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# A Comparative Study of Body Wall Structures of a Pogonophore and an Annelid from a Phylogenetic Viewpoint

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**ABSTRACT**—Pogonophores are tube worms that live in reducing deep-sea waters where sunlight does not penetrate. They are highly adapted for their special habitat in lacking guts and possessing endosymbiotic chemosynthetic bacteria. Because of these peculiar characteristics, it is not yet clear whether they should be classified as annelids or not. Electron-microscopic observations of sections of a Japanese pogonophore (*Oligobrachia mashikoi*) show that the body wall has circular and longitudinal muscular systems. These muscular systems, however, differ from the annelid (*Branchiura sowerbyi*) in these ways: (1) The outer circular muscle of the pogonophore was constructed of smooth muscle cells. In contrast, that of the annelid was composed of obliquely-striated muscle cells, even though the cells were small and bore undeveloped characteristics. (2) The inner longitudinal muscle of the pogonophore was constructed of undeveloped obliquely-striated muscle cells, whereas that of the annelid was composed of well-developed ones. These observations suggest that this pogonophore can not be classified as an annelid, although many previous studies have placed pogonophores in that phylum.

**Key words:** ultrastructure, muscle, body wall, pogonophore

## INTRODUCTION

Reports of the geographical distribution of pogonophores show they were first collected from the bottom of the western Pacific, and were subsequently found along the Atlantic coast from Nova Scotia down to Brazil, and from the Orkney Trench to the Bay of Biscay (Webb, 1969; Margulis and Schwartz, 1988; Smirnov, 2000). They are thought to be highly adapted for a special habitat where sunlight does not penetrate and waters are reducing, because they lack guts and bear chemosynthetic bacteria endosymbiotically in special cells in their bodies (Southward,1982).

Although complete samples have seldom been dredged, many systematic reports discuss morphological observations (Dales, 1962; Westheide, 1985; Margulis and Schwartz, 1988; Ivanov, 1994; Rouse and Fauchald, 1997; Bartolomaeus, 1997; Boore and Brown, 2000; Rouse, 2001), amino acid and DNA sequences (Field *et al*., 1988; Kojima *et al*., 1993; McHugh, 1997; Kojima, 1998). Developmental research has also identified important characteristics comparable with the Oligochaeta (Southward and Galkin, 1997; Southward, 1999). Anatomical details of pogonophore bodies have also been reported (Webb, 1969; George and Southward, 1973; Southward, 1982, 1993; Rose and Fauchald, 1997; Schulze, 2001). These reports show that pogonophore bodies are divided into four sections: the tentacular crown (protosoma), the vestimental region (agonadal mesosoma), the trunk (gonadal mesosoma) and the opisthomere (metasoma). Each region is separated by mesentery and has its own coelom (Webb, 1969). The internal anatomy has not yet been fully described due to the scarcity of complete specimens, and because the internal anatomy was less attractive to study compared to the peculiar profile of the tentacular crown.

Pogonophores have also been reported from Tsukumo Bay, Japan (Imajima, 1973). This Japanese pogonophore was also studied anatomically, and it was reported that the body was covered with chitin tubes buried in the sandy bottom. These tubes were about 40 cm long and 0.2 to 1.0 mm wide (Imajima, 1973). However, the ultrastructural details of pogonophores remain unclear. Thus, it is not yet clear whether pogonophores should be included in the annelids or whether they should be a separate phylum.

Under these circumstances, it is useful to compare the morphology of the muscular systems of pogonophores and annelids to classify the two groups. We have observed and compared the muscular systems in the body walls of a

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pogonophore and an annelid, and discuss these observations to establish if they should be classified into the same phylum or not.

Briefly, the pogonophore body wall consisted of five layers inward from the outer layer: the chitinous surface, the ephitherium, a collagen layer, a thin circular muscle layer, and a thick longitudinal muscle layer. Organization with an outer circular muscle layer and an internal longitudinal layer is quite common in tubular invertebrates, but types of muscle cells and layers in body walls may be adapted in individual phyla of animals.

