

MORPHOLOGY AND MECHANICS OF TONGUE MOVEMENT IN THE AFRICAN PIG-NOSED FROG *HEMISUS MARMORATUM*: A MUSCULAR HYDROSTATIC MODEL

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Accepted 5 January; published on WWW 9 March 1999

Summary

The goal of this study was to investigate morphological adaptations associated with hydrostatic elongation of the tongue during feeding in the African pig-nosed frog *Hemissus marmoratum*. Whereas previous studies had suggested that the tongue of *H. marmoratum* elongates hydraulically, the anatomical observations reported here favour a muscular hydrostatic mechanism of tongue elongation. *H. marmoratum* possesses a previously undescribed compartment of the m. genioglossus (m. genioglossus dorsoventralis), which is intrinsic to the tongue and whose muscle fibres are oriented perpendicular to the long axis of the tongue. On the basis of the arrangement and orientation of muscle fibres in the m. genioglossus and m. hyoglossus, we propose a muscular hydrostatic model of tongue movement in which contraction of the m. genioglossus dorsoventralis, together with unfolding of the intrinsic musculature of the tongue, results in a doubling in tongue length. Electron micrographs of sarcomeres from resting and elongated tongues show that no special adaptations of the sarcomeres

are necessary to accommodate the observed doubling in tongue length during feeding. Rather, the sarcomeres of the m. genioglossus longitudinalis are strikingly similar to those of anuran limb muscles. The ability to elongate the tongue hydrostatically, conferred by the presence of the m. genioglossus dorsoventralis, is associated with the appearance of several novel aspects of feeding behaviour in *H. marmoratum*. These include the ability to protract the tongue slowly, thereby increasing capture success, and the ability to aim the tongue in azimuth and elevation relative to the head. Compared with other frogs, the muscular hydrostatic system of *H. marmoratum* allows more precise, localized and diverse tongue movements. This may explain why the m. genioglossus of *H. marmoratum* is composed of a larger number of motor units than that of other frogs.

Key words: feeding, behaviour, muscular hydrostat, sarcomere, tongue, muscle, ultrastructure, African pig-nosed frog, *Hemissus marmoratum*.

Introduction

Recent studies of feeding behaviour in anurans have described several different mechanisms of tongue protraction during prey capture (Nishikawa, 1997). In many anurans, the tongue shortens during protraction as the m. genioglossus contracts, pulling the tongue pad forward towards the mandibular symphysis (Deban and Nishikawa, 1992). In the marine toad *Bufo marinus* (Nishikawa and Gans, 1996), the tongue elongates by as much as 80% of its resting length during protraction (i.e. it elongates to 180% of its resting length). However, in toads, as in most other anurans, no muscle fibres are present that could elongate the tongue directly via their own shortening. The tongue and jaws accelerate rapidly, and inertia appears to be primarily responsible for tongue elongation (Gray and Nishikawa, 1995; Nishikawa and Gans, 1996). In the pig-nosed frog *Hemissus marmoratum* (Ritter and Nishikawa, 1995), the tongue elongates by as much as 100%

of its resting length during protraction (i.e. it doubles in length). In *H. marmoratum*, however, tongue protraction is too slow for inertia to play a significant role in tongue elongation, and some alternative mechanism must be responsible (Fig. 1).

On the basis of the kinematic analysis of prey capture, muscle denervation studies and gross dissection of the tongue, Ritter and Nishikawa (1995) proposed that *H. marmoratum* uses a hydrostatic mechanism to protract its tongue. This hypothesis was based on the observation that unilateral denervation of the m. genioglossus causes the tongue to bend through an angle of more than 180° towards the inactivated side. These authors assumed that, like other anurans, *H. marmoratum* lacks intrinsic muscles (i.e. muscles that have both their origin and insertion in the tongue) that might be responsible for elongation, and suggested that, rather than functioning as a muscular hydrostat (Kier and Smith, 1985),

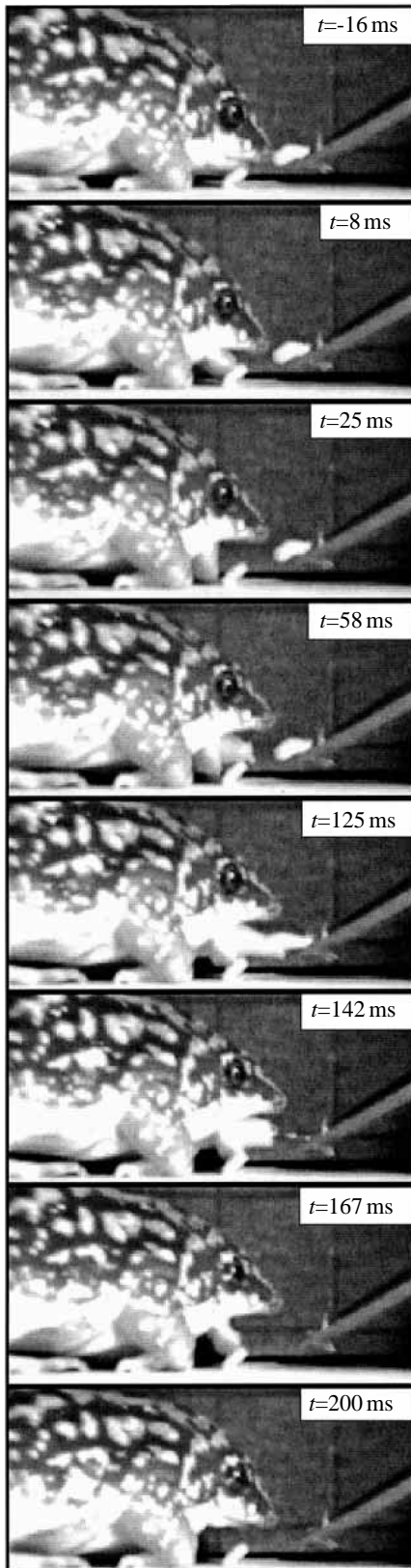


Fig. 1. Video sequence of *Hemisus marmoratum* eating a termite. Numbers on the upper right refer to time (ms) since the onset of mouth opening. Note that the mouth opens fully before the tongue rotates forward ($t=25$ ms). In this sequence, the tongue, once protruded, only elongates to its original resting length ($t=125$ ms) after shortening slightly during the initial stages of protraction, although it is capable of doubling in length.

the tongue might be responsible for tongue elongation during protraction. This hypothesis predicts that a crossed helical array of collagen fibres surrounding the lingual sinus would be present, as well as muscles that would serve to pump fluid into the sinus during tongue protraction.

