Mechanism of tongue protraction in microhylid frogs

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Summary

High-speed videography and muscle denervation experiments were used to elucidate the mechanism of tongue protraction in the microhylid frog *Phrynomantis bifasciatus*. Unlike most frogs, *Phrynomantis* has the ability to protract the tongue through a lateral arc of over 200° in the frontal plane. Thus, the tongue can be aimed side to side, independently of head and jaw movements. Denervation experiments demonstrate that the m. genioglossus complex controls lateral tongue aiming with a hydrostatic mechanism. After unilateral denervation of the m. genioglossus complex, the tongue can only be protracted towards the denervated (inactive) side and the range through which the tongue can be aimed is reduced by 75%. Histological sections of the tongue reveal a compartment of perpendicularly arranged muscle fibers, the m. genioglossus dorsoventralis. This compartment, in conjunction with the surrounding connective tissue, generates hydrostatic pressure that powers tongue movements in *Phrynomantis*. A survey of aiming abilities in 17 additional species of microhylid frogs, representing a total of 12 genera and six subfamilies, indicates that hydrostatic tongues are found throughout this family. Among frogs, this mechanism of tongue protraction was previously known only in *Hemisus* and may represent a synapomorphy of *Hemisus* and Microhylidae.

Key words: tongue protraction, m. genioglossus dorsoventralis, muscular hydrostat, Microhylidae, frog, *Phrynomantis bifasciatus*, feeding, prey capture.

Introduction

Early morphological studies of the feeding system in frogs identified several morphologically distinct tongue types (Magimel-Pelonnier, 1924; Regal and Gans, 1976; Gans and Gorniak, 1982a,b; Trueb and Gans, 1983), indicating that several mechanisms of tongue protraction might be found within anurans. Subsequent functional studies identified three basic mechanisms of tongue protraction: mechanical pulling, inertial elongation and hydrostatic elongation. The first two mechanisms are widespread among frogs (Nishikawa, 1997, 1999), but the third, hydrostatic elongation, has been found only in the monogeneric family Hemisotidae (Ritter and Nishikawa, 1995; Nishikawa et al., 1999).

Mechanical pulling is the primitive mechanism of tongue protraction in frogs. This mechanism is found in all archaeobatrachians (Nishikawa, 1997; Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991), as well as in some meso- and neobatrachian frogs (Deban and Nishikawa, 1992; O'Reilly and Nishikawa, 1995; Nishikawa, 2000). Mechanical pulling is characterized by a modestly protrusible tongue (less than 70% of jaw length), which is protracted by contraction of the m. genioglossus. As the m. genioglossus shortens, the tongue bunches at the front of the jaws and is extended beyond the mandibular symphysis. Unless a prey item is extremely close to a mechanical-pulling frog, the modest extent of tongue protraction requires forward body movement (lunging) in concert with tongue protraction in order for the tongue to come in contact with the prey (Deban and Nishikawa, 1992; Valdez and Nishikawa, 1996).

Inertial elongation is a derived mechanism of tongue protraction among anurans and has evolved at least seven times independently (Nishikawa, 2000). With respect to sheer numbers of species, it is probably the most prevalent mechanism of tongue protraction among living frogs (Nishikawa, 1997, 2000; Nishikawa and Gans, 1995). Inertial elongation is accomplished by tightly coordinated tongue and jaw movements that flip the tongue over the mandibles and extend it well beyond its resting length (Nishikawa, 1992, 2000; Nishikawa and Gans, 1996). The tongue is protracted very fast and is delivered to the target with minimal body movement, allowing the animal to remain relatively cryptic during feeding bouts (Gray, 1997). However, possibly because the tongue movements are ballistic, the frogs are apparently unable to change the trajectory during protraction and have little or no ability to laterally aim the tongue independent of the head.

The third known mechanism of tongue protraction in anurans is hydrostatic elongation. In contrast to the other two

mechanisms of tongue protraction, muscular hydrostatic elongation has been described only once, in the African pig-nosed frog *Hemisus sudanensis* (Ritter and Nishikawa, 1995; Nishikawa et al., 1999). Although this mechanism is similar to inertial elongation in that the tongue is rotated forward over the mandibular symphysis, it differs in that the tongue can be aimed laterally and in elevation relative to the head (Ritter and Nishikawa, 1995). Initially, it was suggested that *Hemisus* used a hydraulic protraction mechanism (Ritter and Nishikawa, 1995). However, a more detailed study of its tongue morphology suggests a muscular hydrostatic mechanism. In *Hemisus*, the tongue has a separate compartment of dorso-ventrally arranged muscle fibers that are surrounded by connective tissue. The connective tissue fibers are arranged to restrict lateral expansion, so that shortening of the dorso-ventral fibers results in elongation of the tongue (Nishikawa et al., 1999).

Molecular and morphological data suggest that *Hemisus* is closely related to frogs of the family Microhylidae (Wu, 1994; Emerson et al., 2000; Haas, 2003). It is therefore interesting that observations of feeding behavior from representatives of several genera within this family indicate that they have a similar tongue protraction mechanism to that seen in *Hemisus* (Meyers et al., 1996; Monroy and Nishikawa, 2000). When capturing prey, microhylids are capable of aiming the tongue independently of head movements. The tongue can be protracted to either the left or right side, allowing them to effectively capture prey positioned over 90° from the midline of the head. Thus, their behavior suggests that microhylids may have a muscular hydrostatic tongue protraction mechanism similar to that seen in *Hemisus*. However, morphological work by Emerson (1976) suggested another possible explanation. She noted that microhylids possess accessory slips of the m. intermandibularis that may be involved in bending the mandible at the mentomeckelian joint during tongue protraction, allowing the tongue to deviate from a straight trajectory.

Here, we examine the mechanism of tongue protraction in microhylids using high-speed videography and muscle denervation techniques. Although we examined 17 species of microhylids, our studies focused on one species in particular: the South African snake-necked frog *Phrynomantis bifasciatus*. The goals of this study were: (1) to determine whether tongue aiming is widespread among microhylids and (2) to elucidate the mechanism(s) that microhylids use to aim their tongue independently of the lower jaw. Our results indicate that all microhylids are capable of lateral tongue movements and that they share a muscular hydrostatic mechanism of tongue protraction with *Hemisus*.