## MATERIALS AND METHODS

The pogonophore examined in this work was a specimen of *Oligobrachia mashikoi* collected from Tsukumo Bay, near the Noto Marine Biological Station. The specimen was collected by dredge from a sandy sea-bottom at a water depth of about 20–25 m. The pogonophore had a tubular body about 0.5 mm in diameter, and bore chitin tubes. The middle portion of the body (the mesosoma) was the material examined in this study. The mesosoma was cut into small pieces about 2 mm in length, and was immediately prefixed for 120 minutes at room temperature. The prefixative solution contained 1.5% glutar-aldehyde and 1.5% paraformaldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4. The prefixed pieces were washed with the buffer several times, and then post-fixed by ice-cold 1% osmium tetroxide in a 0.1 M phosphate buffer at pH 7.4 for 120 min. They were then dehydrated using a series of ethanol solutions (80, 90, 95, and 100%). Resin-embedded specimens were cut into thin sections with a diamond knife, and were subsequently stained with saturated aqueous uranyl acetate and lead nitrate. They were then observed under a JEM-1010 electron microscope.

The comparative material used in this work was the middle portion of an annelid (*Branchiura sowerbyi*) collected from a stream on the Shimane University campus. The material measured about 1 mm in diameter and 10 mm in length. The middle portion was cut into small pieces and fixed using the same procedure as described above for the pogonophore preparation. The prepared specimens were also observed by electron microscopy. Semi-thin senctions of both materials were cut from resin-embedded specimens with a diamond knife, and stained in a “basic fucsin” solution for about 10 min.

## RESULTS

### Light Microscopic Observation

The trunk of the pogonophore featured a thick body wall, and was gutless, as reported in previous studies. The longitudinal muscle layer was extremely thick, and filled the entire body space. Gland cells of unknown function were observed within the epitherium (Fig. 1).

The middle portion of the annelid had a thin body wall, and contained a central alimentary canal and associated accessory glands, along with a large ventral blood vessel and several associated nerves. The longitudinal muscle layer was thin (Fig. 2).

### Electron microscopic observation

Electron microscopic observation of the pogonophore body wall established that it was basically similar in structure to that of the annelid. The pogonophore body wall was composed of five successive layers. These comprised a chitinous outer surface layer, an epitherial layer, a collagen layer, a circular muscle layer, and an innermost longitudinal muscle layer (Fig. 3). Endosymbiotic bacteria measuring about 1.0 μm in diameter were observed within epitherial cells (Fig. 4). These epitherial cells were connected by collagen layer about 0.2 μm in thickness. No basement membrane was recognized beneath the epidermis (Figs. 5 and 6).

The muscular system was constructed of circular muscle lying beneath the epitherium, and longitudinal muscle lying below the circular muscle (Figs. 5 and 6). The circular muscle was observed under collagen layer (inner side of the body wall) (Figs. 5 and 6). The muscle cells were usually trianglular in cross section (Fig. 5). It is noteworthy that structurally these cells were small smooth muscle cells with randomly arranged myofilaments, whether observed in cross section or in longitudinal section (Figs. 5 and 6). The myofilaments were thick, with a diameter of about 44nm.

The longitudinal muscle lay close beneath the circular muscle, and could be divided into two types. The first consisted of smooth muscle cells, and the second was an undeveloped obliquely-striated muscle cells which did not show complete myofibrils (Figs. 6, 7 and 8). The smooth muscle cells had an oval shape, with long axes about 1.0 μm–5.0 μm in cross section. They were situated in the outer region of the longitudinal muscle, and thus were closely related to the circular muscle (Fig. 6). The obliquely-striated muscle cells were situated in the inner portion of the longitudinal muscle, and were elongate-oval in cross section, measuring about 1.4 μm×16 μm (Fig. 7). The structure of these muscle cells is undeveloped compared to those of the annelida (Figs. 7, 8, 11 and 12). That is, the pogonophoran muscle cells did not bear complete myofibrils separated by tubular sarcoplasmic reticular systems, and myofilaments in the defective myofibrils were arranged irregularly (Fig. 8). The numbers of sarcoplasmic reticular systems in the cells were few (Fig. 8). Thick myofilaments were about 27 nm in diameter.