The purpose of the present study was to examine the anatomy, histology and ultrastructure of the tongue of *H. marmoratum*. In particular, we were looking for structures in the musculature and associated connective tissues of the tongue that might be involved in hydrostatic elongation, as well as for structural adaptations of sarcomeres in the tongue muscles that might facilitate the doubling in tongue length that occurs during protraction. In the present study, we report on visible and polarized light microscopy studies of the tongue muscles and associated connective tissue and on transmission electron microscopy studies of sarcomeres in the m. genioglossus muscles of resting and elongated *H. marmoratum* tongues. Our observations refute the hydraulic mechanism proposed by Ritter and Nishikawa (1995) and instead support a muscular hydrostat model of tongue elongation, in which muscle fibres oriented perpendicular to the long axis of the tongue produce elongation directly by their contraction. This result has important implications for motor control of prey capture and helps to explain some unusual features of the feeding apparatus of *H. marmoratum*, such as the relatively large number of motor units in the m. genioglossus (Anderson et al., 1998).

Materials and methods

For histology, three specimens were fixed in neutral buffered formalin for examination in transverse, frontal and sagittal section planes. The floor of the mouth was dissected from the specimens used in frontal and sagittal sections, while the entire head was used for the transverse section series. The specimens were decalcified (S/P decalcifying solution, Baxter, Atlanta, GA, USA). After decalcification, the specimens were dehydrated through a series of alcohols, cleared in a clearing agent (Hemo-D, Fisher Scientific, Pittsburgh, PA, USA) and embedded in paraffin (Paraplast+, Oxford Labware, St Louis, MO, USA). Serial sections were cut at 10 μm thickness on a rotary microtome and mounted on slides. Alternate slides were stained with Milligan's trichrome and with Weigert's haematoxylin counterstained with Picro-Ponceau (Humason, 1979).

The lower jaws of two specimens were prepared for transmission electron microscopy of sarcomeres in the m. genioglossus longitudinalis. The specimens were fixed in a solution of 5% glutaraldehyde with 0.1 mol l⁻¹ sodium

the tongue of *H. marmoratum* is protracted by a hydraulic mechanism. Specifically, Ritter and Nishikawa (1995) suggested that the flow of lymph from the lingual sinus into

cacodylate and 2 mmol l^{-1} calcium chloride. One of the specimens was fixed with the tongue in the resting position. This specimen was anaesthetized in buffered MS222 (tricaine methane sulphonate), and the tongue was fixed in place in the lower jaw. For the other specimen, the length of the resting tongue was measured. The lower jaw was then placed in a dish of Sylgard resin, and the tongue was extended as far as possible using forceps. Insect pins were used to hold the extended tongue in place while it was immersed in fixative. The length of the extended tongue was measured and found to be twice the resting length, which corresponds well with the maximum length change observed during feeding (Ritter and Nishikawa, 1995), although this passive stretching may not precisely mimic the elongation that occurs during active protraction.

Small blocks of tissue were cut from the resting and extended tongue samples in such a way that their orientation in the tongue could be precisely determined during embedding. The samples were postfixed for 40 min at 4°C in a 1:1 mixture of 2% osmium tetroxide and 2% potassium ferricyanide in 0.13 mol l^{-1} cacodylate buffer. The samples were then rinsed in chilled 0.065 mol l^{-1} cacodylate buffer for 15 min, dehydrated through a graded series of ethanols, cleared in propylene oxide and embedded in epoxy resin (Epoxy 812, Ernest F. Fullam, Latham, NY, USA) in an orientation to provide longitudinal sections of the m. genioglossus longitudinalis. Sections of silver interference colour were stained with saturated aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Zeiss EM 10CA transmission electron microscope. The magnification stops of the microscope were calibrated with a grid replica. The thick filament length, the thin filament length and the sarcomere length from both the resting and extended muscle were measured using calipers on photographic prints.

Each value reported here represents the mean of 30 measurements.

Results

Anatomy of the tongue

Anurans generally possess a relatively simple tongue that consists only of two pairs of extrinsic muscles, the m. genioglossus and m. hyoglossus (Horton, 1982; Regal and Gans, 1976). In contrast to most other terrestrial vertebrates, intrinsic muscles are absent in most species and there is no cartilaginous or bony skeleton in the tongue. The tongue as a whole is attached to the floor of the mouth near the mandibular symphysis, and the tongue rotates upwards and forwards over the mandibles during protraction, so that the mucosa is dorsal when the tongue is at rest and ventral when the tongue is fully protracted. The m. genioglossus originates near the mandibular symphysis, inserts posteriorly into the tongue pad and lies just under the mucosa when the tongue is at rest in the oral cavity. The m. hyoglossus originates on the posteromedial process of the hyoid, inserts along the lateral margin of the tongue pad, often interdigitating with the fibres of the M. genioglossus, and lies ventral to the m. genioglossus when the tongue is at rest.

The tongue of *H. marmoratum* is similar to that of typical anurans, with two exceptions: (1) there is no interdigitation of fascicles of the m. genioglossus and m. hyoglossus in the tongue pad; and (2) a novel compartment of the m. genioglossus is present that serves as an intrinsic tongue muscle (see below).