Materials and methods

Most of the animals used in these experiments were obtained from commercial animal dealers, but several individuals also volunteered animals (see Acknowledgements). A total of 20 *Phrynomantis bifasciatus* (Smith 1847), ranging in snout vent length from 35 mm to 52 mm, were used. Animals were housed individually at room temperature in plastic shoe boxes with a substrate of damp paper towels. To examine tongue aiming ability, the frogs were fed either fruit flies or locally collected termites while being filmed with a high-speed video system. The animals then received one of two muscle denervation treatments. After the treatment, they were filmed again to test for an effect of the treatment on their ability to aim the tongue. Differences in aiming before and after treatments were quantified by measuring the angle of the tongue during prey capture events. Although we concentrated our effort on prey capture behavior of *Phrynomantis*, we also examined this behavior in 17 additional species of microhylid frogs. For comparison with species with an inertial elongation mechanism, we also examined aiming ability in *Bufo woodhousii* (Woodhouse toad) and *Rana pipiens* (leopard toad).

High-speed videography

Animals were videotaped with a high-speed video camera (model 660; Display Technologies) with synchronized stroboscopic illumination and a Panasonic AG-6300 video cassette recorder. Feeding sequences were filmed at either 120 fields s^{-1} or 180 fields s^{-1} at room temperature (20–24°C). The frogs were placed on a damp paper towel facing the camera. The camera was elevated above the animal and tilted to an angle of 45°. During prey capture, the lower jaw rotates downward to an angle of approximately 45°, resulting in a perpendicular view of the tongue during protraction. Animals were filmed in several planes, including horizontal and directly overhead, but we found that filming at 45° provided the greatest detail about tongue trajectory and angle.

To initiate tongue aiming, forceps were used to place individual termites around the head. Placement of the termites ranged from directly in front of the animal to positions near the feet and on the forearms. Although the frogs often turn their heads during prey capture, by positioning the termites on the lateral parts of the body we were able to elicit extremes of tongue aiming.

Quantification of aiming

Tongue angle was measured as the maximum angle between the midline of the head (determined by drawing a line down the long axis of the body so that it was placed midway between the eyes and the nares) and the midline of the protracted tongue. One potential problem with measuring tongue angles in this manner is that the angle of the tongue relative to the head will be distorted as the camera angle deviates from perpendicular. To address this concern, we measured known angles drawn on paper with the camera placed at 20°, 45° and 90° (dorsal view). When the camera is placed directly overhead, the angles measured are identical with those drawn on the paper. When the camera angle is at 20°, there is up to a 5° increase in our measurement and at 45° there is a 10° increase. Most of this occurs when the tongue is 45° to either side of the midline. As the tongue angle approaches either the midline or 90°, the actual angle and the measured angle differ by no more than 2°. When *Phrynomantis* reaches peak tongue
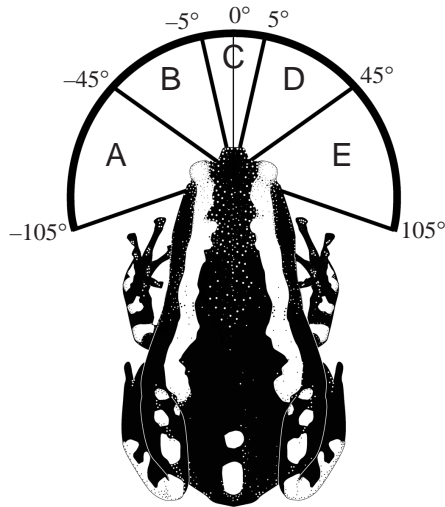
Fig.·1. Tongue aiming ability was quantified by having individuals of *Phrynomantis bifasciatus* aim into five quadrants: (A) left –46° to –105°, (B) left –6° to –45°, (C) 0° to 5° to either side, (D) right 6° to 45°, (E) right 46° to 105°. The quadrant is essentially a bib, with the midline of the head designating 0°. As the head of the animal turns, the quadrant follows this movement so that a line drawn down the midline of the head would always be located at 0°.

Fig. 2. Ventral view of the buccal region of a cleared and stained specimen of *Phrynomantis bifasciatus*. Left and right sides are nearly identical. Major cranial nerves are labeled on the left side and rami of the nerves that innervate the tongue and hyobranchial musculature are labeled on the right side. Branches of the trigeminal nerve (V) innervate the m. submentalis (1) and the m. intermandibularis (2). Branches of the hypoglossal nerve (XII) innervate the m. genioglossus dorsoventralis, longitudinalis and transversalis (3) and the m. hyoglossus (4). The glossopharyngeal nerve (IX) is dorsal to the hypoglossal nerve and innervates other hyobranchial musculature and the tongue pad. Numbers 1 and 3 are located at the approximate sites of nerve transection for denervation of the m. intermandibularis and m. genioglossus lateralis and dorsoventralis, respectively.

protraction, the lower jaw is at an angle of approximately 45° to the horizon, and with the camera positioned at 45° to the horizon we get a perpendicular view of the tongue. Thus, the magnitude of the error in these measurements is always less than 10° and in most cases much less than 10°.

In order to examine tongue aiming ability, we divided the normal aiming range of *Phrynomantis bifasciatus* into five quadrants relative to the head (see Fig. 1). Since, in addition to aiming the tongue laterally, the head can also be rotated in the direction of the prey, all measurements were taken relative to the midline of the head. This prevents us from confounding the effects of tongue aiming relative to the lower jaw and head turning. Tongue angle was measured for at least three feeding attempts in each quadrant. The maximum range of 105° was the greatest angle observed in *Phrynomantis*. Left and right sides were denoted as negative and positive, respectively, to avoid confusion of tongue trajectory after muscle denervation (i.e. after right unilateral genioglossus denervation the tongue is protracted to +45°, even when attempting to capture prey placed at –45°).

