The Structure and Function of a Muscle Articulation-Type Jaw Joint of a Polychaete Worm

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ABSTRACT The arrangement of the musculature and the fibers of the extracellular matrix (ECM) in the flexible jaw joint of the sandworm *Alitta virens* (Annelida, Polychaeta) was studied using dissection and histology. The jaws are capable of a wide range of motions principally related to defense and feeding. The left and right jaws are embedded in and moved by a compact pharyngeal bulb of muscle and ECM that also forms the mouth and esophagus. Eight pharyngeal bulbs were removed and dissected to document gross anatomical features or preserved and embedded in plastic for sectioning in multiple planes. The sections were stained with toluidine blue and basic fuchsin to differentiate muscle and ECM. The sections were then digitized and used to develop a three-dimensional computer illustration. We hypothesize that the muscle and fibers in the ECM are arranged as a muscular hydrostat to support the movement of the jaws. Four specimens were recorded using a digital video camera and a tank with an angled mirror to record lateral and ventral views of jaw movements during locomotion and biting associated with burrow guarding and feeding. Frame by frame kinematic analysis of this video showed that the jaws move symmetrically in a roughly horizontal plane. Although the angle between the jaws increases and then decreases after maximum gape has been reached, the jaws also translate relative to each other such that the axis of rotation is not fixed. Together, these functional morphological and behavioral data identify the jaw mechanism as a flexible joint known as a muscle articulation. As muscle articulations have been previously described only in the beaks of cephalopods and flatworms, this study implies that this type of joint is more common and important than previously recognized. J. Morphol. 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: muscle articulation; joint mechanics; jaws; Annelida; polychaeta; *Alitta virens;* sandworm; king ragworm

INTRODUCTION
Animal Joints

Joints allow relative motion between adjacent rigid skeletal elements or "links." In the biological joint categorization scheme developed by Wainwright et al. (1982) and Alexander (1983), there are two broad structural categories. Sliding joints, common in arthropods and vertebrates, transfer loads directly between abutting links; the shapes of the contacts govern possible movements (Wainwright et al., 1982). Flexible joints are pliable connections between rigid links that allow motion. This flexibility, however, causes these joints to buckle when loaded in compression, which may explain why they are less common than sliding joints (Alexander, 1983). Examples include flexible joints between chiton valves (Wainwright et al. 1982) and the flexible cuticle between stiff subsections of locust tarsi (Alexander, 1983).

The muscular joint between octopus beaks represents a type of flexible joint that resists compression while supporting complex and diverse movements. Because muscle serves a crucial role in support and movement, it has been termed a "muscle articulation." This type of joint relies on a muscular hydrostatic mechanism in which a tightly packed three-dimensional (3D) array of muscle fibers functions both as the effector of movement and as the support for movement. Complex deformations and movements can be created and the stiffness of the entire structure is under active control (Kier and Smith, 1985; Smith and Kier, 1989). Although the structure and function of this joint type has been investigated the beaks of octopus (Uyeno and Kier, 2005, 2007) and in extensible hook bearing proboscides of interstitial flatworms (Uyeno and Kier, 2010), the taxonomic distribution and general principles of form and function have yet to be thoroughly explored. Here,
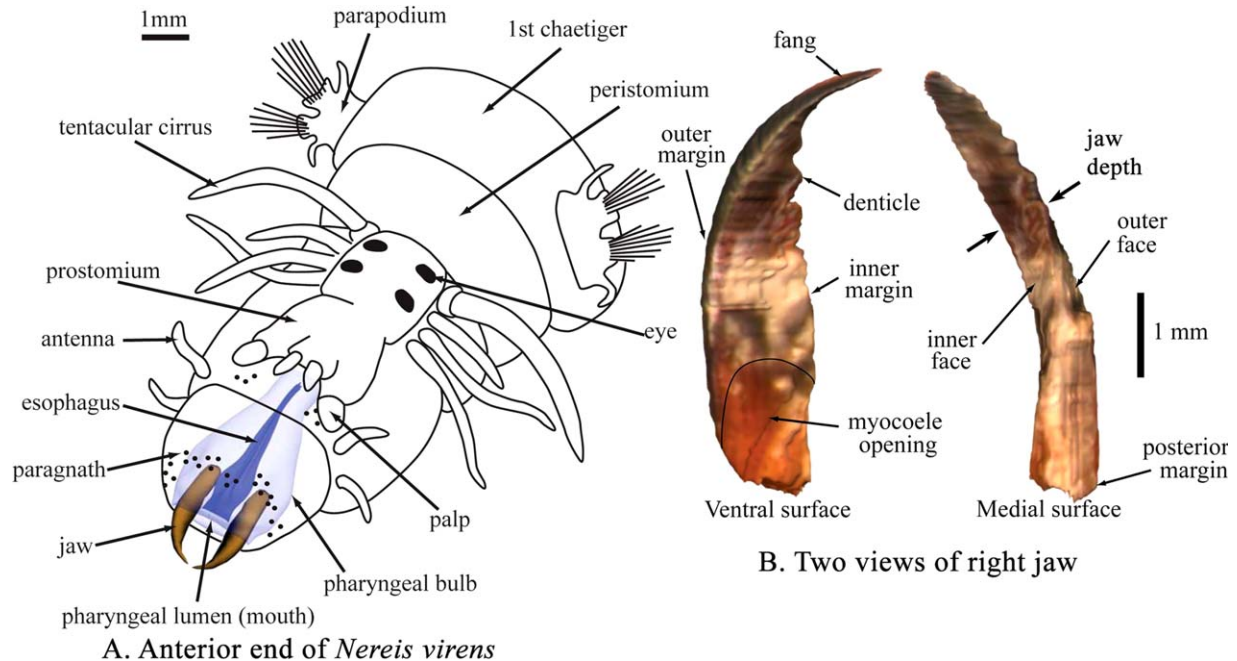
Fig. 1. *Alitta virens*, **A**) A three-quarter dorso-frontal view of the first body segment (chaetiger) and head (peristomium and prostomium) with the pharyngeal bulb (blue) and jaws (gold) everted. **B**) A ventral (left) and medial (right) view of a 3D reconstruction of the right jaw of *A. virens* showing its parts (see text).

we present an analysis of the movement, morphology, and biomechanics of the Nereididae jaw joint to elucidate the general principles of support and movement in this dynamic joint type.