Structure of the tongue muscles

As in other frogs, the m. genioglossus of *H. marmoratum* is medial and unpaired. In contrast to other frogs, the m. genioglossus is composed of two major components in *H.*

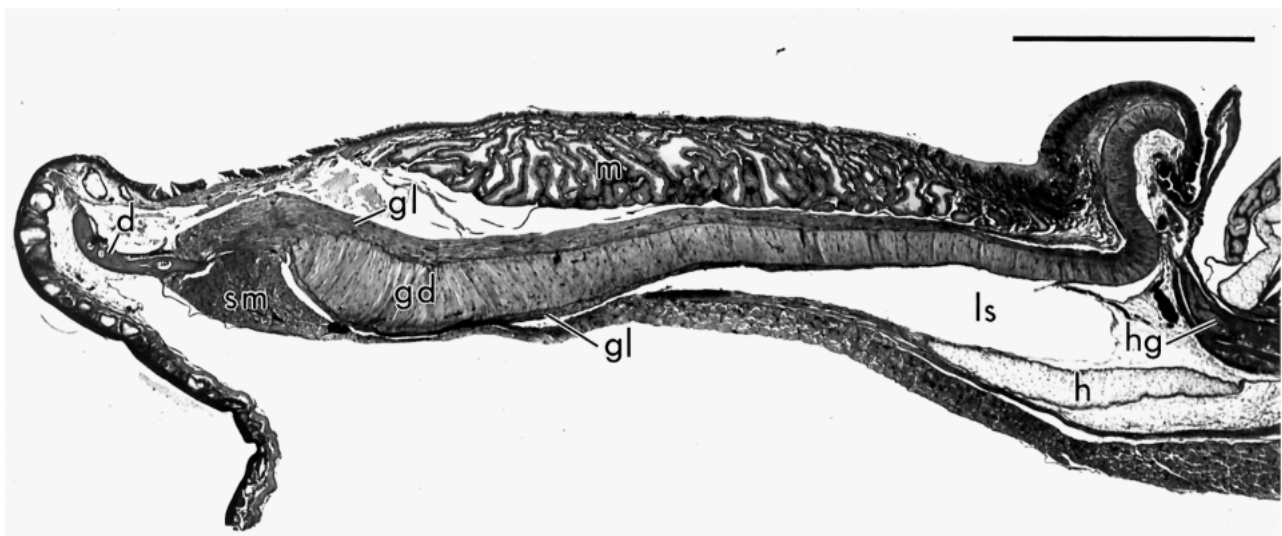


Fig. 2. Photomicrograph of a parasagittal section of the tongue of *Hemisus marmoratum*. The anterior direction is to the left in the micrograph. d, dentary bone; gd, m. genioglossus dorsoventralis; gl, m. genioglossus longitudinalis; h, hyoid cartilage; hg, m. hyoglossus; ls, lingual sinus; m, mucosa; sm, m. submentalis. Scale bar, 1.0 mm. Brightfield microscopy of a $10\text{ }\mu\text{m}$ thick paraffin section stained with Picro-Ponceau and haematoxylin.

marmoratum, the m. genioglossus longitudinalis (present in most frogs) and the m. genioglossus dorsoventralis (new muscle). The m. genioglossus longitudinalis originates on the dentary bone near the mandibular symphysis and runs the length of the tongue to insert on the tongue tip, which is posterior when the tongue is at rest (Fig. 2). The majority of its fibres lie dorsal to the m. genioglossus dorsoventralis when the tongue is at rest, and the dorsal layer tapers towards the lateral margins of the m. genioglossus dorsoventralis. In addition to this robust dorsal layer, two thin slips of longitudinal fibres lie ventral to the m. genioglossus dorsoventralis (Figs 2, 3). These thin ventral layers do not extend to the midline of the tongue or around the lateral margins of the m. genioglossus dorsoventralis.

The m. genioglossus dorsoventralis is a thick mass of muscle lying between the two layers of the m. genioglossus longitudinalis (Figs 2–4). It is medial and unpaired and larger in volume than the m. genioglossus longitudinalis, making up approximately two-thirds of the total genioglossus muscle volume. The fibres of this muscle are oriented directly dorsoventrally, running perpendicular to the tongue axis and to the orientation of the m. genioglossus longitudinalis. At the anteriormost tip of the m. genioglossus dorsoventralis, a few fibres interdigitate with those of the m. genioglossus

longitudinalis. The m. genioglossus dorsoventralis is wrapped by a thick connective tissue capsule consisting of birefringent fibres with staining reactions typical of collagen (Fig. 3). The connective tissue of this capsule provides the origin and insertion points for individual muscle fibres. The fibres of this connective tissue capsule are arranged predominantly transversely to the long axis of the tongue, providing a cylindrical wrapping around the m. genioglossus dorsoventralis (Fig. 5).

The m. genioglossus dorsoventralis does not extend to the anteriormost portion of the m. genioglossus longitudinalis, but instead arises from a thickened connective tissue mass posterior to the origin of the m. genioglossus longitudinalis (Figs 2, 3). The bundle extends to the tongue tip. The fibres of the m. genioglossus dorsoventralis show little variation in fibre direction, but instead are highly aligned perpendicular to the long axis of the m. genioglossus as a whole (Figs 3, 4). This distinct compartment of the m. genioglossus has not been reported previously in frogs.

As in other frogs, the m. hyoglossus originates on the ventral surface of the posteromedial process of the hyoid. This muscle inserts in the tongue in a number of independent fascicles. The most medial fascicle inserts near, but not quite at, the tip of the tongue, whereas the more lateral fascicles insert in a flap of