Morphology

Two preserved individuals of *P. bifasciatus* were sectioned to examine the arrangement of tongue and hyobranchial muscles. Histological sections of the lower jaw and tongue were made in the transverse and sagittal planes. Specimens were decalcified, embedded in paraffin and sectioned serially at 10·µm. Sections were stained using Milligan's Trichrome stain (Humason, 1979). The presence of m. intermandibularis accessory slips was confirmed through gross dissection of two individuals. Photos taken of the dissection with a Nikon Coolpix camera were used to make drawings of the m. intermandibularis musculature. To determine which branches of the hypoglossal nerve innervated the muscles of the lower jaw, we cleared and stained the peripheral nerves of one individual (Fig. 2; Nishikawa, 1987).

Muscle denervation

Animals that received muscle denervation treatments were first anesthetized in 7% ethanol. For most anurans, tricaine methanosulfonate (MS222) is sufficient to anesthetize the animal within 30 min. However, using MS222 it took several hours to fully anesthetize *Phrynomantis bifasciatus*. Using 7% ethanol, the animals could be anesthetized in approximately 30 min. We determined that the animals were under surgical anesthesia when tactile stimulation elicited no response.

Once anesthetized, frogs were placed on the stage of a dissecting microscope. Except for the lower jaw, the entire animal was covered with damp paper towels to prevent dehydration. A small incision was made in the skin above where the nerve branch of interest was located. The

surrounding musculature and blood vessels were teased apart to expose the nerve. To minimize damage to individual muscle fibers, muscles were always teased apart parallel to their long axis. A 1–2 mm section of the nerve was removed and then the incision was closed using Nexaband veterinary surgical adhesive. Post-surgery feeding attempts were made as soon as the animals recovered from anesthesia. To confirm the surgeries before regeneration of the nerve, animals were euthanized within three weeks of the surgery date. Animals were over-anesthetized in 10% ethanol and then fixed in 10% formalin and stored in 70% ethanol.

Two different surgical treatments were performed: denervation of the m. genioglossus and denervation of the m. intermandibularis. The ramus mandibularis of the trigeminal nerve innervates both the m. intermandibularis (posteriorly) and the m. submentalis (anteriorly); it was transected distal to the innervation of the m. intermandibularis. In the second treatment, the hypoglossal nerve branch innervating the m. genioglossus dorsoventralis and longitudinalis was unilaterally transected. Although this nerve also innervates the m. hyoglossus and m. geniohyoideus, we transected the nerve distal to these branches (Fig. 2).

In both treatments, the animals were anesthetized, an incision was made in the skin of the buccal floor, and the intermandibular muscles were teased apart to expose the underlying nerves. The difference between treatments is that in the m. genioglossus treatment, the m. geniohyoideus was also teased apart to expose the ramus hypoglossus of the hypoglossal nerve. Because there was no effect of m. intermandibularis denervation on feeding kinematics or tongue aiming, it is unlikely that the observed effects of m. genioglossus denervation were due to treatment alone. Previous studies of other species support this conclusion, as they revealed no effect of this procedure in sham surgeries in which the hypoglossal nerve was exposed but not transected (Deban and Nishikawa, 1992; Ritter and Nishikawa, 1995).

Statistical analysis

Statistical analysis was accomplished using Statview software on a G3 Power Macintosh computer. We performed an analysis of variance (ANOVA) to determine the effect of unilateral genioglossus denervation on normal tongue protraction. This analysis allowed us to compare feeding attempts before and after denervation when the prey is presented directly in front of the animal. In addition, in one individual we were able to record post-denervation feeding sequences in all of the aiming quadrants. For this individual, a *t*-test was used to examine the effect on aiming in each quadrant after m. genioglossus denervation.

Results

Tongue morphology

The following description of the tongue musculature pertains to the tongue in the resting position (i.e. mucosal layer is dorsal at rest and would be ventral upon tongue protraction). In ventral view, the superficial musculature consists of the m. intermandibularis (im), originating laterally on the mandible and inserting at the midventral fascia. Anteriorly, this muscle is differentiated into slips that run obliquely from the lateral margin of the mandible towards the mandibular symphysis (compare Fig. 3A and Fig. 3B). The m. submentalis (sm) lies dorsal to the m. intermandibularis near the mandibular symphysis, running transversely and connecting the mandibular rami. The hyobranchial protractor, m. geniohyoideus (gh), is composed of lateral and medial slips that originate near the mandibular symphysis and run posteriorly to insert on the posterior edge of the basihyal. The m. hyoglossus is the tongue retractor, which originates on the posterior edge of the posteromedial process of the hyobranchium and inserts at the ventral surface of the tongue pad (at rest). It runs anteriorly along the ventral surface of the hyobranchium before reversing direction and running caudally to insert into the tongue pad (Fig. 4). Unlike in most frogs, the m. genioglossus of *Phrynomantis* is comprised of three separate compartments instead of only one.

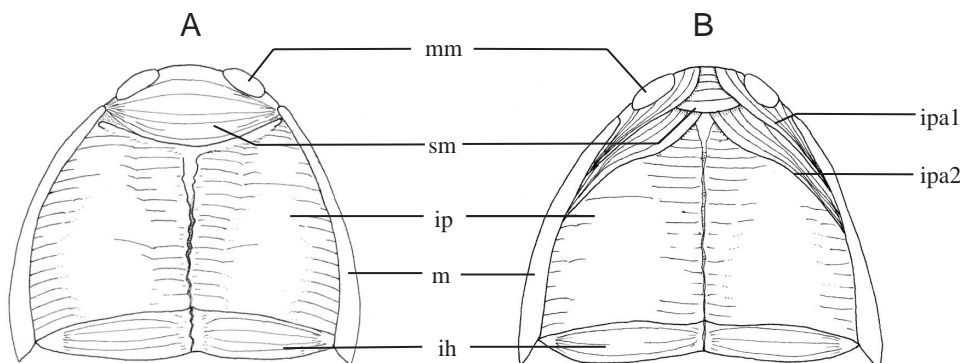As in other frogs, the m. genioglossus longitudinalis (ggl)

