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Morphology and Histochemistry of the Hyolingual Apparatus in Chameleons

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ABSTRACT We reexamined the morphological and functional properties of the hyoid, the tongue pad, and hyolingual musculature in chameleons. Dissections and histological sections indicated the presence of five distinctly individualized pairs of intrinsic tongue muscles. An analysis of the histochemical properties of the system revealed only two fiber types in the hyolingual muscles: fast glycolytic and fast oxidative glycolytic fibers. In accordance with this observation, motor-endplate staining showed that all endplates are of the en-plaque type. All muscles show relatively short fibers and large numbers of motor endplates, indicating a large potential for fine muscular control. The connective tissue sheet surrounding the entoglossal process contains elastin fibers at its periphery, allowing for elastic recoil of the hyolingual system after prey capture. The connective tissue sheets surrounding the m. accelerator and m. hyoglossus were examined under polarized light. The collagen fibers in the accelerator epimysium are configured in a crossed helical array that will facilitate limited muscle elongation. The microstructure of the tongue pad as revealed by SEM showed decreased adhesive properties, indicating a change in the prey prehension mechanics in chameleons compared to agamid or iguanid lizards. These findings provide the basis for further experimental analysis of the hyolingual system. J. Morphol. 249:154–170, 2001.

KEY WORDS: Chameleonidae; morphology; histochemistry; motor-endplate staining; functional morphology

Chameleons have undoubtedly received more attention from anatomists and functional morphologists than any other reptile. The uniqueness of their visual (Harkness, 1977; Ott and Schaeffel, 1995; Ott et al., 1998), hyolingual (Gans, 1967; Rice, 1973), and locomotor (Abu-Ghalyun et al., 1998; Abu-Ghalyun, 1990) systems inspired workers as early as 1733 (Perrault) to investigate the morphology of these animals. In particular, the ballistic tongue projection mechanism of chameleons has stimulated an unusually large body of work. Earlier functional hypotheses were based on the forceful flow of air or blood into the tongue, resulting in its projection (Perrault, 1733; Houston, 1828). However, as more anatomical data became available several hypotheses stressing muscular action were put forward (Cuvier, 1805; Mayer, 1835; Duvernoy, 1836; Kathariner, 1894; Dewevere, 1895). In 1852, Brücke pointed out the importance of the accelerator muscle and proposed that it might be responsible for the ballistic tongue projection. Gnanamuthu (1930) rejected that hypothesis in favor of a hypothesis where the tongue retractors restrain the tongue and projection is the result of lack of activity in this muscle. The first functional studies (Zoond, 1933) attempted to test these hypotheses by transecting or stimulating the muscles thought to be responsible for the observed movements. Later studies used high-speed cinematography to describe the movements of the tongue during projection and again test functional hypotheses (Altevogt and Altevogt, 1954; Bell, 1990; Wainwright et al., 1991).

More recently, Wainwright and Bennett (1992a,b) tested functional hypotheses by recording muscle activity patterns from selected jaw and hyolingual muscles and by stimulating the accelerator in in vitro preparations. Their results clearly contradict the Gnanamuthu (1930) hypothesis and confirm earlier hypotheses stressing the importance of the action of the accelerator muscle during protraction. Moreover, their in vitro experiments point out the importance of the shape of the entoglossal process in chameleons. Van Leeuwen's (1997) quantitative model of the accelerator muscle again confirmed these results by showing how the structure of the muscle and the entoglossal process will result in tongue projection by increasing the normal stress on the entoglossal bone. In both the Wainwright (1992a,b) and Van Leeuwen (1997) articles, the authors assume a number functional properties of the system based on previously published morphological accounts (e.g., Brücke, 1852; Gnanamuthu, 1930;

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Gans, 1967; Bell, 1989) to explain the observed movements and muscle activation patterns. Van Leeuwen (1997), for example, in his model assumed that all fibers in the accelerator muscle were of a single, very fast type (which is a prerequisite for the muscle strain to be constant throughout the muscle, as assumed in the model).

Despite these more recent studies, several peculiarities of the chameleon tongue projection system remain to be explained. The function of the fast hyoid protraction observed just before the onset of projection (Wainwright et al., 1991; Meyers and Nishikawa, 2000), and the function of the hyoid protractor muscles therein (Wainwright, 1992a) have not been addressed. Also, the function of the anterior, noncircular part of the accelerator muscle, and of the activity in the posterior part during tongue retraction, remain to be explained. These muscles show activity patterns conflicting with their proposed function (Wainwright and Bennett, 1992a). Moreover, recent observations of chameleon prey capture (Herrel et al., 2000) show that chameleons do not use the typical agamid or iguanid prey prehension systems but rather "grab" prey items much like an elephant trunk does. This implies that the tongue must be slowed down and stopped just (within a few millimeters) before contacting the prey. As chameleons are extremely accurate and successful when capturing prey (Bell, 1990), the demands exerted on the control of the system are extremely high. In a first step towards an integrated view of how the chameleon controls and uses its tongue, we reexamined the morphological properties of the hyoid, the tongue pad, and hyolingual musculature from a functional point of view. Such data will allow us to postulate and test more specific functional hypotheses concerning the control of the hyolingual apparatus in chameleons.

MATERIALS AND METHODS

Specimens

All specimens used in this study were obtained from commercial dealers. Three *Chameleo jacksonii*, one *C. fischeri*, one *C. oustaleti*, one *C. pardalis* and one *C. calyptratus* were dissected and stained to characterize hyolingual muscles (Bock and Shear, 1972). Drawings were made from all stages of the dissection using a dissecting microscope (Nikon SMZ-10) provided with a camera lucida. Two *C. jacksonii*, one *Brookesia armorata,* and one *B. supraciliaris* were cleared and stained for bone and cartilage using a modified Taylor and Van Dyke (1978) stain.

Additionally, all hyolingual muscles from one *Chameleo oustaleti* and one *C. fischeri* specimen were removed and weighed to the nearest 0.001 g. The muscles were submerged in a 30% aqueous nitric acid solution for 12–24 h until individual fibers could be teased apart using blunt-tipped glass probes. Next, the nitric acid was replaced by a 50% aqueous glycerin solution to stop digestion of the fibers. Fibers were teased apart further and 10–20 fibers were chosen at random. Fibers, as well as an object of known length (for calibration purposes), were drawn using a dissecting microscope (Nikon SMZ-10) equipped with a camera lucida. Drawings were scanned (HP Scanjet II) and fiber lengths were calculated using the public domain NIH-image program (v. 1.61; developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.gov/nih-image/).