The Jaws and Pharynx of Phyllodocida Polychaetes

A biomechanically useful classification system of polychaete feeding strategies proposed by Dales (1962) shows a striking diversity. This diversity may be due to foregut adaptability (Fauchald and Jumars, 1979; Markert et al., 2007) resulting in a remarkable range of pharyngeal morphology (Rouse and Fauchald, 1997). The pharynx is commonly in an axial position and muscularization of the pharyngeal wall to form a fortified pharyngeal bulb is also common (Dales, 1962). This axial muscular bulb is perhaps the most prominent in the Phyllodocida (Rouse and Fauchald, 1997) in which it supports two large jaw elements embedded in muscle that may function as a muscle articulation. The focus of this study is on one of the species in this group, the King Ragworm or clamworm, *Alitta virens* (Sars 1835) *sensu* Pettibone (1963 as *Nereis virens*), Nereididae. These large, common, widely distributed (Wilson and Ruff, 1988; Olive, 1993) marine worms are of considerable ecologic and commercial importance (Brown, 1993, Goerke, 1971; Commito, 1982). They are speciose (Fauchald, 1977; Bakken and Wilson, 2005) and inhabit a variety of habitats (Pettibone, 1963; Wilson, 2000) and

ecological niches. The Nereididae show a range of feeding modes including omnivory (Goerke, 1971), herbivory (Copeland and Wieman, 1924), detritivory, and carnivory (Goerke, 1971; Fauchald and Jumars, 1979). They also absorb dissolved organic matter (Stephens, 1968). *Alitta virens* juveniles are likely detritus feeders and the adults have diverse carnivorous or omnivorous diets, consuming vegetal or animal detritus, diatoms, crustaceans, gastropods, foraminiferans, nematodes, hydrozoans, and polychaetes (Caron et al. 2004).

Jaw Movements in *Alitta virens*

Alitta virens uses pharyngeal bulb eversions and quick, forceful jaw movements in feeding, defense, and burrowing. Despite the variation in diet and feeding modes in the Nereididae, the morphology (Pilato, 1968a) and jaw movements during feeding (Turnbull, 1876; Gross, 1921; Copeland and Wieman, 1924) are similar. During biting, the proboscis is rapidly turned inside out so that the pharyngeal bulb is anteromost (Fig. 1A) with the jaws in a half-opened gape. The jaws are opened and closed to cut algal blades or large prey. Smaller pieces of food are then grasped with the jaws and the bulb is retracted.

Alitta virens also uses its jaws for defense. *A. virens* is extremely thigmotactic (Copeland and Wieman, 1924) and spends most of its time within its burrow (Turnbull, 1876) where it preferentially feeds from the entrance (Fauchald and Jumars,

1979; Wilson and Ruff, 1988) and vigorously defends the burrow against predators and conspecifics (Turnbull, 1876; Gross, 1921; Evans, 1973; Miron et al., 1992a, 1992b; Lewis et al, 2003). The defensive pharyngeal bulb movements are similar to prey capture and food manipulation: rapid extension of the bulb followed by a forceful closing of the jaws as the proboscis retracts. A single bite can cut an adult conspecific in two (Turnbull, 1876).

Jaw Morphology

Jansonius and Craig (1971) established the terminology used here to describe Nereididae jaw structures (Fig. 1B). The two sharp, nonarticulated, hook-shaped jaws resemble open-ended cones with curved anterior tips referred to as fangs. A series of teeth, or denticles, are found along the hardened inner margin formed at the sharp edge of the inner face (Birkedal et al., 2005). The opening at the base of the cone is referred to as the myocoele opening and leads to an inner space known as an antrum or myocoele. The pharyngeal bulb musculature attaches to the inner and outer surface of the jaws at the myocoele opening. Additionally, various locations on the pharynx also bear highly variable numbers of small button-shaped teeth called paragnaths (Fig. 1A; Zghal and Amor, 1986; Bakken et al., 2009). The jaw structures are formed by hardening of specific areas of the pharyngeal epithelium (Paxton, 2004) and are remodeled throughout life (Birkedal et al., 2005). The jaws are composed of glycine- and histidine-rich protein fibers that are biomineralized with inorganic zinc-chloride along wear surfaces. (Lichtenegger et al., 2003, Broomell et al., 2008).

Pharyngeal Bulb Morphology and Functional Hypotheses

Pilato (1968a, 1968b) described the morphology of the pharyngeal bulb and proboscidial eversion and retraction musculature in *Perinereis cultrifera* (Grube 1840), which has similar morphology to *Alitta*. Pilato treats the proboscis (1968b) and the pharyngeal bulb (1968a) mechanisms separately, reflecting their relatively independent functioning. The proboscis includes structures external to the pharyngeal bulb that are responsible for eversions and retractions. Eversions are thought to be hydraulic, using coelomic fluid pressure generated by the body wall musculature. Antagonistic adductor muscles may help orient the proboscis as it is being everted as well as retract it. The pharyngeal bulb is the terminal anterior portion of the foregut with thick muscular walls. The bulb resembles a dorsoventrally flattened prolate spheroid with the left and right jaws embedded on either side of an anterior pharyngeal opening (mouth) that extends through the center of the bulb to exit posteriorly

as the esophagus. The musculature of the bulb is complex with muscle fibers of many orientations.

Pilato (1968a) grouped muscles into those that originate on the jaws and those that do not. The jaw muscles include thirteen pairs of jaw protractors and retractors, jaw base adductors and abductors, jaw fang adductors, and jaw elevator and depressor muscles. The names reflect as yet untested hypotheses of function. Muscles not attached to the jaws include eight orientations of muscle fibers dispersed throughout the bulb named for their arrangement rather than their actions: the superficial circular, transverse and longitudinal fibers, radial fibers, dorsoventral transverse fibers, and posterolateral fibers.

In this study, we analyze the morphology and kinematics of the pharyngeal bulb of *A. virens* to develop hypotheses of the functional role of the muscle and a fibrous sheath of extracellular matrix (ECM). One difficulty in assessing muscular function in a structure with many fiber orientations is in understanding how the contraction of one orientation affects others. This is especially true when fibers interdigitate to form a 3D block of muscle. Further, the function of fibers inserting on the jaws and originating on the surface of the bulb is difficult to interpret; would their contraction move the jaws or deform the bulb? Given these outstanding issues, we identify areas in which additional morphological detail may help refine hypotheses of jaw function. Since only the muscle fibers of the pharyngeal bulb in the Nereididae have been described in detail previously, we also describe the ECM sheath, given its crucial role in energy storage, support, and movement. Finally, we recorded the kinematics of biting during burrow defense to estimate the strains of various muscle fiber orientations during biting. These measurements provide a preliminary test of our functional hypothesis that identifies this joint as a muscle articulation.