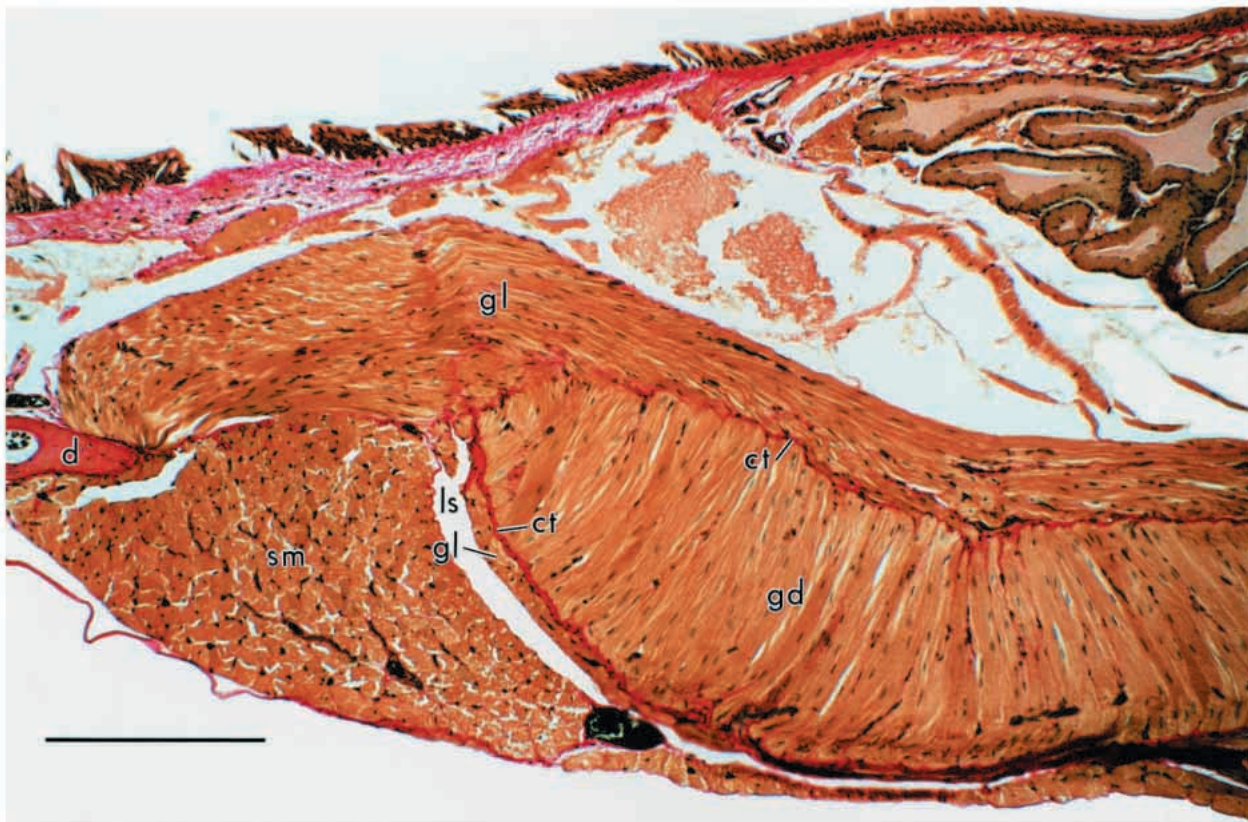


Fig. 3. Photomicrograph of a parasagittal section of the tongue of *Hemisus marmoratum*. The anterior direction is to the left in the micrograph. The connective tissue capsule (ct) surrounding the m. genioglossus dorsoventralis (gd) is visible in the micrograph and is stained red. d, dentary bone; gl, m. genioglossus longitudinalis; ls, lingual sinus; sm, m. submentalis. Scale bar, 0.25 mm. Brightfield microscopy of a 10 μ m thick paraffin section stained with Picro-Ponceau and haematoxylin.

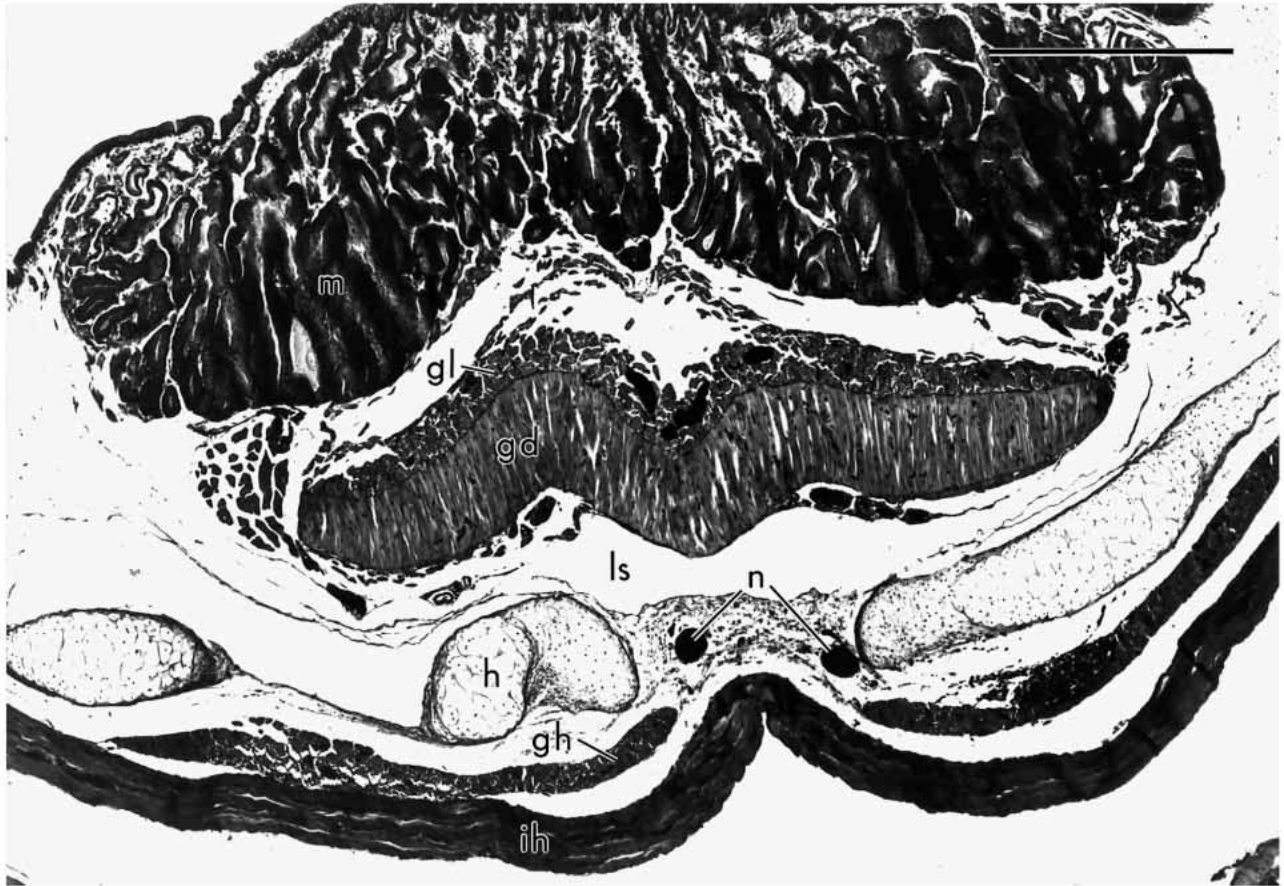


Fig. 4. Photomicrograph of a transverse section of the tongue of *Hemisus marmoratum* showing the tongue, hyoid and associated musculature. gd, m. genioglossus dorsoventralis; gh, m. geniiohyoideus; gl, m. genioglossus longitudinalis; h, hyoid cartilage; ih, m. interhyoideus; m, mucosa; ls, lingual sinus; n, hypoglossal nerves. Scale bar, 0.5 mm. Brightfield microscopy of a 10 μm thick paraffin section stained with Picro-Ponceau and haematoxylin.

tissue at the back of the posterior lobe of the tongue, which is taken up as slack when the tongue is fully protracted (Fig. 2). Folding of the intrinsic musculature at the tip of the tongue is apparent in parasagittal sections (Fig. 2).

