Chapter 18

The Functional Morphology of the Tentacle Musculature of Nautilus pompilius

WILLIAM M. KIER

1. Introduction

The morphology of the musculature of cephalopods, and indeed that of many mollusks, is characterized by a tightly packed three-dimensional arrangement of muscle fibers that lack extensive fluid-filled cavities or hardened skeletal elements. Previous research on the arms and tentacles of squids (Kier, 1982) and the arms of octopuses (Kier, 1987) suggests that the skeletal support of these appendages is provided by a type of hydrostatic skeleton that differs from the classic conception of a hydrostatic skeleton (e.g., Chapman, 1958, 1975; Clark, 1964, 1981; Clark and Cowey, 1958; Wainwright, 1970, 1982) in that the musculature both creates movement and provides skeletal support. These appendages, termed *muscular-hydrostats*, are capable of diverse, complex, and highly controlled movements (Kier and Smith, 1985). This study of the functional morphology of *Nautilus* tentacles was undertaken to explore further the diversity of muscular arrangement and function in cephalopods.

2. Materials and Methods

This study of the morphology and behavior of tentacles of *N. pompilius* Linneaus, 1758 was based on specimens maintained in captivity in the New York

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Aquarium, Brooklyn, New York, by Dr. J. Chamberlain, Brooklyn College of the City University of New York. The animals were transferred from the exhibit area to an aquarium suitable for photography, where 16 mm cine films and still photographs were made of tentacle movements during foraging and feeding. The cine films were analyzed with an NAC Inc. DF-16C 16 mm projector.

Whole tentacles from specimens that had recently died were fixed for 24–48 hr in either Bouin's fixative or phosphate-buffered 2.5% glutaraldehyde and 4% paraformaldehyde. Excellent fixation and staining were achieved, especially with the buffered glutaraldehyde and paraformaldehyde fixative. The tissue was dehydrated through a graded series of ethanols, cleared in xylene, and embedded in paraffin (melting point 56–58°C). Transverse, parasagittal, and frontal serial sections were cut at 7–10 μm with a rotary microtome. Two staining procedures were used: (1) Milligan's Trichrome Stain, with aniline blue substituted for fast green, and (2) Picro-Ponceau with Weigert hematoxylin (Humason, 1979). Both stains show strong contrast between muscle and connective tissue. The sections were studied with direct, phase-contrast, and polarized-light microscopy.

3. Results

3.1. Gross Morphology and Movements of the Tentacles

Nautilus possesses numerous tentacles, classified by Owen (1832) into three groups: (1) one pre- and one postocular tentacle located in front of and in back of each eye, respectively; (2) a variable number of labial tentacles, arrayed on lobes surrounding the buccal mass (some of which are modified to form secondary sexual structures in both male and female animals); and (3) 19 pairs of digital tentacles, surrounding and extending beyond the labial tentacles (Fig. 1). Each digital tentacle consists of an extensible, muscular cirrus enclosed in a protective sheath. The cirri of the labial and digital tentacles are equipped on one side with adhesive annular ridges. The cirri of the ocular tentacles are not adhesive and are thought to be sensory (Griffin, 1900; Willey, 1898a). The morphology and function of the digital tentacles of the chambered nautilus are described in this chapter (Fernandez, 1907; Griffin, 1900; Hamada et al., 1980a; Willey, 1898b) (see also Chapter 17). Descriptions of the structure of the labial tentacles and associated secondary sexual structures of *Nautilus* are provided by Griffin (1898b), Haswell (1896), and Owen (1843); the structure of the ocular tentacles has been described by Griffin (1900) and Willey (1898a, 1902).

The sheaths of the digital tentacles (19 per side) are fused in a mass to form the cephalic sheath. Four of the digital tentacles per side are small and are directed laterally, with sheaths that open closer to the apertural margin of the shell than do the sheaths of the other, larger, and more medial digital tentacles (Fig. 1). The terminal portions (≈ 1 cm) of the sheaths of the digital tentacles are not fused to the cephalic mass and are triangular to quadrangular in cross section. The sheaths taper to a blunt point, where a slitlike or oval opening is located.

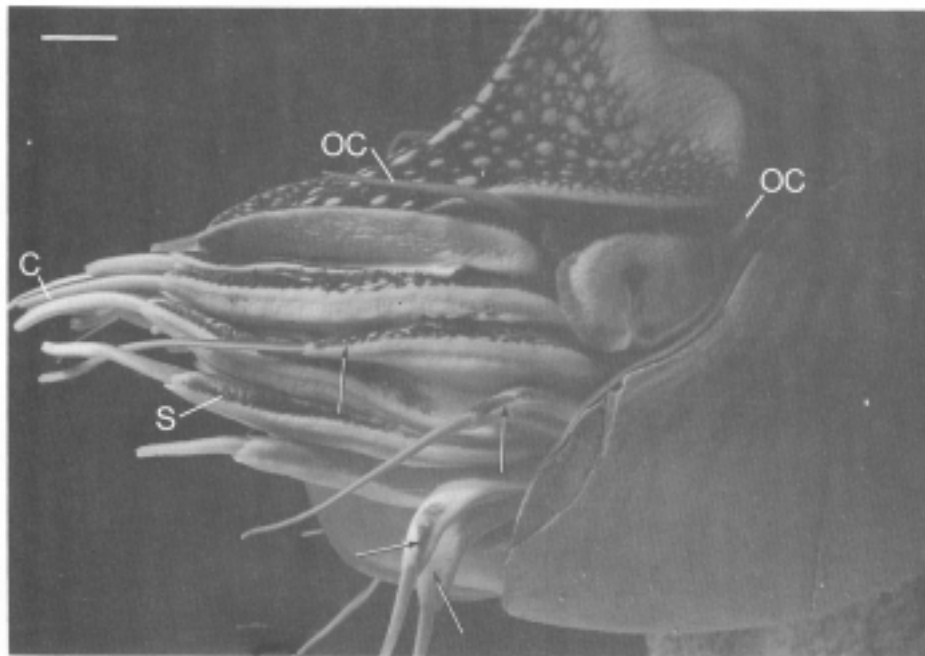The muscular cirri of the digital tentacles can be retracted completely into

