

Observations on musculature in pelagic nemerteans and on pseudostriated muscle in nemerteans

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Abstract

Histological study of 43 pelagic polystiliferans shows that a pseudostriated, or obliquely striated, musculature is a common characteristic of the rhynchocoel wall of seven of eight polystiliferan species examined, representing four of five supposedly widely divergent genera. This kind of muscle tissue was previously reported from pelagic hoplonemerteans by Korotkevich, in 1955, but without a clear description. Both her report and the existence of this muscle type have gone unnoticed in subsequent literature. We also document an earlier, unpublished, ultrastructural discovery of pseudostriation in the body wall of a pelagic polystiliferan and report several more instances in our light microscopic material. We describe an overlooked aspect of the proboscis insertion; that is, the inner longitudinal musculature of the proboscis is confluent with the longitudinal musculature of the rhynchocoel, whereas the outer longitudinal muscle of the proboscis extends to the body wall as proboscis fixators. We note that the so-called circular muscle of the rhynchocoel in some species actually comprises crossed diagonal fibers. We report, for the first time, the presence of crossed diagonal musculature in the body wall of some pelagic polystiliferans.

Introduction

During the last several years we had the unusual opportunity to obtain from moderate deep-sea depths a diverse and abundant collection of living pelagic nemerteans. The resulting collection is superior in quality to all previous collections we have examined. These are the subject of a variety of biological and systematic studies by us (Norenburg & Roe, 1998; Roe & Norenburg, 1998). Some observations we made in the course of anatomical studies may have broad relevance to phylogenetic study of pelagic nemerteans. These warrant reporting separate from more detailed species descriptions, where such observations tend to become lost.

Most of the approximately 100 known species of pelagic nemerteans, with a few notable monostiliferan exceptions (Crandall & Gibson, 1998; Korotkevich, 1961; Norenburg & Roe, 1998), belong to the traditional taxon Pelagica, within the nominal suborder

Polystilifera. Although different taxonomic rankings have been proposed from time to time (cf. Sundberg, 1991) the Pelagica generally has been treated as monophyletic, either by intent or default (e.g., Brinkmann, 1917; Coe, 1926; Crandall, 1993; Gibson, 1988; Korotkevich, 1955). The notable exception is Bürger (1897–1907), who explicitly postulated more than one non-polystiliferan ancestor for polystiliferans. Sundberg (1990), in a cladistic analysis of representative hoplonemerteans, found Polystilifera to be paraphyletic, but Pelagica was a monophyletic sister to the remaining hoplonemerteans. Stiasny-Wijnhoff (1923a) argued that pelagic nemerteans are 'primitive' and ancestral to benthic hoplonemerteans. Therefore, in cladistic terms, her Polystilifera would be paraphyletic, because it excluded monostiliferans. Stricker's (1985) functional analysis of proboscis armature morphology, persuaded him that polystilifery was ancestral to monostilifery. This is consistent with Sundberg's

(1990) cladistic analysis and with the spirit of Stiasny-Wijnhoff's (1923a) arguments.

Our overall understanding of the polystiliferans, arguably, is worse than for any other comparably sized group of nemertean species. Much of the primary literature on pelagic nemerteans is based on poor to atrociously preserved material. Despite this, some insightful anatomical descriptions of these animals have been made in the monographs by Brinkmann (1917), Coe (1926) and Korotkevich (1955). However, comparative anatomy, based on bad preservation and lack of material for many species, has been handicapped by an even smaller set of morphologically tractable characters than the already sparse suite of morphological characters provided by other hoplonemerteans. Whether the apparent simplicity of pelagic nemerteans is a consequence of reduction or ancestral primitiveness is a key mystery in the evolutionary history of the group. Cladistic analyses with the currently available characters offer conflicting evidence or no insight on this problem, depending in part on whether one accepts a phylogeny with hoplonemerteans as the most plesiomorphic extant nemerteans (Norenburg, 1998). Yet, higher-level taxonomic reorganizing of this group persists, without any increase in useful knowledge to direct this activity. In the following presentation we treat the group Pelagica in its traditional sense, which could be either a clade or grade of pelagic polystiliferan hoplonemerteans, and we imply no support for or against monophyly.

Several features of the specimens collected by us illustrate our poor understanding of these features and of pelagic nemerteans. Korotkevich (1955, Figure 16) shows what she describes as 'cross-striated circular muscle' in the rhynchocoel of wall of *Pelagonemertes excisa* Korotkevich, 1955, and reports it as occurring in five additional species. She also reported the presence of 'cross-striation' in longitudinal muscle of the body wall of two species. These observations appear to have been overlooked completely in subsequent non-Russian literature. In this paper, we amplify her description of the striations and we report on (1) presence of 'striated' musculature in additional species of pelagic polystiliferans, (2) aspects of the relationship between proboscis insertion and rhynchocoel (sheath) musculature, (3) topology of circular and crossed diagonal musculature in the proboscis sheath, and (4) the previously unreported presence of crossed diagonal musculature in the body wall of at least some pelagic polystiliferan species.

Materials and methods

Most of the 43 specimens in this study were obtained along a transect (48–56 km long) paralleling the coastline 160 km off shore, north of Pt. Conception, California, in Sept. 1992, March 1993, Sept. 1993, and Feb. 1994. Some specimens were obtained in Santa Cruz Basin, south of Santa Cruz Island, in June 1994, and some were taken in and west of Monterey Bay, California during Sept. 1992 and Dec. 1993 (for details, see Roe & Norenburg, 1998). Animals were collected using a Tucker Trawl modified to recover animals in good condition (Childress et al., 1978). Depths sampled ranged from 0 m (surface) to approximately 3800 m. The two species of protopelagonemertid and one species of *Crassonemertes* were collected about 5 km off Makaha (Waianae), Oahu in May 1993. Specific collecting details are provided with the records of each vouchered specimen.