Histology

For light microscopy the entire head of one preserved *Chameleo jacksonii* and the hyolingual system of three additional preserved *C. jacksonii* and one preserved *C. oustaleti* were prepared for paraffin histology using standard techniques (Humason, 1979). Serial 10 μm sections were made (transverse, sagittal, and frontal) and stained with Masson's trichrome. Additionally, the tongues of a preserved *C. oustaleti* and a *C. jacksonii* were sectioned sagittally and stained with Verhoeff's elastin stain (Bancroft and Stevens, 1977). To determine the orientation of the connective tissue fibers surrounding the m. accelerator and the m. hyoglossus, selected sections through the connective tissue sheets surrounding these muscles were examined under polarized light microscopy. For each muscle, 20 collagen fibers were chosen randomly and the angles between the fiber and the long axis of the muscle were measured using the public domain NIH-image program.

Histochemistry

Muscle samples from the m. sternohyoideus, the m. sternothyroideus, the m. hyoglossus (both from the anterior and posterior regions), the m. mandibulohyoideus 1, the m. mandibulohyoideus 2, the m. mandibulohyoideus 3, the m. genioglossus anterior, the m. genioglossus posterior, the m. accelerator, the m. retractor pouch, the m. intermandibularis anterior, and the m. intermandibularis posterior were obtained from one freshly killed adult *Chameleo melleri*. The muscles were rapidly frozen in isopentane precooled with liquid nitrogen and stored at −80°C until required.

From each muscle a series of transverse sections was cut in a cryostat (−22°C), mounted on dry slides, and air-dried. The sections of each series were stained for one of the following enzymes: alkaline-stable myofibrillar adenosine triphosphatase (mATPase) by preincubation for 15 min at pH 9.4 and incubation for 35 min (37°C), acid stable mATPase by preincubation for 5 min at pH 4.3, and incubation for 35 min (37°C) and succinic dehydrogenase (SDH) with an incubation time of 2 h at 37°C.

Muscle fibers were classified as belonging to one of three types (Table 1) based on histochemical staining intensities. Whereas type I shows characteristics

TABLE 1. Histochemical properties of the hyolingual musculature in chameleons

	FG	FOG	SO/tonic
mATP-ase pH 9.4	++	+/0	0
mATP-ase pH 4.2	+/0	++	0
succinic dehydrogenase	0	+/++	++

Muscle	% FG fibers	% FOG fibers
m. sternohyoideus	70	30
m. sternothyroideus	70	30
m. hyoglossus (anterior)	90	10
m. hyoglossus (posterior)	100	0
m. mandibulohyoideus 1	100	0
m. mandibulohyoideus 2	20	80
m. mandibulohyoideus 3	100	0
m. genioglossus anterior	0	100
m. genioglossus posterior	100	0
m. accelerator	0	100
m. retractor pouch	100	0
m. intermandibularis anterior	40	60
m. intermandibularis posterior	60	40

+ Indicates a reaction; ++ indicates a strong reaction, 0 indicates no enzymatic reaction. FG, fast glycolytic; FOG, fast oxidative glycolytic; SO, slow oxidative.

of slow fibers (most likely tonic), type II can be classified as fast-twitch oxidative glycolytic (FOG) and type III as fast glycolytic (FG) (Peter et al., 1972; Throckmorton and Saubert, 1982; Morgan and Proske, 1984; Herrel et al., 1999).

Motor-Endplate Staining

The following hyolingual muscles of a preserved *Chameleo oustaleti* specimen were removed and stained for motor endplates using the Karnovski and Roots cholinesterase motor endplate stain (as described in Loeb and Gans, 1986): the m. sternohyoideus, the m. sternothyroideus, the m. omohyoideus, the m. hyoglossus, the m. mandibulohyoideus 1, the m. mandibulohyoideus 2, the m. mandibulohyoideus 3, the m. branchiohyoideus, the m. genioglossus anterior, the m. genioglossus posterior, the m. intermandibularis anterior, the m. intermandibularis posterior, the m. accelerator, and the m. retractor pouch.

Electron Microscopy

One *Chameleo melleri* was killed by an overdose of ketamine hydrochloride (Ketalar 100 mg/ml; Parke-Davis, Ann Arbor, MI). For scanning electron microscopy the tongue pad was removed, extensively rinsed in water, and fixed in 6.25% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) for 24 h. After rinsing in buffer, the tongue pad was dehydrated in an increasing ethanol series, critical-point dried, mounted on an aluminum stub, and sputter-coated with gold. Observations were made using a LEO 435VP scanning electron microscope at 15 kV.

RESULTS

In the following description of the hyoid apparatus the terminology of Gnanamuthu (1930) is used. For the hyolingual musculature, the terminology of Houston (1828), Brücke, (1852), Gnanamuthu (1930), and Tanner and Avery (1982) is followed.

Hyoid Apparatus

The hyoid apparatus in chameleons consists of a strongly reduced basihyoid, two pairs of cornua, and an elongated entoglossal process. The entoglossal process is parallel-sided over most of its length and tapers only at the anteriormost 2–3 mm (= anterior 1–1.5% of the length of the entoglossal process). Although the entoglossal process appears to be cartilaginous in histological sections of *Chameleo jacksonii* and *C. oustaleti*, in cleared and stained specimens of *Brookesia perarmorata* and *C. jacksonii* the entoglossal process stains red (bone), indicating some degree of calcification. At the anteriormost tip of the entoglossal process a short tapering cartilaginous element is observed that, at rest, is folded back upon the process (see Figs. 1, 3). Whereas the body of the entoglossal process is composed of typical hyaline cartilage, near the tip of the process a thick layer of dense fibrocartilage is observed. A sheath of connective tissue with a distinct elastin layer at the periphery is firmly attached to the entoglossal process. This sheet extends into, and attaches at the inner aspect of, the accelerator muscle (beyond the actual ring). The anterior pair of cornua (ceratohyals) is completely cartilaginous and short. They articulate with the anterior dorsal side of the basihyoid through a simple U-shaped synovial joint. In all the species examined, the proximal part of the ceratohyal is more robust and articulates with the distal, more flexible part by means of a synovial joint. In some species (*C. fischeri* and *B. supraciliaris*), a triangular flat piece of cartilage attaches to the distal part of the ceratohyal (see also Gnanamuthu, 1930). The ossified second pair of cornua (ceratobranchials) attaches at the posterior side of the basihyoid with a well-defined, saddle-shaped synovial joint. In lateral view the anterior pair of cornua extends upward and is tilted anteriorly; the second pair is attached nearly perpendicularly to the long axis of the hyoid apparatus. Both hyoid arches are attached to each other by a strong layer of connective tissue. Upon projection the entoglossal process is pulled forward, the ceratobranchials are folded back, and the ceratohyals are pointed upwards.