Figure 1. Photograph of live *N. pompilius*. Many of the cirri (C) of the digital tentacles are extended beyond sheaths (S). The labial tentacles are not visible in the photograph, but the ocular tentacles (OC) can be seen in front of and in back of the eye. (→) Four smaller digital tentacles. Scale bar: 1 cm.

the sheaths. The cirri are slender and slightly tapered, with a blunt, rounded tip. The distal portion of the cirrus is approximately triangular in cross section, but the proximal portion is circular. The cirri are encircled with a series of narrow, annular grooves and ridges. The ridges on the oral side of the cirrus (the side facing the mouth) are adhesive and are more pronounced than those on the two aboral faces. The annular grooves and ridges become more closely spaced and less pronounced proximally. The base of each cirrus tapers slightly to insert at the base of the sheath and at this point the tissues are continuous.

The digital tentacles are used to seize and manipulate food and to attach to surfaces. Measurements from cine films of *N. pompilius* show the cirri to be capable of extensions of 90–110% of their fully retracted length. Extension of a cirrus occurs quite slowly, often requiring 5–10 sec or longer. Once extended, the cirri are capable of bending movements in any plane. Bending can occur either sharply at one point on the cirrus or more gently, over the entire extended length. Lateral bending of the free tips of the sheaths was observed during bending of extended cirri. Twisting of the cirri along their long axes (torsion) was also observed (Fig. 2). The total amount of torsion was usually small; the maximum observed was approximately 90° (at the distal tip, relative to the base). Torsion was observed to occur in both directions and commonly occurred during bending. For additional descriptions of the use of the tentacles, see Bidder (1962).
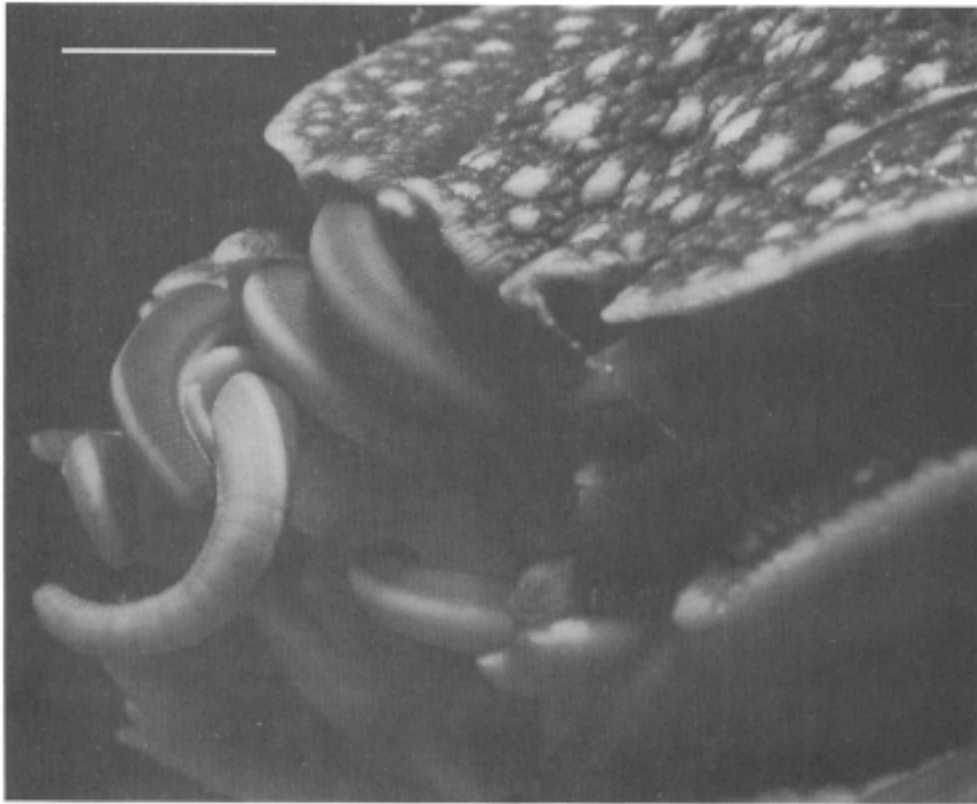
Figure 2. Close-up photograph of torsion and bending in the cirrus of a digital tentacle of a live *N. pompilius*. Scale bar: 1 cm.