Serially sectioned voucher specimens are deposited in the National Museum of Natural History (USNM), Washington, DC, U.S.A. The vouchered material examined includes: *Phallonemertes* cf. *murrayi* (Brinkmann, 1912; = *Planktonemertes murrayi* of Korotkevich, 1955), $n = 6$ (USNM #174019–174024); *Nectonemertes* cf. *mirabilis* Verrill, 1892, $n = 16$ (USNM #174017, 174018, 174025–174038); *Cuneonemertes* cf. *elongata* Coe, 1954, $n = 2$; (USNM #174052, 174053); *Crassonemertes* cf. *robusta* Brinkmann, 1917, $n = 2$ (USNM #174054, 174055); *Crassonemertes* sp., $n = 1$ (USNM #174057); *Proarmaueria* cf. *pellucida* Coe, 1926, $n = 13$ (USNM #174039–174051); and two species of protopelagonemertid, $n = 2 + 1$ (USNM #174058–174060).

Specimens in this study usually appeared alive prior to and at fixation. Specimens coming up in the insulated cod-end consistently were in better condition than those in the netting in front of the cod-end. Individuals were anesthetized either in $MgCl_2$ isotonic with local seawater, or in 0.2% propylene phenoxtyol, and then fixed in Hollande's cupri-picric-formal-acetic fluid, 10% buffered formalin or Streck Tissue Fixative (Streck Laboratories, Inc., Omaha, Nebraska). Specimens for light microscopy were embedded in paraffin (56 °C m. p.), sectioned at 6 or 8 μm and stained with a trichrome method adapted from Mallory, Gomeri and Gurr-McConaill (Crandall, in litt.). About tissues for transmission electron microscopy (TEM) we can say only that they were fixed in phosphate buffered glutaraldehyde, rinsed in buffer and postfixed in osmi-

um tetroxide. One of these tissues is body wall of an unknown pelagic polystiliferan and the other is body wall and proboscis sheath of *Phalloneustes cf. murrayi*. The latter was stored in 70% ethanol for about 10 months prior to embedding. These tissues were embedded in plastic resin and thin-sectioned as described by Stricker & Folsom (1998).

Results

Striated muscle

Proboscis sheath

Striation visible with routine histological preparation is present in the circular muscle (CM) and longitudinal musculature (LM) of the proboscis sheath in seven of the nine polystiliferan species examined (Figure 1A–F). The two exceptions are *Phalloneustes cf. murrayi* and *Cuneoneustes cf. elongata*. Striation is present in six *Nectoneustes cf. mirabilis*, 12 of 13 *Proarmaueria cf. pellucida* specimens, all three *Crassonneustes* specimens, and in three specimens of two protopelagonemertid species. In what we assume to be optimal optical sections, the striation appears to be a simple alternation of light and dark bands within a muscle fiber. In *Nectoneustes* muscle fibers are about 5 μm in diameter; their light and dark bands are about 0.7 μm and 0.6 μm wide respectively, but this varies with specimens and could not be measured effectively with light microscopy. In a few instances, where muscle cells are isolated from each other and staining is optimal, the striation is reminiscent of typical striated muscle, including the presence of a central light band in the dark band (Figure 1C). In some cases, the dark bands appear to form a helical strand running through the length of a muscle fiber (Figure 1A, B). In other cases, each dark band appears to form an incomplete ring, with its two ends meeting at an offset. Often, in the same sections with striated muscle fibers, other fibers do not have evident striation but do have a somewhat twisted longitudinal array of very fine fibrils (Figure 1A). The two conditions seem to be mutually exclusive for a given fiber, as though they are allomorphs. A full range of variation in pattern can be found within a given specimen, and some variation is present in all specimens. In some cases the striation is evident through wide staining variation among slides from a single specimen, whereas in other instances striation may be barely or not at all discernible under

strong staining conditions, while being readily evident in the most weakly stained slides of the same specimen. In other cases, we cannot detect striation in specimens that we attribute without hesitation to a species where it is clearly present in other specimens, as is the case with *Nectoneustes*. In one instance (*P. cf. murrayi*, USNM #174020), a very faint striation can be detected in the LM of the anterior proboscis, but this is an indirect manifestation, visible only as a regular pattern of light and dark staining. Visualization of the striation appears to be sensitive to fixation conditions and/or staining procedures. Poorly defined dense bodies, irregularly aligned in TEM of badly fixed rhynchocoel muscle, provide equivocal evidence of striation in *Phalloneustes cf. murrayi* (Figure 1F). We have been unable to observe muscle striation in re-examining the specimens comprising Coe's large taxonomic range of sectioned pelagic specimens in the USNM collection.

Body wall

Stephen Stricker (in litt.) had discovered previously with TEM, but not reported, that the body-wall musculature of an unspecified pelagic polystiliferan appears to be obliquely striated (Figure 2). With light microscopy of our paraffin-embedded specimens we discern striation in body-wall muscle in two specimens of *P. cf. murrayi* (Figure 3A), two *N. cf. mirabilis* (Figure 3B), and one specimen each of the two protopelagonemertid species, all of which have relatively robust body-wall musculature compared to the other species. The striation of the body-wall musculature is never as distinctive as that of the proboscis sheath and appears to be at a finer scale; the individual muscle fibers are thinner than those of the sheath, and spacing of striations appears smaller. No striation of body-wall muscle was evident with TEM of the same block of *P. cf. murrayi* tissue in which there was some evidence of striation of the rhynchocoel muscle (but the poor quality of fixation must be borne in mind).

Proboscis insertion

The proboscis insertion of the several species examined follows the general hoplonemertean plan. Muscle fibers or bundles (also called 'fixators') radiate outward from the point where the proboscis and rhynchodeum meet, pass over the anterior face of the cerebral ganglia, and insert among the LM of the body wall (Figure 4). The primary difference is the amount of musculature involved. In some cases there may be very few dis-

