Journal of Nematology 32(3):245–252. 2000.

Ultrastructure of the Z-organ and Parts of the Female Genital Tract in *Xiphinema coxi coxi*[1]

M. R. Cho,[2] R. T. Robbins,[3] and K. S. Kim[3]

Abstract: Ultrastructure of the Z-organ and associated apophyses in *Xiphinema coxi coxi* was studied by transmission electron microscopy to determine their structural origin and relationship with other parts of the genital tract. The Z-organ of *X. coxi coxi* is oval-shaped, ca. 30 μm long and 16 μm wide. It is clearly distinguished from the other parts of the female genital tract by its thick muscular outer wall, epithelium-lined lumen, and 4-5 centrally located apophyses. Each apophysis is continuous with the epithelial lining of the Z-organ, suggesting that it originated from epithelium. The apophyses appear as thickened and densely folded masses forming numerous interlaced pores and (or) chambers containing mucous-like materials and electron-dense crystals. These apophyses are characteristic of a typical Z-organ; no globular structures characteristics of the pseudo-Z-organ were observed. The thickness of the muscular layer of the oviduct and uterus varied with position. The overall Z-organ ultrastructure of this study, including body wall and internal apophyses, was comparable to the typical Z-organ of *X. ifacolum*. This suggests that *X. coxi coxi* should be classified as a *Xiphinema* species that contains the typical Z-organ.

Key words: apophyses, genital tract, morphology, nematode, oviduct, pseudo-Z-organ, transmission electron microscopy (TEM), typical Z-organ, ultrastructure, uterus, *Xiphinema coxi coxi,* Z-organ.

Female genital tract differentiation into a Z-organ (Z-differentiation) was first described in *Xiphinema ebriense* Luc, 1958. Similar uterine differentiations have also been reported in *Axonchium* Cobb, 1920 and *Labronema* Thorne, 1939 (Grimaldi-De Zio et al., 1979). In the genus *Xiphinema* Cobb, 1913, two types of Z-differentiations were distinguished (Luc, 1973)—the typical Z-organ and the pseudo-Z-organ—and this motivated taxonomists to pay closer attention to the comparative morphology of the genital tract of *Xiphinema.* The structure of the typical Z-organ is quite distinct from the other parts of the uterus. It has a strong outer wall of circular muscles, an internal sclerotized wall with five longitudinal striations, and a sclerotized central lumen typically with four or five well-developed refringent apophyses. The pseudo-Z-organ is structurally more variable between species than the typical Z-organ, and is not so distinct or clearly demarcated from adjacent parts of the uterus. The pseudo-Z-organ has weakly developed circular muscles, and sclerotization of the internal wall is either weak or absent. It usually contains globular bodies that vary in size, number, and form between species (Luc, 1973). Various other uterine differentiations, including spiniform structures (spines) and crystalline structures, have been reported in more than 70 *Xiphinema* species (Kruger, 1988). A series of polytomous keys identifies 13 *Xiphinema* species with typical Z-organs, 41 species with pseudo-Z-organs, 15 species with pseudo-Z-organs and spines, and 17 species with spines only (Loof and Luc, 1990, 1993; Loof et al., 1996). The Z-differentiation is found typically in didelphic *Xiphinema* species and has taxonomic value for specifies determination (Luc, 1975). Kruger (1988) compared the Z-differentiations of 14 *Xiphinema* species and presented a comprehensive literature review on Z-differentiation. He suggested that the use of the term pseudo-Z-organ and the artificial distinction between apophyses and globular structures be discontinued to minimize confusion and ensure stability in the literature. The distinction between the typical Z-organ and the pseudo-Z-organ is more related to the structure of the Z-organ wall than to the formations filling the lumen (Loof and Luc, 1990). However, ultrastructural studies of

Received for publication 20 March 2000.
[1] Published with the approval of the director of the Arkansas Agricultural Experiment Station. This work was supported in part by grant #0403-05414-24-1199 from the US-USDA-CSRS-84-14384/Robbins.
[2] Division of Horticultural Environment, National Horticultural Research Institute, Suwon, 441-440, Korea.
[3] Professors, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.
E-mail: rrobbin@comp.uark.edu
This paper was edited by Ken Evans.

the typical Z-organ in *X. ifacolum* (Bleve-Zacheo et al., 1984) demonstrated clear differences between both the structure of the body wall of the Z-differentiation and the internal compositions in the lumen.