MATERIALS AND METHODS

Twelve adult (~200 mm length) *Alitta virens* (Sars, 1835) specimens were donated by Seabait (Maine) L.L.C. (Franklin, ME). They were maintained in glass tube artificial burrows (10 mm inner diameter, 200 mm length) in a recirculating artificial seawater system (21°C, 35–38 ppt salinity, 10h light: 14h dark) and were fed shrimp or squid *ad libitum*.

Eight specimens were processed for histology. They were anaesthetized with 1.5% ethanol in seawater (adapted from O'Dor et al., 1990) until they did not respond to touch. A ventral incision from the 6th body segment to the peristomium tip was used to expose the pharyngeal bulb. In two specimens, the anterior ends were then cut off at the 6th body segment and fixed whole for use in gross anatomical dissections. In five specimens, the pharyngeal bulbs were dissected free from the proboscis walls and the protractor and retractor muscles and then fixed for 48 h in buffered formalin in seawater (10% v/v, Kier, 1992). These bulbs were embedded in glycol methacrylate plastic (JB-4, Structure Probe, West Chester, PA) and serially sectioned in transverse, parasagittal, and frontal planes using either tungsten carbide or Ralph glass knives (Bennett et al.,
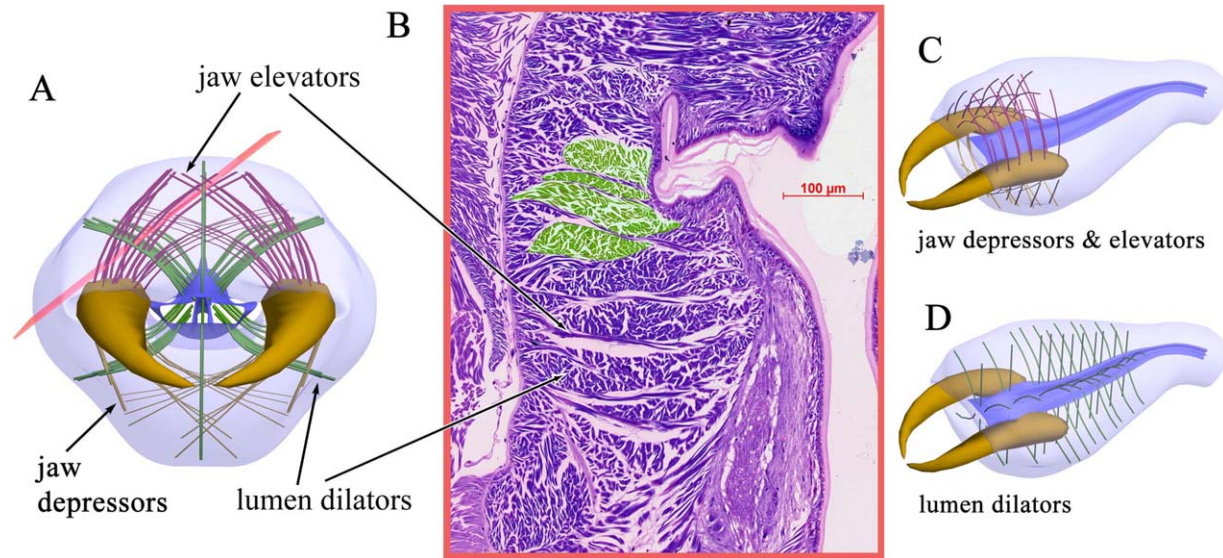
Fig. 2. *Alitta virens*, **A**) An anterior schematic view of the trajectories of the dorsal jaw elevator (mauve), ventral jaw depressor (gold) and radial lumen dilator (green) muscle fibers. The pink oblique frontal section plane indicates the approximate section corresponding to the histological micrograph in B. **B**) The micrograph shows the interdigitation of the jaw elevator and radial lumen dilator muscle fibers. Brightfield micrograph of sections stained with Toluidine blue/Basic fuchsin. The portions of the section highlighted in green illustrate the interdigitation of the lumen dilators (green) and the jaw elevator muscles interspersed between. **C**) and **D**) An antero-dorsal three-quarter left side schematic view of the pharyngeal bulb showing the trajectories of the jaw elevator and depressor muscle fibers (C) and the radial lumen dilator muscle fibers (D). Note the lumen of the esophagus (dark blue) extending longitudinally from the mouth between the jaws to exit the pharyngeal bulb posteriorly.

1976) at a thickness of 2.5 μm. Every fifth section was collected and stained using either a Toluidine blue stain (2% Toluidine blue O (C.I. 52040) in 2% sodium borate; modified from Burns, 1978) or a 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 differentiate muscle and collagenous fibers. Sections were examined by brightfield and polarized light microscopy (Zeiss AxioPhot with AxioCam HRc digital camera).

Modeling software (Anim8or freeware, http://www.anim8or.com) was used to create a 3D schematic illustration of the muscles and ECM fibers. Digitized section contours were aligned to photos of the intact bulb taken from multiple planes. The contours were fit within the model by matching points ($\approx$150) to measurements of corresponding points in the actual bulb. Jaws dissected from one bulb were digitized using a custom microscope stage that rotated each jaw through 360° in 22.5° increments. Digitized spline contours of the jaws were used as input into space-carving software (Octcarve; Wong and Cipolla, 2001) to create high-resolution 3D jaw elements. A full 3D schematic that included the jaws, pharyngeal bulb surfaces and representative muscle fibers (of exaggerated thickness and reduced number to better illustrate orientations of fiber groups) was virtually assembled in Autodesk 3D Studio Max (San Rafael, CA) for export as an interactive 3D model (see Supporting Information Fig. 1) via the Tetra 4D 3D PDF converter (Tech Soft 3d, Bend OR).

The remaining four specimens were used to record pharyngeal movements. A glass aquarium (150 mm height $\times$ 150 mm width $\times$ 600 mm length) with a mirror angled at 45° below allowed simultaneous lateral and ventral video recordings of specimens guarding their glass tube burrows. Periodic placement (approximately every 5 min) of a glass probe within 50 mm of the opening elicited a defensive lunge and bite. Digital video recordings of bites performed as part of this burrow guarding behavior [using a commercial Sony Handycam with NightShot lowlight sensitivity–30 frames per second (approximately 58–70 frames per bite); 530 lines of horizontal resolution] were analyzed frame by frame using Adobe Photoshop

CS2 (Adobe Systems, San Jose, CA) and Image J (NIH, http://rsbweb.nih.gov/ij/) to measure changes in jaw orientation.