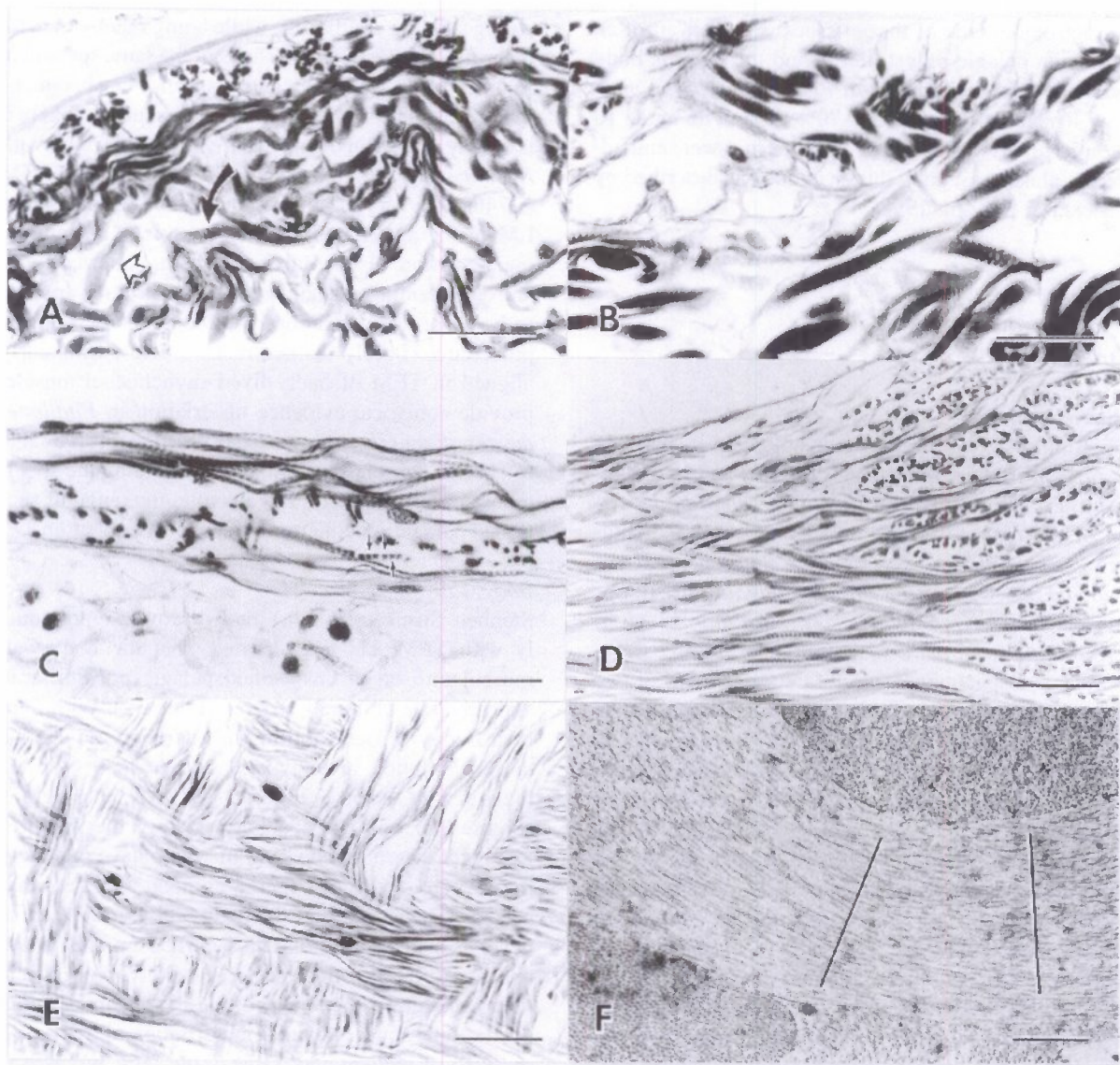


Figure 1. A. *Nectonemertes cf. mirabilis*. Transverse section of rynchocoel musculature, showing organized striation (solid arrow) and irregular strands (open arrow) in muscle fibers. Scale = 40 μm . B. *Nectonemertes cf. mirabilis*. Enlargement of Fig. 1A. Scale = 20 μm . C. *Proarmaueria cf. pellucida*. Transverse section of proboscis sheath, showing striations and discrete bands (arrows) in oblique muscle fibers. Scale = 40 μm . D. Protospelagonemertid (USNM #174058). Transverse section of ventral portion of proboscis sheath (near transition of dorsal blood vessel, where longitudinal fibers tend to be lacking), showing striated circular and/or oblique muscle fibers. Scale = 50 μm . E. Protospelagonemertid (USNM #3174060). Longitudinal, tangential section of proboscis sheath, showing striated longitudinal fibers and interlacing of thick, multifiber bundles of longitudinal and circular and/or oblique muscle fibers. Scale = 50 μm . F. *Phallonemertes cf. murrayi*. TEM thin section; poorly fixed rynchocoel muscle showing hint of aligned dense bodies near vertical lines. Scale = 0.5 μm .

crete bundles of fibers and in other cases there may be a relatively complete diaphragm formed. In all of the pelagic polystiliferans examined here, the insertion muscles insert among either the inner or the entire LM of the body wall – which, in the latter case, usually is so thin that ‘inner’ and ‘outer’ are not practically distin-

guishable. In our *Nectonemertes*, *Phallonemertes*, one of our *Crassonemertes*, and both protospelagonemertids, fixators are arranged in a conspicuous radial array (Figures 5A and B). These gradually proceed posteriorly, forming a ‘divided LM’ in principle, though this