Although the structure of the Z-differentiation has been shown to be remarkably constant within a species (Bleve-Zacheo et al., 1984; Luc, 1975), some controversy has arisen in the identification of the type of Z-differentiation that occurs in *X. coxi*. Several authors have described the differentiation as a pseudo-Z-organ (Brown et al., 1983; Grimaldi-De Zio et al., 1979; Luc, 1975), whereas others have described it as a typical Z-organ (Sturhan, 1984; Tarjan, 1964). However, it was reported that the Z-organ of *X. coxi* Tarjan, 1964 occupies an intermediate position between pseudo-Z-organ, characterized by globular bodies, and typical Z-organ, characterized by sclerotized apophyses (Coomans, 1964). Clear difference in the morphology of Z-differentiation has been an important diagnostic criterion between *X. coxi coxi* Tarjan, 1964 and the species *X. malawiense* Brown, Luc, and Saka, 1983, *X. limbeense* Brown, Luc, and Saka, 1983, *X. pseudocoxi* Sturhan, 1984, and subspecies *X. coxi europaeum* Sturhan, 1984 (Brown et al., 1983; Cho and Robbins, 1990; Sturhan, 1984).

The objective of our study was to determine the ultrastructural characteristics of the Z-differentiation in *X. coxi coxi* and to compare our findings with homologous regions in other *Xiphinema* species. This paper also presents ultrastructure of parts of the genital tract adjacent to the Z-differentiation.

Materials and Methods

Xiphinema coxi coxi females were extracted from soil collected from an alfalfa field near the Nematology Laboratory at the University of Florida, Gainesville. The genital region from living females was dissected in modified Karnovsky's fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer at pH 7.0. After dissection, the specimens were fixed for 24 hours at 4°C in freshly prepared fixative. The specimens were rinsed twice in the cacodylate buffer for 20 minutes before postfixation in 1% osmium tetroxide for 8 hours at 4°C. After rinsing briefly in the buffer, specimens were stained *en bloc* in 0.5% aqueous uranyl acetate for 24 hours at 4°C. Prior to dehydration, the specimens were embedded in 2% agar to facilitate handling and orientation of the specimens in the final block (Wright and Jones, 1965). Specimens were dehydrated in an ethanol series of 30%, 50%, 70%, 80%, 95%, and three changes of 100% followed by two changes of propylene oxide to ensure complete dehydration. The specimens were embedded in Spurr's medium and sectioned with a model 2088 LKB ultrotome (Switzerland). Silver/gold sections were double-stained with 2% uranyl acetate and lead citrate before viewing with a Jeol 100 CX transmission electron microscope (Japan). Light microscope observations were made using a Nikon Optiphot-2 with Nomarksi differential interference contrast (Japan).

RESULTS

The Z-organ in *Xiphinema coxi coxi* is a distinct oval-shaped tube ca. 30 µm long with a maximum width of 16 µm situated between the distal end of the uterus and the proximal end of the oviduct. The Z-organ is composed of a thick muscular outer wall surrounding a thin epithelial layer and containing 4-5 apophyses in the central lumen (Figs. 1,2,5). Twelve cells that form part of the Z-organ outer wall are seen laterally to the Z-organ lumen (Fig. 1). Each of the 12 cells is a portion of 12 rings, each consisting of six cells, that make up the outer wall. Light microscope observations (counting nuclei) confirmed that the wall of the Z-organ consists of ca. 72 strong circular muscle cells, arranged in a series of 12 rings with six cells per ring, perpendicular to the axis of the uterus. Neither a constriction nor a sphincter was observed at either end of the Z-organ, but the Z-organ area was distinct from the other parts of the oviduct and uterus by its conspicuous oval shape and the thick muscular wall. The muscular wall of

