

Morphology of the Muscle Articulation Joint Between the Hooks of a Flatworm (Kalyptorhynchia, *Cheliplana* sp.)

THEODORE A. UYENO* AND WILLIAM M. KIER

*Department of Biology, University of North Carolina at Chapel Hill, CB #3280, Coker Hall,
Chapel Hill, North Carolina 27599-3280*

Abstract. Schizorhynch kalyptorhynchs are meiofaunal turbellarian predators that possess an eversible proboscis that can be armed with two stout hooks. The hooks grasp and manipulate prey using a wide range of rotations and translations. These diverse motions are possible because the hook supports may function as a muscle articulation type joint—that is, a joint formed of muscle and connective tissue that connects, separates, and moves the microscopic hooks. We analyze the morphology of the flexible joint in a species of *Cheliplana* by using three types of microscopy: light, laser scanning confocal, and transmission electron. Radial myofilament bundles are present in the core of the hook supports, and lateral divaricator muscle fibers are located on their lateral surfaces. We develop a novel model for movements of the proboscis and describe the tensile function of the basement membrane that surrounds each hook support's medial glandular region. Contraction of divaricator muscle fibers antagonized by contraction of radial myofilaments causes the lateral bending of the hook supports and opening of the hook apparatus. Relaxation of the divaricator fibers and maximal contraction of the radial myofilaments, which put the medial basement membranes in tension, may cause medial bending in the hook supports and closing of the hook apparatus. During proboscis retraction, closure may also be aided by the compression of the hook apparatus as the proboscis is drawn through the rostral pore. The study provides new insights into the principles of support and movement in muscle articulations.

Introduction

Joints permit relative motions between the rigid links that provide skeletal support. Joints typically allow translation and rotation while resisting compressive and tensile forces. Two categories of animal joints are recognized: sliding and flexible (Wainwright *et al.*, 1982; Alexander, 1983). Sliding joints, such as vertebrate and arthropod articulated joints, transfer loads directly between abutting links. The shapes of these contacting surfaces help limit range of motion. Flexible joints are less common and consist of a pliable connection between rigid links. As such, these joints (such as the distal leg segments of smaller insects and the joints between polyplacophoran [chiton] valves) have limited ability to resist loading in compression (Wainwright *et al.*, 1982).

Morphological (Uyeno and Kier, 2005) and functional (Uyeno and Kier, 2007) studies of the octopus beak joint suggest that it represents a newly recognized class of flexible joint that can bear compressive loads without buckling. Termed a “muscle articulation,” this joint uses muscle and connective tissue to connect the links while simultaneously separating them, providing motive force, and resisting compressive forces dynamically. Because the beak joint of cephalopods represents the only muscle articulation examined thus far, the goal of this study is to begin to identify the general principles of support and movement in these joints by examining an example from another organism, a predatory schizorhynch kalyptorhynch flatworm with two beak-like hooks mounted on an anterior, eversible, and muscular proboscis.

Free-living flatworms (“turbellarians” of the phylum Platyhelminthes) are common members of the taxonomically diverse meiofauna (Martens and Schockaert, 1986). Meiofaunal organisms live in the water-filled interstices

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* To whom correspondence should be addressed at Department of Biological Sciences Northern Arizona University, PO Box 5640, Flagstaff, AZ 86011-5640. E-mail: Ted.Uyeno@nau.edu