Fig. 3. Ventral view of the superficial throat musculature in two anurans. (A) Undifferentiated m. intermandibularis posterior of a typical frog. (B) Differentiation of the m. intermandibularis posterior into two separate accessory slips in *Phrynomantis bifasciatus*. mm, mentomeckelian element; sm, m. submentalis; ip, m. intermandibularis posterior; m, mandible; ih, m. interhyoideus; ipa1, m. intermandibularis posterior accessory 1; ipa2, m. intermandibularis posterior accessory 2.
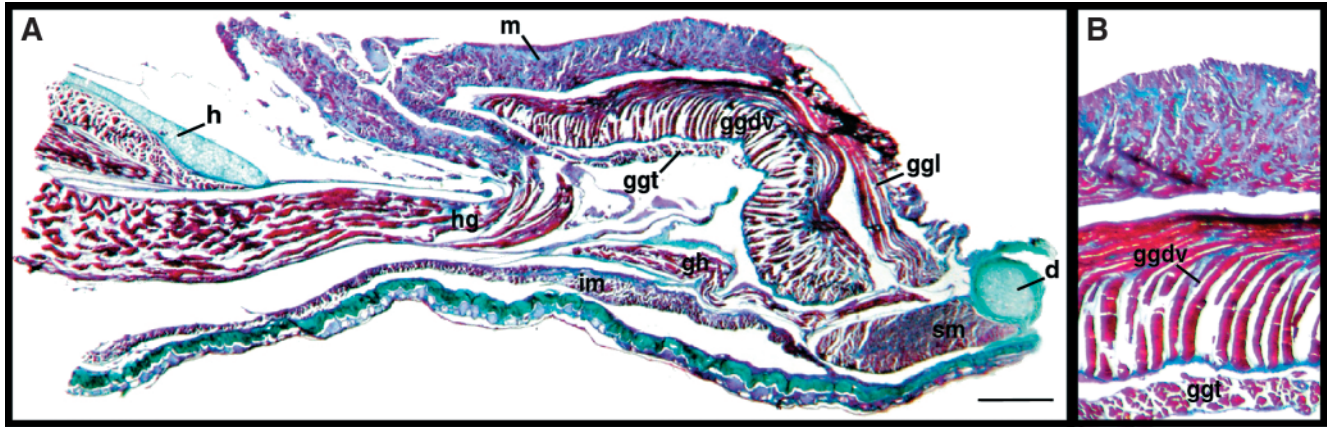
Fig. 4. Sagittal section of the tongue of *Phrynomantis bifasciatus*. (A) Note that the fibers of the m. genioglossus dorsoventralis are directed longitudinally and then dorso-ventrally. (B) Magnified view of the m. genioglossus dorsoventralis. Single fibers run in both the longitudinal and vertical planes. d, dentary; gh, m. geniohyoideus; ggdv, m. genioglossus dorsoventralis; ggl, genioglossus longitudinalis; h, hyobranchium; hg, m. hyoglossus; im, m. intermandibularis; ggt, m. genioglossus transversalis; m, mucosal layer. Scale bar, 1 mm.

originates at the mandibular symphysis. It is attached to the mandible by a thin band of fascia and runs postero-dorsally along the ventral surface of the mucosa. However, unlike in other frogs, it does not spread extensively into the tongue pad or interdigitate with fibers of the m. hyoglossus. In addition to the ggl, there is an m. genioglossus dorsoventralis (ggdv) that lies ventral to the ggl and shares a similar origin and connective tissue attachment. Near its origin, the ggdv fibers run posteriorly into the tongue tip, but as they proceed posteriorly they turn and are directed ventrad, inserting into a thick layer of surrounding connective tissue. Finally, there is an additional intrinsic muscle, the m. genioglossus transversalis (ggt), which originates laterally from connective tissue and runs transversely beneath the ggdv (Fig. 4).

Tongue aiming

The ability to aim the tongue is well developed in *Phrynomantis*, although there appears to be individual variation in the propensity to aim the tongue. A comparison of aiming ability in the different quadrants revealed that all individuals were able to aim in each quadrant. In addition to the tongue being protruded at an angle, the head may also be moved towards the prey item when the tongue is aimed laterally about the head. This strategy increases the range over which the frogs are able to capture prey and effectively allows them to capture prey items off the forearms. Although the animals are able to capture prey items over a wide range, qualitative observations of prey capture suggest that prey capture success decreases at extreme angles.

Although individuals of *Phrynomantis* exhibit little variation in aiming ability (Table 1), there are clear species differences within the Microhylidae. All microhylid species examined were able to aim the tongue independent of head movements. However, the maximum tongue angles measured for the species varied greatly, with the most extreme angles (>100°) measured in *Phrynomantis* and *Dermatonotus muelleri*

Table 1. *Mean ± s.d. of tongue angles in each quadrant for six individuals of* Phrynomerus bifasciatus, *together with the results of a* t-*test examining the effect on aiming after right unilateral M. genioglossus denervation on one individual*

Individual	Quadrant A (−105° to −46°)	Quadrant B (−45° to −6°)	Quadrant C (−5° to +5°)	Quadrant D (+6° to +45°)	Quadrant E (+46° to +105°)
Normal					
1	−57.0±10.9	−33.8±8.5	0.4±0.5	+32.2±8.4	+62.0±14.8
2	−51.2±3.0	−32.8±8.9	0.8±0.8	+32.4±6.1	+57.8±4.3
3	−60.0±1.4	−33.0±3.4	2.0±2.1	+37.3±4.6	+67.5±20.0
4	−60.2±18.2	−36.4±6.9	0.2±0.4	+32.6±3.5	+84.2±21.3
5	−52.2±5.6	−30.4±8.2	1.0±1.4	+35.4±8.1	+51.0±5.5
6	−55.3±11.4	−30.5±9.5	0.8±1.3	+36.8±5.1	+62.3±6.3
After right unilateral M. genioglossus denervation					
3	+30.0±16.5*	+38.0±9.4*	+34.6±4.0*	+38.6±5.9	+64.3±19.0

*Aiming was significantly (*P*<0.05) affected in quadrants A, B and C, in which the tongue consistently deviated towards the intact side (animal's right side).

(Mullers' termite frog; Table 2; Fig. 5). The most extreme tongue angles were measured from animals showing the greatest propensity to aim. Hence, we may not have elicited maximum aiming attempts in some species. Unlike microhylids, *Rana pipiens* and *Bufo woodhousii* exhibited little or no ability to aim the tongue (Table 2). Although *R. pipiens* was able to aim the tongue up to 5°, this is substantially less than in all the microhylids examined.