RESULTS
Jaw Morphology

The left and right conic jaws have bilateral symmetry, are relatively thin laterally (termed "jaw depth"; Fig. 1B) and twist along their length, forming an inward and slightly ventrally pointing fang. The jaws are dorsoventrally compressed forming a ventral and a dorsal face (usually referred to as an "inner" and "outer" face because of the length-wise twist) and thus a sharp inner (or medial) and a broader outer (or lateral) margin. The myocoele opening is slightly flared and leads to a large myocoele that extends somewhat into the denticles. There are approximately six free denticles that are separated by narrow spaces along the inner margin. The free denticles have a strong inclination toward the fang. The posterior end of each jaw, approximately half of its length, is embedded within the tissue of the pharyngeal bulb.

Pharyngeal Bulb Musculature

Like *P. cultrifera*, the pharyngeal bulb of *A. virens* resembles a bilaterally symmetrical, dorsoventrally flattened prolate spheroid with the foregut lumen extending through the center along the long axis (e.g., Fig. 2C,D, Supporting Information Fig. 1). The muscular proboscis wall is attached to the pharyngeal bulb at a location lateral to where
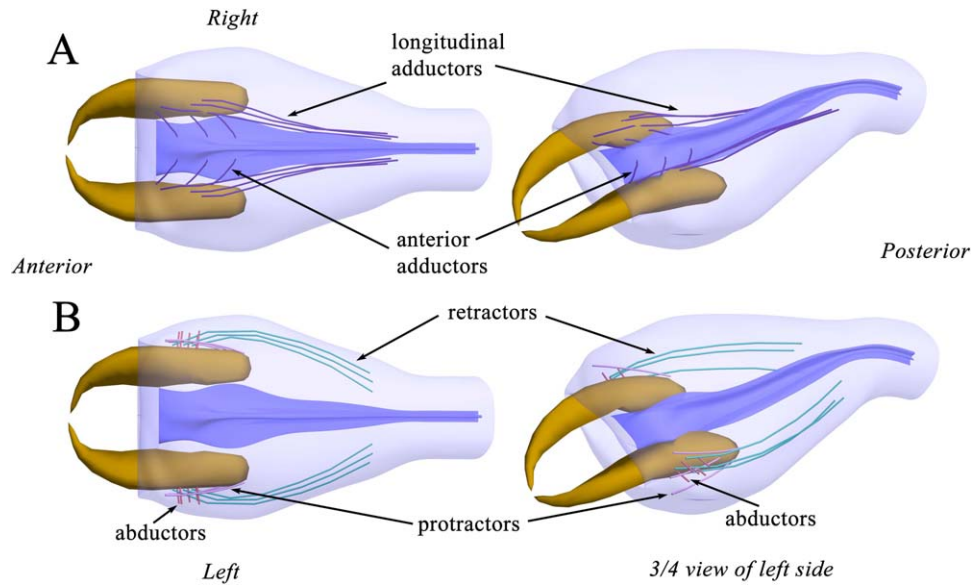
Fig. 3. *Alitta virens*, dorsal (left) and antero-dorsal three-quarter left side views (right) of the bulb schematic. **A**) The anterior and longitudinal adductor muscle fibers (dark blue). **B**) The jaw abductor (pink), jaw protractor (light purple), and jaw retractor (light green) muscle fibers.

the jaws protrude. Because many of the muscle fiber orientations resemble those of *P. cultrifera* (Pilato, 1968a), we use the same terminology for *A. virens*. Muscle fibers are shown schematically in the 3D illustration and are thus fewer in number and larger in size than in life.

The jaw elevator and depressor muscle fibers (Fig. 2A,C, Supporting Information Fig. 1; URL: XXX) are interspersed between other muscle fiber orientations [e.g., the lumen dilators (Fig. 2B, green highlight identifies the interspersing pattern)]. The left and right fibers extend over the midline to cross each other. Other muscle fiber groups, such as the superficial longitudinal and transverse fibers and the lumen dilators, pass between the elevator and depressor muscle fibers. The jaw elevators (Fig. 5C) originate on the outer (dorsal) face of the left and right jaws and extend medio-dorsally in a curved radiating pattern where they insert on the fibrous ECM sheath of the pharyngeal bulb. The jaw depressors (Fig. 4B) originate on the inner (ventral) face of the left and right jaws and extend in a similar radiating pattern to insert on the ECM sheath of the bulb's ventral surface.

The lumen dilators (Fig. 2A,D, Supporting Information Fig. 1; URL XXX) are radial muscle fibers that originate on the lumen walls of the foregut and extend radially to insert on the ECM sheath covering the bulb's outer surface.

The jaw adductors (Fig. 3A, Supporting Information Fig. 1) originate along the medial posterior surfaces of the jaws including both external jaw surfaces as well as the surface within the myocoele opening. The fibers are divided into anterior and longitudinal adductors. The anterior adductors (Fig. 4B) insert along the lateral surfaces of the anterior portion of the foregut within the pharyngeal bulb. The longitudinal adductors extend toward the posterior of the pharyngeal bulb where they insert on the basal bulb muscle fibers at the posterior end of the bulb and the origin of the esophagus.

The jaw abductor, protractor, and retractor muscle fibers (Figs. 3B and 4A,B, Supporting Information Fig. 1) interdigitate with each other. They all originate on the lateral-most posterior surfaces of the jaws. The abductors are short fibers that extend radially from their origin to insert on the ECM sheath surrounding the pharyngeal bulb. The protractors originate on the extreme posterolateral edges of the jaws and extend anteriorly to insert on the ECM sheath lateral to where the jaws emerge from the bulb musculature. The retractors are longitudinal fibers that extend from their origin to insert on the basal bulb muscle fibers at the posterior end of the pharyngeal bulb.