Musculature

In the following paragraphs, a short description of the origin and insertion of the hyolingual muscles will be given (see Figs. 1–3), followed by a

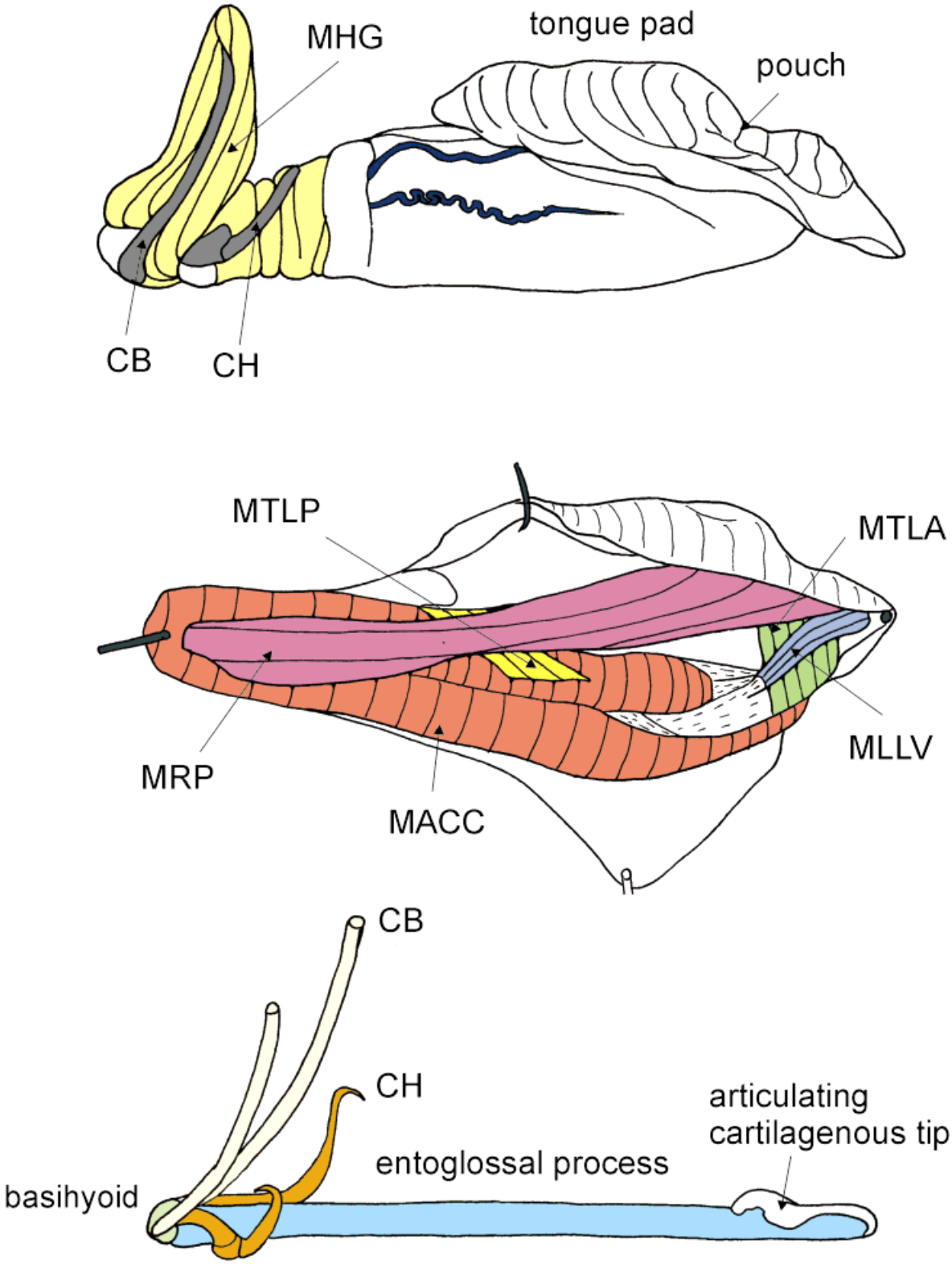

Fig. 1. Top: Lateral view on the hyolingual apparatus of a male *Chameleo oustaleti* after removal of the hyoid musculature. Middle: Lateral view on the dissected tongue after removal of the m. hyoglossus and the hyoid apparatus. Bottom: Lateral view on the hyoid of a male *C. oustaleti*. CB, ceratobranchial; CH, ceratohyal; MACC, m. accelerator; MHG, m. hyoglossus; MLLV, m. longitudinalis lingua anterior; MRP, m. retractor pouch; MTLA, m. transversalis lingua anterior; MTLP, m. transversalis lingua posterior.