A specialized, crossed-helical array of connective tissue was not observed in association with the lining of the lingual sinus nor was there any hypertrophy of the lingual veins or arteries (Figs 2, 3).

Electron microscopy of the m. genioglossus longitudinalis

The muscle fibres of the m. genioglossus longitudinalis (Fig. 6) are nearly identical in ultrastructure to those described previously in anuran limb muscles (Gordon et al., 1966). There is no evidence of supercontraction in the resting tongue sample. I-bands are clearly visible, and there is no evidence of thick filaments projecting into adjacent sarcomeres. The general arrangement of the sarcomeres is similar to that reported in other frog muscles, including similar myofibril dimensions (thick filament length $1.64 \pm 0.09 \mu\text{m}$, thin filament length $0.95 \pm 0.05 \mu\text{m}$; means \pm s.d., $N=30$) and a similar disposition of the systems of transverse tubules and sarcoplasmic reticulum. The sarcomere length was

$2.00 \pm 0.06 \mu\text{m}$ in the resting tongue sample and $3.33 \pm 0.26 \mu\text{m}$ (means \pm s.d., $N=30$) in the extended sample (Fig. 6).

Discussion

Muscular hydrostat model

In *Hemisus marmoratum*, the tongue protractor muscle (m. genioglossus) consists of two compartments, one in which the muscle fibres are oriented parallel to the long axis of the tongue, as in other frogs (m. genioglossus longitudinalis corresponding to m. genioglossus medialis of other species), and a novel one, never before described, in which the fibres are oriented dorsoventrally within the tongue (m. genioglossus dorsoventralis). We believe both compartments are important in tongue protrusion. From its fibre orientation, we infer that the m. genioglossus longitudinalis of *H. marmoratum* initiates protrusion and rotates the tongue over the mandibles so that the tip faces forwards, as in most other frogs. In this position, the larger bundle of the m. genioglossus longitudinalis is ventral to the m. genioglossus dorsoventralis. After the m. genioglossus longitudinalis has rotated the tongue into place, we suggest that the m. genioglossus dorsoventralis then plays

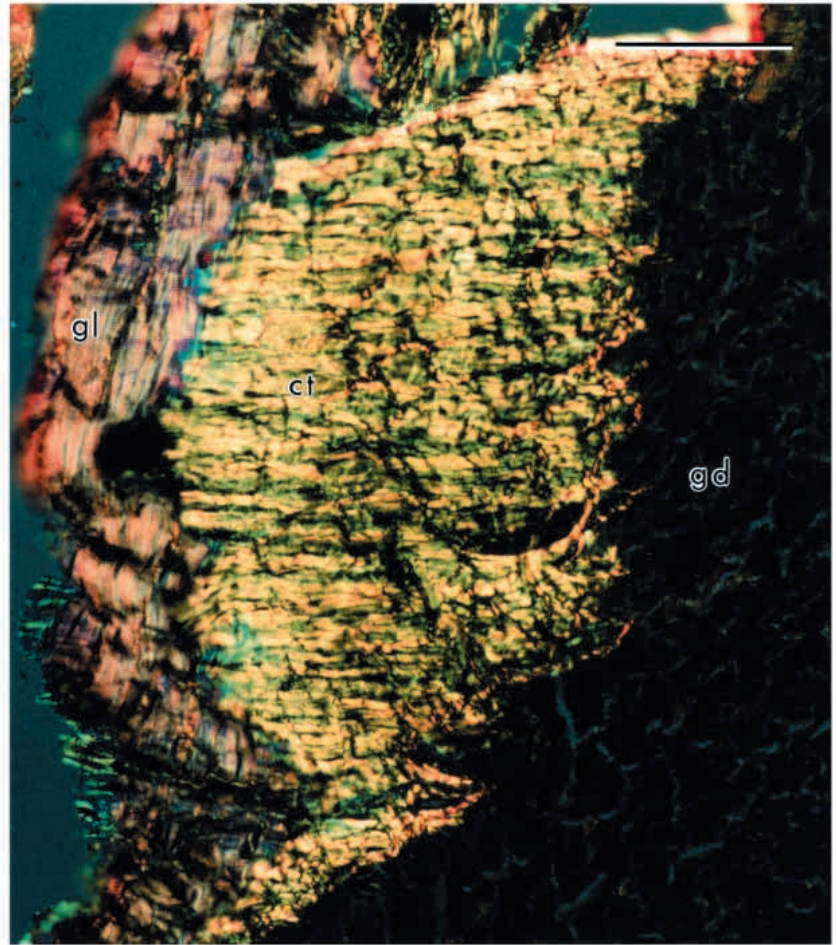


Fig. 5. Photomicrograph of a frontal section of the tongue of *Hemisus marmoratum*. The long axis of the tongue is oriented vertically in the micrograph. The section grazes the birefringent connective tissue fibres (ct) of the capsule enclosing the m. genioglossus dorsoventralis (gd). Fibres of the m. genioglossus longitudinalis (gl) are visible to the left. The connective tissue fibres are oriented horizontally in the micrograph and are thus perpendicular to the long axis of the tongue. Scale bar, 100 μ m. Polarized light microscopy of a 10 μ m thick paraffin section stained with Picro-Ponceau and haematoxylin.

the major role in tongue elongation. The m. genioglossus dorsoventralis is the only muscle in *H. marmoratum* that can elongate the tongue directly by its own shortening.