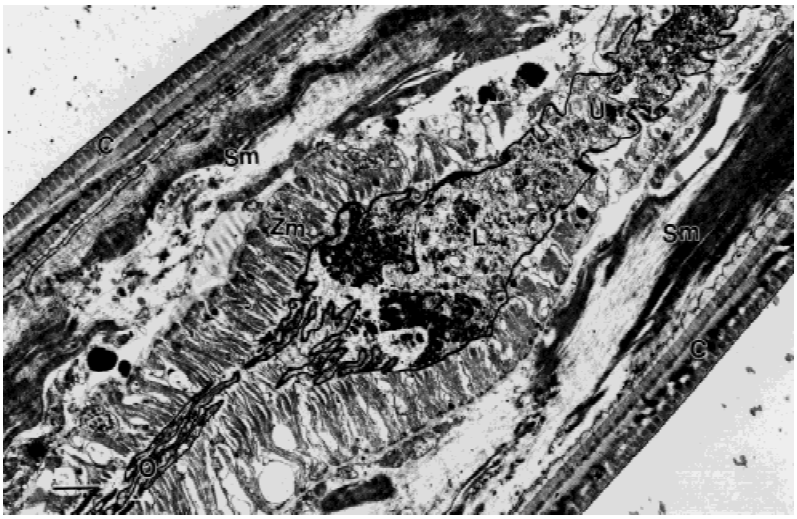

FIG. 1. A low magnification of a longitudinal section through the Z-organ between uterus (U) and oviduct (O) of *Xiphinema coxi coxi*. The Z-organ has a wide bulging lumen (L) with two apophyses visible. The muscle (Zm) surrounding the Z-organ is much thicker than that of the uterus. Note the external circle (C) and somatic muscle (Sm). Bar = 500 nm.

the Z-organ was thickest at the distal end (Figs. 1,2).

The Z-organ lumen was lined with a highly folded epithelial layer (Figs. 1,2). A maximum of 5 apophyses was observed in cross section near the center of the Z-organ (Fig. 5). The size of each apophysis was ca. 3 μm wide by 4 μm long, and apophyses appeared somewhat spherical in transverse section (Fig. 5). In longitudinal sections, apophyses appeared to have angular margins with pits on the surface (Figs. 1,2). Each apophysis was continuous with the epithelial lining of the Z-organ (Fig. 5). The structures of the apophyses and the epithelial lining appeared to be identical in electron density and texture (Fig. 3). The apophyses in some sections appeared to be