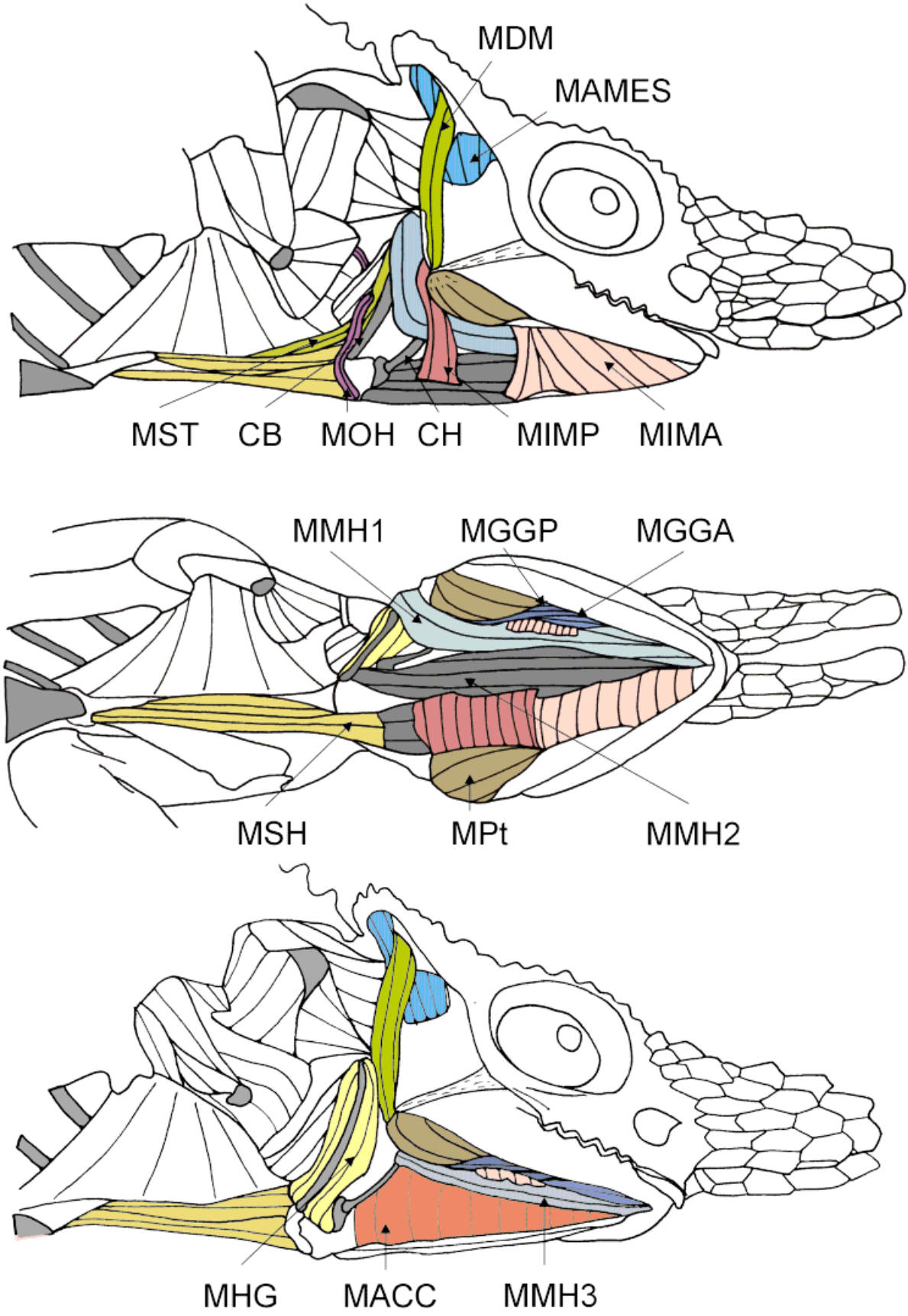
MDM
MAMES
MST
CB
MOH
CH
MIMP
MIMA
MMH1
MGGP
MGGA
MSH
MPt
MMH2
MHG
MACC
MMH3

Figure 2.

short account on the distribution of motor endplates (Fig. 4) and a summary of the histochemical properties of the muscle (see Table 1, Fig. 4). For more detailed descriptions of the myology of the chameleon hyolingual system we refer the reader to Houston (1828), Brücke (1852), Mivart (1870), Kathariner (1894), Camp (1923), Gnanamuthu (1930, 1936), and Tanner and Avery (1982).

Retractor muscles

M. sternohyoideus (MSH). This muscle originates at the posteriormost ventral side of the xiphisternum and inserts at the posterior and distalmost ventral side of the basihyoid. Isolated motor endplates are scattered throughout the muscle and three distinct aggregation sites are observed towards its insertion. Compared to other muscles the MSH shows relatively few motor endplates. The MSH consists of both FG and FOG fibers which are randomly distributed throughout the muscle. The majority of the fibers are of the FG type.

M. sternothyroideus (MST). This muscle consists of two distinct slips. The posteriormost originates at the connective tissue band situated at midbody, directly anterior to the xiphisternum, and inserts at the posterior upper half of the second cornua. The anterior slip originates at the connective tissue situated at midbody at the level of the forelimbs and is hidden in ventral view by the posterior slip. This anterior slip inserts at the posterior aspect of the tip of the second cornua. Motor endplates are aggregated into several distinct zones, more or less equally spaced throughout the muscle. Additionally, a fairly large number of isolated endplates are observed throughout the center portion of the muscle. Similar to the MSH, the MST consists of both FG and FOG fibers. Although both fiber types seem to be randomly distributed throughout the muscle, about 70% of the fibers are of the FG type.

M. omohyoideus (MOH). The m. omohyoideus originates at the lateral, posterior side of the basihyoid. The muscle runs dorsad, curves around the m. sternothyroideus, dives back to ventral, curves beneath the m. episternocleidomastoideus, and inserts at the anterior aspect of the ventral side of the scapula. The motor endplates are distributed into two distinct oblique longitudinal bands, one starting near the origin of the muscle and one starting about half-way down the muscle. All endplates appear to be of the en-plaque type. No fiber typing was performed on this muscle.

M. hyoglossus (MHG). The m. hyoglossus originates at the medial aspect of the second hyoid cornua over its entire length. Near its origin the muscle is very bulky. The muscle belly narrows, passes under the articulation of the first cornua with the basihyoid, and passes forward. Once past the first cornua the muscle is folded into itself until it reaches the posterior side of the m. accelerator. The m. hyoglossus continues to run alongside the m. accelerator (under the strong layer of connective tissue surrounding the m. accelerator) for about one-fourth of the length of the latter and inserts firmly onto its lateral aspect. Near its origin on the hyoid, motor endplates are distributed in distinct transverse bands. More anteriorly, the arrangement changes to an oblique longitudinal banding pattern that appears at regular intervals through the entire midsection of the muscle. However, near the insertion of the muscle endplates are scarcer and occur in small circular aggregations. All endplates examined were of the en-plaque type. Sections from two distinct areas within the MHG were examined for fiber types. The posterior part of the muscle (near the hyoid) consists exclusively of FG fibers. However, the anterior part of the muscle contains both FG and FOG fibers. In total, about 90% of the fibers present show a histochemical profile corresponding to an FG fiber.

Protractor muscles

M. mandibulohyoideus 1 (MMH1). This muscle runs adjacent to and is attached for most of its length to the MMH3. It originates lateral to the jaw symphysis and inserts on the tip of the first cornua of the hyoid apparatus. The motor endplates are most numerous near the insertion at the hyoid. A central band of endplates is also observed. All fibers show a histochemical staining pattern corresponding to FG fibers.

M. mandibulohyoideus 2 (MMH2). The MMH2 is a flat, strap-like muscle that originates by means of a short aponeurosis near the jaw symphysis. The muscle runs posteriad and inserts at the entire ventral aspect of the basihyoid. The motor endplates are most abundant and aggregated near the insertion of the muscle. About midway through the muscle another fairly large aggregation of endplates is visible.