Effect of m. intermandibularis denervation

To determine whether mandibular bending plays a role in tongue aiming, we transected the ramus of the trigeminal nerve innervating the m. intermandibularis. After bilateral transection of the m. intermandibularis, the tongue is still able to protract normally, and prey capture sequences are qualitatively similar to sequences recorded before denervation (compare Fig. 6A and Fig. 6B). In addition, feeding attempts after surgery revealed no deficits in the ability to aim to the extreme angles seen before surgery. One individual consistently aimed more than 58°, with one attempt at 90°. Although we did not test for differences, it

appeared that prey capture success rate did not differ from normal feeding sequences.

Effect of m. genioglossus denervation

Unilateral denervation of the m. genioglossus in *P. bifasciatus* resulted in deficits in the ability to both aim the tongue and to capture prey. When the nerve branch innervating the m. genioglossus dorsoventralis and m. genioglossus longitudinalis is transected on the right side, the tongue is flipped out of the mouth and bends towards the denervated side (right side) upon protraction. Even when the frog attempts to feed on prey placed directly in front of it (0°), its tongue deviates towards the denervated side (ANOVA, $F=262.2$, $P=0.0001$; compare Fig. 6A and Fig. 6C). Prior to denervation, the tongue deviated only 3.4±2.4°. However, after denervation, the tongue is protracted at a mean angle of 43±9.4° towards the denervated side. Regardless of which side of the m. genioglossus is denervated, animals are never able to aim the tongue towards the active side after unilateral denervation.

In the one individual that attempted to aim in all the

Table 2. *Species in which tongue aiming ability was examined, including the number of animals observed, presence or absence of aiming, maximum tongue angle when aiming, mean angle of tongue deviation after unilateral m. genioglossus denervation (when prey is presented directly in front of the frog) and direction of deviation after unilateral denervation*

Species	N	Aiming	Maximum tongue angle	Unilateral m. genioglossus	Side of deviation
Bufonidae					
Bufo woodhousii	3	No	0	0	No deviation
Ranidae					
Rana pipiens	3	Little	5	20	Active side
Microhylidae					
Brevicipitinae					
Breviceps mossambicus	7	Yes	83	–	–
Callulina kreffti	4	Yes	52	–	–
Probreviceps sp.	1	Yes	44	–	–
Cophylinae					
Platypelis tuberifera	1	Yes	29	–	–
Dyscophinae					
Dyscophus guineti	5	Yes	53	6	Inactive side
Microhylinae					
Dermatonotus muelleri	5	Yes	109	47	Inactive side
Gastrophryne olivacea	5	Yes	77	7	Inactive side
Gastrophryne carolinensis	2	Yes	48	–	–
Hypopachus variolosus	1	Yes	42	–	–
Kaloula pulchra	5	Yes	32	9	Inactive side
Microhyla achatina	3	Yes	53	–	–
Microhyla pulchra	9	Yes	–	–	–
Phrynomerinae					
Phrynomantis bifasciatus	20	Yes	103	44	Inactive side
Phrynomantis microps	3	Yes	–	–	–
Scaphiophryninae					
Scaphiophryne calcarata	1	Yes	23	–	–
Scaphiophryne gottlebei	3	Yes	–	–	–
Scaphiophryne marmorata	2	Yes	62	–	Inactive side
Scaphiophryne pustulosa	7	Yes	33	22	Inactive side

quadrants after denervation, there was a significant deficit when attempting to capture prey in quadrants A, B and C (Table 1). As mentioned above, when attempting to capture prey in quadrant C (0°), the tongue deviated towards the denervated side, as also occurred when the animal attempted to aim the tongue into quadrants A and B. Interestingly, in *Phrynomantis* we noticed no deficit in the ability to aim towards the denervated side after surgery, and prey could still be captured at the extreme angles seen before surgery (Table 1).

We also unilaterally denervated the m. genioglossus in at least one individual of several other species of microhylids, a bufonid (*B. woodhousii*) and a ranid (*R. pipiens*) (Table 2). Not surprisingly, in all other species of microhylids that were examined, we found a similar effect in which the tongue always deviated towards the denervated side. There appear to be species differences in the angle at which the tongue deviates after denervation (Table 2), varying from 7.1° in *Gastrophryne olivacea* (Great Plains narrowmouth toad) to 46.9° in *Dermatonotus muelleri*. These results are in sharp contrast to those seen in both *B. woodhousii* and *R. pipiens*. In *B. woodhousii*, unilateral denervation resulted in a shortening of tongue protraction distance, but the tongue did not deviate from a straight trajectory. However, in *R. pipiens*, the tongue deviated on average 20° towards the intact side instead of towards the denervated side as was seen in the microhylids.