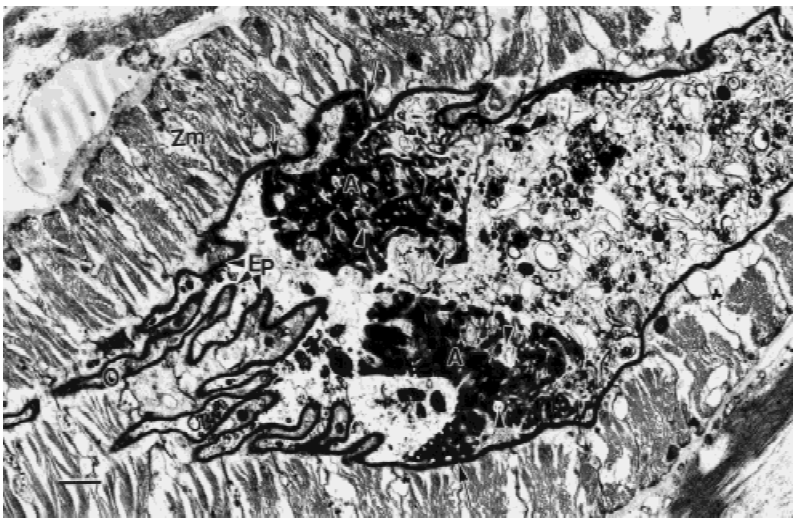

FIG. 2. Higher magnification of the Z-organ in Fig. 1, with the epithelial layer (Ep) lining the lumen of the Z-organ appearing to be highly sclerotized and becoming a thick ''wall'' with many infoldings. The apophyses (A) have many small pores, channels, and (or) chambers (arrowheads) of various sizes and shapes. In several places, the apophyses are continuous with the epithelial wall (arrows). Bar = 1,000 nm.

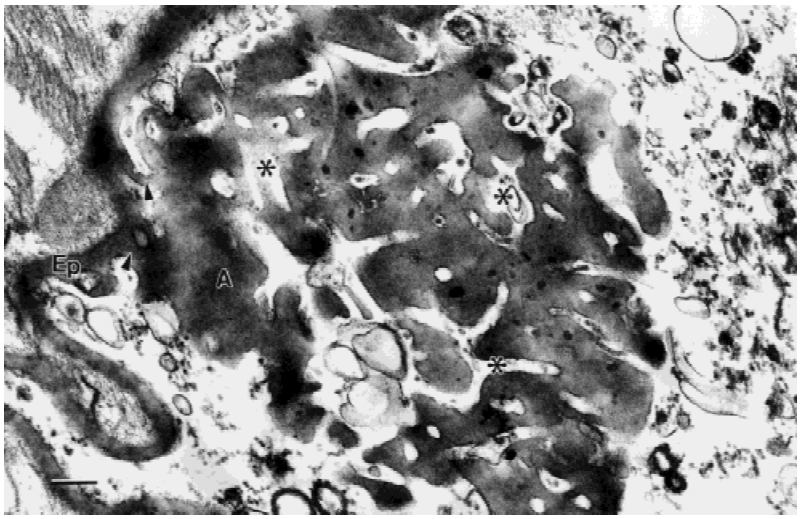

FIG. 3. Higher magnification of an apophysis showing the sclerotized, smooth, sheath-like matrix that is interrupted by many pores, channels, and (or) chambers (asters). The texture and electron density of the epithelial wall (Ep) and apophysis (A) are identical in appearance, and the epithelium and apophysis are continuous with one another (arrowheads). Bar = 250 nm.

partially fused with interlaced masses of thickened and intensely folded epithelial lining (Figs. 3,5). The matrix of the apophysis was amorphous, interrupted by a number of electron-lucent pores, channels, or chambers of various shapes and sizes exhibiting a sponge-like surface (Figs. 2–4). Mucous-like substances, membranous remnants, and extremely electron-dense crystals of various sizes and shapes were observed in some of the chambers (Figs. 3,4), which opened into the lumen of the Z-organ.

An egg, located within the greatly extended Z-organ viewed in cross section, occupied almost all the body diameter except for the external cuticle and the somatic musculature (Fig. 7). The characteristic structural components of the Z-organ, such as the thick outer muscle layer, the epithelial layer lining the lumen of the Z-organ, and