Fig. 2. Top: Lateral view of the head of a male *Chameleo fischeri* after removal of the skin , the m. sternohyoideus, part of the m. sternothyroideus, and the m. constrictor colli. Middle: Ventral view of the head of a male *C. fischeri* after removal of the skin, the m. constrictor colli (both sides), the right m. sternohyoideus, the right m. sternothyroideus, the right m. intermandibularis anterior, and the right m. intermandibularis posterior. Bottom: Lateral view of the head of a male *C. fischeri* after removal of the skin, the m. sternohyoideus, the m. sternothyroideus, the m. constrictor colli, the m. intermandibularis (anterior+ posterior), the m. mandibulohyoideus (I and II), and the m. branchiohyoideus. CB, ceratobranchial; CH, ceratohyal; MACC, m. accelerator; MAMES, m. adductor mandibulae externus superficialis; MDM, m. depressor mandibulae; MGGA, m. genioglossus anterior; MGGP, m. genioglossus posterior; MHG, m. hyoglossus; MIMA, m. intermandibularis anterior; MIMP, m. intermandibularis posterior; MMH1, m. mandibulohyoideus I; MMH2, m. mandibulohyoideus II; MMH3, m. mandibulohyoideus 3; MOH, m. omohyoideus; MPt, m. pterygoideus; MSH, m. sternohyoideus; MST, m. sternothyroideus.

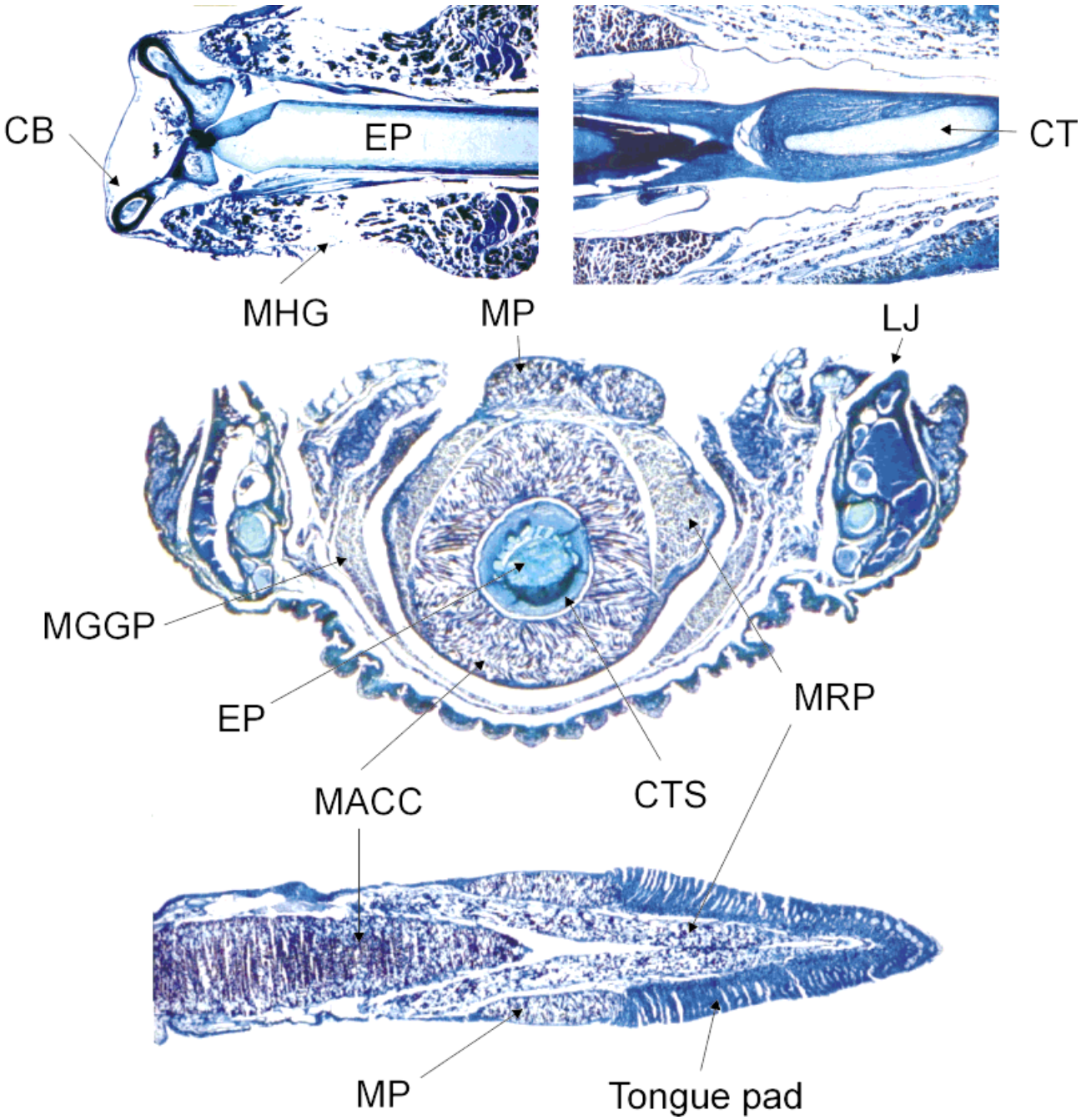

Fig. 3. Top left: Frontal section through the posterior part of the hyolingual apparatus of *Chameleo jacksonii*. Note the articulation of the ceratobranchials with the basihyoid. Top right: Frontal section through the anterior part of the hyolingual apparatus in *C. jacksonii*. Note the articulating cartilaginous piece in front of the actual entoglossal process and the associated dense fibrocartilage. Middle: Transverse section through the posterior part of the hyolingual system in *C. jacksonii*. Note the well-developed m. accelerator, the thick pouch retractor muscles, and the distinct m. pulvinares. Bottom: Frontal section through the hyolingual apparatus in *C. jacksonii*. Note the well-developed pouch retractor muscles inserting onto the inner aspect of the everted pouch. CB, ceratobranchial; CT, cartilaginous tip; CTS, connective tissue sheet surrounding the entoglossal process; EP, entoglossal process; MACC, m. accelerator; MGGP, m. genioglossus posterior; MHG, m. hyoglossus; MP, m. pulvinaris; MRP, m. retractor pouch.

Beyond that, isolated endplates seem to be spread throughout the muscle. The MMH2 consists of a fiber mosaic of both FG and FOG fibers. Most fibers, however, show a histochemical profile corresponding to FOG fibers.