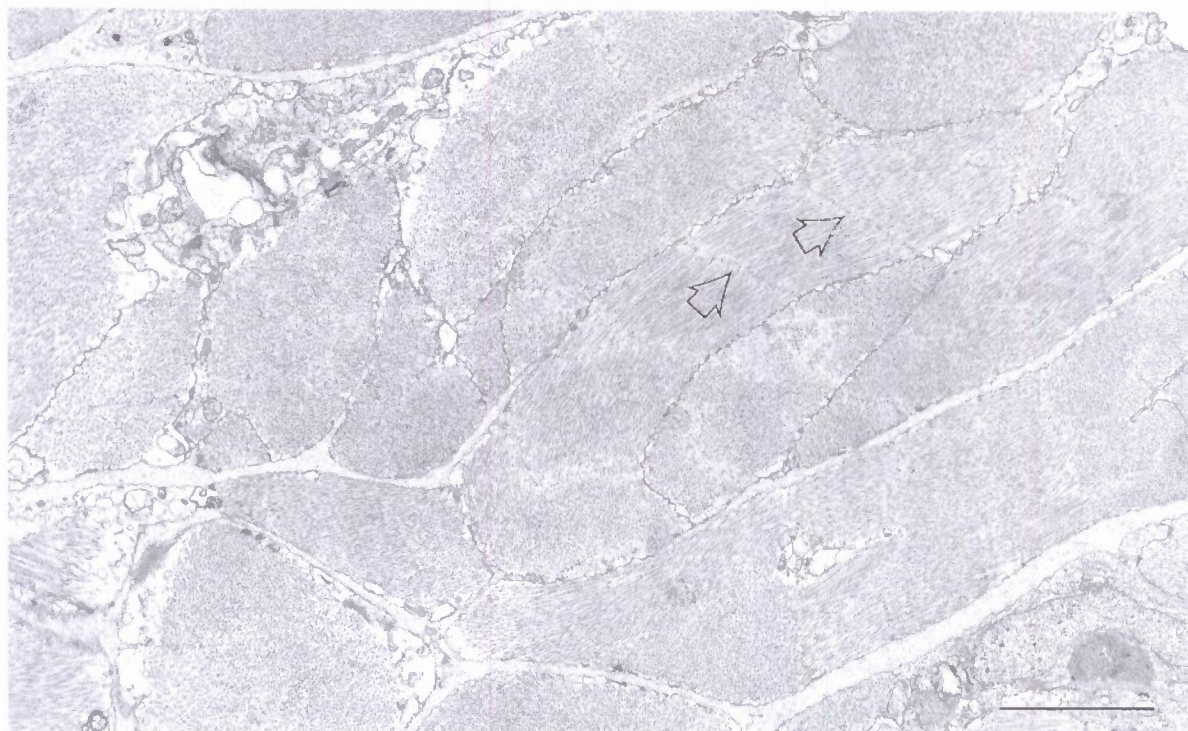


Figure 2. Unknown pelagic polystiliferan. TEM thin section; body-wall muscle showing aligned dense bodies (open arrows). Scale = 0.5 μm .

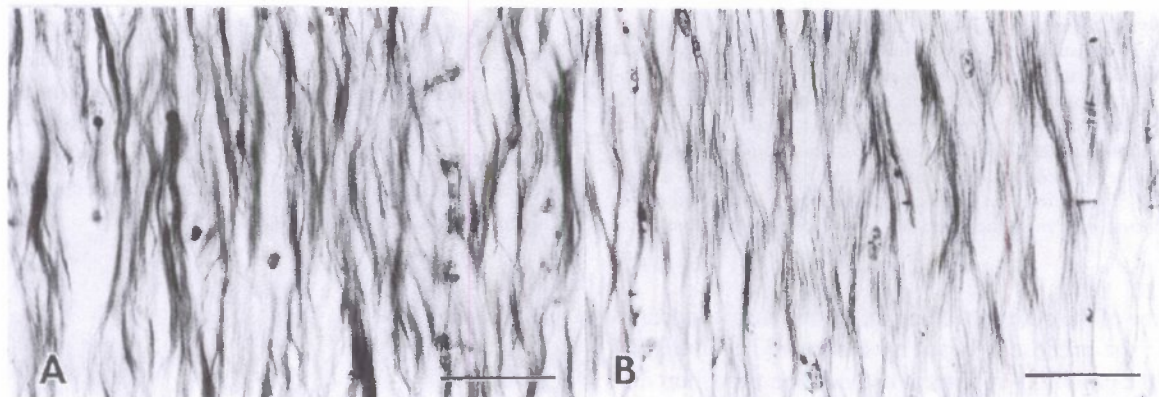


Figure 3. Tangential longitudinal section; body-wall muscle showing fine striations in longitudinal muscle fibers. A. *Phallonemertes cf. murrayi*. B. *Nectonemertes cf. mirabilis*. Scale = 40 μm .

is not nearly as definitive as it is in certain monostiliferans (cf. Kirsteuer, 1974).

The proboscis nerves, in the pelagic nemerteans examined here, arise from the cerebral ganglia individually or from one or more pairs of stem nerves that ramify before entering the proboscis. They form a nerve plexus about the musculature of the anterior proboscis sheath immediately posterior to the proboscis

insertion (Figure 5C, D). This feature makes it particularly convenient to follow the course of the two proboscideal LM strata. In all except one of the species we examined, the inner LM (i.e., nearest the rhynchocoel lining) of the proboscis is confluent with the LM of the proboscis sheath, whereas the outer LM extends to the body wall. In one *Proarmaueria* (USNM #174045) the proboscis insertion is especially weakly developed and

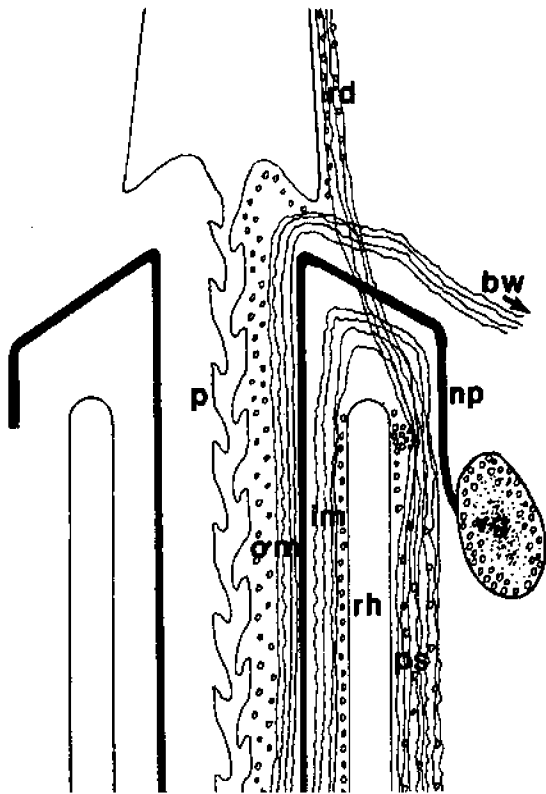


Figure 4. Schematic cartoon of generalized proboscis insertion of pelagic polystyliferan, showing inner and outer strata of proboscis longitudinal muscle separated by nerve plexus (heavy line) respectively confluent with rhynchocoel and body-wall longitudinal muscle. Abbreviations: bw, muscle extending toward body wall; im, inner longitudinal and circular muscle strata of proboscis; np, proboscis nerves and plexus; om, outer circular and longitudinal muscle strata of proboscis; p, proboscis; ps, proboscis sheath; rd, rhynchodeum wall; rh, rhynchocoel; vg, ventral cerebral ganglion.

some of the outer LM fibers of the proboscis appear to join the proboscis sheath musculature. The inner LM of the proboscis appears to extend for a very short distance along the anterior rhynchocoel wall, but it quickly becomes interwoven with CM. The inner CM of the proboscis is separated from the inner LM by extracellular matrix (ECM) and abuts the rhynchothelium directly. (We use the term rhynchothelium to refer to the lining of the rhynchocoelic cavity, which includes the lining of the proboscis.) In contrast, immediately adjacent is the CM of the proboscis sheath, but it is separated from the proboscis rhynchothelium by ECM and it is not separated from the LM. At this transition, the sheath CM usually forms a relatively distinctive sphincter, posterior to which the CM is interwoven with LM (Figure 5E).