The annelidan body wall was composed of epitherial, collagen and muscle layers (Figs. 9 and 10). This conformation is similar to that of the pogonophore body wall, but the arrangement of the layers is quite different. The annelidan layers were arranged in the following order from exterior to interior: epitherial layer, outer collagen layer, circular muscle, inner collagen layer and longitudinal muscle (Figs. 9 and 10). It is also noteworthy that the circular muscle was composed of obliquely-striated muscle cells, and the circular muscle was surrounded by extensive collagen layer (Figs. 9 and 10). The circular muscle seemed to be composed of obliquely-striated muscle cells of two sizes. One was small, and contained thicker myofilaments, and the other was relatively large and had thick myofilaments measuring about 27nm in diameter (Fig. 10).

The longitudinal muscle was also composed of obliquely-striated muscle cells, but of a well-developed type

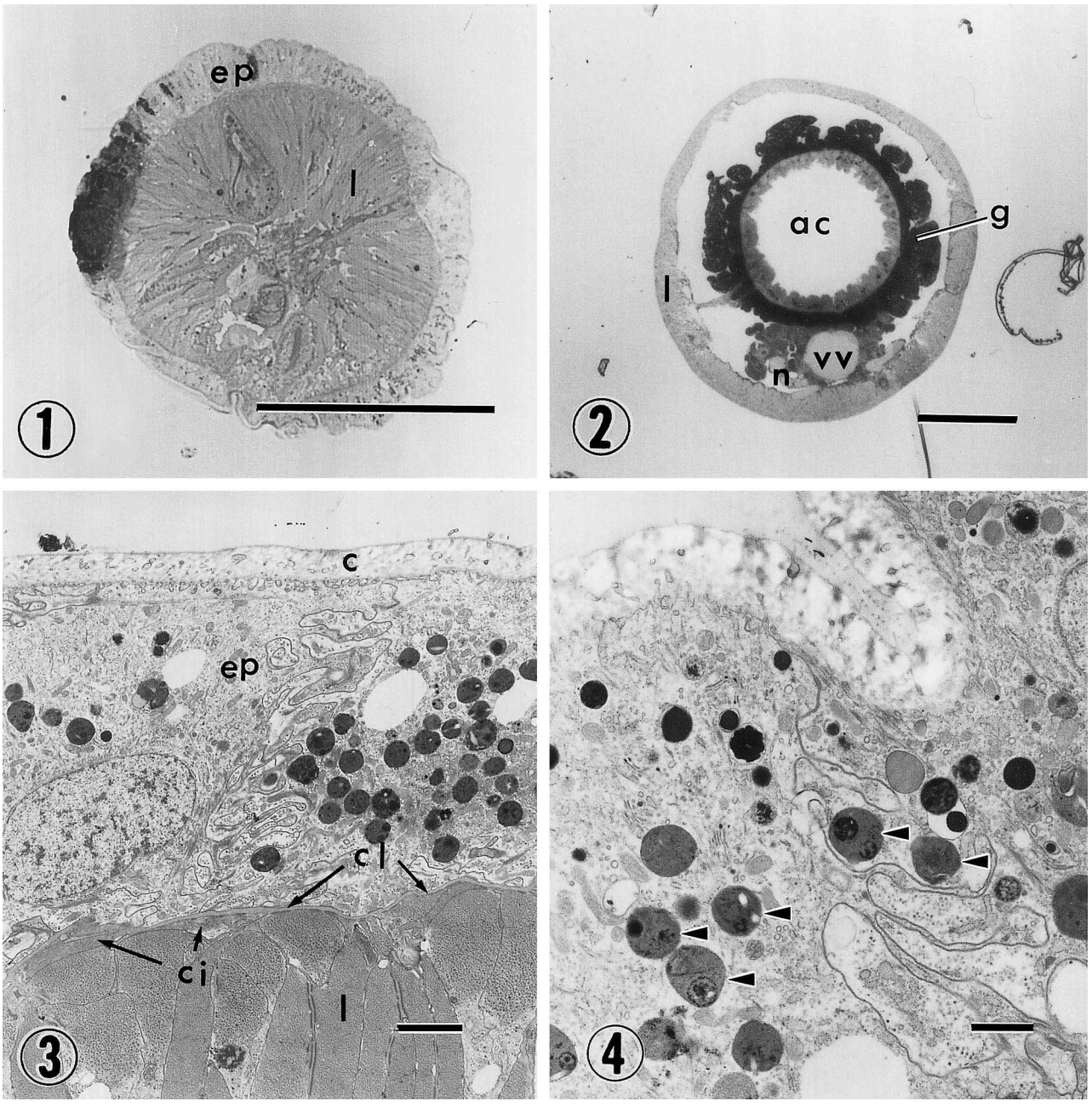


**Fig. 1.** Light micrograph of a cross section of the middle portion (mesosoma) of a pogonophore. No gut is visible. Note the thickness of the body wall. ep, epitherium; l,longitudinal muscle. Scale bar indicates 200 μm.
**Fig. 2.