## 3.2. Microanatomy of the Digital Tentacles

The axial nerve cord lies within the musculature and runs longitudinally down the cirrus in a subcentral location on the oral side (Figs. 3 and 4). The nerve cord is enlarged at the location of each oral adhesive ridge along the length of the cirrus (Fig. 5a). Nerves branch off laterally and orally from the axial nerve cord at the location of each oral adhesive ridge and extend within the trabeculae of the radial muscles (Fig. 5b) to the oral side of the cirrus, where they connect with a peripheral network of nervous tissue (described below). The nerve cord itself is enveloped by a thin sheath of fibrous connective tissue. Adjacent to the nerve cord on the oral side is a thick-walled artery. A larger and thinner-walled vein is located toward the outer surface on the oral side of the cirrus. Surrounding the axial nerve and occupying the core of the cirrus is a mass of radially arranged muscle fibers. The radial muscle fibers are arranged perpendicularly to the long axis of the cirrus. Radial muscle fibers extend from the central muscle mass of the cirrus to the periphery in longitudinally oriented sheets. Similar extensions of the central muscle mass of the arms of *Octopus* were termed *trabeculae* by Graziadei (1965a), and the term is therefore adopted for extensions of the radial

Figure 3. Schematic cutaway view of the cirrus of a digital tentacle of *N. pompilius*. Key: (AN) axial nerve cord; (AR) artery; (COM) crossed oblique muscle; (EP) epidermis; (LM) longitudinal muscle; (LN/LM) longitudinal nerve/LM network; (OAR) oral adhesive ridge; (OM) oblique muscle; (RM) radial muscle; (TR) trabeculae of radial muscle; (V) vein. Scale bar: 0.5 mm.
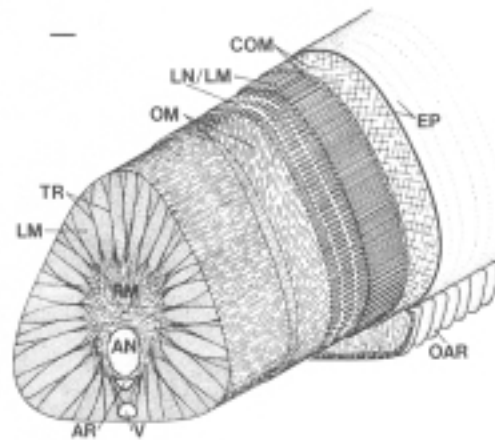
muscle in the cirrus of *Nautilus* (Figs. 3, 4, and 5). The trabeculae divide the surrounding longitudinal muscle into bundles and branch as they approach the periphery of the cirrus, where they insert on a layer of connective tissue immediately beneath the epidermis (see below). Both the longitudinal muscle and radial muscle are divided by and associated with a fine network of connective tissue (Figs. 5a and 6). The muscle fibers that make up the radial musculature in the center of the cirrus are not tightly packed, even when the shrinkage resulting from