Topology of proboscis sheath muscle

Longitudinal sections of proboscis sheath from *N. cf. mirabilis*, *P. cf. murrayi*, and the proarmueriid indicate that the 'circular' muscle of the sheath consists primarily or exclusively of obliquely oriented fibers crossing each other (Figure 6A–C). The sheath musculature of the protopelagonemertids is so massively developed that it is difficult to determine the overall orientation of the 'circular' musculature. In one of the specimens, it appears that most of the inner fibers are circular in orientation, whereas the outermost fibers are crossing, obliquely oriented fibers. We do not have longitudinal sections of the proboscis sheath for the remaining species.

Oblique body-wall muscle

Oblique, or diagonal musculature is present between the outer CM and inner LM of the body wall in *Phaltonemertes*, *Nectonemertes* (Figure 6D), and the two protopelagonemertid species (Figure 6E). In each it is a relatively wide-mesh network that is never more than one fiber thick and is discernible only in tangential sections along the surface of the body wall. Differences are in size of the mesh and the number of muscle fibers comprising individual strands of the network. These differences correlate, more or less, with robustness of the overall body-wall musculature, which in these species is from several to many fibers thick. Body-wall musculature of the other four species is only one to few fibers thick and often is discontinuous, especially along the lateral margins of the body.

Discussion

Striation

Many features that figure prominently in taxonomy and systematics of other nemerteans are significant in the Pelagica by their absence – for example, cerebral sensory organs, cephalic glands, ocelli, and nephridia. However, ontogeny and organization of the musculature of the proboscis and rhynchocoel have loomed large in speculation on evolutionary relations among nemertean groups ever since the seminal paper by Wijnhoff (1914). The presence of striated muscle in pelagic nemerteans, and its taxonomic and anatomical distribution could have profound implications about phylogeny within the nemerteans. Conversely, our

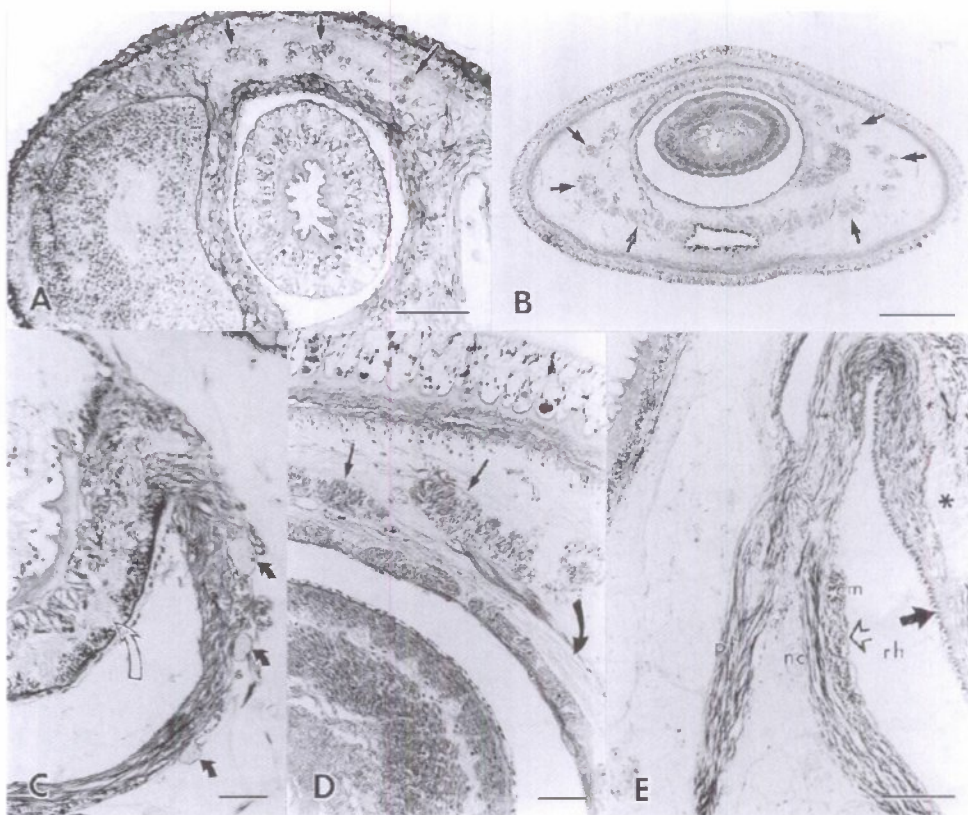


Figure 5. A. *Nectonemertes cf. mirabilis*. Transverse section of cerebral region, showing individual proboscis insertion fixators (arrows) approaching body wall. Scale = 200 μm . B. *Crassonemertes* sp. Transverse section of cerebral region, showing radial array of fixator muscle bundles (arrows) of proboscis insertion. Scale = 500 μm . C. *Cuneonemertes cf. elongata*. Transverse and slightly oblique section through proboscis sheath near its anterior limit, showing ramified proboscis nerves (solid arrows) along dorsal wall and ramified nerves connected by a commissure (open arrow) along the ventral wall, which is slightly closer to actual proboscis insertion. Scale = 50 μm . D. *Crassonemertes* sp. Transverse section in same region as Figure 5B, showing plexus of proboscis nerves (curved arrow) encircling proboscis sheath, with some proboscis fixator muscle bundles (arrows) external to that. Scale = 50 μm . E. Protapelagonemertid (USNM #174060). Sagittal section through proboscis insertion, showing inner and outer muscle strata of proboscis (solid arrow), separated by nerve plexus (*), and respectively confluent with proboscis sheath (hollow arrow) and proboscis fixator (pf) extending to body wall; a few longitudinal muscle fibers crossing the nerve plexus extend between the proboscis sheath and the rhyndocoel wall. Note the distinct bundle of inner circular muscle fibers (CM) near the anterior of the proboscis sheath, and the complete absence of circular muscle fibers through the curvature that connects the sheath and proboscis; nc, proboscis nerve commissure; rh, rhyndocoel. Scale = 200 μm .

understanding of the evolutionary history of this character, via a well-corroborated phylogeny, might provide some new insight to the functional significance of muscle striation.