** Light micrograph of a cross section of the middle portion of an annelid. A digestive duct is recognized at the center, and a large blood vessel is present on the ventral side. Compare the thickness of the body wall with that of the pogonophore. ac, alimentary canal; g,gland cell; l,longitudinal muscle; n,nerve; vv, ventral blood vessel. scale bar 200 μm.
**Fig. 3.** Electron micrograph of a cross section of the body wall of a pogonophore. The body wall is composed of five layers. c, chitinous surface; ci, circular muscle; cl, collagen layer; ep, epitherium; l, longitudinal muscle. x 4,800, scale bar 2 μm.
**Fig. 4.** Electron micrograph of epitherial cells of a pogonophore. Many endosymbiotic bacteria are present in the epitherial cells (arrowhead). x 9,500, scale bar 1μm.

(Figs. 9, 10, 11 and 12). The muscle consisted of two types of obliquely-striated muscle cells. One showed a smaller cross section (1.0 μm×1.9 μm), and had unusually thick myofilaments (54nm in diameter), whereas the other showed larger cross section (1.7 μm×27 μm) and normally-sized myofilaments (27nm in diameter) (Fig. 9). The smaller muscle cells were always attached to the collagen layer situated beneath the circular muscle. The larger cells, however, did not occur in the outer surface portion of the longitudinal muscle (Fig. 9). The longitudinal muscle itself was enveloped by a layer of endotherium, which separated it from the coelecom (Fig. 11). The large obliquely-striated

