublateral glands

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are situated in the stoma region. In *Eleutherolaimus* Filipjev, 1922, an additional pair of ventrosublateral glands with orifices at the level of the nerve ring was described. Riemann (13) referred to a paper by zur Strassen (17) in which the latter author described ventrosublateral gland openings close to the anterior end of the pharynx in *Siphonolaimus*. On the basis of these observations, Riemann (13) proposed classifying Linhomoeidae and Siphonolaimidae in Siphonolaimoidea and to differentiate this superfamily from Monhysteroidea by the position of the pharyngeal gland outlets, among other characters. This proposition was accepted by Lorenzen (11).

Jacobs et al. (9) described three pharyngeal gland cells (one dorsal and a pair of ventrosublateral) in *Diplolaimella dievengatensis* Jacobs, Van de Velde, Geraert & Vranken, 1990, occurring at the base of the pharynx. The ventrosublateral nuclei were referred to as S_2N_1 and S_2N_2 in the text (p. 9–10) as well as on the illustration (Fig. 10H,J) (9). Another pair of smaller but still prominent nuclei were located just behind the nerve ring. These nuclei were referred to as S_1N_1 and S_1N_2 in the text (p. 10), where it was mentioned that "these were first interpreted as gland nuclei"; but S_1N_1 and S_1N_2 were still explained as "anterior set of ventrosublateral gland nuclei" in the legend to Fig. 10. Thus, there remains some ambiguity about the ventrosublateral nuclei.

Jacobs and Heyns (7) described five pharyngeal glands in *Monhystera coomansi* Jacobs & Heyns, 1992: one dorsal gland with nucleus (DN) at the base of the pharynx and the outlet (DO) at the base of the stoma, a first pair of ventrosublateral glands with nuclei (S_1N) just in front of the nerve ring and outlets (S_1O) at the base of stoma, and a second pair of ventrosublateral glands with nuclei (S_2N) between level of DN and nerve ring and outlets (S_2O) at level of nerve ring. Jacobs et al. (8) re-described three *Monhystera* species and gave the same interpretation as Jacobs and Heyns (7), with the only difference being

the positions of S_1N (at level or just posterior to nerve ring) and S_2N (near base of pharynx).

MATERIALS AND METHODS

Specimens for light microscope (LM) and scanning electron microscope (SEM) observations were fixed with hot ($\pm 60^\circ\text{C}$) 4% formaldehyde. Nematodes were extracted with centrifugal flotation from freshwater sediment of Lake Tana, Ethiopia. Ludox (50% silicasol [SiO_2] colloid solution) was used as an extraction agent. For SEM, nematodes were hand-sectioned longitudinally under a binocular microscope; the longitudinal sections then were dried with the critical point drying procedure and attached to self-adhesive tape, coated with gold, and studied under SEM, according to the procedures of Eyuaem and Coomans (6). Nematodes for transmission electron microscopy (TEM) were isolated from "Sluice Dock" Ostend, a man-made marine lagoon near the Belgian coast, and cultured according to Vranken et al. (16). Specimens were picked from petri dishes, cooled in an ice-bath to stretch, and then killed and fixed in an ice-cooled fixative composed of 1.5% acrolein, 3% glutaraldehyde, and 1.5% paraformaldehyde in 0.2 M sodium cacodylate buffer. After fixation and rinsing, the specimens were post-fixed in 2% osmium tetroxide, followed by *en bloc* staining in 2% uranyl acetate. Other details of the procedures of nematode preparation for TEM were those described in Van de Velde and Coomans (15).

RESULTS

Light microscopic observations on different Monhysterids, viz. *Monhystera* cf. *gabaza* Joubert & Heyns, 1980; *M. somereni* Allgén, 1952; four undescribed *Monhystera* spp.; *Monhystrella macrura* (de Man, 1880) Andrassy, 1981; *M. lepidura altherri* (Juget, 1969) Jacobs, 1987; six undescribed *Monhystrella* spp.; *Eumonhystera dispar* (Bastian, 1865) Andrassy, 1981; *E. filiformis* (Bastian, 1865) Andrassy, 1981; *E. pseudobulbosa* (von Daday, 1896) Andrassy, 1981; *E.*

mwerazii (Meyl, 1957) Andrásy, 1981; and *E. vulgaris* (de Man, 1880), Andrásy, 1981, did not yield conclusive evidence about the exact position of the pharyngeal gland outlets and nuclei. The dorsal gland nucleus (DN) is situated near the base of the pharynx, and the cell extends to the stoma region. The ventrosublateral sectors are often vacuolar, which makes it difficult to locate the gland nuclei. However, a pair of nuclei in sublateral to lateral position was found in most specimens slightly behind the level of DN. A second pair of such nuclei was seen, though less frequently, anterior to the level of DN at less than two pharynx widths from pharynx base. Still other nuclei occur and, apart from DN, it is impossible to decide whether the above-mentioned nuclei are really S_2N and S_1N . Glandular tissue in the ventrosublateral sectors was observed up to the nerve ring (Fig. 1).