Discussion

The mechanism of tongue protraction in Phrynomantis

Three mechanisms of tongue protraction (mechanical pulling, inertial elongation and hydrostatic elongation) have been identified in living frogs (Nishikawa, 2000). To date, only the African genus *Hemisus* has been shown to protract its tongue using a hydrostatic mechanism. The tongue movements of *Hemisus* are characterized by relatively slow protraction velocity and the ability to modulate protraction distance, height and azimuth. Unilateral denervation of the m. genioglossus causes the tongue to deviate strongly towards the denervated (inactive) side (Ritter and Nishikawa, 1995). Histological preparations of the tongue of *Hemisus* reveal a novel component of the m. genioglossus, the m. genioglossus dorsoventralis (ggdv). The ggdv is composed of muscle fibers that are arranged
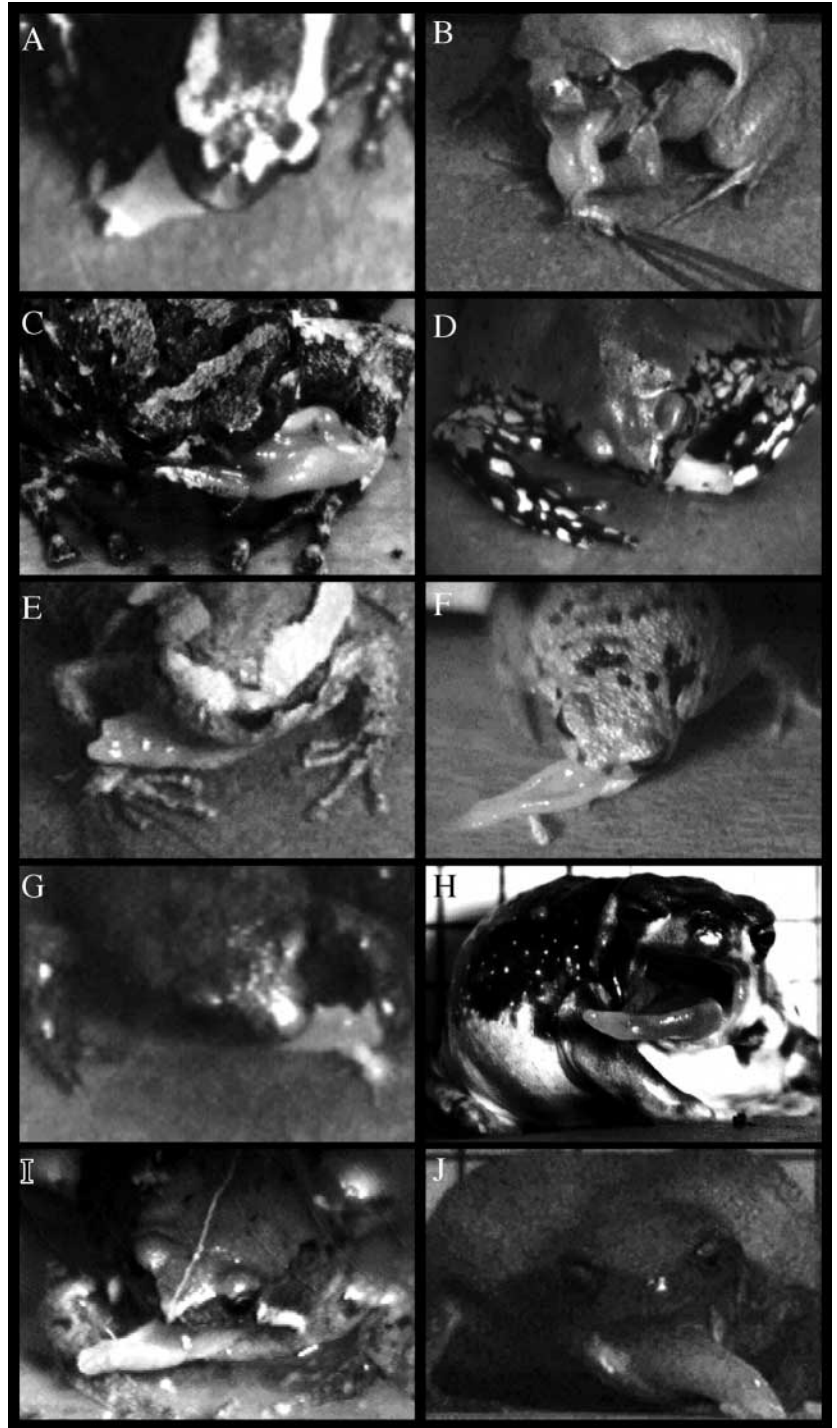

Fig. 5. Examples of tongue aiming in microhylid frogs: (A) *Phrynomantis bifasciatus*; (B) *Dyscophus insularis*; (C) *Scaphiophryne marmorata*; (D) *Dermatonotus muelleri*; (E) *Kaloula pulchra*; (F) *Callulina* sp.; (G) *Gastrophryne olivacea*; (H) *Breviceps adspersus*; (I) *Microhyla* sp.; (J) *Probreviceps* sp. Note the angle of the tongue in relation to the midline of the head. All pictures were taken with the camera positioned at 45°, except H and J, which were head-on profiles.

perpendicular to the long axis of the tongue and are surrounded by a sheet of connective tissue (Nishikawa et al., 1999). Because muscles maintain a constant volume as they change

shape (Kier and Smith, 1985), shortening of the dorso-ventral fibers must be directed into either lateral expansion, forward elongation of the tongue or both. When the fibers of ggdv are recruited in *Hemisus*, the thick sheet of connective tissue surrounding this muscle resists lateral expansion and causes tongue elongation. It is thought that asymmetrical recruitment of the m. genioglossus pushes the tongue towards the side with relatively lower recruitment.