The deep longitudinal muscle fibers (Fig. 5A, Supporting Information Fig. 1; URL XXX) are found between the posterior margin of the jaws and the posterior end of the bulb. Some of these fibers may be traced to the same origins as retractor and longitudinal adductor muscle fibers, however, the aggregate cross sectional area of these fibers is less than the area of longitudinally oriented fibers that insert on the posterior end of the bulb. As such, we describe the deep longitudinal muscle fibers as a unique group that may insert within the ECM of the muscles that insert on the posterior margins of the jaws.
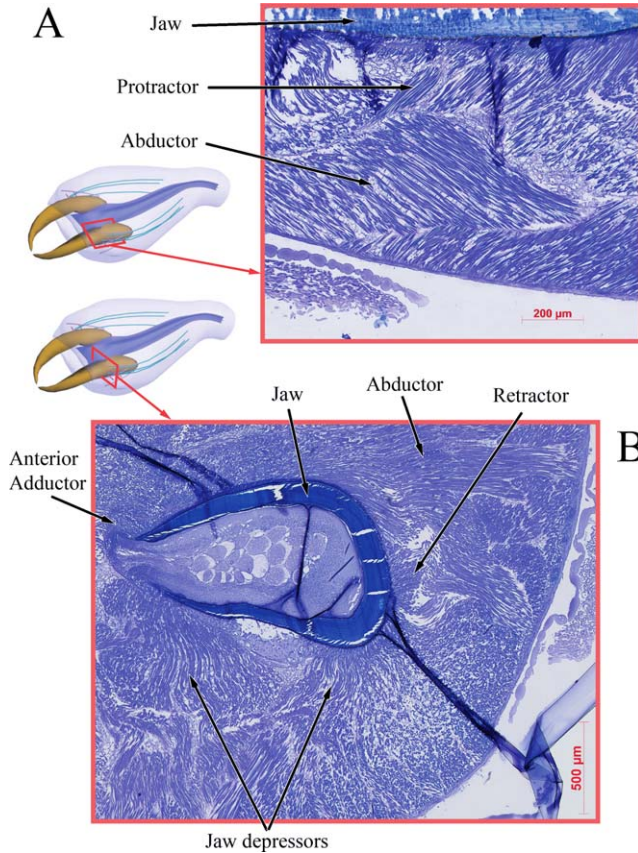
Fig. 4. *Alitta virens*, **A**) An oblique longitudinal (frontal) section through the left jaw (as indicated by the box drawn on the bulb schematic to the left). The dark blue material at top in histological section to the right is the cut surface of the jaw and between the jaw and the left lateral surface of the bulb are obliquely cut protractor muscle fibers (angling left/anteriorly) and abductor muscle fibers (angling right/posteriorly). **B**) The bulb schematic inset shows the plane of the transverse section through the left jaw shown in the micrograph below. The jaw appears as a horseshoe in this section as the myocoele opening is an oblique ellipse relative to the long axis of the jaw. Two bundles of jaw depressor muscle fibers can be seen extending from the jaw toward the ventral surface of the bulb. Abductor muscle fibers are shown extending laterally from the jaw to the left surface of the bulb. Retractor muscle fibers are shown curving into the plane of the micrograph (posteriorly). Section folds are visible in A and B and are due to the presence of the hard jaw materials. Brightfield micrographs of sections stained with Toluidine blue.

The superficial longitudinal and transverse muscle fibers (Fig. 5B,C, Supporting Information Fig. 1) are deep to the ECM sheath at the surface of the bulb. The transverse fibers are separated into dorsal and ventral halves such that their origins and insertions are located along the lateral surfaces of the bulb. The longitudinal fibers have an anterior origin on the ECM sheath at the opening of the pharynx and extend to insert on the posterior end of the pharyngeal bulb and its basal bulb muscle fibers (Fig. 6A,B, Supporting Information Fig. 1). As these fiber orientations are closely associated with the surface of the bulb, they interdigi-
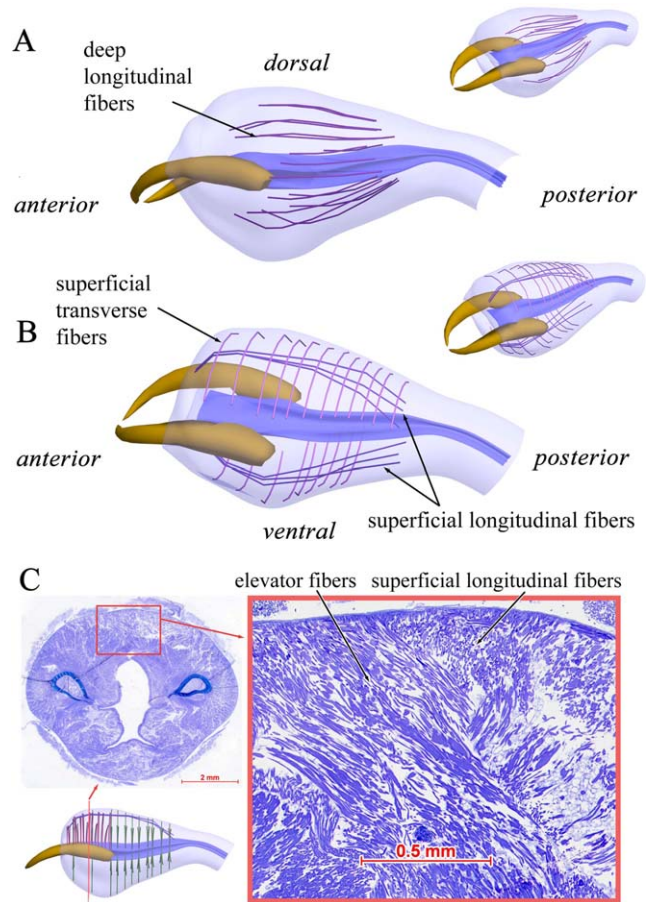
Fig. 5. *Alitta virens*, **A**) A postero-lateral view of the pharyngeal bulb schematic showing the general trajectories of the deep longitudinal muscle fibers (dark purple). These fibers likely have a number of origins (see text) and insert in the area of the basal bulb muscle fibers. **B**) A slightly posterior, dorso-lateral view of the general arrangement of the superficial longitudinal (light mauve) and the superficial transverse (light purple) muscle fibers. These muscle fibers are closely associated with the fibrous ECM sheath (see Fig. 5C). See insets in A) and B) for antero-dorsal three-quarter left side views. **C**) The inset on the lower left shows the pharyngeal bulb schematic with the jaw elevator fibers (pink), the radial lumen dilator fibers (green) and the superficial longitudinal fibers (light mauve). The red plane indicated on the bulb shows the approximate location of the transverse histological section shown inset on the upper left. The red box shows the location of the magnified area shown to the right. The magnified area shows the crossing left and right elevator fibers and the superficial longitudinal fibers. Brightfield micrograph of section stained with Toluidine blue.

tate with the perpendicular fibers (most notably the lumen dilators) that insert on the ECM sheath.