Turbeville (1991) reported muscles with 'characteristics of obliquely striated muscle' from three species of Paleonemertea, but detailed anatomical distribution was not specified. His (ibid.) and other TEM investiga-

tions to date reported only invertebrate smooth muscle for hetero- and hoplonemertean. It should be noted here, *inter alia*, that Norenburg (unpublished poster at 'The Origins and Relationships of Lower Metazoa,' London, 1983) observed strikingly 'striated' larval muscles (Figure 7) in a heteronemertean pilidium larva. Korotkevich (1955) had noted the presence of 'cross-striated' rhyndocoelic CM in *Nectonemertes major*

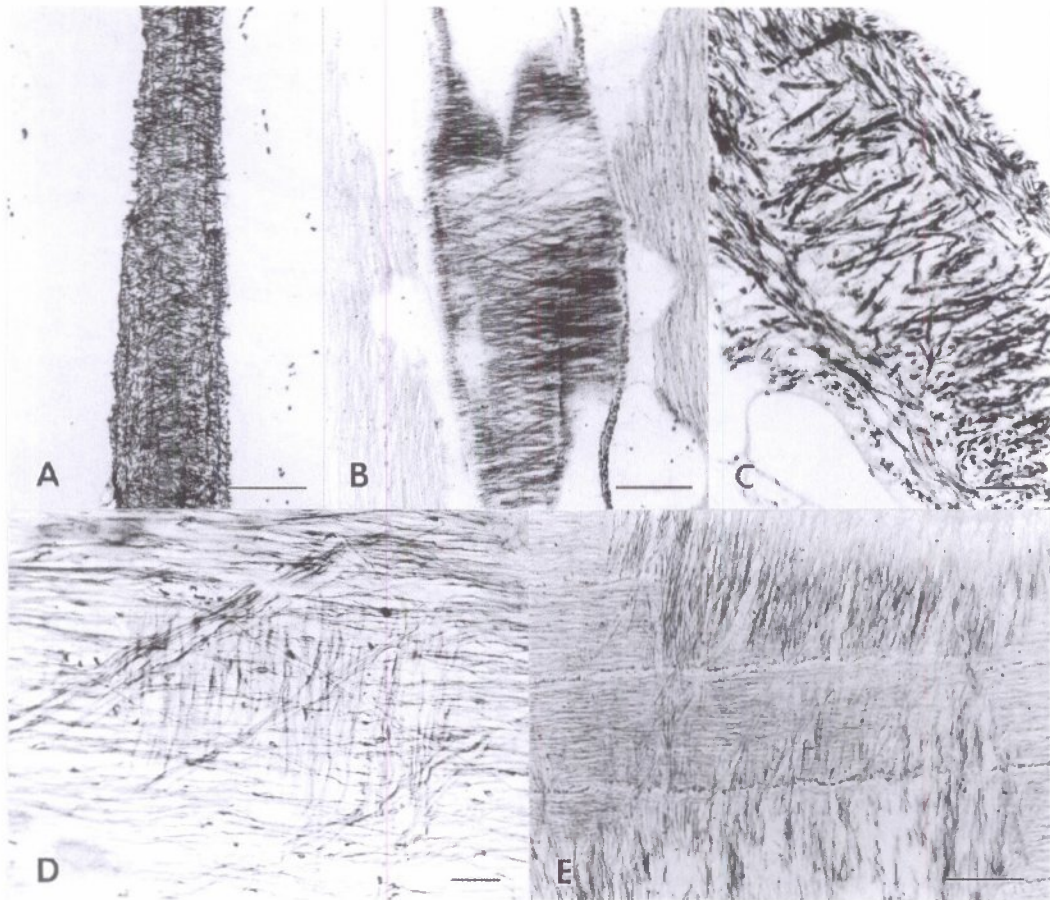


Figure 6. A–C. Longitudinal sections through proboscis sheath, showing significant presence of oblique muscle fibers; most apparent circular fibers are actually oblique. A. *Phallonemertes cf. murrayi*. Scale = 200 μm . B. *Nectonemertes cf. mirabilis*. Scale = 200 μm . C. *Proarmaueria cf. pellucida*. Scale = 50 μm . D. *Nectonemertes cf. mirabilis*. Longitudinal tangential section of body wall, showing oblique muscle fibers between inner circular and outer longitudinal musculature. Scale = 50 μm . E. Protipelagonemertid (USNM #174060). Longitudinal tangential section of body wall, showing oblique muscle fibers between inner circular and outer longitudinal musculature. Scale = 200 μm .

Korotkevich, 1955 (p. 77), *Neoarmaueria* (formerly *Mesarmaueria*) *crassa* (Korotkevich, 1955; p. 87), *Pelagonemertes excisa* (p. 22, Figure 16), *Pelagonemertes brinkmanni* Coe, 1926 (p. 115), *Pelagonemertes oviporus* Korotkevich, 1955 (p. 117), and *Pelagonemertes laticauda* Korotkevich, 1955 (p. 123). She (ibid.) also had noted muscle 'cross-striation' in 'histological cross sections of the body wall' (therefore, probably CM) of *Pelagonemertes brinkmanni* (p. 115), and in the body-wall LM of *Pelagonemertes oviporus* (p. 117). In our paraffin sections of the body

wall we have observed striation only in LM. Striation of the body-wall muscle is not evident in most of our specimens of *Phallonemertes* and *Nectonemertes*. We infer from this that its appearance, within the limits of our light microscopic capacity, is strongly dependent on preparative procedures. We believe that differences in the initial stages of fixation likely account for some of the variability in demonstrating the striation of musculature, but we have no clear correlations. Even in cases of apparent poor fixation, where muscle tissue is swollen and stained irregularly, striation still is evi-