M. mandibulohyoideus 3 (MMH3). Although hard to discern from the MMH1 in a superficial examination, this muscle seems to be a distinct entity. It originates between MMH1 and MMH2 near the jaw symphysis and inserts on the upper third of the

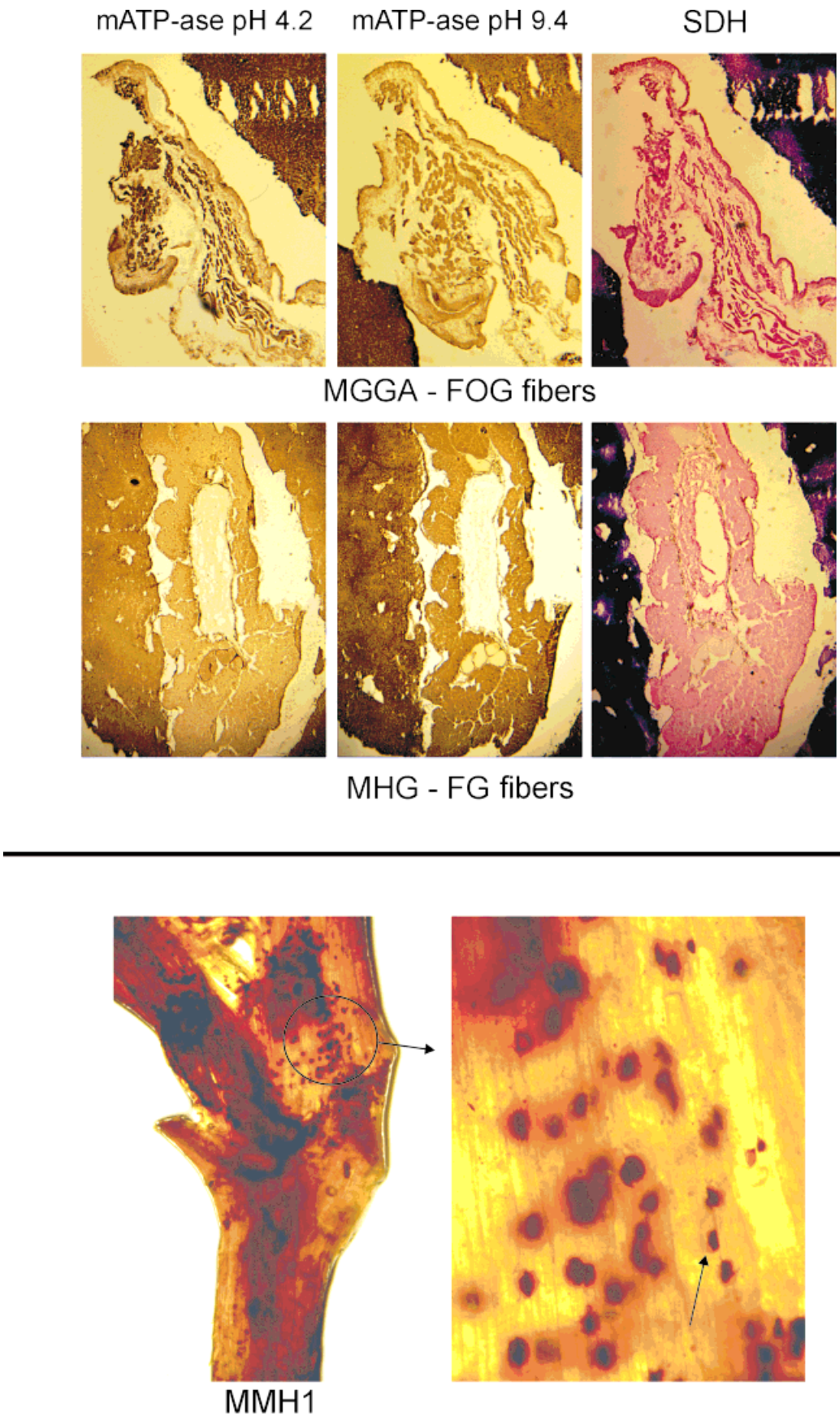

Fig. 4. Top: Histochemical staining of the m. genioglossus anterior (MGGA) and the posterior part of the m. hyoglossus (MHG) in an adult *Chameleo melleri*. Note that whereas the MGGA is composed of only fast-oxidative glycolytic fibers (FOG), the MHG is composed of fast glycolytic fibers (FG) only. Fiber types can clearly be discriminated on their staining intensity for the enzymes used: acid stable myofibrillar ATP-ase (mATP-ase at pH 4.2), alkaline stable myofibrillar ATP-ase (mATP-ase at pH 9.4), and succinic dehydrogenase (SDH). Bottom: representative muscle (m. mandibulohyoideus I, MMH1) stained for the presence of motor endplates. Note how all the endplates are of the en-plaque type (arrow on right).

second cornua. As in the MMH1, there are two distinct aggregation sites of endplates, one near the insertion, and one at about one-third of the muscle. All fibers show a histochemical staining pattern corresponding to FG fibers.

M. branchiohyoideus (MBH). Originates by means of a short aponeurosis at the posterior side of the upper third of the first cornua and inserts at the anterior aspect of the upper fourth of the posterior cornua. The motor endplates are all situated near the origin of the muscle. No fiber typing was performed on this muscle.

M. "genioglossus" anterior (MGGA). This is the most anterior of the two genioglossus-like muscles. It originates tendinously at the inner anterior fourth of the mandible and inserts onto the buccal floor at the level of the basihyoid. Motor endplates are concentrated near the origin of the muscle in a wide diagonally oriented band. All fibers of the MGGA are of the FOG type.

M. "genioglossus" posterior (MGGP). This more posteriorly situated muscle originates at the inner aspect of the mandible, just anterior to the m. pterygoideus. The fibers run posteriad and insert on the mouth floor in the throat region. The aponeurosis by which it inserts appears to extend backwards almost to pectoral girdle. The motor endplates are concentrated into two distinct areas, one near the origin of the muscle, and one just past halfway down. The second aggregation is less dense and stretches into the posterior part of the muscle. The MGGP consists of a single fiber type, corresponding to an FG fiber.

"Intrinsic" musculature

M. accelerator (MACC). The m. accelerator surrounds the entoglossal process and is itself surrounded by a strong sheet of connective tissue connecting it to the tongue pad at the dorsal side and the m. hyoglossus laterally. The m. accelerator is cylindrically shaped for three-quarters of its length. The anterior quarter of the muscle consists of both a dorsal and ventral bundle of muscle fibers. The dorsal bundle is continuous with the m. accelerator and continues to just posterior to the pouch. The ventral part extends all the way to the tip of the tongue, tapering to the front. At about the place where the dorsal part ends, a vertically oriented connective tissue septum separates the ventral part. In both the dorsal and ventral parts the fibers are oriented perpendicular to the long axis of the muscle. In the posterior third, the muscle fibers are helically arranged (see also Van Leeuwen, 1997). Motor endplates could only be discerned just below the thick connective tissue sheet covering the muscle and are distributed evenly throughout the muscle. The fibers of the MACC are all of the FOG type.