We hypothesize that the tongue of *H. marmoratum* functions as a muscular hydrostat that maintains a constant volume as it changes shape (Kier and Smith, 1985). When the m. genioglossus dorsoventralis shortens, it will decrease the thickness of the tongue and, if the volume of the tongue is constant, this change in shape will then be translated into tongue elongation. Most muscular hydrostats possess muscle fibres that are oriented to cause an increase in length by contracting in width and height simultaneously, causing a geometric increase in length as diameter decreases (i.e. the change in length is proportional to the square of the decrease in diameter; Kier and Smith, 1985). *H. marmoratum*, however, possesses muscle fibres that are oriented in only two dimensions, longitudinal and vertical. Because transversely arranged muscle fibres are absent, contraction of the m. genioglossus dorsoventralis will cause tongue elongation only if an increase in diameter is prevented. The robust, transversely aligned connective tissue capsule that surrounds the m. genioglossus dorsoventralis provides a means of resisting lateral expansion. Since the width of the tongue is constant, however, contraction of the m. genioglossus dorsoventralis will cause a linear, rather than geometric, increase in length.

Previous studies (Ritter and Nishikawa, 1995) have reported a maximum tongue elongation of 100%. If the intrinsic tongue musculature is not folded at rest, this 100% elongation would require contraction of the m. genioglossus dorsoventralis to 50% of its resting length. This result presents a potential problem with respect to muscle fibre shortening. Striated muscles of vertebrates typically contract to only 60–70% of their resting length, although Carlson and Wilkie (1974) suggest that they may contract to as much as 50%. In addition, the longitudinal fibres of the m. genioglossus may be stretched by as much as 100% of their resting length during elongation.

There are at least two possible ways in which these dimensional changes could be accommodated. The first possibility is that the musculature of the tongue may be folded when the tongue is retracted, so that elongation of the tongue as a whole involves both unfolding and elongation of the intrinsic muscle mass. Folding of the intrinsic muscle mass, in particular at the tip of the tongue, was commonly observed in parasagittal sections (Fig. 2). Unfolding during elongation would reduce both the amount of contraction required by the m. genioglossus dorsoventralis and the elongation that must be accommodated by the m. genioglossus longitudinalis. We estimate from histological sections that unfolding of the intrinsic musculature at the tongue tip would increase the tongue length by approximately 25% (from 4 to 5 mm in

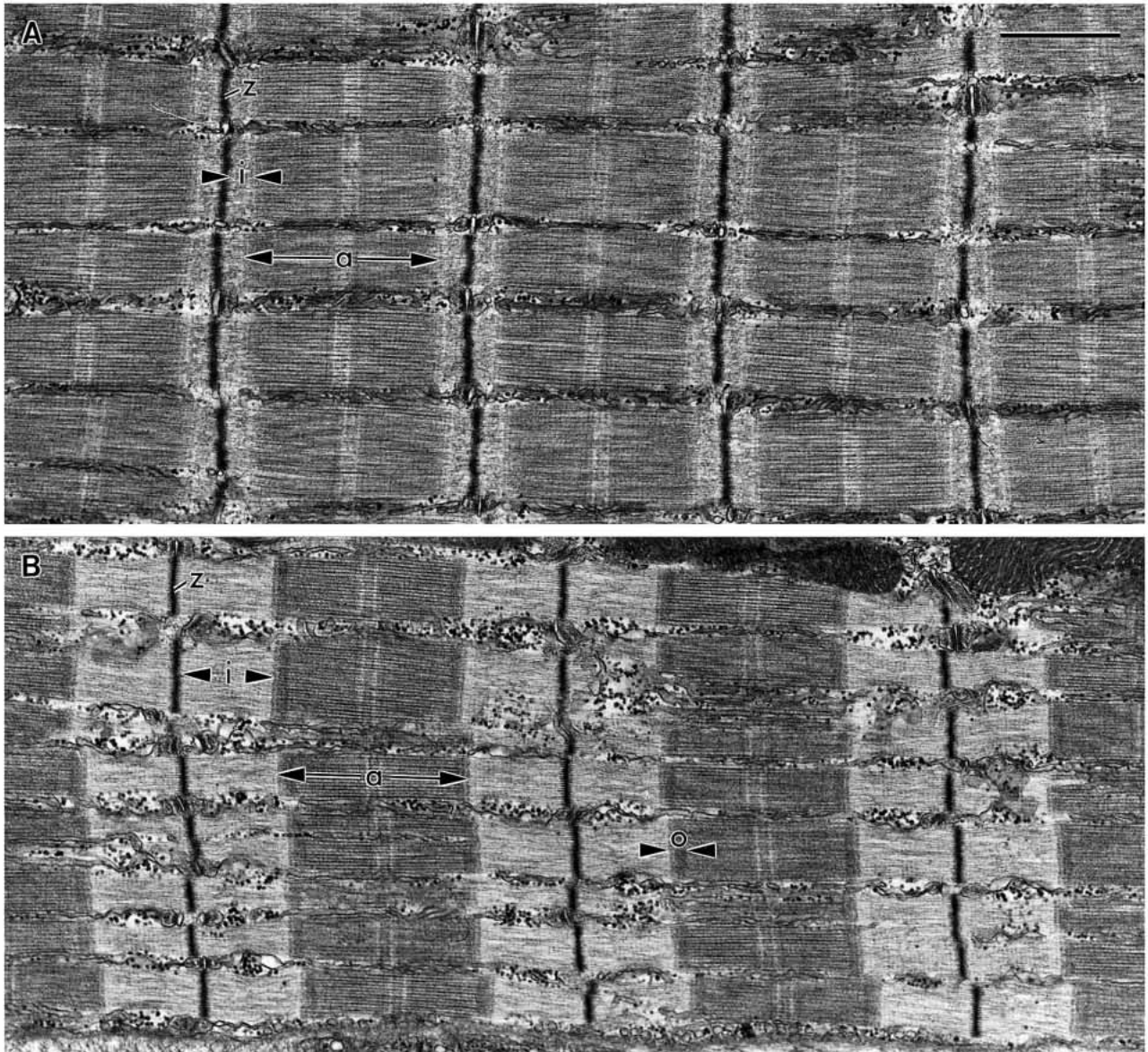


Fig. 6. Electron micrographs of longitudinal sections of the m. genioglossus longitudinalis from resting (A) and extended (B) tongues of *Hemisus marmoratum*. Note the extreme elongation of the sarcomeres in the extended tongue, with only minimal overlap (o) between thick and thin filaments. a, A-band; i, I-band; z, Z disc. Scale bar, 1 μm .