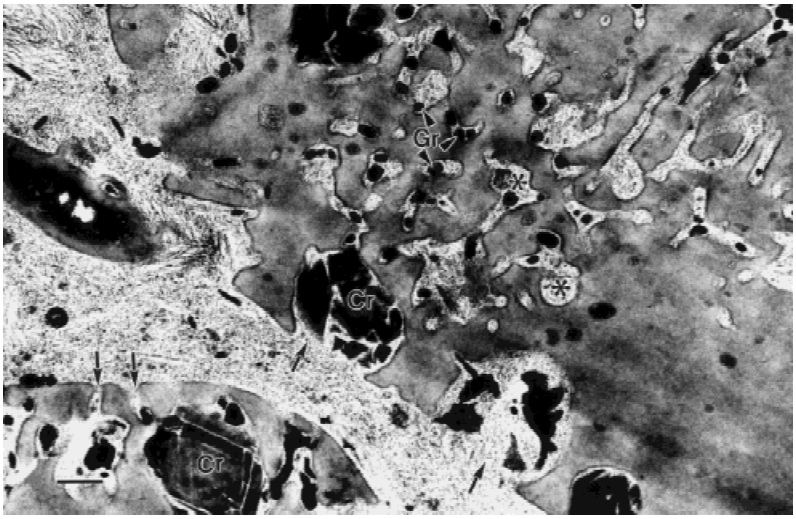

FIG. 4. A portion of an apophysis with many pores, channels, or chambers containing electron-dense crystals (Cr) and granules (Gr). The chambers open to the Z-organ lumen (arrows). Bar = 250 nm.

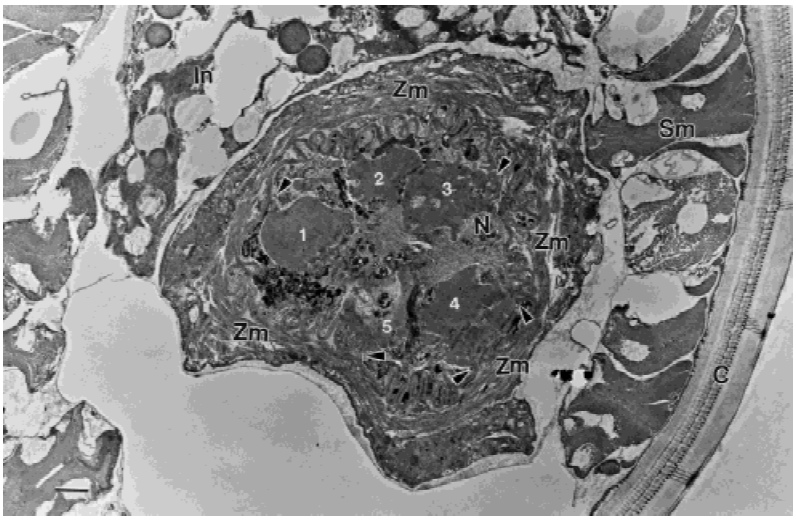

FIG. 5. Transverse section of a Z-organ containing five (1,2,3,4,5) apophyses, which are continuous with the edges of epithelial wall infoldings (arrowheads). Longitudinally sectioned myofibrils (Zm) surrounding the entire circumference of the Z-organ are clearly visible. A portion of the intestine (In) is also present. Somatic muscle = Sm. Bar = 1,000 nm.

apophyses in the lumen, were not obvious, apparently because they were greatly stretched by the presence of the mature egg (Fig. 7). These structures appeared to be replaced by a thin and extremely electron-dense layer, with no discernible structural details between the somatic muscle and the egg shell (Fig. 7). The egg, in section, appeared oval and was surrounded by a shell that appeared as an electron-lucent band tightly appressed to the wall (Fig. 7). The egg was filled with yolk granules, which appeared as extremely electron-dense, spherical granules of various sizes (Fig. 7).

The lumen of the distal portion of the oviduct near the Z-organ was much narrower than that of the Z-organ and was highly convoluted (Fig. 6). No apophyses or