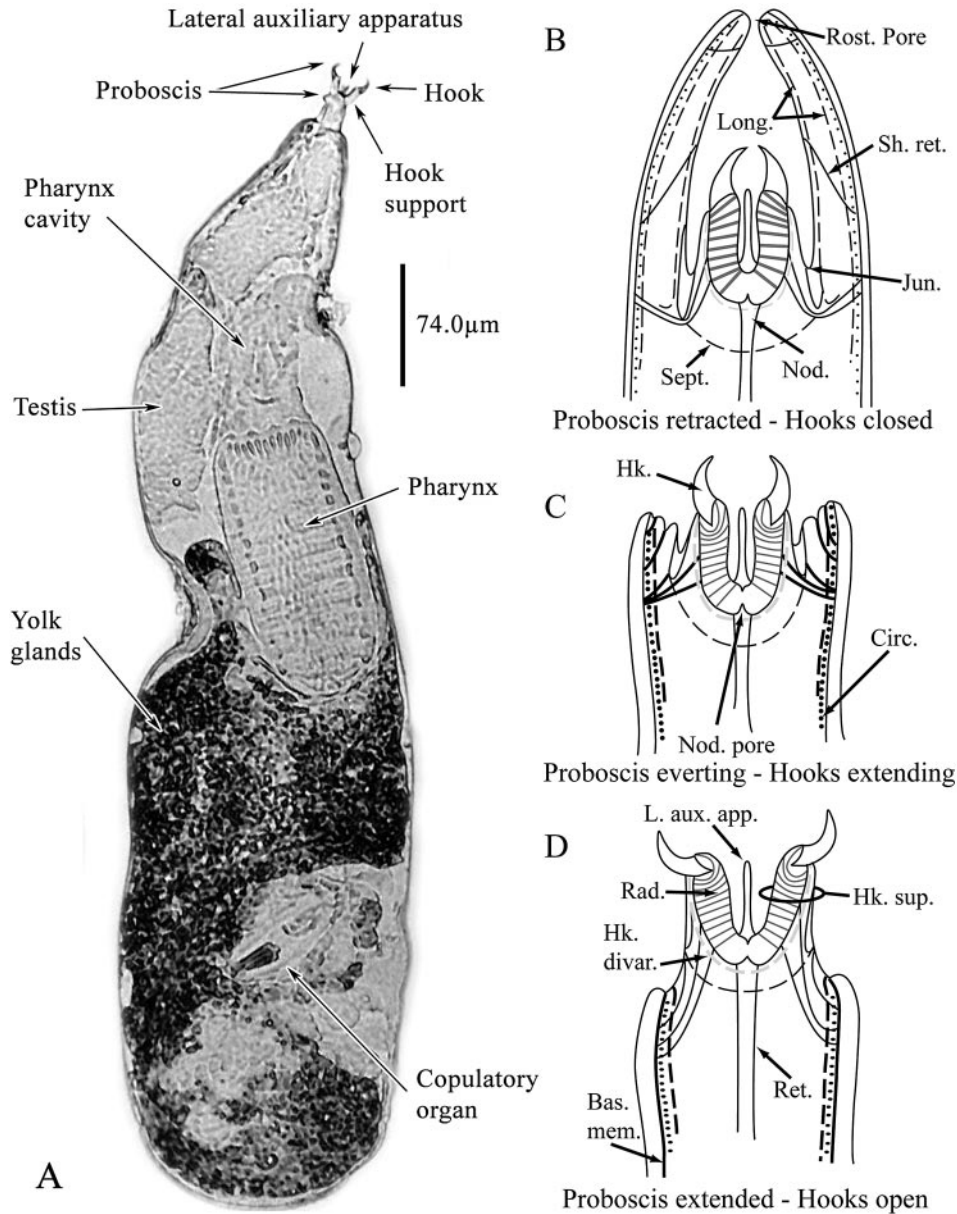


Figure 1. (A) Micrograph of unidentified *Cheliplana* species from Northern California. Photograph provided by and used with permission of Dr. Matthew Hooe, University of Maine. (B–D) Karling's (1961) karkinorhynch proboscis opening sequence: (B) illustrates the retracted proboscis resting position with hooks closed, (C) shows the sheath being retracted and the hooks opening, (D) shows the hooks opened and the proboscis fully extended. Adapted from fig. 67 of Karling (1961), with permission from Wiley-Blackwell. (*Bas. mem.*, basement membrane; *Circ.*, circumferential muscles; *Hk.*, hook; *Hk. divar.*, hook divaricator muscle fibers; *Hk. sup.*, hook support; *Jun.*, junction; *L. aux. app.*, lateral auxiliary apparatus; *Long.*, longitudinal muscle; *Nod.*, nodus; *Nod. pore*, nodal pore; *Rad.*, radial myofilaments; *Ret.*, retractor muscle; *Rost. pore*, rostral pore; *Sept.*, septum; *Sh. ret.*, sheath retractor muscle.)

between grains of marine sediments and exhibit numerous morphological specializations (Remane, 1933; Mare, 1942; Giere, 2009). They typically have highly miniaturized bodies with lengths between 200 and 3000 μm and are often soft and cylindrical, facilitating movement through small interstices (Swedmark, 1964; Ricci and Balsamo, 2000).

The predatory turbellarian studied here belongs to the monophyletic (Littlewood *et al.*, 1999) suborder Kalyptorhynchia Graff, 1905 (Fig. 1A).

Kalyptorhynchs possess an additional specialization: an anterior eversible proboscis that is separate from the midventral pharynx (Karling, 1947, 1949, 1950, 1961; Beauchamp, 1961;

Schilke, 1970; Doe, 1976). These eversible proboscides are often used to remove prey from smaller gaps between sand grains (Meixner, 1938). After grasping the prey, the flatworm then moves to larger "feeding" spaces and folds in half to transfer the food to the pharynx (Karling, 1961; Martens and Schockaert, 1986). Meixner (1925) described eukalyptorhynch body musculature as particularly suited to the task of bending the anterior body and proboscis toward the pharynx.

Kalyptorhynchs are subdivided into eukalyptorhynchs, which have proboscides with cone-shaped tips (Karling, 1961), and schizorhynchs, which have proboscides with anterior tips that are split (Rieger *et al.*, 1991). Gnathorhynch eukalyptorhynchs and schizorhynchs have independently evolved, well-developed, fully sheathed, and retractable proboscides that are armed with stout hooks used as pincers to capture prey (Meixner, 1928; Karling, 1961; Doe, 1976; Rieger *et al.*, 1991). Similar to the beaks of octopuses, these hooks are embedded in tissue and thus do not articulate with each other or with other stiff skeletal elements. In this study, we examine the morphology of proboscis and hook of a schizorhynch in order to explore its potential function as a muscle articulation.

We use terminology common to Doe (1976), Karling (1961, 1989), and De Vocht and Schockaert (1999). The two dorsoventrally opposed halves of the unarmed schizorhynch proboscis are referred to as *tongues* (or lips) or *hook supports* (or base units, muscle pads, muscle bulbs, or hook carriers) if the structure bears a sclerotized part or *hook*. Tongues, such as those found in the family Schizorhynchidae (Schilke, 1970), resemble tapering tentacles composed of myofilament bundles oriented transversely to their long axes. Contraction of these myofilament bundles is thought to flatten the tongues, curl them medially, and wrap them around the prey. Antagonistic longitudinal divaricator muscle fibers are believed to straighten or shorten the tongues (Karling, 1961; Rieger *et al.*, 1991).

Hook supports are found in three schizorhynch families and likely evolved from tongues (Beauchamp, 1961; Karling, 1961). The morphology of the sclerotized parts, which are specializations of the basement membrane (Doe, 1976), vary: In the Nematohynchidae, Schilke (1969) described small, straight, needle-like teeth carried on tongue tip. The Karkinorhynchidae (Fig. 1B, C) has more substantial incurved hooks at the tips of relatively short hook supports (Karling, 1961; Rieger *et al.*, 1991). In *Cheliplana*, a species of which we study here, the hooks may bear a single tooth-shaped process along their inside or medial edges (Karling, 1961). The largest and most well-developed hooks are found in the family Diascorhynchidae (Beauchamp, 1961; Karling, 1961), where they resemble jaws, sometimes with several interlocking medial processes (Rieger *et al.*, 1991).

The proboscides of the genus *Cheliplana* are held within an anterior sheath formed by the infolding of the body wall

and everted through an anterior *rostral pore* (Rieger *et al.*, 1991). Eukalyptorhynch sheaths (De Vocht, 1989, 1991) are mostly cellular with several syncytial belts through which a number of glands extend. The sheath extends from the pore to the *junction*, the point where the sheath joins the base of the proboscis. In schizorhynchs, the *nodus*, a muscle-free reticulum that contains a connective tissue framework, forms the posterior end of the proboscis (Karling, 1961; De Vocht and Schockaert, 1999). The posterior end of the proboscis in most schizorhynchs (the differentiation is less obvious in karkinorhynchs) is divided from the surrounding parenchyma of the body by a *septum* (Fig. 1B) of extracellular matrix and muscles that demarcate a *post-rostral bulb* region (Karling, 1961).

As schizorhynchs have no specific protractor muscles, the proboscis is everted (Fig. 1B, C) by the retraction of the rostral wall and by the pressure generated by contraction of the superficial circumferential body muscles (Karling, 1961; Rieger *et al.*, 1991). While everted, the two opposable hooks may open and close (Beauchamp, 1961) with independent, asymmetric motions (Karling, 1961) and with a wide range of motion along the dorsoventral axis (M. Hooge, University of Maine; pers. comm.). The proboscis is withdrawn into its sheath using retractor muscles that insert on the nodus and originate within the body beyond the post-rostral bulb septum (Karling, 1961).

Opening and closing hook movements occur only in the mid-sagittal plane (Karling, 1961) and are generated by the dorsal and ventral hook supports. The symmetrical hook supports contain *radial myofilament bundles* (or longitudinal muscle rows; see below) and lateral *divaricator muscle fibers* (or external longitudinal, or hook, abductor muscle fibers) that generate hook movement (Doe, 1976).