Scanning electron microscopy of longitudinal sections of an undescribed *Monhystera* sp. revealed five outlets (Fig. 2). The dorsal outlet (DO) is a small (about 0.7- μm -long) slit just behind the small dorsal tooth (Fig. 2B). A first pair of ventrosublateral gland outlets (S_1O) occurs at 52–54% of the pharynx length, at the level of the nerve ring (Fig. 2A,C); a second pair (S_2O) occurs at 83–85% of the pharynx length (Fig. 2A,D). S_1O and S_2O are about 1- μm -long slits (Fig. 2). The tissue opposite S_2O is different from the remainder of the pharyngeal region. This area appears granular as opposed to the fibrillar (=muscular) overall appearance of the pharynx (Fig. 2A).

Transmission electron microscopy confirmed the position of DO and S_1O in *Geomonhystera disjuncta* (Bastian, 1865) Jacobs, 1987 (Fig. 3A,B) and *Diplolaimella divengatensis*. In the posterior part of the pharynx there are elaborate cytoplasmic areas composed of rough endoplasmic reticulum with wide cisternae and secretory granules. In the most basal part (at cardia level), the dorsal gland ends in a narrow middorsal extension (Fig. 4A). To the left and to the right of this extension, as well as

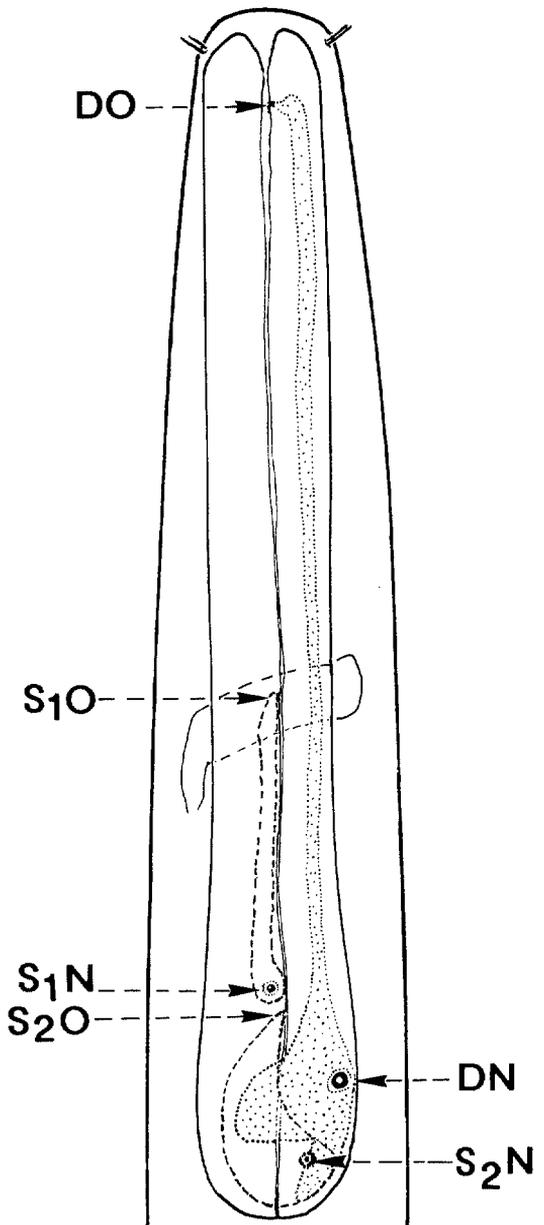


FIG. 1. Schematic representation of pharyngeal glands in monhysterids. DN: Dorsal gland nucleus, DO: Dorsal gland outlet, S_1N : Nuclei of the first pair of ventrosublateral glands, S_1O : Openings of the first pair of ventrosublateral glands, S_2N : Nuclei of the second pair of ventrosublateral glands, S_2O : Outlets of the second pair of ventrosublateral glands.