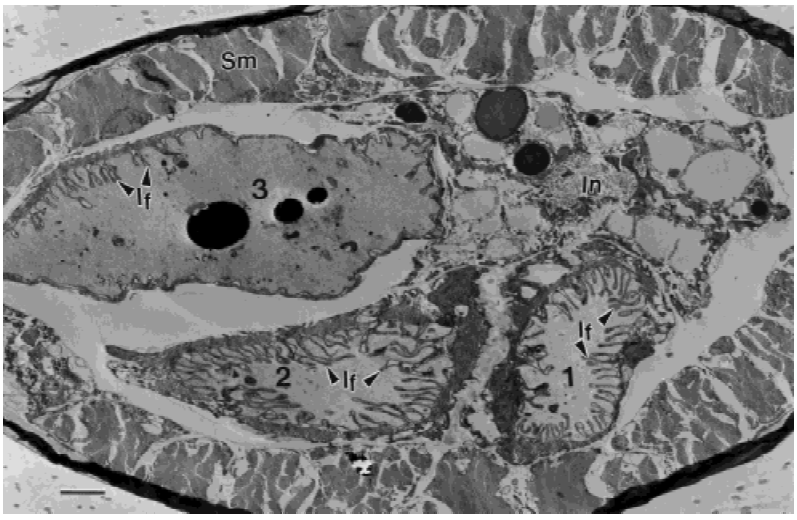

FIG. 6. Transverse section of the oviduct immediately distal to the Z-organ that shows the oviduct section in three (1,2,3) separate positions due to the convolutions of the duct. The lumen exhibits different degrees of epithelial wall infolding at the three positions (If). The lumen of the intestine (In) is also shown. Somatic muscle = Sm. Bar = 500 nm.

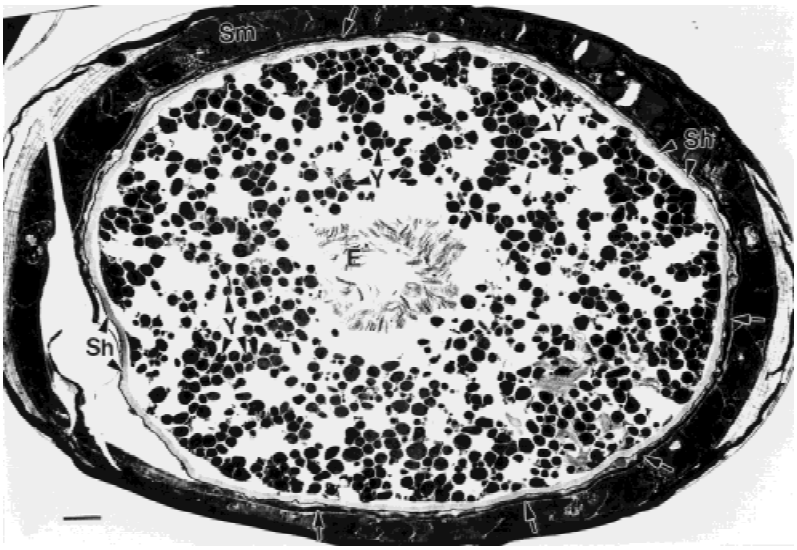

FIG. 7. A transverse section of the Z-organ containing an egg (E). Structures characteristic of the Z-organ, such as the layers of thick muscle, epithelial wall, and apophyses, are not evident. These layers are apparently stretched extensively due to the presence of the egg and appear simply as an electron-dense band (unlabeled arrows) between the eggshell (Sh) and the external somatic muscle layer (SM). The egg is filled with electron-dense yolk granules (Y) of various sizes. Bar = 500 nm.

other material present in the Z-organ occurred in the lumen of the oviduct. However, the epithelial layer is highly plicated, demonstrating the presence of many membrane infoldings throughout the entire circumference of the oviduct lumen (Fig. 6).

DISCUSSION

The Z-differentiation of *X. coxi coxi* observed in this study generally agrees with the description of the typical Z-organ (Luc, 1958, 1973) and also with the original Z-organ (Tarjan, 1964) observed in type *X. coxi* females. Ultrastructure of the Z-differentiation of *X. coxi coxi* clearly shows the presence of apophyses in the lumen and, in this respect, it resembles the typical Z-organ of *X. ifacolum* (Bleve-Zacheo et al., 1985). No globular bodies, characteristic of the structure of the Z pseudo-organ observed in *X. diversicaudatum* (Bleve-Zacheo et al., 1984) and *X. meridianum* (Van de Velde et al., 1990), were present in *X. coxi coxi* specimens examined in this study. This indicates that *X. coxi coxi* should be classified taxonomically as possessing the typical Z-organ, not as a species with the pseudo Z-organ (Brown et al., 1983; Grimaldi-De Zio et al., 1979; Luc, 1975) or a type intermediate between the typical Z-organ and the pseudo Z-organ (Kruger, 1988).