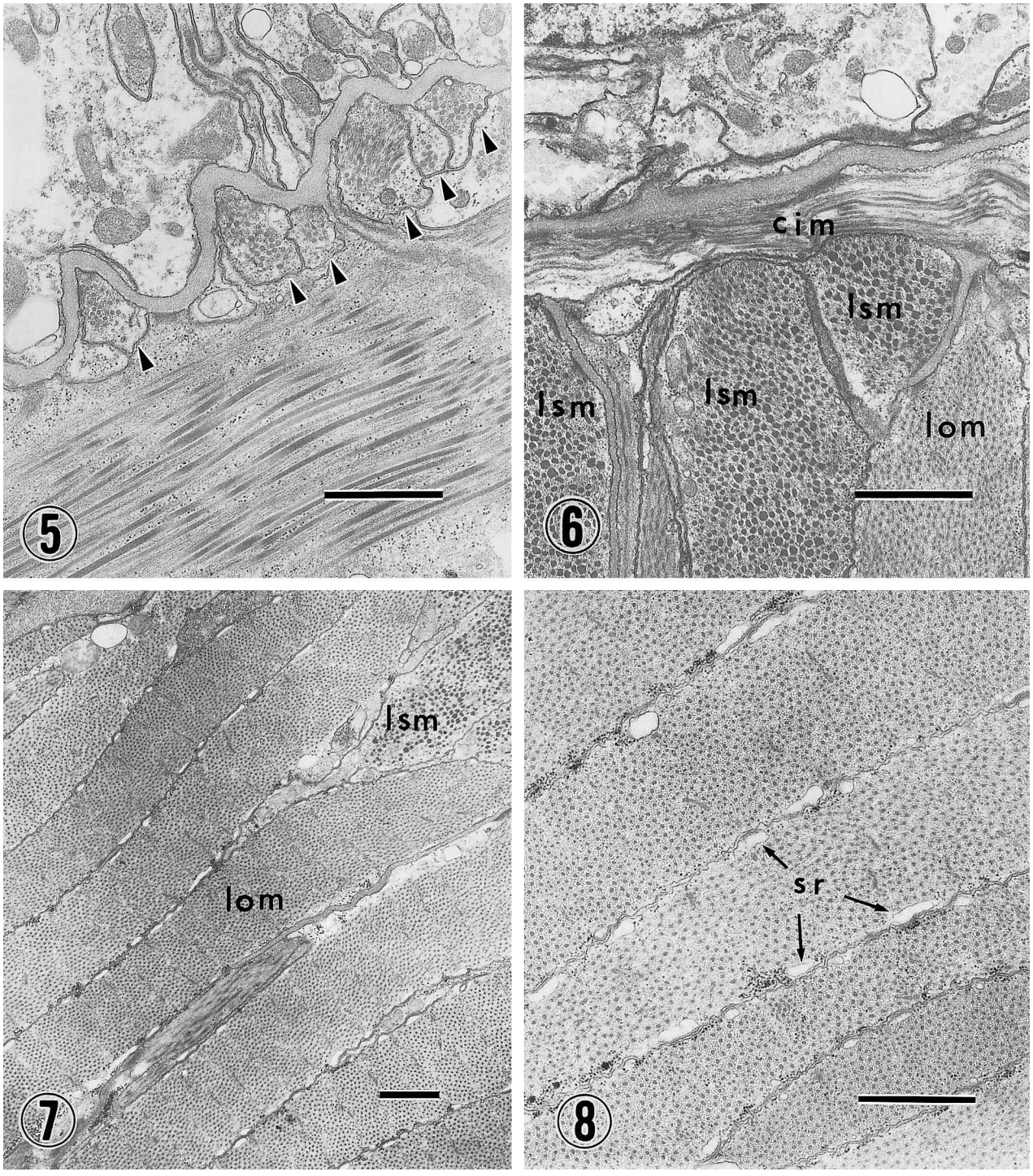


**Fig. 5.** Electron micrograph of a longitudinal section of the pogonophore body wall. The circular muscle cells are situated under the collagen layer, but are not surrounded by the layer. The circular muscle is constructed of smooth muscle cells (arrowhead). x 19,000, scale bar 1 μm.

**Fig. 6.** Electron micrograph of a cross section of the pogonophore body wall. The circular muscle cells are not surrounded by collagen layer. Smooth muscle cells are observed in the outer part of the longitudinal muscle, and are closely associated with the circular muscle. cim, circular muscle cell; lom, obliquely-striated muscle cell in the longitudinal muscle; lsm, smooth muscle cell in the longitudinal muscle. x 19,000, scale bar 1 μm.

**Fig. 7.** Electron micrograph of a cross section of pogonophore longitudinal muscle. Obliquely-striated muscle cells have an elongated oval shape. lom, obliquely-striated muscle cell in the longitudinal muscle; lsm, smooth muscle cell in the longitudinal muscle. x 9,500, scale bar 1 μm.

**Fig. 8.** Enlarged view of pogonophore obliquely-striated muscle cells. Myofibrils are not evident, but indistinct units of thick myofilaments are visible. The sarcoplasmic reticular systems are not well-developed. sr,sarcoplasmic rericula. x 19,000, scale bar 1 μm.