Morphological evidence suggests that *Phrynomantis* also utilizes hydrostatic elongation for tongue protraction. The tongue of *Phrynomantis* is anatomically similar to that of *Hemisus* in that both a longitudinal and a dorso-ventral component of the genioglossus muscle are present. However, the tongue of *Phrynomantis* differs in several respects from that of *Hemisus*. First, the m. genioglossus longitudinalis and m. genioglossus dorsoventralis both have a connective tissue
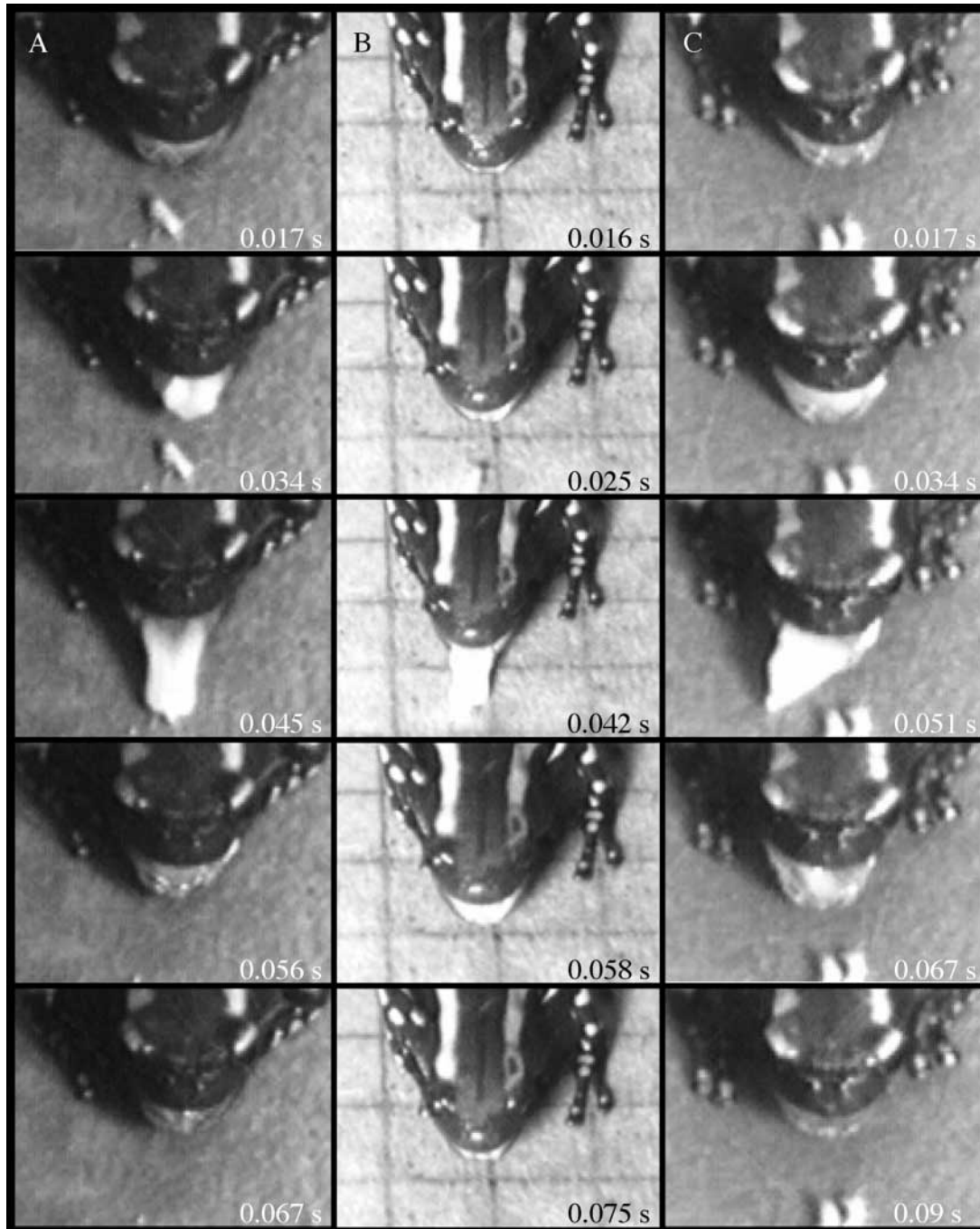

Fig. 6. Tongue protraction in *Phrynomantis bifasciatus* when prey is placed directly in front of the animal. (A) Normal feeding. There is no deviation of the tongue when attempting to capture prey. (B) After right unilateral m. submentalis and m. intermandibularis denervation, the tongue is protracted normally. (C) After right unilateral m. genioglossus (both longitudinalis and dorsoventralis) denervation, the tongue deviates towards the right (inactive) side. Animals are no longer able to capture prey placed directly in front of the head or towards the active side.

origin on the mandible. Second, in *Phrynomantis*, individual fibers of the m. genioglossus dorsoventralis are directed longitudinally and dorso-ventrally (Fig. 4), whereas in *Hemisus* they are only in the dorso-ventral plane (compare Fig. 4 in the present study with fig. 3 in the study by Nishikawa et al., 1999). The functional significance of this arrangement in *Phrynomantis* remains unclear, since it seems that a strictly dorso-ventrally arranged compartment would be the most effective in lengthening the tongue.

Denervation experiments in *Phrynomantis* also support a muscular hydrostatic mechanism. After unilateral denervation of the entire m. genioglossus (gg) complex, the tongue is protruded towards the denervated (inactive) side when animals attempt to feed on prey lying directly in front of them. This is the expected result if a hydrostatic pushing mechanism is being utilized in tongue protraction. If inertial elongation were at play, the tongue would either show no lateral deviation (as in *B. woodhousii*) or would be pulled to the active side (as in *R. pipiens*). In combination with the anatomical data, deviation to the inactive side after unilateral denervation supports the use of a muscular hydrostatic mechanism of tongue protraction in *Phrynomantis*.

The mechanism of tongue aiming in Phrynomantis

At least three different mechanisms could be used by microhylids to aim their tongues laterally independent of head movements, including: (1) rotating the base of the tongue by contraction of accessory slips of the m. intermandibularis; (2) pulling the tongue to the side by differential contraction of longitudinal fibers of the m. genioglossus; or (3) pushing the tongue to one side with hydrostatic pressure generated by dorso-ventral fibers of the m. genioglossus (as previously described in *Hemisus*; Nishikawa et al., 1999). We will describe each potential mechanism in turn and discuss the evidence for and against its playing a role in laterally directed tongue protraction in microhylids.

In the plesiomorphic m. intermandibularis of frogs, all of the fibers are arranged in parallel, forming a sheet that runs laterally from the mid-ventral line to the mandibles and the rod-shaped mentomeckelian bones. By contrast, the m. intermandibularis of some frogs can include one or more accessory slips with fibers that run from the mandibles to the fascia covering the submentalis, which in turn lies directly below the base of the tongue pad (e.g. Trewavas, 1933; Liem, 1970; Tyler, 1971; Emerson, 1976). Accessory slips of the m. intermandibularis are well developed in many microhylids (see figs 2–5 in Emerson, 1976). Emerson (1976) suggested that these accessory slips of the m. intermandibularis might play a role in tongue protraction but did not specifically speculate on a potential role in lateral tongue movement. We hypothesized that differential activation of the right or left accessory slips might pull the mentomeckelian bones and tilt or rotate the base of the tongue. Tilting or rotation of the tongue base could plausibly contribute to lateral movements. Our results do not support this functional hypothesis, because when the m. intermandibularis complex was denervated unilaterally, there

was no measurable deficit in the range of lateral protraction to either side. These results unambiguously indicate that the complex anatomy of the m. intermandibularis and unusually shaped mentomeckelian bones in microhylids are not functionally related to the ability to protract the tongue laterally.