Fig. 2). Further extension of the tongue from 5 to 8 mm (a doubling of the original length) would then require shortening of the m. genioglossus dorsoventralis to approximately 63% of its resting length and would cause elongation of the m. genioglossus longitudinalis by only 60%. Thus, we believe that unfolding of the intrinsic musculature is an important component of tongue projection in *H. marmoratum*.

The second possibility is that the tongue musculature may be modified for supercontraction, as has been described for the retractor muscles of the chameleon tongue (Rice, 1973). The electron microscopical analysis, however, did not reveal any obvious ultrastructural specialization for long-range elongation and contraction. Indeed, the ultrastructure of the sarcomeres of this muscle is more or less identical to that described

previously for the frog semitendinosus muscle (Gordon et al., 1966). In particular, there was no evidence of a mechanism of supercontraction. Although the samples from the resting tongue show shortened sarcomeres, the sarcomere length is typical of vertebrate striated muscle (Gordon et al., 1966). The electron micrographs of fibres from the elongated tongue, however, show highly extended sarcomeres, with minimal overlap between thick and thin myofilaments. This minimal overlap between thin and thick filaments implies significantly reduced force production by the m. genioglossus longitudinalis when the tongue is fully protracted and suggests that mechanisms such as passive elastic restoring forces may aid in the initiation of tongue retraction.

While the actual range of elongation and shortening may

differ somewhat from the artificial elongation that was required for electron microscopy in the present study, measurements of sarcomere length in tongues fixed in resting and elongated positions suggest that the m. genioglossus longitudinalis is elongated by approximately 65 % during the maximal tongue elongation of 100 %. This figure is in good agreement with the estimate of elongation of the m. genioglossus longitudinalis described above and provides additional evidence that folding and unfolding of the intrinsic muscle mass of the tongue is important in creating the observed dimensional changes of the tongue during feeding.

Two additional features of the tongue of *H. marmoratum* appear to facilitate length changes during prey capture. These are the amount of endomysial connective tissue and the orientation of collagen fibres within the tongue as a whole, as measured qualitatively from scanning electron micrographs of tongues digested with NaOH (Webster, 1996). In a preliminary study, Webster (1996) compared the amount and orientation of endomysial connective tissue in three anurans: *Hyla cinerea* (family Hylidae), a frog with a tongue that shortens during protraction (i.e. a mechanical puller); *Bufo marinus* (family Bufonidae), a toad that uses inertia to elongate the tongue (i.e. an inertial elongator); and *Hemisis marmoratum*. He found that *Hyla cinerea* had the most connective tissue, that *B. marinus* was intermediate and that *H. marmoratum* had the least endomysial connective tissue in the tongue that might resist elongation. The orientation of collagen fibres also differed among taxa. *Hyla cinerea* had the largest proportion of fibres with low orientation angles (approaching 0°) relative to the long axis of the tongue, *B. marinus* was intermediate, and *H. marmoratum* had the most fibres with high orientation angles (approaching 90°). Connective tissue fibres with low angles (approaching 0°) actively resist elongation of the tongue (Kier and Smith, 1985). In *H. marmoratum*, most of the collagen fibres were oriented nearly perpendicular to the long axis of the tongue (modal angle 80°), where they do not resist tongue elongation but instead resist increases in tongue diameter.

We conclude that a muscular hydrostatic mechanism is the most likely explanation for tongue elongation in *H. marmoratum*. Ritter and Nishikawa (1995) postulated a hydraulic mechanism of tongue elongation in *H. marmoratum* because they overlooked the presence of the dorsoventral compartment of the m. genioglossus, which is apparent in histological sections but not in gross dissection. In their hydraulic model, they proposed that movement of lymph from the lingual sinus into the tongue was responsible for tongue elongation. In the present study, an examination of the connective tissues of the tongue under polarized light revealed no specialized connective tissues surrounding the lingual sinus. In the absence of crossed helical arrays of connective tissue surrounding the sinus, inflow of lymph would increase the diameter of the tongue and oppose elongation. Furthermore, the muscles surrounding the lingual sinus do not appear to be arranged so as to be able to force lymph into the tongue, and no specialized vasculature was observed that might be used to

inflate the tongue during protraction. There is, therefore, no morphological evidence to support the hydraulic model of tongue elongation in *H. marmoratum*.

In theory, several muscles could contribute to tongue retraction in *H. marmoratum*. These include the m. genioglossus longitudinalis, the m. genioglossus dorsoventralis (by relaxing) and the m. hyoglossus. In all frogs that have been studied except *H. marmoratum*, the m. hyoglossus is responsible for tongue retraction, and denervation of this muscle completely abolishes retraction of the tongue (Tso et al., 1995). In *H. marmoratum*, however, the tongue retracts completely after denervation of the m. hyoglossus, although it follows a somewhat different trajectory on its way back into the mouth. This observation suggests that the tongue is retracted in part by the m. genioglossus longitudinalis, with the m. hyoglossus controlling the trajectory of tongue retraction. Passive elastic restoring forces may also contribute to tongue retraction, and measurement of the passive mechanical properties of the tongue would therefore be of interest, particularly given the observation of minimal overlap of thick and thin filaments in the sarcomeres of the m. genioglossus longitudinalis at full elongation.

Implications for motor control

The m. genioglossus dorsoventralis has not been described previously in any anuran species, although a very similar compartment appears to be present in all microhylids that have been studied (D. C. Cannatella, personal communication). In some anuran phylogenies (see, for example, Ford and Cannatella, 1993), this compartment may represent a synapomorphy of the families Hemisotidae and Microhylidae. In both these taxa, this muscle appears to confer the ability to elongate the tongue hydrostatically during prey capture, an ability that is absent in all other anurans that have been studied to date.

The dorsoventral compartment of the m. genioglossus and the related ability to elongate the tongue hydrostatically are associated with several novel functions in terms of motor control, which appear to be absent from frogs that use either mechanical pulling (i.e. muscle shortening) or inertia to protract their tongue. These include the ability to protract the tongue slowly, thereby increasing capture success, and the ability to aim the tongue in azimuth and elevation relative to the head.