Figure 4. Micrograph of a transverse section of the cirrus of a digital tentacle of *N. pompilius*. See the Fig. 3 caption for details. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 0.5 mm.
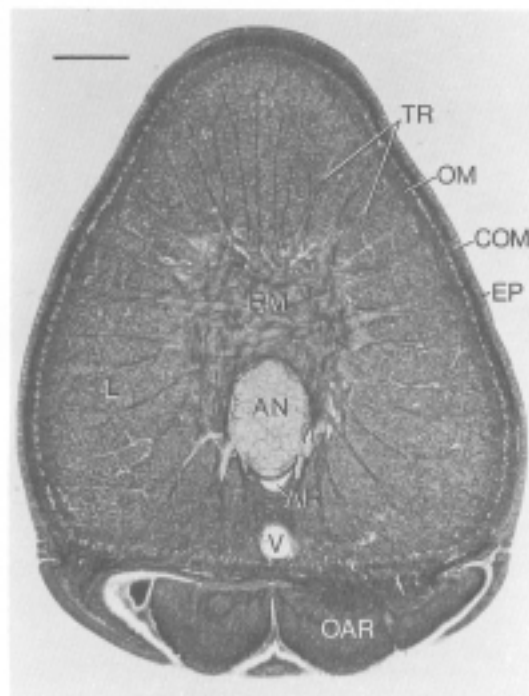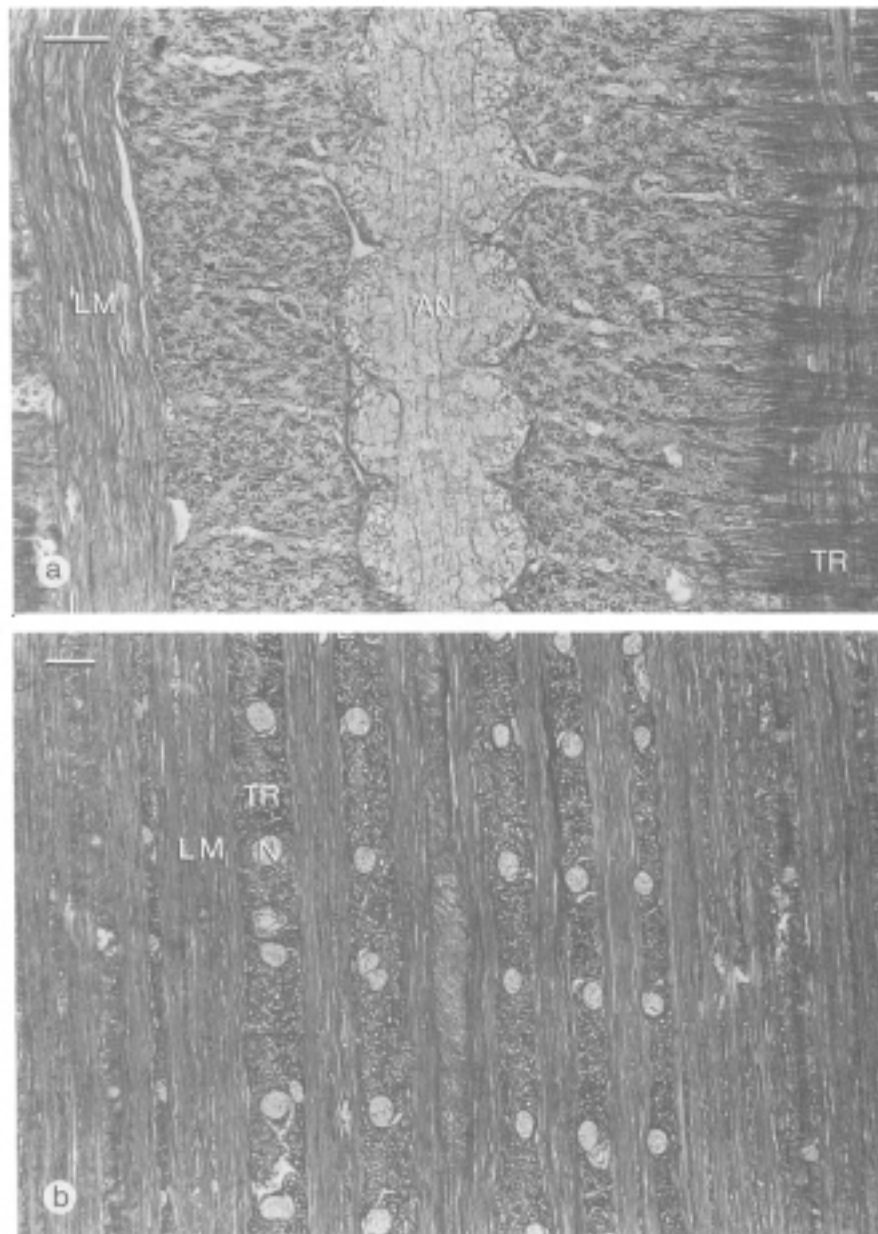
Figure 5. (a) Micrograph of a frontal section of the cirrus of a digital tentacle of *N. pompilius* at the level of the axial nerve cord. The nerve cord (AN) runs vertically in the micrograph and has greater diameter at the location of each adhesive ridge along the length of the cirrus. Surrounding the nerve cord is the radial muscle mass. Note that the radial muscle fibers, many of which are cut in cross section, are not closely packed but, instead, are separated by connective tissue (less densely stained). Longitudinal muscle (LM) is visible at the far right and far left of the micrograph. Trabeculae (TR) of the radial muscle mass are visible at right. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 100 μm. (b) Micrograph of a frontal section of the cirrus of a digital tentacle of *N. pompilius*. This section is closer to the oral side of the cirrus than the section in (a). Bundles of longitudinal muscle (LM) can be seen running vertically in the micrograph. Between the bundles of longitudinal muscle are the trabeculae (TR) of the radial muscle mass with muscle fibers in cross section. Note the numerous nerves (N) in cross section in the trabeculae. Each transverse alignment of a group of nerves occurs at the location of an oral adhesive ridge. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 100 μm.
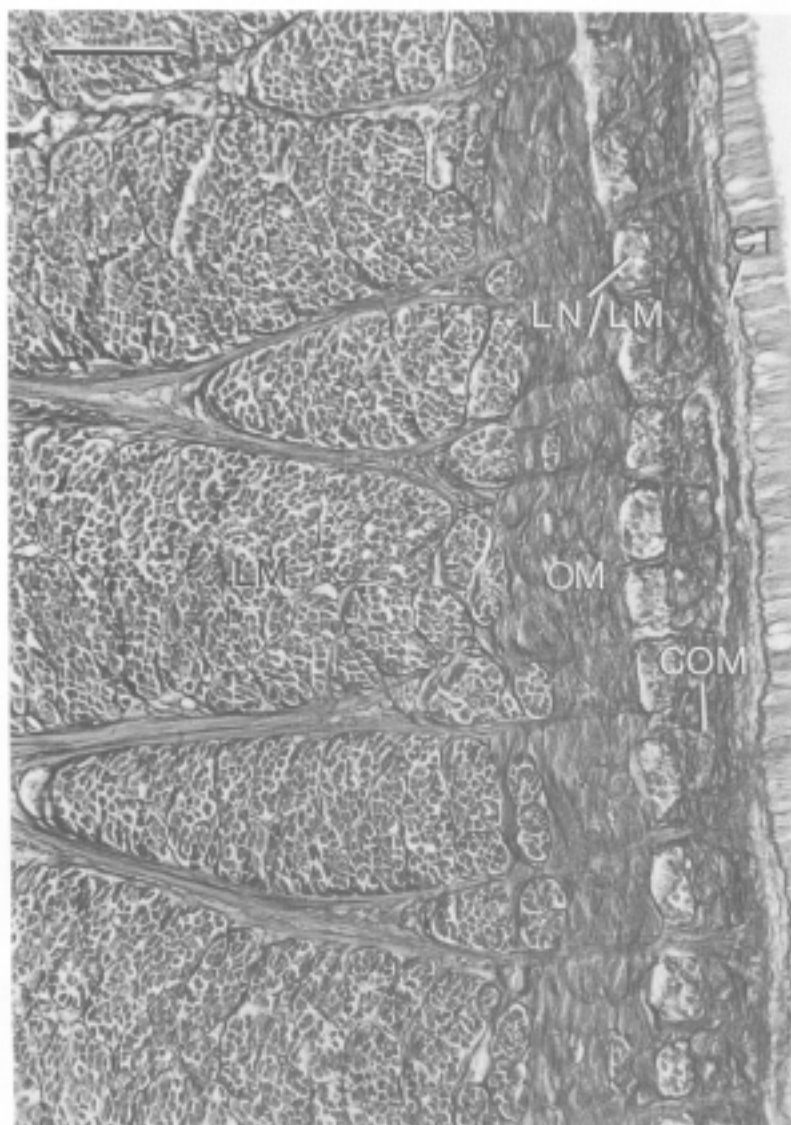
Figure 6. Micrograph of a transverse section of the cirrus of a digital tentacle of *N. pompilius*. The trabeculae of the radial muscle mass extend from the central radial muscle mass and branch to insert on connective tissue (CT) immediately beneath the epidermis. The trabeculae pass through (from left to right in the micrograph) the longitudinal muscle [(LM) cut in cross section], the oblique muscle layer [(OM) cut in oblique section], the longitudinal nerve/longitudinal muscle (LN/LM) bundle network, and finally the thin crossed oblique muscle layer (COM) underneath the connective tissue and epidermis. Note the connective tissue network in the longitudinal muscle. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 100 μm.