Electron micrographs taken by Doe (1976; see Fig. 3B) of transverse sections below the hooks show that each hook support is composed of (A) lateral divaricator muscle fibers, (B) a glandulo-muscular core surrounded by an electron-dense matrix that is continuous with the hook inner wall, and (C) a medial glandular region. Radial myofilaments are found within the electron-dense wall of the glandulo-muscular core and extend between the medial glandular region and the lateral divaricator muscle fibers. Each glandulo-muscular core includes a stack of radial myofilament bundles that extends from the hook support base to its anterior end (Karling, 1961). The bases of the hook supports are firmly attached to the proboscis bulb septum by the nodus (Karling, 1961). All karkinorhynchs have divaricator muscle fibers with a longitudinal orientation and forming the lateral surfaces of the hook supports (Karling, 1961). They originate on the nodus and insert on the outer hook margins. Adductor muscles are present in diascorhynch proboscides, but not in those of karkinorhynchs (*e.g.*, Fig 1B, C, *Cheliplana*; Karling, 1961; Schilke, 1970). Two lateral accessory apparatuses are attached to the nodus between the hook

supports and are constructed of gland and muscle cells and have sensory cells at each anterior tip (Doe, 1976).

In this study, we analyze the morphology of the proboscis in *Cheliplana* in an attempt to answer the following questions. What soft tissue components adduct and abduct the hooks? How are compressional forces transmitted across the joint as the hooks manipulate prey items? How are the various muscles of the proboscis antagonized or supported? We place particular emphasis on the connective tissue elements, given their crucial role in other muscle articulations and in support and movement in general.

Materials and Methods

Specimens of an unidentified species of *Cheliplana* were collected, partially prepared (see below), and supplied to us by Dr. M. Hooge (University of Maine). The undescribed species was identified as belonging to the genus *Cheliplana* Beauchamp, 1927, based on body morphology, reproductive morphology (Hooge, pers. comm.), and proboscoidal features (Karling, 1983). The specimens were extracted by Dr. Hooge, using magnesium sulfate anesthetization (Sterner, 1971), on 1 February 2004, from samples of fine-grained subtidal sand from San Felipe Bay (30°54'21.6"N, 114°42'35.5"W), San Felipe, Baja California, Mexico. Five specimens selected for histological study were relaxed in isotonic magnesium sulfate, fixed in warm Stefanini's fixative (Stefanini *et al.*, 1967), washed in Millonig's phosphate buffer (Millonig, 1961), fixed in phosphate-buffered 1% (v/v) osmium tetroxide, and dehydrated in acetone. Dehydration was accelerated with microwave radiation (Giberson and Demaree, 1995). Specimens in vials were placed on ice in a microwave oven with two water-filled 300-ml beakers as water ballasts. The specimens were dehydrated by two irradiations at 650 W, 7-s each and separated by a 20-s interim. The specimens were embedded in EMBed/Araldite epoxy resin (Mollenhauer, 1964) and then shipped to us. We cut serial sections for both light and transmission electron microscopy. Semithin sections 0.5- to 1.5- μm thick were cut using triangular Latta-Hartmann glass knives (Latta and Hartmann, 1950). The sections were stained with toluidine blue/basic fuchsin stain (1% toluidine blue O [C.I. 52040] in 1% sodium borate and 0.1% basic fuchsin [C.I. 42510]; modified from Blaauw *et al.*, 1987) to provide some contrast between muscle and connective tissues. The sections were examined using brightfield and phase contrast microscopy. Transverse thin sections of 30 nm were cut on a Reichert-Jung Ultracut E ultramicrotome with a diamond knife and stained for 25 min in 50% ethanol/50% saturated aqueous uranyl acetate and for 4 to 5 min in lead citrate. The sections were examined and photographed using a JEOL 1200EXII transmission electron microscope.

Dr. Hooge prepared whole mounts of six worms to reveal

musculature by staining F-actin with fluorescently labeled phalloidin (Alexa 488; Molecular probes, Eugene, OR; Hooge, 2001). The specimens were fixed for 1 h in 4% (w/v) formaldehyde, rinsed in PBS (phosphate-buffered saline), attached to a coverslip treated with poly-L-lysine, simultaneously permeabilized, and stained for 45 min with phalloidin-Alexa diluted in 0.2% (v/v) Triton X-100 in PBS, and mounted under a second coverslip with Fluoromount-G (Southern Biotechnology Associates, Inc.; Birmingham, AL). We visualized these specimens using laser scanning confocal and differential interference contrast microscopy. Since the specimens were pressed laterally between two coverslips (a squash preparation), the pharyngeal bulb was in an everted position with the dorsal and ventral hooks oriented in a plane parallel to the coverslips and perpendicular to the optical axis of the confocal microscope. Three-dimensional images of the pharyngeal bulb musculature were produced by stacking parasagittal images. Contours drawn from these three-dimensional images, along with measurements from the histological sections, were used to generate a three-dimensional reconstruction of the proboscoidal bulb using 3D modeling software (Anim8or; Glanville, 2006).

Results

Hooks and lateral auxiliary apparatuses

The hooks are slender, hollow, and medially curving cones that are 17.5 to 20.0 μm in length and oval in cross-section (Figs. 2, 3). They possess a flared posterior opening with thickened walls where they are attached to the anterior tips of the hook supports (Fig. 3A). At the level of the flared base, the hook diameter is about 2.5 μm (Fig. 2B). This diameter tapers to about 1.25 μm at the midpoint of the shaft and continues to taper to an inward-curving closed point (Fig. 2A). The curve in the shaft approximates a 90° bend in the mid-sagittal plane, and thus the tip section is perpendicular to the shaft base. The hooks of this species lack medial processes.

The lateral auxiliary apparatuses (Figs. 1D, 3C, D) are present to the left and the right of the hook supports and are about 10 μm long and 0.8 μm in diameter. The posterior end of each apparatus is connected to the nodus at the base of the hook supports. Fluorescent imaging shows that the lateral auxiliary apparatuses contain no myofilaments (Fig. 3C, D).