In contrast to inertial elongators, which must protract their tongues rapidly to achieve elongation, hydrostatic elongators may protract their tongues either slowly or rapidly. There appears to be a speed *versus* accuracy trade-off in the prey capture behaviour of frogs, in which high accuracy is associated with slower prey capture movements (Nishikawa, 1999). In terms of prey capture, *H. marmoratum* is the slowest and most accurate (100 %) of the anuran species that have been studied to date (Ritter and Nishikawa, 1995), whereas *B. marinus* is the fastest and least accurate, capturing even slow-moving prey only approximately 30 % of the time. Toad tongues reach velocities of 1000–4000 mm s⁻¹ and

accelerations of more than 30 times gravity during protraction. In contrast, the tongue of *H. marmoratum* reaches maximum velocities of 240 mm s^{-1} (approximately 15 times slower than that of *B. marinus*) and accelerations of 3.5 m s^{-2} (more than 85 times slower than that of *B. marinus*).

Because the tongue moves more slowly, the muscular hydrostatic system described here allows more precise, localized and diverse tongue movements. For this reason, we would expect that the dorsoventral compartment of the m. genioglossus should be composed of a large number of relatively small motor units. Independent recruitment of portions of the muscle would allow for precise, localized and modulated movement. In addition, sequential recruitment during tongue protraction may help to support the tongue against gravity during the slow and gradual protraction. In contrast, the m. genioglossus of inertial elongators such as *B. marinus* or *Rana pipiens* would be expected to be composed of a small number of large motor units that are activated simultaneously to produce a large instantaneous acceleration when the tongue is launched from the mouth. As predicted, approximately 250 motor neurones innervate the m. genioglossus of *R. pipiens* (Stuesse et al., 1983), whereas approximately 950 motor neurones innervate the m. genioglossus of *H. marmoratum* (Anderson et al., 1998). Gradual and modulated protraction also confers the advantage that the tongue trajectory can be corrected on-line within a gape cycle. In contrast, the tongue trajectory of inertial elongators such as *B. marinus* is determined at launch, is not corrected in flight and is constrained to fall on a straight line (Nishikawa and Gans, 1996).

Perhaps more importantly, with sufficiently fine subdivision of the neuromotor system, the muscular hydrostatic mechanism may provide not only for elongation but also for bending in both the lateral and dorsoventral directions (Kier and Smith, 1985). Various combinations of the action of the m. genioglossus longitudinalis, m. genioglossus dorsoventralis or m. hyoglossus could achieve bending in multiple directions. In theory, the m. genioglossus dorsoventralis should provide support for bending by resisting longitudinal compression, as described for other muscular hydrostats by Kier and Smith (1985). When combined with activity of the m. genioglossus dorsoventralis, activity of the m. genioglossus longitudinalis or the m. hyoglossus should bend the tongue, when protruded, either ventrally or dorsally, respectively. Unilateral contraction of the dorsoventral muscles will cause greater elongation on one side, bending the tongue to one side while elongating. Unilateral contraction of the longitudinal or hyoglossus muscles on a single side will bend the tongue laterally if shortening is resisted by the m. genioglossus dorsoventralis. Bending requires independent action of subsets of muscle fibres both within and between muscle groups, once again suggesting that motor units should be small in *H. marmoratum*.

All anurans studied to date readily aim their heads relative to their bodies during feeding. In addition, some anurans can adjust tongue protraction distance to prey distance (Deban and

Nishikawa, 1992; O'Reilly and Nishikawa, 1995). Not only can *H. marmoratum* adjust its tongue protraction distance to prey distance but it is also the only anuran species studied to date that aims its tongue relative to its head in both azimuth and elevation (Ritter and Nishikawa, 1995). Microhylids appear to be intermediate between *H. marmoratum* and other frogs in that they can aim their tongues relative to the head in distance and azimuth but not in elevation (Jaeger and Nishikawa, 1993; Meyers et al., 1996).

In *H. marmoratum* (Ritter and Nishikawa, 1995) and the microhylid *Phrynomerus bifasciatus* (Meyers et al., 1996), when unilateral denervation of the m. genioglossus is performed, the tongue bends towards the inactivated side. In *P. bifasciatus*, the tongue deviates by up to 90° from the target, whereas in *H. marmoratum* it deviates by more than 180° . In contrast, the amplitude of tongue movement is reduced after unilateral denervation of the m. genioglossus in *B. marinus*, but the direction of tongue protraction is unaffected (K. C. Nishikawa, personal observation). These experiments are consistent with the proposed muscular hydrostatic mechanism of tongue protraction, since the genioglossus muscles on the denervated side would be inactive, and thus greater elongation would occur on the opposite side of the tongue as a result of the normal activity of the genioglossus muscles (causing bending towards the denervated side).

Elevation of the tongue could be controlled by activation of the m. genioglossus longitudinalis, the m. hyoglossus, or both. Both morphological relationships and experimental evidence suggest that the m. genioglossus longitudinalis is responsible for the control of tongue elevation during protraction, whereas the m. hyoglossus is responsible for the control of tongue elevation during retraction (Ritter and Nishikawa, 1995). In *H. marmoratum*, the trajectory of the tongue during protraction is unaffected by bilateral denervation of the m. hyoglossus, whereas during retraction the tongue trajectory exhibits a significant upward deviation after bilateral hyoglossus denervation.

During the evolution of *H. marmoratum* and microhylids, it appears that the m. genioglossus has conserved its basic function in protracting the tongue during prey capture. In contrast, the mechanics of tongue protraction have changed markedly from mechanical pulling in basal anurans, to inertial elongation in several unrelated taxa, to muscular hydrostatic elongation in *H. marmoratum* and microhylids. These mechanical changes have allowed precise and modulated movement in multiple planes, in contrast to inertial elongators, such as *B. marinus*, which have evolved their own suite of neurological adaptations for controlling their ballistic tongues (Nishikawa and Gans, 1992, 1996).

We thank S. Wainwright for encouraging our collaboration on muscular hydrostats and for hosting a sabbatical for K.C.N., D. Ritter for comments on an earlier version of the manuscript, A. van Nievelt for assistance in the preparation of the histological material, B. Olsen for assistance in the preparation of material for electron microscopy and E. Craft

and S. Whitfield for assistance with photography. This work was supported by grants IBN 9809942 (to K.C.N.), IBN 9407616 (to K.K.S.) and IBN 9219495 (to W.M.K.) from the National Science Foundation, and grant R25-GM56931-01 (to K.C.N.) from the National Institutes of Health.

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