histological techniques is taken into account. Instead, individual muscle fibers and bundles of muscle fibers are separated from one another by the fine connective tissue network. The connective tissue is especially apparent in frontal sections such as Fig. 5a, but can also be seen in transverse sections (Fig. 6).

Enclosing the longitudinal muscle on each side of the cirrus is a layer of oblique muscle fibers (Figs. 3, 4, and 6). The oblique muscle layer is thickest laterally and thins aborally and especially orally. Parasagittal serial sections of the cirrus show that the handedness of the oblique muscle fibers on one side of the cirrus is opposite to that on the other side. If an oblique muscle fiber from either oblique muscle layer is traced from the oral side to the aboral side of the cirrus, it follows an oblique course from distal to proximal (Fig. 7a). The muscle fibers from the oblique layer on each side of the cirrus cross and interdigitate orally and aborally. No distinct connective tissue structure is present at their origin and insertion. The fiber angle of the oblique muscle layers (the angle that a muscle fiber makes with the long axis of the cirrus) ranged from 40 to 50° in the cirri examined. (However, the state of extension or retraction of the cirri was not known.)

Just outside the perimeter formed by the oblique muscle layers is an unusual array of longitudinal muscle bundles that are associated with a network of nervous tissue (Figs. 6 and 7). The longitudinal muscles of this network lie adjacent to and outside the longitudinally arrayed nervous tissue. Muscle fibers from the trabeculae of the radial muscle mass extend between the longitudinal nerve/muscle bundles. In addition to the longitudinally arrayed nerve bundles, circumferential nerve bundles connect each longitudinal nerve bundle around the periphery (Fig. 7). The circumferential nerve bundles have the same periodicity as the oral adhesive ridges. Nerve branches from the axial nerve cord connect to this peripheral array of nervous tissue at the positions of the circumferential nerve bundles.