Proboscoidal musculature

Hook supports—glandulo-muscular cores. Each glandulo-muscular core is formed of two stacks of about 18 radial bundles of myofilaments layered one on top of the other (Fig. 3A, C). Because they are not surrounded by a sarcolemma, we refer to them here as myofilament bundles. It is

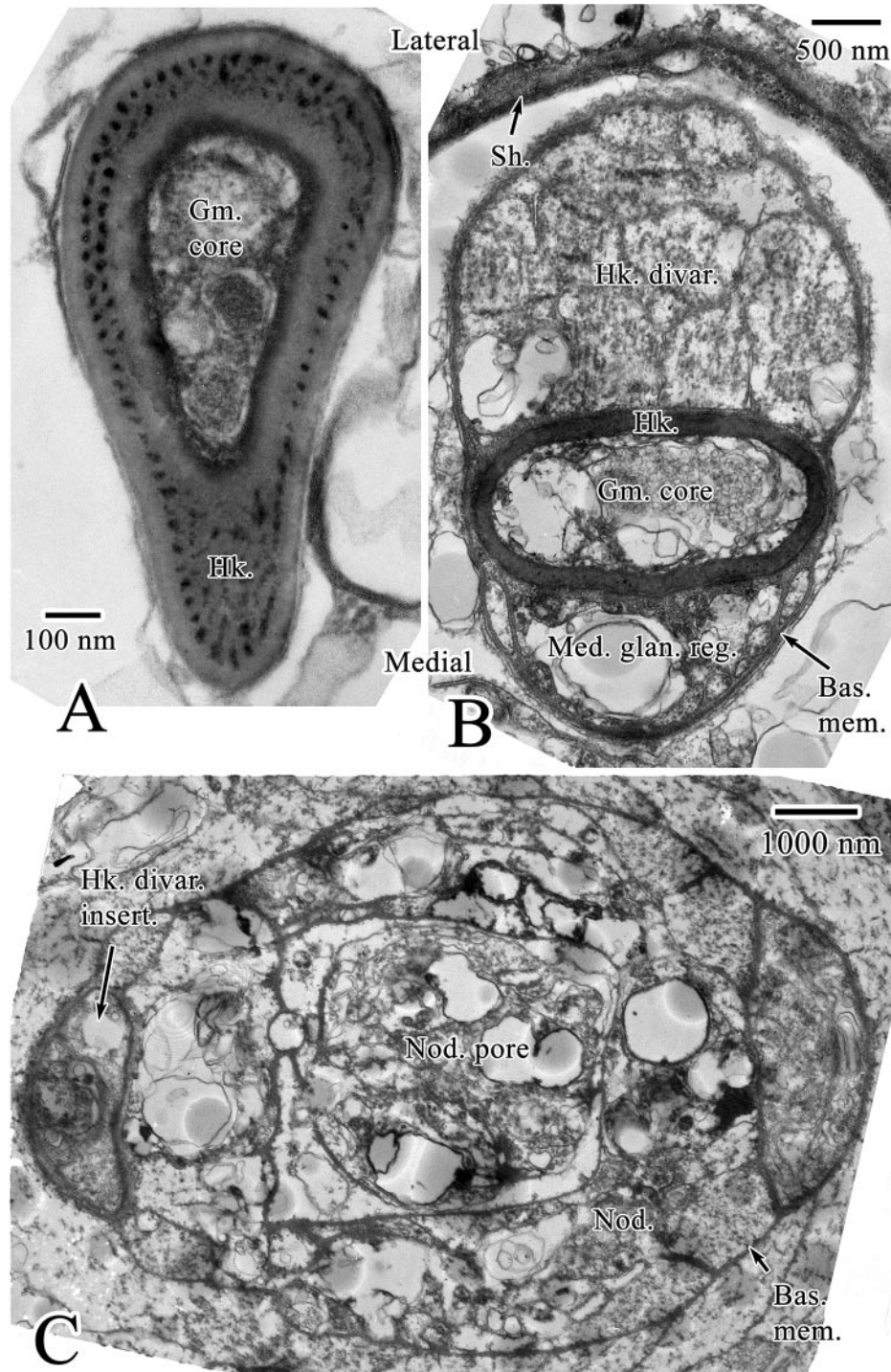


Figure 2. Transmission electron micrographs of cross-sections through the proboscis of *Cheliplana* sp. showing features of the hooks and basement membranes. (A) Section through hook near tip. (*Gm. core*, glandulo-muscular core; *Hk.*, hook.) (B) Section through hook support near base of the hook showing the basement membrane surrounding the medial glandular region. Note that the divaricator muscle cross-sectional area appears quite large because the section is slightly oblique. (*Bas. mem.*, basement membrane; *Hk. divar.*, hook divaricator muscle fibers; *Med. glan. reg.*, medial glandular region; *Sh.*, sheath.) (C) Section through proboscis through the anterior end of the nodus near the insertion point of the hook divaricator muscles. Note that the nodal pore is filled by space and glandular material. (*Hk. divar. insert.*, hook divaricator insertion; *Nod.*, body of nodus; *Nod. pore*; nodal pore.)