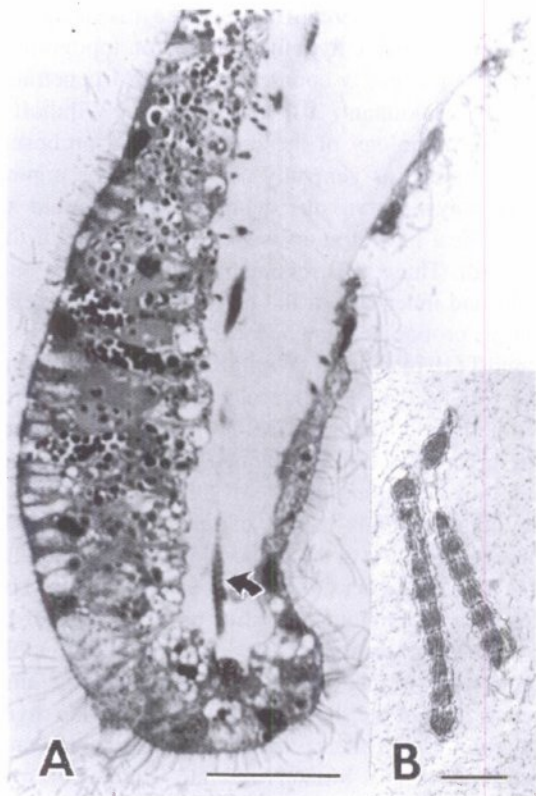


Figure 7. Lineiform pilidium larva. A. Section parallel to oral-aboral axis (1- μ m thick plastic section) of lateral lobe (species unknown), showing striated larval muscle (arrow). Scale = 20 μ m. B. TEM section of larval muscle. Scale = 5 μ m.

dent in some instances, although it is not as sharply defined. Clearly, a determination that striation is absent is fraught with uncertainty. Stricker's electron micrographs of body-wall muscle show evidence of oblique striation in fibers that are orthogonal to each other. We infer from this that both the longitudinal and circular fibers of his specimen are striated. In most of the specimens where we find evidence of striation in muscle of the proboscis sheath, it is evident in both 'circular' and longitudinal fibers. We anticipate that the same is true of Korotkevich's specimens but that she did not see striation in the LM because she worked mostly with transverse sections of the body. Rosenbluth (1972) suggested that oblique striation easily may be, and probably has been overlooked, even with TEM, when sectioned tissue has not been orientated suitably. There seems to be little consensus on exactly what constitutes oblique striation.

So-called, obliquely striated muscle is found in a variety of invertebrates (e.g.; Kalyptorhynchia – De

Vocht, 1989; Nematoda – Hope, 1969; Gnathostomulida – Rieger & Mainitz, 1977; Annelida – Rieger & Rieger, 1975; Rosenbluth, 1972). There is, as yet, little evidence how or if muscle striation in these forms is related. Turbeville (1991) evokes Miller's (1975) work on length-tension characteristics of muscle, which suggests that smooth and obliquely striated muscles might be favored in organisms capable of significant extension. It is evident from observing living pelagic nemerteans that they and their body wall are among the least extensible among nemerteans, though this may not be true for the rhynchocoel. Rieger et al. (1991) consider that oblique striation, apparent in some specialized turbellarian muscles, may be related to rapid contraction. Korotkevich (1955) made the same argument with respect to the potential for rapid eversion of the proboscis. However, the proboscis of most nemerteans is capable of rapid eversion; that of living, freshly caught pelagic nemerteans seems not to be notably different (pers. obs.). A functional interpretation for 'why' pelagic nemerteans have striated muscle does not emerge easily from the available evidence. For instance, *Phalloneustes* has striated body-wall muscle but appears not to have striated rhynchocoelic muscle. In contrast to many benthic nemerteans, the body-wall muscle of most pelagic nemerteans is relatively weak and may not provide as much hydraulic assistance to the rhynchocoel musculature. However, some protopelagoneurids have relatively strong body-wall muscle plus massively developed rhynchocoel muscle, and both are striated.

The broad distribution of muscle striation in pelagic Polystilifera, as now seems to be the case, suggests that it is homologous within this group. Yet, the present study indicates that it may be lacking in some species or in some muscle systems among the polystiliferans. The fact that striated muscle is known from some palaeoneurids, a heteroneurid and from pelagic polystiliferans presents three primary possibilities: 1) convergence among subclades in the three major groups, 2) striated muscle is plesiomorphic for nemerteans and has been lost in some clades (possibly including one or more pelagic polystiliferan clades), or 3) muscle striation is present to some degree in all nemerteans and it is not easily recognized, even with TEM. The first two possibilities can be tested and distinguished from each other only with a comprehensive phylogeny for nemerteans. Sundberg's (1990) morphology-based cladistic analysis of hoplonemerteans supported the hypothesis that pelagic polystiliferans are the plesiomorphic sistergroup to the remaining hoplone-

merteans; this is supported by a morphology-based cladistic analysis of the phylum (Norenburg, 1998). Thus, a reasonable working hypothesis seems to be that muscle striation is plesiomorphic for hoplonemerteans and for the phylum. However, this will require more definitive characterization of muscle cytology from a spectrum of nemertean taxa where striation presently must be presumed to be absent. Our results cause us to question whether striation truly is lacking when it is not visible with light microscopy or not easily evident with TEM.

Proboscis insertion and sheath muscle

Our observations on well-developed proboscis insertions suggest that the LM of the proboscis may represent two constructs, with the outer LM related to the body-wall LM and the inner LM related to the rhynchocoel LM. This possibility appears not to have been fully appreciated previously. Brinkmann (1917: pl. 15, Figure 10) illustrates approximately that arrangement but makes no further mention of it. Coe (1926: 166) describes the LM of the sheath as being continuous with that of the proboscis and later states that the LM of the proboscis gives rise to the insertion muscles.