A thin, interlaced layer of crossed oblique muscle fibers wraps the longitudinal bundles of muscle and nervous tissue (Figs. 3 and 7b). This layer was incorrectly identified by Griffin (1900) as a circular muscle layer. The crossed oblique muscle layer is wrapped by a thin layer of fibrous connective tissue that is in turn covered by a simple columnar epithelium on the aboral side of the cirrus. A large number of goblet cells are found in the epithelium. The epithelium covering the adhesive ridges on the oral side of the cirrus is different from that on the aboral surface. The epithelial cells that line the grooves between adhesive ridges are cuboidal, with large nuclei. There is an abrupt transition on the proximal side of each ridge from cuboidal to tall, columnar epithelial cells that comprise the surface of the adhesive ridge (Fig. 8). The nuclei of these cells are basal, and the terminal one third of each columnar cell is filled with intensely staining granules (red in Milligan's, black in Picro-Ponceau with hematoxylin) (see Chapter 17). The musculature of the adhesive ridges inserts on a thick basement membrane underlying the tall columnar epithelium (Fig. 8). Muscle fibers project from this insertion in trajectories that are perpendicular to the oral surface of the cirrus and converge to pass between the longitudinal nerve/muscle bundles. After passing between the longitudinal nerve/muscle bundles, the musculature of the adhesive ridge becomes incorporated into the musculature of the cirrus and is commonly observed to be continuous with the trabeculae of the radial muscle mass.
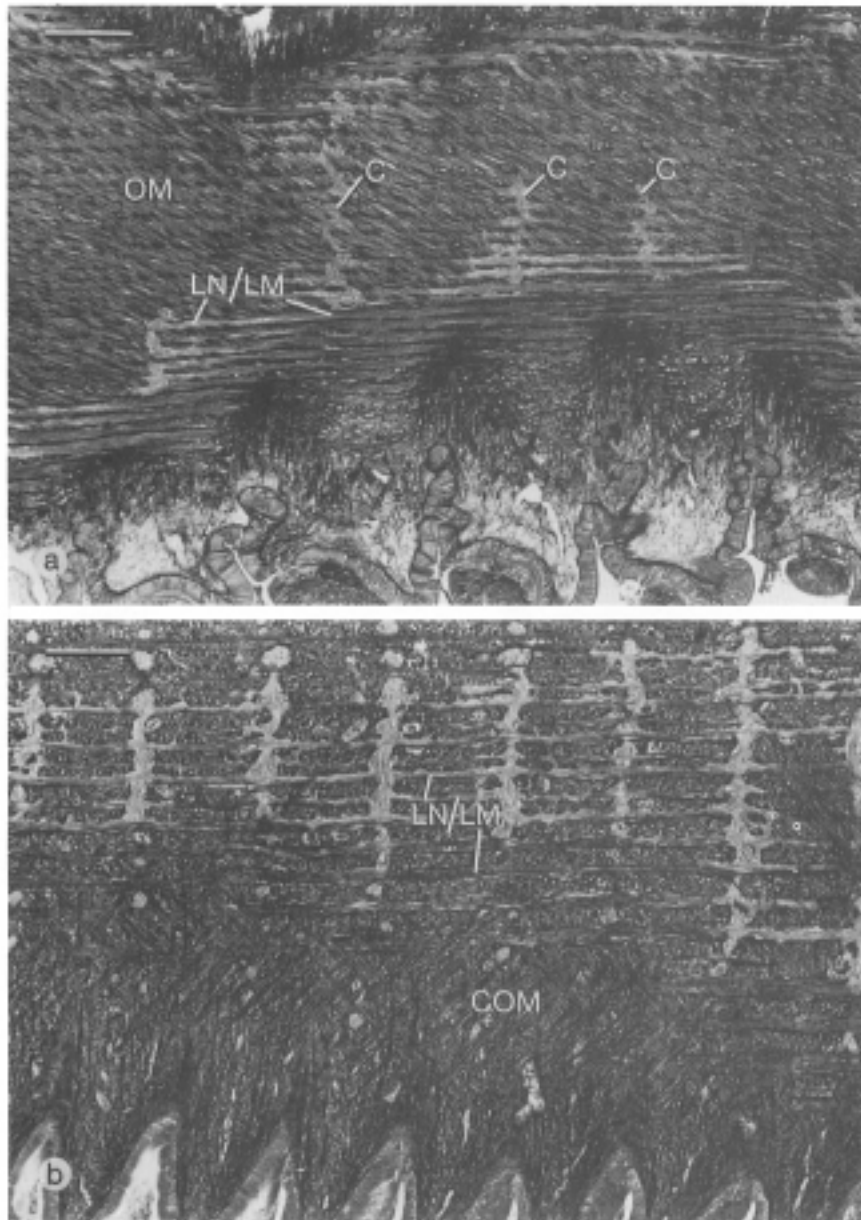
Figure 7. (a) Micrograph of a parasagittal section of the cirrus of a digital tentacle of *N. pompilius*. The long axis of the cirrus is horizontal. The oral adhesive ridges can be seen at the bottom of the micrograph. The oblique muscle layer (OM) runs across the upper part of the micrograph. The longitudinal nerve/longitudinal muscle (LN/LM) network is also visible. Circumferential nerve bundles (C) of this network have the same periodicity as the oral adhesive ridges. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 200 μm. (b) Micrograph of a frontal section of the cirrus of a digital tentacle of *N. pompilius*. The long axis of the cirrus is horizontal. The oral adhesive ridges are visible at the bottom of the micrograph. The upper portion of the micrograph is deeper into the cirrus than the lower portion. The crossed oblique muscle layer (COM) is visible. The longitudinal nerve/longitudinal muscle (LN/LM) network is visible in the upper portion of the micrograph. Note the circumferential nerve bundles of this network (oriented vertically in the micrograph). Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 200 μm.
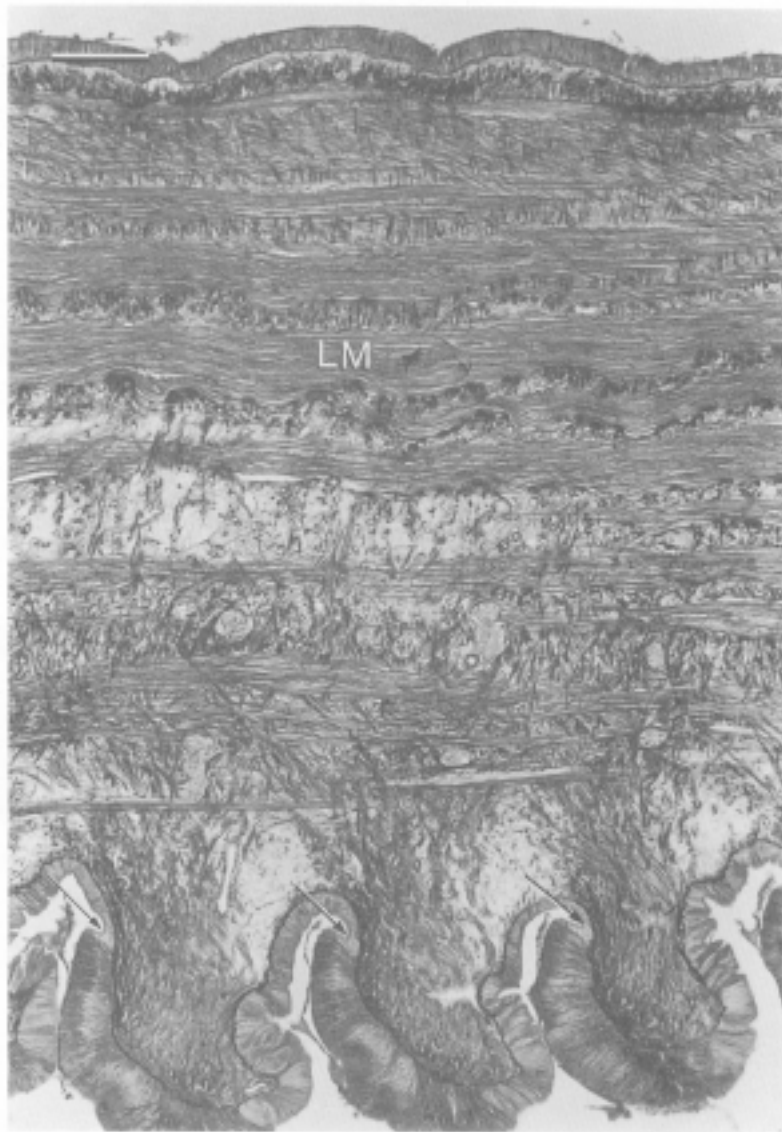
Figure 8. Micrograph of a parasagittal section of the cirrus of a digital tentacle of *N. pompilius*. The long axis of the cirrus is horizontal. The oral adhesive ridges are at the bottom of the micrograph. Note the abrupt transition in the epithelium from groove to ridge (→). The musculature of the adhesive ridges inserts on a thick basement membrane underlying the epithelium and extends up into the musculature of the cirrus. Longitudinal muscle (LM) bundles are also visible. Direct microscopy of paraffin section stained with Milligan's Trichrome. Scale bar: 200 μm.