M. "retractor pouch" (MRP). The pouch retractor muscle originates at the dorsolateral aspect of posterior third of the m. accelerator. The paired muscle runs anteroventrad and inserts medially on the inner side of the so-called dimple or pouch (Fig. 1). The pouch consists of an invagination of the tongue pad, just anterior to the circular portion of the m. accelerator. The motor endplates in the pouch retractor muscle are concentrated in a central area, about half-way through the muscle, and are of the enplaque type. The MRP consists of a single fiber type, corresponding to an FG fiber.

M. longitudinalis linguae ventralis (MLLV). This small paired muscle originates at the anteriormost internal aspect of the tongue pad (just posterior to the bifurcated tongue tip). The fibers run posteriad and insert on the lateral aspect of the noncircular ventral portion of the m. accelerator. Due to the small size of the muscle, no motor-endplate staining or fiber typing was done. We do not adopt the terminology applied to this muscle by Gandolfi (1908, m. longitudinalis linguae extensoris) as the muscle is clearly not an extensor of the tongue.

M. pulvinaris (MP). This helically arranged, paired muscle is confined to the tongue pad sensu stricto. It arises at the posteriormost aspect of the tongue pad and gradually increases in diameter towards the front. The muscles from both sides run anteriad, pass laterally to the pouch, and gradually taper to end just anterior to the latter structure.

M. transversalis linguae anterior (MTLA). This short, paired muscle is situated in the anterior tongue, medial to the MLLV. The fibers originate at the dorsal surface of the noncircular portion of the MACC and run dorsad to insert at the inner aspect of the anterior tongue pad (just anterior to the pouch).

M. transversalis linguae posterior (MTLP). This paired muscle originates at the laterodorsal aspect of the MACC, just posterior to the beginning of the circular portion. The fibers run dorsad and slightly posteriad and insert at the medial inner aspect of the tongue pad, posterior to the pouch.

Intermandibularis musculature

M. constrictor colli (MCC). The MCC is a very thin muscle that originates at the dorsal nuchal fascia, runs ventrad, curves medially posterior to the lower jaw, and inserts at the midventral fascia. Due to its fragile nature, the muscle could not be dissected out whole, and thus no motor-endplate staining or fiber typing was performed.

M. intermandibularis anterior (MIMA). This muscle originates broadly on the inner side of the mandible and inserts at the midventral fascia (which is attached to the jaw symphysis). It covers the anterior one of the two genioglossus-like muscles. The motor endplates occur in a fairly narrow band near the jaw, running the entire width of the muscle. The MIMA contains two distinct fiber types, one corresponding to a FG fiber and the other to an FOG fiber. About 60% of the fibers are of the FOG-type, but both types seem to be randomly distributed throughout the muscle.

M. intermandibularis posterior (MIMP). This muscle originates by means of a short aponeurosis at the posterior side of the lower jaw, near the origin of the m. depressor mandibulae. Although the muscle

is rather thick and fleshy near its origin, it soon becomes largely aponeurotic and inserts on the midventral fascia. Motor endplates are widely scattered throughout the muscle and seem to be of the enplaque type. Similar to the MIMA, the MIMP contains both FG and FOG fibers, which are distributed in a mosaic. However, in the MIMP the majority of the fibers are of the FG type.

Connective Tissue

The orientation of the connective tissue sheet surrounding the m. accelerator was examined under polarized light. Using the birefringent properties of the connective tissue fibers, their orientation was determined. Both on the ventral and dorsal side of the m. accelerator the connective tissue fibers are arranged in a crossed helical array, with the angle of the fibers being 65 ± 5° to the long axis of the muscle in its relaxed state (Fig. 5A–D). The connective tissue fibers surrounding the m. hyoglossus, as well as those in the thick connective tissue sheet interconnecting both sides, are oriented at 90 ± 4° to the long axis of the muscle in its relaxed state (Fig. 5E,F).

Morphometric Analysis

Tables 2 and 3 summarize some quantitative aspects of the hyolingual musculature in *Chameleo fischeri* and *C. oustaleti*. In both species, the m. accelerator and the m. hyoglossus are the largest muscles and compose 30–34% of the total hyolingual muscle mass. In the smaller *C. fischeri* specimen, the pouch retractor muscle is the next biggest muscle, accounting for about 7%. However, in the larger *C. oustaleti* this muscle only composes about 4% of the total hyolingual muscle mass and is markedly smaller than the hyoid protractors (Tables 2, 3). Muscle fibers are relatively short in most muscles, with the exception of the hyoid pro- and retractors in both species. As the fibers in the m. hyoglossus in the larger *C. oustaleti* are smaller than those in the *C. fischeri* examined, the physiological cross section of this muscle is relatively larger in the larger animal. Similarly, due to the relatively long fibers, the physiological cross sectional areas of the hyoid muscles (both pro- and retractors) in both species are rather small compared to the tongue pro- and retractors (m. accelerator and m. hyoglossus).

Tongue Pad: Microstructure and the Occurrence and Distribution of Taste Buds

In the following description of tongue surface morphology, we adopt the terminology of Schwenk (1985) and Rabinowitz and Tandler (1986). The chameleon tongue can be subdivided into three distinct areas: the tongue tip, the foretongue, and the hindtongue. The tongue tip is the bifurcated anterior end of the tongue (note that the bifurcation is almost absent in chameleons) and the area just adjacent and posterior to it. The tongue tip is characterized by a smooth surface at low magnification (Fig. 6). At higher magnification the dense, closely packed papillae (with little or no microstructure) are visible. These papillae, which seem randomly oriented at the bifurcated tip, are aligned in transverse rows towards the foretongue. After extensive investigation of this area only a few taste buds were detected.

The foretongue of chameleons consists of the area that is invaginated to become the typical pouch during prey capture (Herrel et al., 2000). The entire foretongue is homogeneous in structure and consists of transversely oriented rows of densely packed reticular papillae with prominent microstructure. The density of the packing decreases somewhat at the mid to posterior side of the foretongue (= area that forms the bottom of the pouch) and occasionally free plume cells (Rabinowitz and Tandler, 1986) are observed at the edges. No taste buds were observed in this area. Towards the posterior side of the foretongue, the papillary arrangement in transverse rows disappears laterally. The hindtongue of chameleons is mostly devoid of papillary structures and consists of a smooth epithelial structure. This part of the tongue surrounds the accelerator muscle and is situated behind the tongue pad sensu stricto. At high magnification, prominent microstructure can be observed (Fig. 6). In contrast to the anterior parts of the tongue, taste buds are more prominent on the hindtongue (Fig. 6).