The basal bulb musculature marks the posterior end of the pharyngeal bulb and is formed by an array of fibers that originate and insert on the dorsal and ventral portions of the ECM sheath (Fig. 6A,B, Supporting Information Fig. 1). These fibers curve anteriorly as they approach the surface of the bulb such that the overall array resembles a
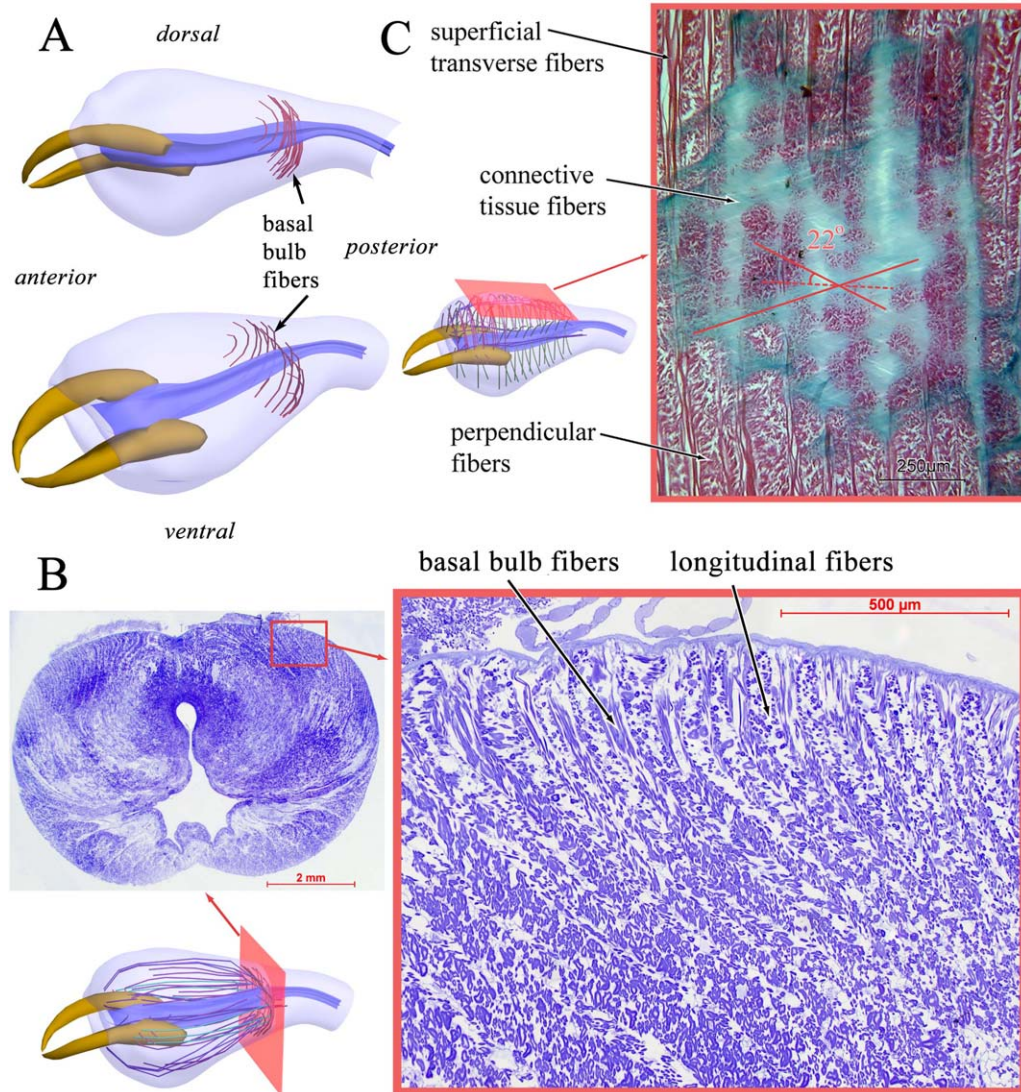
Fig. 6. *Alitta virens*, **A**) A slightly ventral postero-lateral (top) and an antero-dorsal three-quarter left side view (bottom) of the pharyngeal bulb schematic showing the arrangement of the basal bulb fibers (dark red). These fibers are oriented dorsoventrally and mark the end of the pharyngeal bulb. **B**) The inset of the lower left shows the pharyngeal bulb with the trajectories of the longitudinally arranged fibers [the dorsal and ventral superficial longitudinal fibers (light mauve), the jaw retractors (teal), the jaw abductors (dark blue), and the deep longitudinal fibers (dark purple)] and the basal bulb muscle fibers (dark red). The red plane indicated on the bulb shows the approximate location of the transverse histological section shown inset on the upper left. The red box shows the location of the magnified area shown to the right. The magnified area shows the highly interdigitated basal bulb (obliquely cut) and longitudinal fibers (transversely cut). Brightfield micrograph of section stained with Toluidine blue. **C**) The inset on the left shows a slightly antero-dorsal left lateral view of the pharyngeal bulb with the trajectories of the radial lumen dilator fibers (green), superficial longitudinal fibers (light mauve), and superficial transverse fibers (light purple). The obliquely frontal red plane indicates the approximate level of the section appearing to the right. The histological micrograph shows the superficial transverse fibers, the perpendicular fibers (these could be basal bulb fibers, jaw elevator or depressor fibers, lumen dilator fibers, or abductor fibers depending on where the grazing section is taken) that insert on the fibrous ECM sheath, and its fibers that are arranged in a crossed-fiber array at an angle of 22° to the longitudinal axis of the bulb. Polarized light micrograph of paraffin section stained with Milligan's Trichrome stain.

cup with the opening oriented toward the jaws (Fig. 5A). There is an interruption at the center of the medial fibers (the center of the cup's base) through which the esophagus exits the pharyngeal bulb. The dorsoventrally oriented basal bulb fibers interdigitate with fibers of many other orientations; lateral (the posterior-most superficial transverse fibers), radial (lumen dilator fibers), and longitudinal (superficial longitudinal fibers, jaw retractor fibers, and jaw adductor fibers).