In section, the muscle cells of the cirrus have the appearance of regular, obliquely striated muscle (terminology of Millman, 1967). Transverse sections of the muscle fibers show the cells to be made up of a central core, presumably of mitochondria, surrounded by a mass of myofilaments that show a banding pattern characteristic of obliquely striated muscle (see Kier, 1985) [regular, obliquely

striated muscle is distinguished from irregular, obliquely striated muscle by the presence of a mitochondrial core in the former and the absence of such a core in the latter (Millman, 1967)]. In an ultrastructural study of Nautilus, Hochachka et al. (1978) found regular, obliquely striated muscle in cirrus number two of the spadix (a modified labial tentacle of the male), in the funnel musculature, and in the retractor muscles. Obliquely striated muscle is the most common type in cephalopods (Kawaguti and Ikemoto, 1957; Kawaguti, 1962; Cloney and Florey, 1968; Florey, 1969; Socastro, 1969; Gonzalez-Santander and Socastro Garcia-Blanco, 1972; D. V. Ward and Wainwright, 1972; Moon and Hulbert, 1975; Amsellem and Nicaise, 1980; Mommsen et al., 1981; Bone et al., 1981; Chantler, 1983; Nicaise and Amsellem, 1983; Kier, 1985).

4. Discussion

The morphology of the musculature of the cirri of Nautilus is similar in many respects to that of other muscular cephalopod appendages, such as the arms and tentacles of many squids (Kier, 1982) and the arms of octopuses (Kier, 1987). Therefore, discussion of the functional role of the muscular morphology of the cirri is allied with the proposals outlined previously for squids and octopuses. The most important biomechanical feature of these "muscular-hydrostats" is that they are constant in volume. The musculature and body fluids of these appendages are composed primarily of an aqueous liquid that is practically incompressible at physiological pressures. In addition, there is no evidence of flow of fluid into or out of the cirri, and no gas-filled spaces are present. In a structure of constant volume, a change in one dimension will cause a compensatory change in at least one other dimension. The following analysis of the function of the musculature of the cirri of Nautilus is based on this principle.

Elongation of the cirri probably results from the contraction of the radial musculature. Radial muscle contraction decreases the cross-sectional area of the cirrus, and because the cirrus is constant in volume, a decrease in cross section results in an increase in length. The displacement resulting from radial muscle contraction is amplified mechanically, because of the relationship between the diameter and the length of initially elongate, constant-volume structures like the cirri (see Kier and Smith, 1985). To illustrate the effects of this mechanical amplification, consider a constant-volume cylinder 4 mm in diameter and 80 mm long, the approximate dimensions of a retracted cirrus of one of the specimens studied. (Because the cirri can be completely retracted within the sheaths, measurements of the dimensions of retracted cirri could not be taken from the cine films of live animals and were determined by dissection of preserved specimens instead.) An increase in the length of such a cylinder by 100%, or 80 mm (a typical extension observed), is caused by a decrease in diameter of slightly less than 1.2 mm. The displacement produced by radial muscle contraction is thus amplified mechanically and is analogous to the mechanical amplification observed in support systems that use hardened skeletal elements to provide leverage.