The second potential mechanism would involve the m. genioglossus pulling the tongue to one side as it is protracted. The fibers of the ggl originate on the mandible tips and run caudally along the dorsal surface of the resting tongue pad. Asymmetrical recruitment of these fibers could pull the tongue either to the right or left side (as in *R. pipiens*), the tongue deviating towards the side showing greater activation. If this mechanism were present in microhylids, we would expect that unilateral denervation of the m. genioglossus would cause the tongue to bend towards the intact (active) side. Although we were unable to denervate the ggl and ggdv individually in *Phrynomantis*, unilateral denervation of both muscles caused the tongue to deviate towards the inactive side. While it is possible that the ggl, when acting alone, pulls the tongue as it does in *R. pipiens*, the overriding effects of ggdv suggest that the ggl is not determining tongue trajectory.

The third potential mechanism would involve using the hydrostatic pressure generated in the ggdv to aim the tongue to one side or the other during protraction. Because the pressure generated in the ggdv pushes the tongue out of the mouth, differential activation of the ggdv would bend the tongue towards the less active side. This mechanism is consistent with the results of the denervation experiments. Unilateral gg denervation invariably reduced the range of motion of the tongue to part of the range on the denervated (inactive) side.

Although the results of unilateral gg denervation in *Phrynomantis* are similar to those in *Hemisus*, some important differences should be noted. The tongue of *Hemisus* initially moves directly forward after unilateral gg denervation such that prey can still be captured directly in front of an individual as long as it is not far from the mouth (Ritter and Nishikawa, 1995). By contrast, after unilateral gg denervation, the trajectory of the tongue of *Phrynomantis* is initially to the denervated side. Thus, food directly in front of the animal cannot be captured. If *Hemisus* misses prey directly anterior to the head after unilateral gg denervation, the tongue tip follows a semi-circular trajectory and eventually runs into the side of the head, close to 180° off course (Ritter and Nishikawa, 1995). By contrast, the tongue of *Phrynomantis* travels in a relatively straight line and lands at approximately 45° off course (Fig. 6C). Ritter and Nishikawa (1995) did not determine if *Hemisus* could capture prey positioned to either side of the head after unilateral gg denervation. Our results indicate that *Phrynomantis* can still accurately aim the tongue through a limited range on the denervated side.

The results of the unilateral gg denervation experiment suggest that both sides of the ggdv are active during protraction regardless of where the tongue is aimed. If prey are presented on the intact (active) side, the tongue is still protruded when

attempting to feed. If the ggdv were stimulated unilaterally during aiming, then we would expect no tongue protrusion when attempting to aim towards the active side after unilateral gg denervation. This hypothesis could be further explored by recording bilateral muscle activity from both ggl and ggdv or by selectively denervating each muscle compartment.

The evolution of tongue protraction in microhylids

Previous authors have noted internal compartments of the gg from gross dissection in several other microhylids, including *Callulops stictogaster* (irumbofoie callulops frog; Burton, 1983), *Breviceps* sp., *Cophixalus ornatus* (ornate rainforest frog) and *Austrochaperina robusta* (chirping land frog; Horton, 1982). The fact that all microhylids surveyed to date can aim the tongue laterally and react similarly to unilateral gg denervation suggests that the ggdv muscle and hydrostatic elongation are common features of all microhylids. Although the ggdvs of *Hemisus* and microhylids differ, in that the former has no mandibular origin, they may represent a morphocline in the development of an internal genioglossus compartment. The similarities in morphology and the fact that *Hemisus* consistently falls out near Microhylidae in recent phylogenetic hypotheses (Ford and Cannatella, 1993; Wu, 1994; Emerson et al., 2000; Hass, 2003) suggest that the ggdv may be a derived character of *Hemisus* and Microhylidae (Nishikawa et al., 1999; Emerson et al., 2000). Intrinsic tongue muscles have also been reported in the tongue of *Rhinophrynus dorsalis* (Mexican burrowing toad). However, these fibers are thought to be derived from the m. hyoglossus and only play a role in changing tongue shape, with tongue protraction being powered mainly by hyobranchial movements (Trueb and Gans, 1983).

The results of the present study reveal considerable variation in aiming prowess among microhylids. This variation in performance suggests that important morphological variation in the tongue musculature among microhylids awaits description. Variation in the arrangement of connective tissue and collagen fibers in the tongue may also play an important role in elongation and aiming. The orientation of the connective tissue fibers determines the direction of shape change (Kier and Smith, 1985) and may also influence the extent of tongue elongation (Zepnewski and Nishikawa, 2000). Fiber angles less than $54°44'$ tend to inhibit elongation, whereas those greater than $54°44'$ facilitate elongation. In *Hemisus* (Nishikawa et al., 1999), collagen fibers surrounding the m. genioglossus are oriented at an angle of nearly $80°$, resisting lateral expansion and facilitating elongation. Although we did not measure fiber angles in *Phrynomantis*, we would predict them to be greater than $54°44'$. Connective tissue orientation might also explain species differences in tongue deviation angles after unilateral transection. Initially, we suspected that post-transection tongue angle could be predicted by the maximum aiming angle observed during normal feeding, so that species with the largest aiming angles also exhibited the largest deviations after transection. However, this is not necessarily the case. For example, *Gastrophryne*

olivacea aims to a slightly greater extent than *Scaphiophryne marmorata* (marbled rain frog) but has a mean deviation angle substantially less than that of *S. marmorata* (Table 2). The underlying mechanism for these differences in behavior remains unclear. It is likely that a combination of morphological characteristics, including connective tissue and muscle fiber orientation, are important, and detailed anatomical studies are needed.

In summary, it appears that microhylid frogs protract the tongue using a muscular hydrostatic mechanism. This mechanism was previously known only in *Hemisus*. While protraction is probably accomplished by recruiting the lateral and dorso-ventral portions of the m. genioglossus, lateral displacement is due to the ggdv. Due to the orientation of the surrounding connective tissue, muscle contraction of the ggdv results in lateral rather than longitudinal displacement. This mechanism of protraction increases the range of possible movement relative to that of tongues protracted by mechanical pulling or inertial elongation. Feeding behavior of *Phrynomantis* and 17 other species of microhylids, representing six subfamilies, suggests that this general mechanism is used by all microhylids. The presence of an intrinsic component of the m. genioglossus, a hydrostatic elongation protraction mechanism and lateral tongue aiming may be synapomorphies of Microhylidae and *Hemisus*.

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