The Extracellular Matrix Sheath of the Pharyngeal Bulb

A thin sheath of fibrous ECM surrounds the pharyngeal bulb (Fig. 6C). This thin layer is
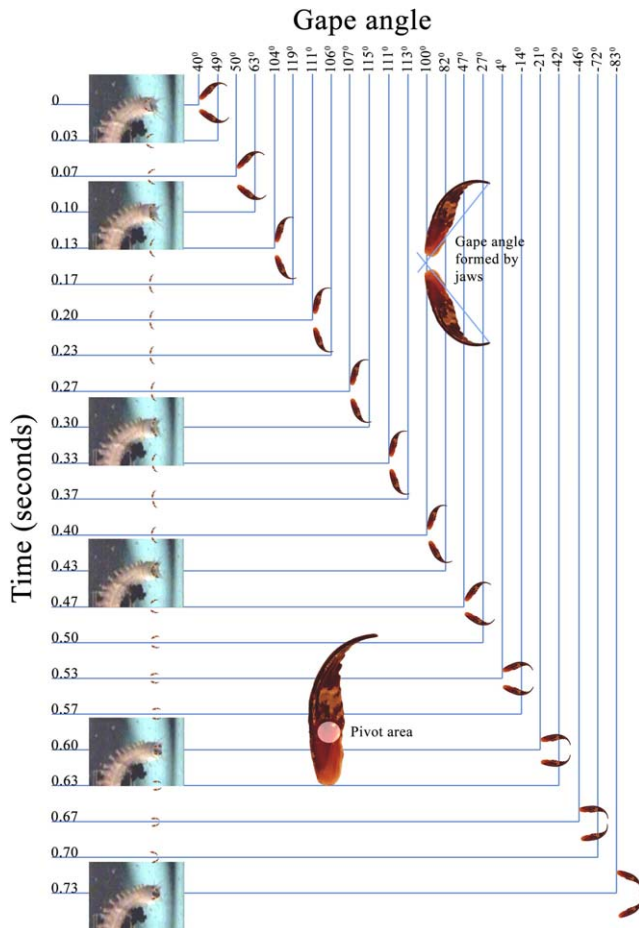
Fig. 7. *Alitta virens*, video frames of jaw movements during a typical burrow defense bite. Shown on the abscissa are the jaw positions measured as gape angle formed between the jaws during sequential video frames indicated by time on the ordinate. The upper inset shows how the angle was measured between the jaws and the lower inset shows the pivot area on the jaw.

closely apposed to the underlying musculature and contains fibers that are highly birefringent and show staining characteristics typical of collagen. The fibers are arranged in parallel arrays in a crossed-fiber arrangement with an angle of 22° relative to the long axis of the pharyngeal bulb. No analogous sheath was observed in the foregut lumen wall.

Jaw Movements During Defense of Burrows

When perturbed, *A. virens* retracts within its burrow and then defends the opening by quickly extending out a short distance and performing a bite (duration: 2–3 s). Typical bites can be described by tracking the exposed fang position and orientation in a series of ventral view video fields (Fig. 7). Four animals and 15 bites of this type were recorded (3–4 bites each). Of these, the sequence shown in Figure 7 represents the bite with the widest gape range, although common

phases of movement could be distinguished in all bites recorded.

Below we describe a generalized defensive biting motion. Relative movement phases, rather than specific durations and displacements, are reported because these phases can be performed with considerable variation in the speed, timing, and linear and angular displacements of the jaws. All jaw movement seems to occur within an approximately midfrontal plane. Although lateral views could not be obtained, we estimate that the plane of jaw movements can be pitched ventrally by up to 10° relative to the horizontal.

The bite begins with proboscis eversion. There is slight jaw rotation (opening) as the posterior end of the bulb is everted past the prostomial opening. As a result, when the anterior halves of the jaws appear, they are already opened with a gape of approximately 40° (e.g., Fig. 7, 0 s). Then, as the bulb is fully everted, the jaws begin a lateral translation that correlates with increasing diameter of the pharyngeal opening (Fig. 7, 0.03–0.1 s). After the jaws separate, the gape between the fangs is further widened by jaw rotation up to an angle of 115° (the angle formed between the tips and the center of the bases of the jaws; Fig. 7, upper inset, 0.3 s). During this movement, the center of rotation is not fixed but may occur anywhere within an area just posterior of the longitudinal center of each jaw (Fig. 7, lower inset).

Jaw closure (Fig. 7, 0.4–0.73 s) occurs when the jaws rotate past the point where they are parallel (Fig. 7, 0.73 s, −83°). In burrow defense bites, jaw closing usually occurs simultaneously with retraction of the proboscis. In bites associated with feeding, repeated rotations and translations open and close the jaws to grasp and manipulate the food. Simultaneous closure of the jaws and retraction of the pharyngeal bulb requires that the jaws rotate toward the medial axis so that their fangs touch or even cross over each other as they enter the peristomium.

DISCUSSION
Summary and Analysis of Novel Morphological Findings

We have observed two important aspects of Nereididae pharyngeal bulb morphology that have not been reported previously. First, a sheath formed of parallel arrays of ECM fibers (presumably collagenous) surrounds the pharyngeal bulb and is organized in a crossed-fiber arrangement with a fiber angle of 22° relative to the bulb longitudinal axis. Second, many of the muscle fibers are not present in large discrete bundles, but instead are dispersed throughout the solid muscular pharyngeal wall and interdigitate with fibers of other orientations. This 3D muscle fiber arrangement has the characteristics of a muscular hydrostat (Kier and

Smith, 1985; Smith and Kier, 1989) that serves the multiple functional roles required of a muscle articulation.

The Fibrous ECM Sheath

The sheath surrounding the pharyngeal bulb is likely to be of considerable importance to the function of the bulb because its fibers may constrain shape changes and store elastic energy. Similar connective tissue fiber arrays provide structural reinforcement, control shape, transmit stresses, and store elastic energy in many soft-bodied invertebrates (Thompson and Kier, 2001).

Clark and Cowey (1958) noted that shape change in pressurized, soft-bodied cylindrical vermiform animals is limited and controlled by tension-bearing fibers arranged as left- and right-handed helical arrays similar to the sheath surrounding the pharyngeal bulb described here. They developed a simple geometrical model that can be applied to aid the interpretation of the functional role of the pharyngeal sheath. Since the fiber angle measured in this study was 22°, it is likely that an important function of the sheath is in limiting elongation of the pharyngeal bulb while still accommodating expansion of the pharynx lumen. The function of the lumen dilators (see below) in expanding the pharynx lumen requires that both a) the length of the pharynx be controlled (so that lumen dilator contraction does not simply cause elongation of the bulb) and b) the overall diameter of the bulb increase, which can only occur if the fiber angle is less than 54°44'. The rationale is as follows. Dilator muscle contraction will thin the bulb wall surrounding the pharynx. Since the volume of the bulb wall is constant, a decrease in thickness of the wall must result in an increase in the internal and external diameter, expanding the volume of the internal lumen. The crossed-fiber array sheath can accommodate this increase in diameter (and the increase in total enclosed volume) with an increase in fiber angle, which at this fiber angle will also result in a small decrease in overall length of the bulb. The limit to expansion would only be reached in the extreme case of the fiber angle approaching 54°44'. Note that dilation of the pharyngeal bulb requires that the fiber angle be less than 54°44'; increase in diameter of a hypothetical pharyngeal bulb with fibers oriented greater than or equal to 54°44' would be prevented by the fibers unless the enclosed volume decreased. Because collagen fibers can accommodate strains of approximately 0.10 beyond resting length (Vogel, 2003) they frequently serve as elastic energy storage mechanisms (e.g., cephalopod mantles (e.g., Thompson and Kier, 2001), suckers (e.g., Kier and Smith, 2002), and fins (e.g., Johnsen and Kier, 1993). Thus, it is possible that the sheath may return energy elastically as it antagonizes the musculature that widens the esophageal lumen.