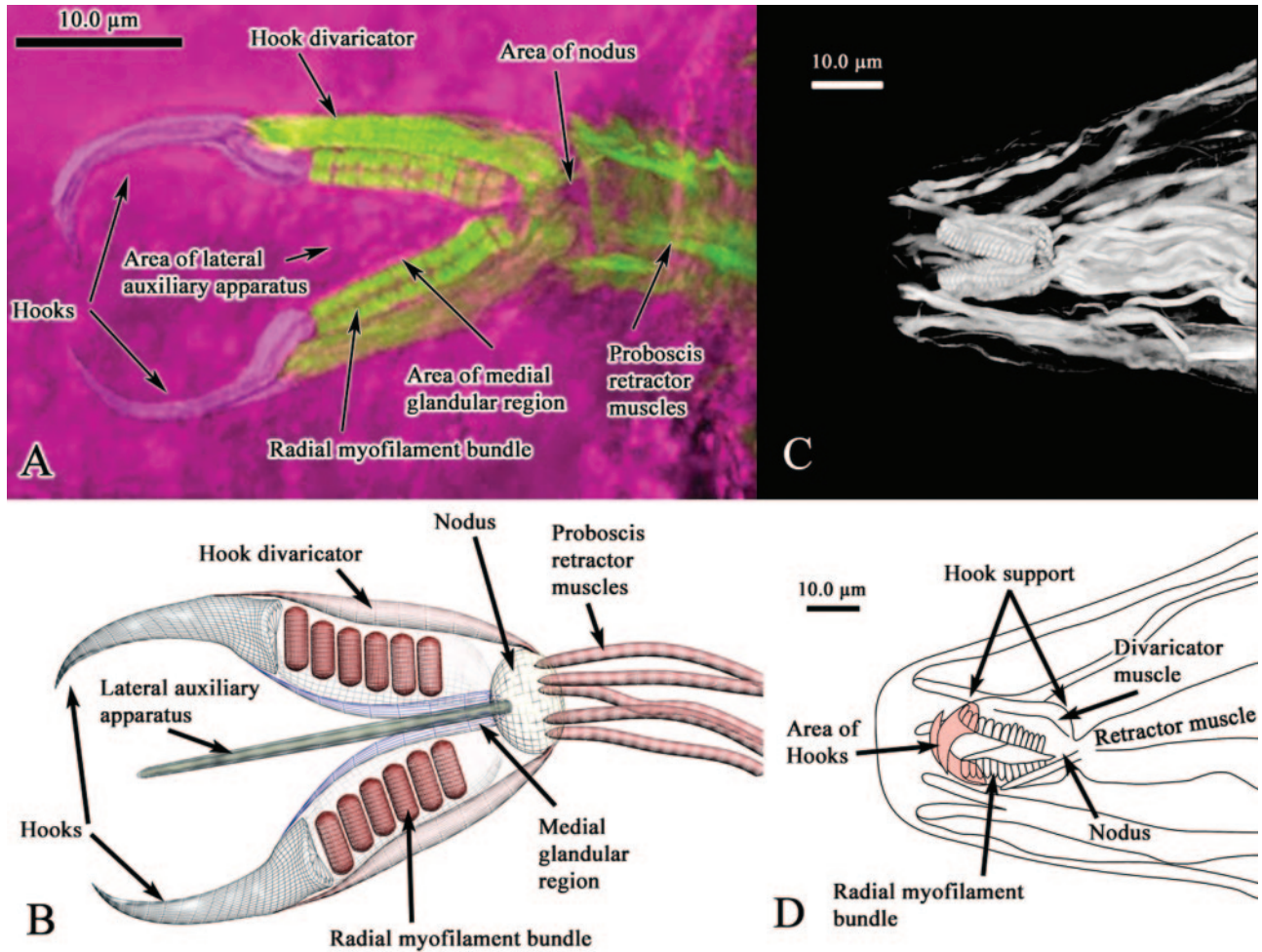


Figure 3. The proboscis of *Cheliplana* in lateral view. (A) This micrograph is composed of a stack of mid-sagittal laser confocal z-axis fluorescent images overlaid with a differential interference contrast image to visualize the hooks. The lateral auxiliary apparatuses and the medial glandular regions are not visible here, but their positions are noted. (B) Three-dimensional computer model showing mechanical elements. (C) A confocal reconstruction showing the three-dimensional distribution of fluorescently labeled F-actin in the musculature. (D) A sketch outlining the features present in C. The transverse alignment of the radial myofilament bundles and the longitudinal orientation of the proboscis retractor and hook divaricator muscle fibers are clearly seen.

unclear how many of these myofilament bundles are included within a single muscle fiber. When the hook supports are straight, the long axis of both stacks is roughly parallel to the long axis of the hook support. The long axes of the layered stacks of myofilament bundles are oriented in planes approximately perpendicular to the longitudinal axis of the hook support. They are not, however, parallel to each other or to the mid-sagittal plane (Fig. 3C); instead they are obliquely arranged. There is an indentation at the midpoint of each bundle of myofilaments that gives it a chevron-shaped appearance. Overall, the posterior end of each hook support is connected to the nodus, and the anterior end fits within the flared opening of the hooks.

Hook supports—divaricator muscles. The longitudinal, lat-

eral divaricator muscle fibers are the only other muscle group in the hook supports (Figs. 2, 3, 4). These fibers appear to be striated and have a longitudinal orientation when the hook supports are straight and extend along the lateral sides of the glandulo-muscular cores. The divaricator muscle fibers originate on the nodus external to the origin of the glandulo-muscular cores and insert on the external posterolateral margin of the flared hook base.

Retractor muscles. The retractor muscles are extrinsic to the hook supports and can be seen best in the three-dimensional reconstructions generated by confocal microscopy (Fig. 3C). These medial fibers appear not to be striated and are oriented parallel to the long axis of the body and proboscis. The retractor muscles insert on the connective tissue nodus that sur-