Branching of the radial muscle bundles as they extend outward from the central, radial muscle mass of the cirrus (see Figs. 4 and 6) may be important in

distributing the load produced by radial muscle contraction. Distributed loads rather than point loads are common in support systems that lack rigid skeletal elements, such as the cirrus (Wainwright *et al.*, 1976).

The longitudinal muscle is probably responsible for retraction of the cirrus. During elongation and shortening of the cirrus, the longitudinal muscle operates over a range of extension and contraction of approximately 100% of its length at rest. The operating range of retractor muscles is discussed in Kier and Smith (1985) and Kier (1987).

During elongation and shortening, the radial and longitudinal muscles operate independently, but bending movements of the cirrus probably require simultaneous activity of portions of the radial and longitudinal musculature. Bending movements are produced by contraction of longitudinal muscle on one side of the cirrus. This unilateral longitudinal muscle contraction will cause bending only if the longitudinal compressional force tending to shorten the cirrus is resisted (Kier, 1982; Kier and Smith, 1985). Because shortening increases the diameter of the cirrus, shortening due to longitudinal muscle contraction can be prevented by resisting increase in diameter. Constant diameter may be maintained by contractile activity of the radial muscles of the cirrus. Thus, bending requires simultaneous, coordinated activity of the radial and longitudinal musculature. Note that with appropriate nervous control, the same arrangement of musculature used for creating bending movements also can be used for extension and retraction.

The cirri were observed to be capable of bending movements in any plane. Such bending requires longitudinal muscle arrayed around the entire circumference, and as Fig. 4 shows, such a situation obtains in the cirrus. In addition, much of the longitudinal muscle is situated peripherally, away from the central axis of the cirrus. This location is of interest, because a greater moment arm for bending the cirrus is provided by a peripheral rather than a central arrangement of longitudinal muscle.

Torsional movements of the cirri are probably caused by contraction of the oblique musculature (Kier, 1982; Kier and Smith, 1985). The direction of torsion depends on the handedness of the contracting oblique muscle. For example, contraction of oblique muscle arranged as a right-hand helix will create counterclockwise torsion of the distal portion of the cirrus relative to the proximal portion when viewed from the proximal end of the cirrus, looking distally. Both right- and left-handed oblique muscle is present, and torsion of the cirri in either direction was observed. Figure 4 shows that the oblique musculature is situated peripherally in the cirrus, away from the central axis. This arrangement provides a larger torsional moment through which to apply torque than a more central location would.

In addition to creating a torsional force, helical or oblique muscle fibers may also create force for elongation or shortening, depending on their fiber angle. A theoretical model of a constant-volume cylinder wrapped by helically arranged, extensible fibers (Kier and Smith, 1985) predicts that in addition to creating a torsional force, oblique muscle fibers with a fiber angle greater than 54°44' create a force for elongation, and oblique muscle fibers with a fiber angle less than 54°44' create a force for shortening. Oblique muscle fibers with a fiber angle equal to 54°44' create a torsional force and do not create force for elongation or shortening.

The fiber angles measured in oblique muscles of the cirri approximate 50°, suggesting that the oblique muscles contribute primarily to torsional movements and play a minor role, if any, in creating changes in length of the cirri. It should be noted, however, that the state of extension or retraction of the cirri sampled was not known. Because the fiber angle increases and decreases as the cirri shorten and elongate, respectively, it is possible that the oblique muscle could contribute to length changes of the cirri at the extremes of extension and retraction.

The arrangement of the musculature of *Nautilus* cirri is similar to that of the arms and tentacles of many squids and octopuses. The details of the arrangement are different, however, and may be correlated with differences in function. The radial musculature of the cirrus, suggested here to be responsible for elongation, is not as extensive or tightly packed as the transverse musculature of the tentacles of loliginid squid, which may be responsible for elongation (Kier, 1982). The difference in extent and packing of muscle is perhaps significant, because squid tentacles are extended rapidly and forcefully (15–35 msec), whereas the cirri of *Nautilus* are extended comparatively slowly (5–10 sec). In both appendage types, the displacement created by the radial and transverse musculature is mechanically amplified, due to the geometric relationships of these initially elongate, constant-volume structures.

The oblique musculature of the cirri is less extensive and complex than the oblique musculature of many squid and octopus arms (Kier, 1982; Kier, 1987). Squid and octopus arms are wrapped by two layers of oblique muscle, each forming complete right- and left-handed helical muscle/connective tissue systems. The oblique musculature of the cirri primarily consists of a single oblique layer in which the handedness of one side is opposite that of the other. The relatively small amount of torsion observed in the cirri compared with that in octopus and squid arms may be correlated with the less extensive oblique musculature of the cirri.

In *Nautilus* cirri, as in octopus arms, the same arrangement of muscle is capable of creating changes in length and also bending movements, with appropriate nervous control. The pattern and form of bending observed are variable, suggesting complex motor control and a complex, subdivided neural system. Little is known about sensory input and motor control of the cirri, and in this regard, the elaborate longitudinal nerve/muscle and associated circumferential connections described here deserve further study.