Muscular Hydrostat of the Pharyngeal Bulb

The pharyngeal bulb may function as a muscle articulation because the musculature likely performs multiple functions including rotating and translating the jaws open and closed, providing support to the muscles that generate these movements, bearing the compressional forces between the jaws, and serving as a pivot for each jaw. These functions are likely provided by a 3D, interdigitating array of muscles organized as a muscular hydrostat. This hypothesis requires that, in addition to considering individual fiber function, the simultaneous actions of multiple fiber orientations must also be considered. We describe below a novel functional interpretation of the pharyngeal bulb morphology as a muscular hydrostat with specialized, but interconnected, anterior and posterior halves.

The posterior portion of the pharyngeal bulb may resist longitudinal compression and act as a bolster or support for the muscles that move the jaws. The dorsoventrally oriented basal bulb muscle fibers are oriented perpendicularly to a group of longitudinal fibers (Figs. 5A,B and 6B; the deep and superficial longitudinal fibers) and the posterior ends of the longitudinal adductor and retractor fibers (Fig. 3A,B). Additionally, most of the basal bulb fibers are arranged obliquely relative to the lumen dilator muscle fibers in this area and the basal bulb and longitudinal fibers are perpendicular to the superficial transverse fibers. Because fibers are oriented in all three dimensions, active control of all three dimensions can occur, as in other muscular hydrostats. The orientation of the basal bulb muscle fibers suggests that their isolated contraction would dorsoventrally flatten the posterior end of the bulb. The cocontraction of other muscle fiber orientations, however, may result, not in movement, but in increased pressure and thus stiffness of this zone. This stiffening of the posterior half of the bulb could provide the support required for anterior muscle fibers that move the jaws.

As the jaws are embedded in the anterior half, the muscle fibers responsible for moving the jaws, creating pivots, and resisting compression are located here. Although there is no obvious partition between the anterior and posterior portions of the musculature of the pharyngeal bulb, the differences in fiber orientations may reflect a difference in function. Cocontraction of the lumen dilator muscle fibers and the superficial transverse muscle fibers should result in an elongation of the bulb. If length is controlled by the surrounding ECM sheath, then the walls of the bulb (and the lumen) should increase in diameter until the ECM

sheath is placed in tension. This motion could explain lateral translation of the jaws. When the fibers of the ECM sheath are in tension, the muscles can generate no significant additional shape change. Instead the cocontracting muscles increase the pressure and stiffness of the structure and may provide the structural support for pivoting of the jaws (Fig. 7, lower insert) and the support that allows longitudinal adductor and abductor muscle fibers to rotate the jaws open and closed. The jaws are embedded within the bulb wall and interrupt the course of the radial lumen dilator fibers. The radial muscles on either side of the jaws are represented by the anterior adductor (Fig. 3A) and abductor (Fig. 3B) muscle fibers. Thus, there is a full complement of radial fibers that surround the jaws and that may function to thin the pharyngeal walls by bringing the inner foregut and outer bulb surfaces closer together as described above. This circumferential increase may be antagonized by the superficial transverse fibers. Thus, the lateral translation of the jaws and the widening of the mouth during the initial stages of a bite may be caused by this increase in circumference.

The jaws were observed to rotate laterally to widen the gape in the second stage of the bite. We hypothesize that the tissue of the anterior bulb wall becomes stiff because the radially arranged fibers are antagonized by simultaneously active superficial transverse fibers. This stiffening of the tissue may support the position of the jaws and provide a pivot against which the longitudinal adductors may act in closing and the abductors in opening. The stiffened posterior half may resist compression of the bulb due to contraction by these longitudinal fibers.

Observations of Pharyngeal Bulb Function

Although we are unable to test directly the hypotheses of muscle function proposed above, observations of jaw movements provide insights into the role of the musculature during support and movement. During burrow defense bites (Fig. 7), the jaws are rotated and translated in a roughly frontal plane, perhaps indicating that the jaw elevator and depressor muscle fibers stabilize dorsoventral jaw movements. As the bite begins, translation of the jaws away from the bulb midline increased the gape between the fangs. This occurred simultaneously with the dilation of the pharyngeal opening, suggesting that the translation may be caused by radial muscle fiber contraction. The width of the gape was then increased by jaw rotation in which the symmetrical left and right pivots were located in an area posterior to the midpoint of each jaw's long axis (Fig. 7, lower inset). Since the longitudinal abductor muscle fibers originate immediately anterior to this location, this suggests that the posterior half of the jaw is supported while the

anterior portion is adducted or abducted by the longitudinally arranged muscle fibers. Because the jaw movements observed here did not involve biting an object, it is likely that little reaction force was transmitted through the pharyngeal wall. Jaw opening concurrent with an increase in the diameter of the pharynx suggest that the radial muscle fibers of the anterior bulb may provide the stiffening required to support these movements.

CONCLUSIONS

The pharyngeal bulb of *A. virens* resembles the octopus beak joint (Uyeno and Kier, 2005, 2007) in a number of aspects, suggesting that the bulb is also a muscle articulation. The muscle and fibrous ECM are arranged as a muscular hydrostat that serves common and multiple roles: the soft tissues separate and connect the jaws; they bear compressional forces transferred across the joint; and they generate the force that moves the jaws.

This study, in combination with previous research, suggests that muscle articulations are an important joint mechanism; they are used as joints in four invertebrate phyla [inarticulate brachiopods (Trueman and Wong, 1987) cephalopods (Uyeno and Kier, 2005, 2007), kalyptorhynch turbellarian flatworms (Uyeno and Kier, 2010) and jawed polychaetes] and serve critical functions typically related to feeding and defense. The mouthparts of onychophorans (Manton and Harding, 1964) and some wood-boring beetle larvae (Nieves-Aldrey et al., 2005) show characteristics that suggest that they may also function as muscle articulations. A diversity of invertebrates with bodies nearly devoid of rigid parts thus may rely on this flexible soft-tissue joint type to orient the teeth, hooks, blades, anvils, and spades that often represent the only rigid or mineralized body elements and to move them with force in complex motions critical to survival and reproduction.

ACKNOWLEDGMENTS

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