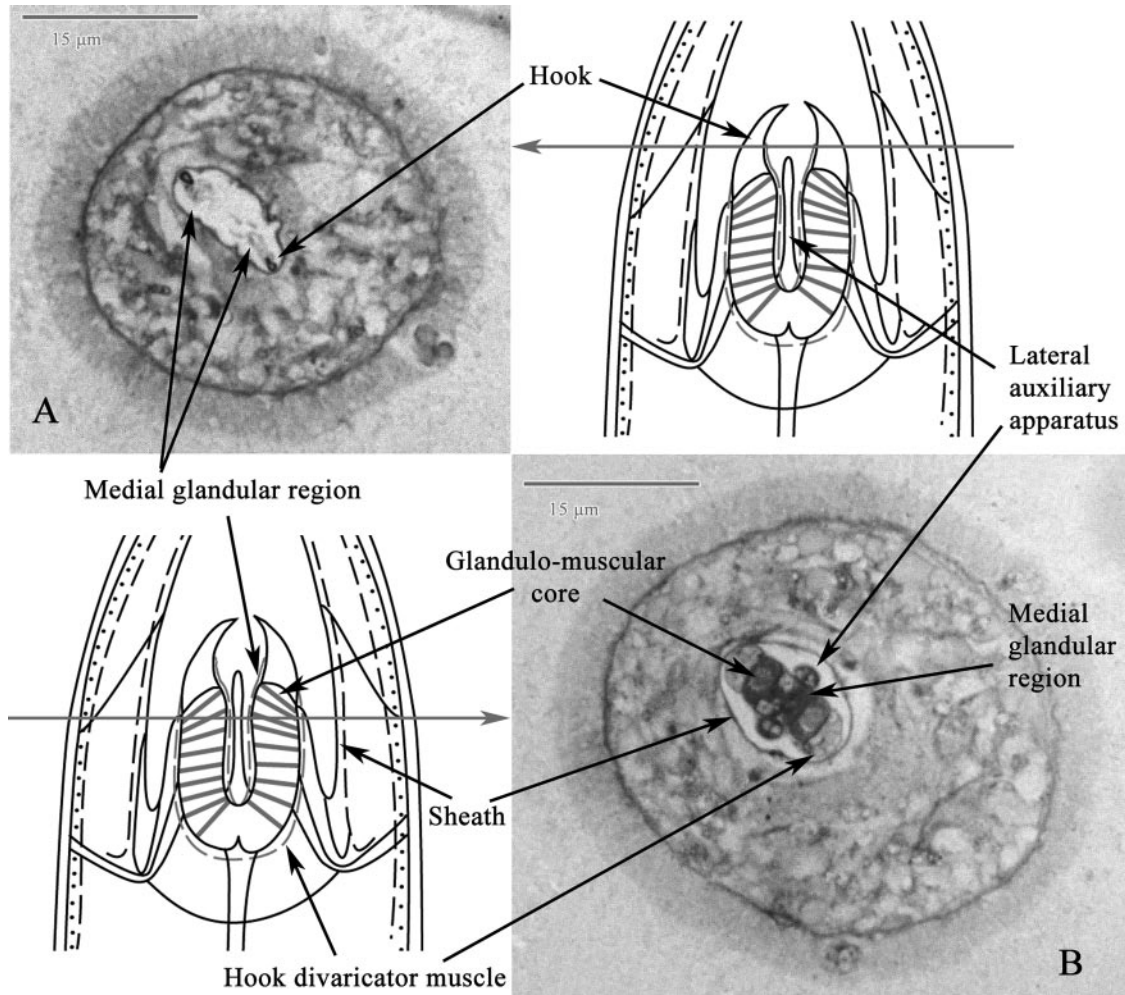


Figure 4. Transverse histological sections through the proboscis of *Cheliplana* at the levels indicated by the grey horizontal arrows shown on drawings of the lateral aspect of the proboscis. Section A is more anterior than section B. Note in (A) the anterior portions of the basement membranes surrounding the medial glandular region close to where they attach to the medial surface of the hook. (Brightfield microscopy of EMBed/Araldite epoxy resin section; toluidine blue/basic fuchsin stain.)

rounds the nodal pore (Fig. 2C). The retractor muscles extend into the body, where they originate on the body wall.

Connective tissue elements of the proboscis

In histological sections, the connective tissue elements exhibit staining characteristics similar to those of collagen. However, because Doe (1976) noted that the hook supports contain glandular tissue, which may also stain with toluidine blue/basic fuchsin, we examined the putative tensile connective tissue elements with transmission electron microscopy for electron-dense extracellular matrix (Fig. 2).

Nodus. In confocal microscope images of fluorescently labeled musculature, the nodus appears as a small space (Fig. 3) in the area where the radial myofilament bundles, the

divaricator muscle fibers, and the retractor muscles are connected at the base of the proboscis. In histological sections (Fig. 4), this space is occupied with tissue with staining characteristics similar to those of collagen. Transverse transmission electron microscopy sections through this area show a glandular region surrounded by an electron-dense extracellular matrix (basement membrane) (Fig. 2C).

Medial longitudinal tensile element. Surrounding the medial glandular area is a thick basement membrane (Fig. 2B). This electron-dense extracellular matrix becomes thicker toward the anterior tips of the hook supports. The medial glandular area extends from the nodus to the posteromedial surfaces of the hook shafts. As Doe (1976) noted, the hook support, including the divaricator muscle fibers, the glandulo-mus-

cular core, and the medial glandular area are all surrounded by a basement membrane. The medial glandular area thus appears firmly attached to the glandulo-muscular core, and sliding between the two elements is unlikely. In histological sections (Fig. 4), these basement membranes show staining characteristics similar to those of collagen.

Discussion

The proboscis of *Cheliplana* likely functions as a muscle articulation. This is because flexible tissues (musculature and connective tissue) of the joint allow the hooks to open and close with a wide range of motion that need not be dorsoventrally symmetrical (Karling, 1961; Beauchamp, 1961). Karling (1961) noted that while hook abduction (opening) seems well-understood, a clear mechanism of hook adduction (closing) is not apparent from the organization of the flexible tissue. Below, we evaluate the hypotheses of hook opening and closing reviewed by Karling (1961) and present our modified hypothesis of function (Fig. 5) based on the morphological analysis of the proboscis of *Cheliplana* as a muscle articulation.

Hypothesis of opening

Karling (1961) and Schilke (1969) suggested that lateral divaricator muscle fibers contract to rotate the hook tips open while the radial myofilament bundles contract to provide skeletal support and resist the compressional forces exerted by the divaricator muscles (Fig. 1B–D). As the radial myofilament bundles of the glandulo-muscular core in *Cheliplana* are transverse to the long axis of the hook supports, their orientation is appropriate to provide such skeletal support. Our findings thus support the proposals of Karling (1961) and Schilke (1969).

This proposed mechanism requires that the glandulo-muscular cores antagonize the divaricator musculature so that the hook supports bend rather than simply shorten. How might this occur? Doe (1976) showed that the basement membrane that surrounds the glandulo-muscular core is continuous with the inner wall of the hook itself and thus represents the mechanical tensile connective tissue link between the hook and hook support. The basement membrane may also constrain shape change in the hook supports. Contraction of the radial myofilament bundles must reduce the radial (dorsoventral) thickness of the glandulo-muscular core. If the basement membrane that surrounds the core constrains increases in the transverse dimension of the core, then the only shape change possible is an increase in the length of the glandulo-muscular core. This radial myofilament contraction may, in coordination with contraction of the lateral divaricator musculature, bend the glandulo-muscular core laterally, resulting in the opening of the hooks.

Hypotheses of closing

Karling (1961) noted that in karkinorhynchs, the hook apparatus closing mechanism is uncertain because their proboscides lack adductor muscles. Retraction of the proboscis through the narrow rostral pore seems to represent one mechanism of closure. As the retractor muscles draw the proboscis through the rostral pore, the hook apparatus is compressed and the hooks are forced together. However, as Karling (1961) noted that the hook supports themselves could generate openings and closings, he proposed three functional hypotheses for closing of the hook apparatus in the everted proboscis.

The first proposed closing mechanism relies on the elasticity of the nodus to antagonize movements of the hook supports. The nodus connective tissue fiber arrangement would in this proposal store energy that would be used to return the hook supports to a closed position after opening. We found the nodus to be a relatively small structure at the base of the hook supports to which the hook support base, the divaricator muscle fibers, and the proboscis retractor muscles are all closely attached. Because of its size, its basal position, and the inelasticity of connective tissue within the basement membrane, the nodus may function primarily as an anchor point.

The second hypothesis suggests that transverse musculature below the nodus in some karkinorhynchs supplements the mechanism of the first hypothesis by providing additional closing force. We found no muscle fibers other than those of the retractor muscle posterior to the node in *Cheliplana* (Fig. 3C). Furthermore, if these transverse muscle fibers were located posterior to the nodus, which may serve as a fulcrum, the posteriorly located transverse muscle fibers would open, not close, the hooks.

A third hypothesis can be derived on the basis of Karling's (1961) illustration of movements in a karkinorhynch proboscis (Fig. 1B–D). The radial myofilament bundles of the hook supports are shown extending from the outer medial surface of the hook base to its outer lateral surface. Thus contraction of these myofilament bundles might rotate the hooks closed. This seems unlikely in *Cheliplana*, since in transmission electron microscopy sections taken near the opening of the hook (Fig. 2B), glandular material and no radial myofilament bundles are found (Fig. 2B); and in confocal fluorescence images (Fig. 3A) the radial myofilament bundles do not appear to extend to within the hooks.