In addition to the presence (Z pseudo-organ) or absence (typical Z-organ) of the sphincters between the uterus proper and each end of the Z-differentiation, the major difference between the two Z-organ types appears to be the occurrence of ''sclerotized refringent apophyses'' in the typical Z-organ and of globular bodies in the pseudo Z-organ (Bleve-Zacheo et al., 1985; Coomans, 1964; Luc, 1973). The matrix of apophyses consists of electron-dense, amorphous sheath-like material interrupted by the presence of numerous pores and (or) chambers of various shapes and sizes containing (primarily) electron-dense granules and crystalloids. The matrix of globular bodies, on the other hand, consists of ''non-sclerotized'' amorphous material that incorporates a variety of inclusions, such as vesicles of various sizes, tubular slits, electron-dense granules, and crystals (Bleve-Zacheo et al., 1984; Van de Velde et al., 1990). Apophyses usually exhibit sharply linear and (or) angular boundaries, perhaps due to the sclerotization,

whereas the globular bodies have smooth and roundish outer boundaries.

The apophyses of *X. coxi coxi* and *X. ifacolum* (Bleve-Zacheo et al., 1985) are continuous with the thick inner wall of the Z-organ (epithelial tissue wall, Bleve-Zacheo et al., 1985) and are probably formed from material synthesized by the layer of epithelial cells that line the lumen of the Z-organ. A structurally intact layer of this epithelium was not evident, probably because the layer is very thin and fragile and, therefore, had been preserved poorly during the process of tissue preparation for electron microscopy. The occurrence of disrupted cell organelles, such as ribosomes and pieces of rough endoplasmic reticulum, between the muscle cells and folded inner wall of the Z-organ in both *X. coxi coxi* and *X. ifacolum* (Bleve-Zacheo et al., 1985) indicates the presence of such an epithelial layer. In addition to the continuity of the apophyses with the inner wall of the Z-organ, both structures have similar electron density and structural texture, suggesting they are of similar material and may have a single origin. For these reasons, apophyses have been considered to be proliferations of the ''cell wall'' of the single-layered-epithelium that lines the lumen of the Z-organ (Bleve-Zacheo et al., 1985). The origin of the apophyses appears to be the same as that of the globular bodies described by Bleve-Zacheo et al. (1984) and Van de Velde et al. (1990) in the pseudo Z-organ of *X. diversicaudatum* and *X. meridianum,* respectively, based on the morphological relationship between the globular bodies and the inner wall of the pseudo Z-organ.

The function of the typical Z-organ or the pseudo Z-organ is still unknown although some speculations have been made. Cytochemical studies indicated that matrix material of apophyses and globular bodies contains no pepsin or pronase digestive proteins and is, therefore, suggested to be made of glycoproteins that function like sponges (Bleve-Zacheo et al., 1984, 1985). However, the contents of the pores, chambers, tubules, and (or) vesicles that are present in the apophyses and the globular bodies were digested by the enzymes, which led to the suggestion that they are secretions containing material involved in egg-shell deposition.

Based on a number of ultrastructural studies, including the present study of *X. coxi coxi,* apophyses characteristic of the typical Z-organ and the globular bodies characteristic of the pseudo Z-organ are distinct structures that can be used as diagnostic features in *Xiphinema* taxonomy and classification. However, why *Xiphinema* species have two different structural systems that have the same or similar functions is not clear; each structure may have additional characteristic functions.

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