over the entire width of both ventrosublateral sectors, occurs the cytoplasm of the second pair of ventrosublateral glands. In this cytoplasm are two nuclei, one each in the left and right adradial dorsal sectors;



FIG. 2. SEM photographs of *Monhystera* sp.. A) Longitudinal section of pharynx. Arrow-heads point to outlets of both pairs of ventrosublateral glands, arrows point to glandular tissue at S_2O level. B) Stoma showing dorsal tooth (black arrow) and dorsal gland outlet (white arrow). C) Outlet of a ventrosublateral gland of the first pair (arrow). D) Outlets of second pair of ventrosublateral glands (arrows). Scale bars equal $10\ \mu\text{m}$ in A, $1\ \mu\text{m}$ in B-D.

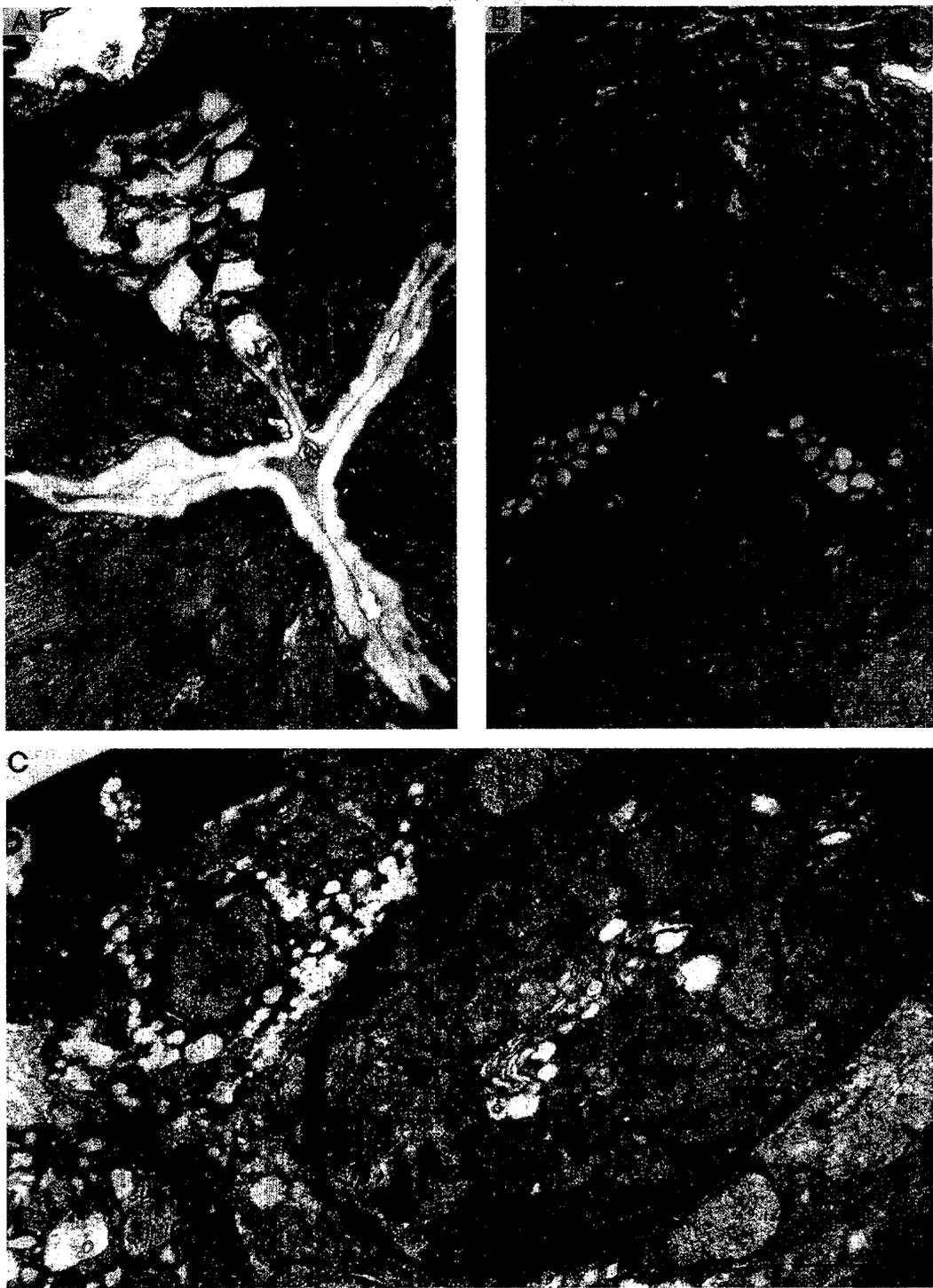


FIG. 3. TEM photographs of *Geomonhystera disjuncta*. A) Dorsal gland outlet (arrow). B) First pair of ventrosublateral gland outlets (arrowheads). C) Gland nuclei at base of pharynx. DN: dorsal gland nucleus; N.R.: = nerve ring; S₂N: nuclei of second pair of ventrosublateral glands. Scale bars equal 0.5 μm in A and B, 1 μm in C.



FIG. 4. *Geomonhystera disjuncta*. A) Cross-section at base of pharynx showing the two subdorsally located SN. B) Cross-section at level of DN, arrows indicate boundary between ventrosublateral gland tissue and dorsal gland tissue. CA: cardia; DG: dorsal gland extension; DN: dorsal gland nucleus; SN: ventrosublateral gland nuclei. Bar equals 1 μm .

they represent the S_2N (Figs. 3C; 4A). More anteriorly, the cytoplasm of the second pair of ventrosublateral glands is shifted ventrally due to a considerable extension of the dorsal gland, which now occupies the whole dorsal sector and the lateral parts of the ventrosublateral sectors. At this level DN is found (Figs. 3C; 4B).

DISCUSSION

The observations reported here are in agreement with some of the literature on *Monhystera* (7,8) for the presence of five pharyngeal glands and the position of DO and DN, but not for the other details. There is agreement with Jacobs et al. (9) in the position of S_2N in *Diplolaimella*, despite some ambiguity in that study about the ventrosublateral nuclei and the number of pharyngeal glands.

The three outlets and nuclei described by Chitwood and Chitwood (2) for Monhysterida (DO at or close to the stoma, SO close to the level of the nerve ring, DN and a pair of SN at the base of the pharynx) were found in this study. To our knowledge, the two additional outlets in the posterior region of the pharynx have not been reported previously in any study of Monhysterida.