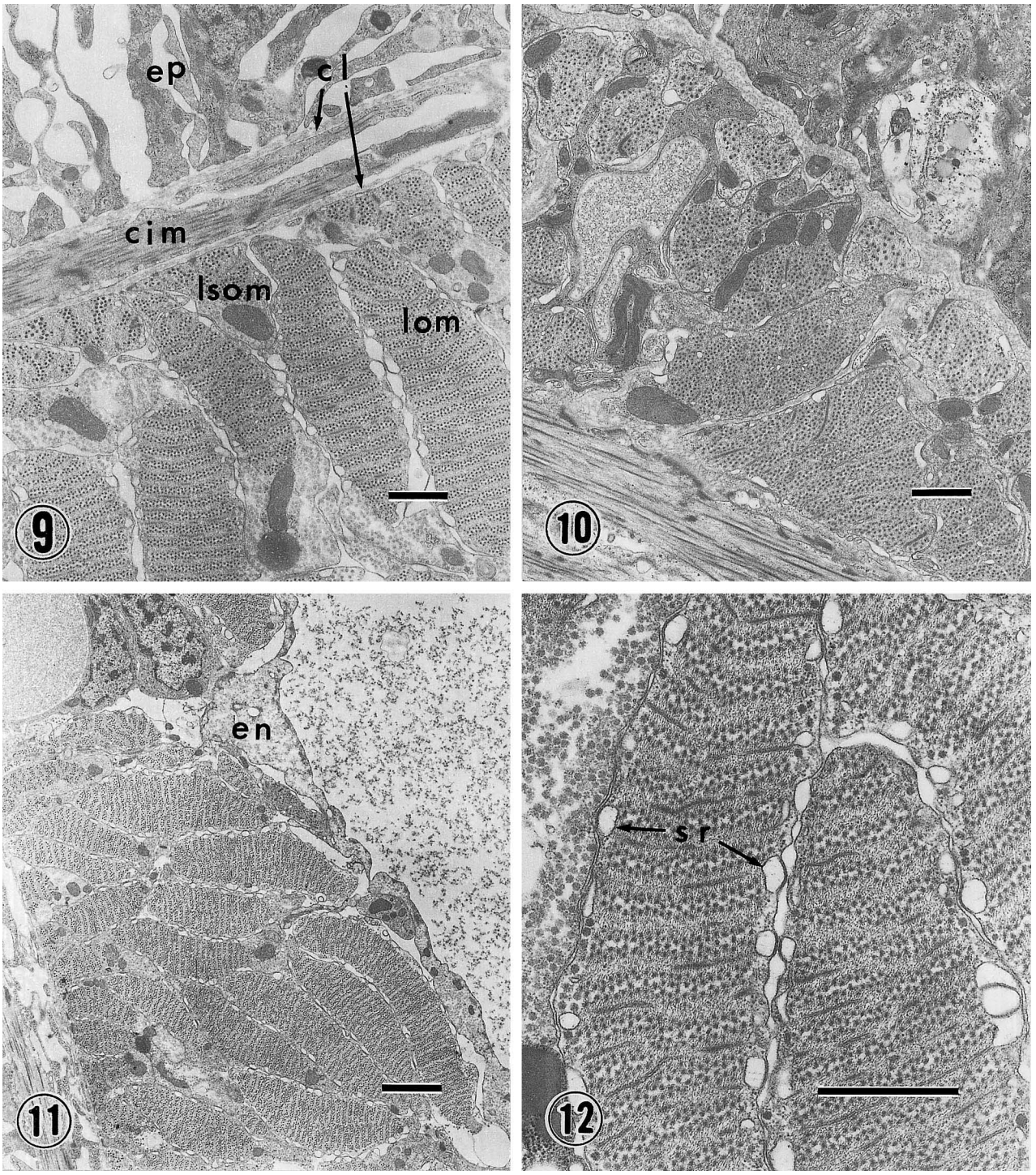


**Fig. 9.** Electron micrograph of a cross section of annelid body wall. This photograph shows four layers of cells, although the body wall is composed of five layers. cim, circular muscle; cl, collagen layer; ep, epitherium; lom, obliquely-striated muscle cell in the longitudinal muscle; lsom, small obliquely-striated muscle cell in the longitudinal muscle. x 9,500, scale bar 1 μm.

**Fig. 10.** Electron micrograph of a longitudinal section of annelid body wall. The circular muscle cells are situated in the collagen layer, and are completely surrounded by these layers. The circular muscle seems to be constructed of two types of obliquely-striated muscle cells (small- and medium-sized). x 9,500, scale bar 1 μm.