Karling (1961) noted that the two lateral accessory apparatuses may help in protraction of the proboscis but ascribed little function in opening or closing. Doe (1976) showed that they contain glandular tissue, are stiffened by muscle and a robust basement membrane, and bear sensory cells at the tip. Thus the apparatuses may serve an important sensory, rather than mechanical, role. However, one possibility is that their medial

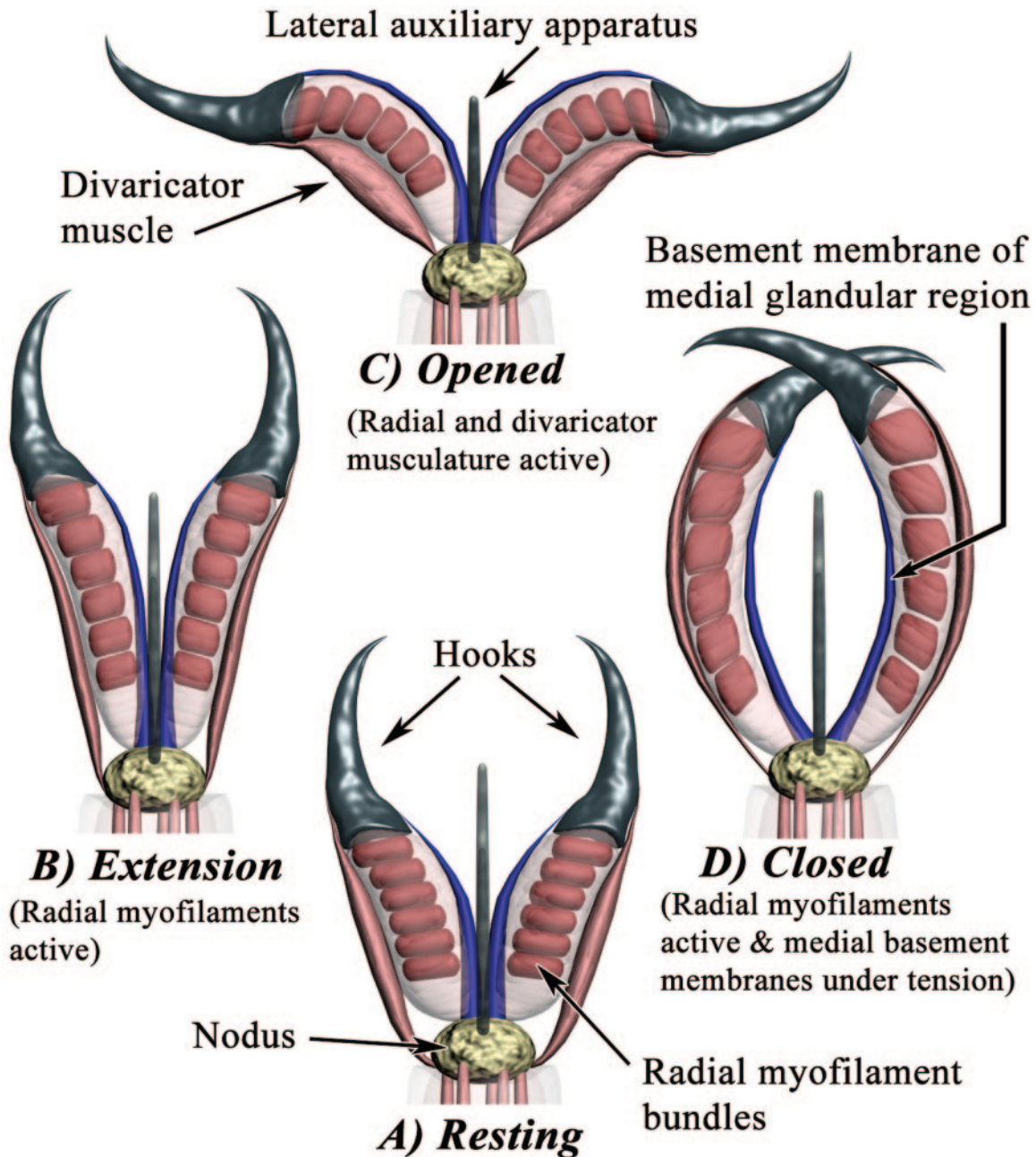


Figure 5. The proposed mechanism for the opening and closing of the proboscis hooks of *Cheliplana*. (A) Resting phase; all muscles relaxed. (B) Extension phase; radial myofilament bundles activate and elongate and stiffen the glandulo-muscular core of the hook supports; hooks extend. (C) Opened phase; lateral divaricator muscle fibers activate and bend the glandulo-muscular core. (D) Closed phase; maximal extension of the glandulo-muscular cores loads the basement membranes surrounding the medial glandular regions in tension and forces the glandulo-muscular cores to bend medially. The return to resting phase requires relaxation and elongation of the radial myofilament bundles. The glandulo-muscular cores and their radial myofilament bundles may return to resting dimensions by the contraction of the antagonistic divaricator muscle fibers. The contraction of divaricator muscle fibers compresses the long axis of the glandulo-muscular cores to the length at which the basement membranes of the medial glandular region are no longer under tension.

connection to the nodus allows the force of prey contact to be transmitted to the center of the nodus and thus force the medial elements of the hook supports backward to initiate closing.

Because medial and lateral elements are anchored together to the nodus, however, compression of the apparatuses may simply push the proboscis posteriorly.

While the above hypotheses may be valid for other schizorhynchids, they do not appear to be adequate explanations for closing in *Cheliplana*. Below, we describe a hypothesis that relies on the tensile properties of the medial glandular region basement membrane originally described by Doe (1976).

A novel functional hypothesis of proboscis movement

To describe the proboscis of *Cheliplana* as a muscle articulation, we must identify how the soft tissue elements are organized to support each other and to create appropriate movement of the hooks. We consider each hook support as a beam with a medial tensile element (the connective tissue basement membrane surrounding the medial glandular region), a core of variable length (the glandulo-muscular core with its radial myofilament bundles), and a lateral contractile element (the divaricator muscle fibers). These three elements allow the beam to bend in both the medial and lateral directions by varying the length and location of the neutral axis and by switching the sides under compression and tension. Details of these proposed roles are presented as a sequence of steps as the hooks are rotated and translated during opening and closing movements (Fig. 5A–D).

Resting. The radial and divaricator musculature of the hook supports are inactive. When at rest (Fig. 5A), the hook supports are probably straight and shortened along the anterior-posterior axis, with the radial myofilament bundles of the hook supports in an elongated state.