Wijnhoff (1914; Stiasny-Wijnhoff, 1923b) postulated, and Brinkmann (1917) agreed, that the inner (subrhynchothelial) CM of the hoplonemertean proboscis is a new construction (i.e., autapomorphy) among nemerteans. More recent evidence that ICM also occurs in the proboscis of other nemerteans (e.g., Turbeville, 1991; Montalvo et al., 1997) contradicts this hypothesis. In Wijnhoff's (1914; Stiasny-Wijnhoff, 1923b) view, the outer CM and inner LM of the proboscis, together with the inner LM and outer CM of the rhynchocoel, may be derived ontogenetically from the inverted body-wall musculature, with the rhynchocoel resulting from a delamination within the inverted LM. Brinkmann (1917: 159) asserted that pelagic nemerteans contradicted that theory. He considered the interwoven rhynchocoel musculature to be characteristic of the 'primitive' genera of pelagic nemerteans. He argued that even in an 'advanced' form like *Nectonemertes*, in which the rhynchocoel possesses an outer CM and inner LM, one can discern the 'ancestral' (*ursprungliche*) position of the CM and LM just behind the proboscis insertion – that being, an inner CM and an outer LM. Brinkmann (1917: 159) declared the inner (subrhynchothelial) CM of the proboscis is continuous with that of the proboscis sheath, where he asserts it is penetrated by LM derived from

the body wall. Coe (1926: 166) repeated this viewpoint. This is a reasonable hypothesis based on topography, but one can as easily counter that the CM penetrates the LM. Brinkmann (1917) believed that Wijnhoff's thesis on homology of the body-wall and proboscis muscle strata was generally correct. But, he argued that in polystiliferans the delamination is within an inner CM, a new stratum without homologue in the body wall. Thus, in Brinkmann's view, only the outer CM and inner LM of the body wall are represented in the proboscis-rhynchocoel complex. In contrast, Wijnhoff (1914; Stiasny-Wijnhoff, 1936) believed the outer CM of the rhynchocoel has its homologue in the innermost body-wall CM, seen in some paleone-merteans or in what she postulated to be its homologue, the dorsoventral musculature of other nemerteans. In longitudinal sections of some of our specimens there is apparent continuity of the proboscis subrhynchothelial CM and the inner CM of the proboscis sheath. The latter forms a pronounced sphincter just posterior to the proboscis insertion. However, we think it notable that the proboscis CM abuts the rhynchothelium, whereas ICM of the sheath is separated from the rhynchothelium by ECM, as is the case in other nemerteans (Turbeville, 1991; Montalvo et al., 1998). Stiasny-Wijnhoff (1936, p. 117) noted that in most eurentantic (benthic) polystiliferans the proboscis sheath near the brain includes only LM, and that CM appears only farther posterior. She also noted that in some eurentantic species this anteriormost region includes only CM. She concluded (*ibid.*, p. 118) that this inner CM of the sheath is a derivative of the proboscis inner (subrhynchothelial) CM, as distinct from the outer CM of the proboscis sheath, which she considered a derivative of an ancestral body-wall ICM.

Whereas the proboscis nerves in many monostiliferans emanate from the ventral cerebral ganglia as just one pair of nerves (*pers. obs.*), and branch within the proboscis proper, this is not the case in the pelagic polystiliferans examined here. At least some cratene-mertids and perhaps a few other monostiliferans resemble the pelagic polystiliferans in this character (*pers. obs.*; Frank Crandall, *pers. comm.*), but it has not been adequately documented or studied in these other groups to allow for detailed comparison here.

Topology of proboscis sheath muscle

Our observations convinced us that it is not always practicable to distinguish oblique or circular orientation in rhynchocoel muscle, especially from trans-

verse sections. Descriptions of the rhynchocoel sheath musculature vary by author, and in most cases are based exclusively on transverse sections. For instance, Brinkmann (1917) appears not to distinguish between CM and oblique muscles; he describes only LM and CM from the proboscis sheath of *Phallone-mertes murrayi*. Our longitudinal sections of presumed conspecific forms show principally what Coe (1926) refers to as spiral (oblique) muscle. Coe (1926) appears to be the first to have made a consistent distinction between oblique and CM. Nevertheless, it is obvious from examining his sectioned material that in many or most cases it is improbable that he could or did distinguish between the two (unpubl. obs.). The variety of topological relationships of rhynchocoel CM, LM and spiral muscle in pelagic polystiliferans exceeds that seen in any other group of nemerteans. The variety observed may represent weakly constrained morphogenetic events (= plasticity) or significant phylogenetic divergence. Either scenario is consistent with a view that the Pelagica, and therefore the Polystilifera, is paraphyletic. Our observations offer no new insight on the possible relationship of polystiliferan rhynchocoel muscle and the wickerwork rhynchocoel musculature of Cratenemertidae. However, Sunberg's (1990) and Norenburg's (1998) analyses clearly favor the hypothesis that, within the hoplonemerteans, the monostiliferan bilayered rhynchocoel musculature is a derived state whereas an interwoven state is plesiomorphic.

Body-wall oblique muscle

Reports of oblique or diagonal musculature in the body wall of hoplonemerteans used to be rare. However, evidently it has been grossly under-reported in nemertean descriptions (Norenburg, 1986). More recent work continues to increase rapidly the number of species from which it is reported (pers. obs.; Crandall, 1993). To our knowledge, it has not been reported from pelagic polystiliferans. Nevertheless, it is present in the relatively muscular body walls of *Phallonemertes*, *Nectonemertes* and the protopelagonemertids. As is the case for a number of monostiliferans, ascertaining its presence sometimes requires exceedingly careful examination of sections that are tangential to the body wall. Thus, the presence of this oblique musculature cannot be taken to have any particular phylogenetic importance at this time, because assertions of absence must be considered suspect when rigorous documentation is lacking.

Conclusion

This study demonstrates that key aspects of polystiliferan muscle organization are poorly documented and prone to misinterpretation, as is so often the case in nemertean anatomy. The presence of striated musculature in pelagic polystiliferans is an important datum in considering evolution of this character among nemerteans and other invertebrates. The fact that its visualization is variable encourages caution in using this character. The present study provides morphological evidence for possible independent ontogenetic origins of the two LM strata in the hoplonemertean proboscis. Our work also reconfirms the complex variability of muscle pattern in the proboscis insertion and proboscis sheath of polystiliferans. Despite Brinkmann's (1971) and Stiasny-Wijnhoff's (1923a, b; 1936) best efforts to organize this apparent jumble of patterns into a coherent phylogenetic picture, a better hope for reconciling these patterns is in using cladistic analysis to derive independent phylogenetic hypotheses. Because of the evident paucity of suitable morphological data for polystiliferans, developing a well-supported phylogenetic hypothesis is likely to require molecular data.

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