On the basis of our combined observations from LM, SEM, and TEM, we describe the glandular system of *Monhystera* as follows: DO at base of dorsal buccal tooth or at the base of the stoma when a dorsal tooth is absent; S_1O at level of nerve ring, S_1N in posterior pharynx region at level of the slight basal expansion (almost two pharynx widths anterior to pharynx base); S_2O at 83–85% of pharynx (i.e., slightly posterior to S_1N), S_2N slightly behind level of DN. This arrangement is in agreement with the generally accepted idea that SO never open at the anterior end of the pharynx in Chromadoria, but at variance with observations by zur Strassen (17), Riemann (13), and Jacobs and Heyns (7).

Other authors have asserted that uninucleate pharyngeal glands may be branched

and then may occupy other sectors than the one to which they belong (3,14); this branching also was seen in our TEM observations. Therefore, it is clear that the only reliable criterion for determining the outlets is the position of the outlets themselves, not the presence of glandular tissue on its own. For this reason the interpretation of Riemann (13) for some Monhysterida should be checked and that of Jacobs and Heyns (7) should be corrected on the basis of the information presented here.

The pharyngeal gland pattern described above may be the typical plan for Monhysterida. This arrangement is similar to that described for *Caenorhabditis elegans* by Albertson and Thomson (1), and DO at base of stoma, S_1O at base of metacarpus, and S_2O and all nuclei (DN and two pairs of SN) in the basal bulb. Chitwood and Chitwood (2) described only three glands in *Rhabditis*. The pattern with the DO close to or inside the stoma, the S_1O about halfway along the pharynx, the S_2O at the base of the pharynx, and all gland nuclei in the posterior region of the pharynx may well be the basic pattern for the Class Chromadorea (including the subclass Secernentia in our interpretation) as opposed to the Class Enoplea with the DO and S_1O in or close to the stoma. If this pattern is accepted as typical, then taxa still classified under Chromadorea but with an Enoplea pattern of pharyngeal gland outlets (e.g., Linhomoeidae, Desmoscolecida) do not belong in Chromadorea. However, it is advisable to make such transfers only after adequate observations on these taxa are performed.

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