Extension. Following eversion of the proboscis through the rostral pore, likely resulting from increased hydraulic pressure generated by contraction of the body wall circular muscles, partial contraction of the hook support radial myofilament bundles results in protrusion of the hooks (Fig. 5B). Contraction of the radial myofilament bundles decreases the cross-section of the glandulo-muscular core and thereby increases its length. This is because the lateral dimension of the core is constrained by the basement membrane. Karling (1961) noted that the dorsoventral dimension of each hook support decreases during extension.

Opening. Opening movements (Fig. 5C) require the hooks to translate and rotate in the midsagittal plane so that their curved tips divaricate and are brought nearly parallel to the longitudinal axis of the proboscis. The connective tissue nodus may anchor the hook supports as they move. The translation and rotation required to open the hooks may be accomplished by the co-activation of both the radial myofilament bundles and the divaricator muscle fibers. Contractions of the divaricator muscle fibers bend the hook supports laterally and thus translate and rotate the hooks. This bending of the hook supports can only occur, however, if the

radial myofilament bundles of the glandulo-muscular cores resist the compressional forces created by the divaricator muscle fibers, which would otherwise simply shorten the hook supports.

Closing. Hook closure (Fig. 5D) may occur in two phases. In the first phase, the divaricator muscles relax while the radial myofilament bundles of the glandulo-muscular core remain activated. The glandulo-muscular core therefore becomes straight because it remains stiff (as the radial myofilament bundles are active) without being bent (because the divaricator muscle fibers are inactive). In the second phase, further elongation of the glandulo-muscular core, caused by maximal contraction of the radial myofilament bundles, results in the elongation of the hook supports beyond the length to which the basement membrane surrounding the medial glandular region may extend. The connective tissue of this basement membrane may limit further lengthening of the medial, but not the lateral, regions of the hook supports and result in bending of the supports toward the midline and closing of the hooks.

Retraction. Relaxation of the radial myofilament bundles in the glandulo-muscular core causes the hook supports to retract from any other position back to the resting position (Fig. 5A). This is because without the antagonistic support provided by the glandulo-muscular core, either the activation of the divaricator muscle fibers or the tension within the basement membrane of the medial glandular region will return the hook supports to their shorter resting length.

Conclusion

Karling's (1961) mechanism for hook opening (or tongue divarication) is probably applicable to most schizorhynchids. In *Cheliplana*, the glandulo-muscular core is solid and essentially constant in volume, and its lateral cross-sectional dimension may be constrained by the basement membrane described by Doe (1976). Thus Karling (1961) is likely correct in that contraction of the radial myofilament bundles may result in a decrease in cross-section and increase in length of the hook support. Simultaneous contraction of the lateral divaricator muscle fibers results in a lateral bending, and thus opening of the hooks.

Karling (1961) described the closing mechanism in karkiorhynchid proboscides as being puzzling because of their lack of adductor muscles. We suggest that the proboscis of *Cheliplana* is a muscle articulation with soft tissues arranged in a manner that supports opening and closing. As a muscle articulation, the hook support musculature probably generates the force necessary for hook movement; hook opening forces may be generated by the divaricator muscle fibers and radial myofilament bundles, and closure may be the result of the hook support bending as its glandulo-

muscular core lengthens because of asymmetric forces on either side. In the hook supports, as in the octopus buccal mass studied previously (Uyeno and Kier, 2005, 2007), a compression-bearing element is formed of soft tissue. In the hook support, the compression is resisted by the radial myofilament bundles that cause the glandulo-muscular core to stiffen during contraction. Compressive forces may be transmitted from one hook to the other through the stiffened hook supports connected at their bases by the nodus. The hook supports may be stiffest when the extension force of the glandulo-muscular core, the tension in the basement membrane of the medial glandular region, and the hook retraction forces generated by the lateral divaricator muscle fibers are balanced.

It may be a common property of muscle articulations that a given orientation of soft tissue fibers typically has multiple functions. As an example, the radial myofilament bundles of the glandulo-muscular core are thought to resist compression and also help adduct the hooks. In muscle articulations, arrangements of soft tissue (in this case both muscle and connective tissues) work synergistically to provide skeletal support. Although this morphological analysis of the proboscis of *Cheliplana* reveals similarities to the muscle articulation of the octopus beak studied previously, novel characteristics were also observed. The proboscis mechanism does not involve a complex arrangement of muscle fibers in many orientations. Instead, two discrete and perpendicular muscles (the glandulo-muscular core radial myofilament bundles and the lateral longitudinal divaricator muscle fibers) and the tension-bearing basement membrane of the medial glandular region are connected by the nodus to form a muscle articulation with relatively few components. Karling (1961) and Schilke (1970) noted that other schizorhynchids (*e.g.*, Diascorhynchidae) have adductor muscles. In *Cheliplana*, the arrangement of the basement membrane provides for adduction without requiring separate metabolically expensive adductor muscles.

As we were limited in this study to morphological descriptions only, future experimental studies of the kalyptorhynch proboscis are needed to test these functional hypotheses. Video recording of hook movements during opening and closing behaviors will provide useful insights. Measurements of the bending angles and muscle length changes of the hook supports and of the displacements of the hook during abduction may be instructive in testing the functional roles of the muscular components of the proboscis. Additional possible experiments include using fluorescent calcium indicators to visualize active muscle contraction (*e.g.*, Ito *et al.*, 1988) while recording corresponding changes in microscopic tension during divarication of the hook supports (Anazawa *et al.*, 1992). It would also be useful to investigate the neural control of hook movements to understand how the radial myofilament bundles are activated to produce partial hook support elongations. While these fur-

ther studies of microscopic structures might require specialized techniques, the transparency of these animals may aid *in vivo* investigations of muscle function.

Kalyptorhynchids may represent a good model system to study the evolution of a muscle articulation. Karling (1961) noted that unarmed schizorhynch proboscides, from which armed karkinorhynch proboscides may have evolved, are used to wrap and adhere to prey items. Contractions of transverse myofilament bundles of the tongues are thought to produce the wrapping motion. A medial tension-bearing element such as that described here has, however, not yet been reported. Furthermore, the proboscides of other schizorhynchids (*i.e.*, the Diascorhynchidae) are well-developed, with strong adductor and abductor muscles and interlocking jaws that are capable of strong biting motions. A comparative biomechanical analysis of unarmed schizorhynchids, armed karkinorhynchids, and armed diascorhynchids may provide insight into not only the evolution of these animals, but also the evolution of an increasingly complex muscle articulation from a simple muscular hydrostat precursor. Finally, little is known about the control of proboscis behavior in schizorhynchids (or indeed in muscle articulations in general), and thus a comparative analysis may reveal changes in the requirements for control as the schizorhynch proboscis has evolved.

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