**Fig. 11.** Electron micrograph of a cross section of annelid longitudinal muscle. The longitudinal muscle is separated from the coelecom by an endotherium. en, endotherium. x 4,800, scale bar 2 μm.

**Fig. 12.** Enlarged view of annelid obliquely-striated muscle cells. Complete myofibrils are evident, and the sarcoplasmic reticular systems are well-developed. sr,sarcoplasmic rericula. x 22,800, scale bar 1 μm.

muscle cells were well-developed compared to those of the pogonophore (Fig. 12), and bore complete myofibrils composed of a regular arrangement of about 50-70 thick myofilaments (Fig.12).

## DISCUSSION

Many studies have reported the anatomical and histological characteristics of pogonophores, but accounts of their ultrastructure are few. The ultrastructural reports available deal with general descriptions of fine structures of the animal (Webb, 1969; Southward, 1993), or the fine structures of extremely specialized organs which are the basis of pogonophore classification, such as the setae (George and Southward, 1973; Bartlomaeus, 1997) and chaetae found on the body surface (Schulze, 2001). Consequently, this report may be the first detailed examination of the muscular systems of pogonophore body walls.

Tubular motile organs and tissues in higher animals contain two muscular systems. Pogonophore body walls also have two muscular systems, and thus in this respect are similar to the tubular organs of higher animals.

Smooth muscles are generally considered to develop for slow but enduring contraction, whereas obliquely-striated muscles are considered to be less efficient for this function (Lanzavecchia, 1977; Lanzavecchia *et al*., 1985). Ultrastructures of obliquely-striated muscles are characterized by: (1) myofibrils in which myofilaments are surrounded by sarcoplasmic reticular systems are not found in the cells, but a regular arrangement of myofilamentous units, not surrounded by sarcoplasmic reticular systems, is observed; (2) cells lack the triads of sarcoplasmic reticular systems; (3) actin binding “rods” are organized instead of Z-disks (Ikemoto, 1963). Thus, obliquely-striated muscle cells are not arranged in regular order compared to cross-striated muscles. Furthermore, obliquely-striated muscle cells can be classified into many types by their structures. These vary from a undeveloped type resembling smooth muscle cells to a well-developed type resembling cross-striated muscle cells. It is reasonable to consider the undeveloped type as less efficient, and the well-developed type can contract speedily and efficiently.

The muscular system in the body wall of the pogonophore examined differed from the annelid in these ways: (1) The outer circular muscle of the pogonophore was constructed of smooth muscle cells. In contrast, that of the annelid was composed of obliquely-striated muscle cells, even though the cells were small and bore undeveloped characteristics. (2) The inner longitudinal muscle of the pogonophore was constructed of undeveloped obliquely-striated muscle cells ultrastructurally, whereas that of the annelid was composed of well-developed ones.

From our observations, we found that the circular muscles in the pogonophore and in the annelid differ significantly in their situation. The circular muscle in the pogonophore was situated at the surface of the longitudinal muscle, just under the collagen layer, and was in close contact with the longitudinal muscle. That of the annelid was situated in the collagen layer, and individual cells were surrounded by collagen layer.

We established two differences in the body wall muscular systems of pogonophores and annelids: (1) Muscle cells in both the circular and longitudinal muscles in pogonophore body walls are undeveloped compared to those in annelids. (2) The circular muscle in pogonophores contacts the longitudinal muscle, whereas the annelida circular muscle is surrounded bycollagen layer. These results suggest that pogonophore body walls are built of simpler and more undeveloped muscular systems than are those of annelids. These differences may be markers to classify pogonophores as distinct from annelids. From these differences, we consider that pogonophores are systematically lower animals than annelids.

It is not yet clear whether the observed differences between pogonophores and annelids reflect the difference of phylum, or whether they were caused by evolution or adaptation for their habitats. However, we support the former option on the basis of the ultrastructural observations described above. The muscular systems of pogonophores and annelids can be clearly classified into two distinct types by the organization of their body walls.

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