DISCUSSION

Morphology

A thorough examination of the hyolingual musculature shows that the chameleon tongue is much more complex than is usually assumed in functional studies. The intrinsic musculature is highly complex and consists of at least five distinct sets of muscles (see Bell, 1990). Although all these muscles presumably function to shape the tongue pad, no functional studies have yet examined their function during prey capture or transport (but see Herrel et al., 2000). Even well-studied muscles such as the m. accelerator are morphologically more complex than previously assumed. Whereas it had been known that anteriorly the muscle is separated into a dorsal and a ventral noncircular part, it was previously unknown that the anterior ventral part is separated from the main body of the muscle by a distinct connective tissue septum. Functionally, this anterior ventral part can thus be considered a separate entity. The muscle activity patterns recorded from this muscle are also radically divergent from those recorded from the remainder of the muscle (Wainwright and Bennett, 1992a). However, the functional significance of this part of the muscle remains obscure.

TABLE 2. Quantitative properties of the hyolingual system in a male Chameleo fischeri *(body mass: 16.97 g; snout-vent length: 92.9 mm; head length: 30.53)*

Muscle	Mass (g)	Fiber length (mm)	Phys. XS (cm^2)	Rel. mass (%)
CB-MF	0.0023	3.10	0.0074	0.56
MACC	0.1250	1.68	0.7438	30.38
MBH	0.0066	5.17	0.0128	1.60
MGGA	0.0170	5.56	0.0310	4.13
MGGP	0.0049	5.01	0.0098	1.19
MHG	0.1205	4.73	0.2547	29.28
MRP	0.0307	8.22	0.0373	7.46
MIMA	0.0148	1.88	0.0789	3.60
MIMP	0.0080	5.07	0.0158	1.94
MMH1	0.0171	10.49	0.0163	4.16
MMH3	0.0094	9.24	0.0102	2.28
MMH2	0.0084	11.20	0.0075	2.04
MOH	0.0028	4.55	0.0062	0.68
MSH	0.0291	9.94	0.0293	7.07
MST	0.0149	6.15	0.0242	3.62
Tongue Pad	0.1107			

Relative mass is expressed as the mass of the muscle relative to the entire hyolingual muscle mass. CB-MF, small muscle slip running from the ceretobranchial to the mouth floor, which was only observed in this specimen.

The extrinsic musculature is also very complex but largely resembles the condition seen in agamid and iguanid lizards. Major differences are the position and attachment of the m. sternothyroideus and the presence of an m. genioglossus-like muscle attaching to the mouth floor. As argued by Schwenk (1986), this genioglossus-like muscle is most likely homologous to the m. genioglossus in other lizards. In *Sphenodon,* slips of the m. genioglossus insert on the buccal floor, as observed in chameleons (Osawa, 1898; Schwenk, 1986).

Despite the importance of the shape of the hyoid in tongue projection (Wainwright and Bennett, 1992b), the entoglossal processes of the chameleons examined in this study are largely cylindrical, with only a minor taper at the anteriormost aspect. However, significant taper is observed at the articulating cartilaginous piece, positioned in front of the actual entoglossal process. In order to function as proposed previously (Wainwright and Bennett, 1992b), the articulating piece must be unfolded and brought in-line with the entoglossal process at the beginning of the tongue projection cycle. Presumably this automatically occurs as a result of the activation of the m. accelerator during the early phases of tongue protrusion. Clearly, this hypothesis needs to be tested with simultaneous emg and cineradiographic recordings.

The articulation of the ceratobranchials with the reduced basihyoid is also of great functional interest. These structures articulate in such a way that there are two positions where stress on the joint is minimal: one with the ceratobranchials sitting nearly perpendicular to the long axis of the hyoid (rest position) and one with the ceratobranchials lying parallel to the long axis of the hyoid (extended position). When moving the ceratobranchials backwards from the rest position, force needs to be applied to overcome the initial resistance of the system. Once a certain extension is reached, the system will automatically jump to the next state with the ceratobranchials in the extended position (see also Meyers and Nishikawa, 2000). This passive property of the hyoid might explain the fast hyoid protraction observed at the onset of tongue projection (Wainwright et al., 1991; Meyers and Nishikawa, 2000). The function of this fast hyoid protraction, however, remains unknown. We hypothesize that the fast hyoid protraction might function as a mechanical trigger for the actual onset of tongue projection.

Observations of chameleons capturing prey indicate a recoil of the tongue with adhering prey. Upon return of the tongue to the mouth, the tongue pad with prey moves back faster than it can be reeled in by the tongue retractor, resulting in it being frequently catapulted back beyond the head of the animal (Bell, 1990; Fig. 7). It should be noted, however, that such a recoil is only observed on near-maximal efforts. Based on these observations we suspected the presence of elastic recoil. After inspection of the hyolingual apparatus, we identified the thick connective tissue sheet connecting the tongue to the hyoid apparatus as a potential candidate for the storage of elastic strain energy. Sections of the hyolingual apparatus stained for elastin showed the presence of an outer layer of elastin in this connective tissue sheet. Still, the remainder of this connective tissue sheet seems to be composed of collagen. We hypothesize that the connective tissue sheet with the elastin covering it acts as a mechanism preventing damage to the tongue retractors by mechanically limiting the maximal tongue projection distance.

TABLE 3. Quantitative properties of the hyolingual system in a male Chameleo oustaleti *(body mass: 96.92 g; snout-vent length: 150.0 mm; head length: 55.26 mm)*

Muscle	Mass (g)	Fiber length (mm)	Phys. XS (cm^2)	Rel. mass (%)
MTLA	0.0025	2.16	0.0114	0.10
MTLP	0.0028	2.24	0.0125	0.12
MACC	0.8256	4.02	2.0547	34.66
MGGA	0.0359	6.40	0.0561	1.51
MGGP	0.0338	8.15	0.0415	1.42
MST	0.0041	9.14	0.0045	0.17
MRP	0.1032	4.89	0.2113	4.33
MHG	0.7495	2.29	3.2799	31.46
MIMP	0.0545	10.81	0.0504	2.29
MMH1	0.3002	11.34	0.2648	12.60
MMH2	0.1648	12.01	0.1372	6.92
MOH	0.0148	8.31	0.0178	0.62
MLLV	0.0052	2.54	0.0203	0.22
MSH	0.0852	10.95	0.0778	3.58
Tongue Pad	0.5882			

Relative mass is expressed as the mass of the muscle relative to the entire hyolingual muscle mass.

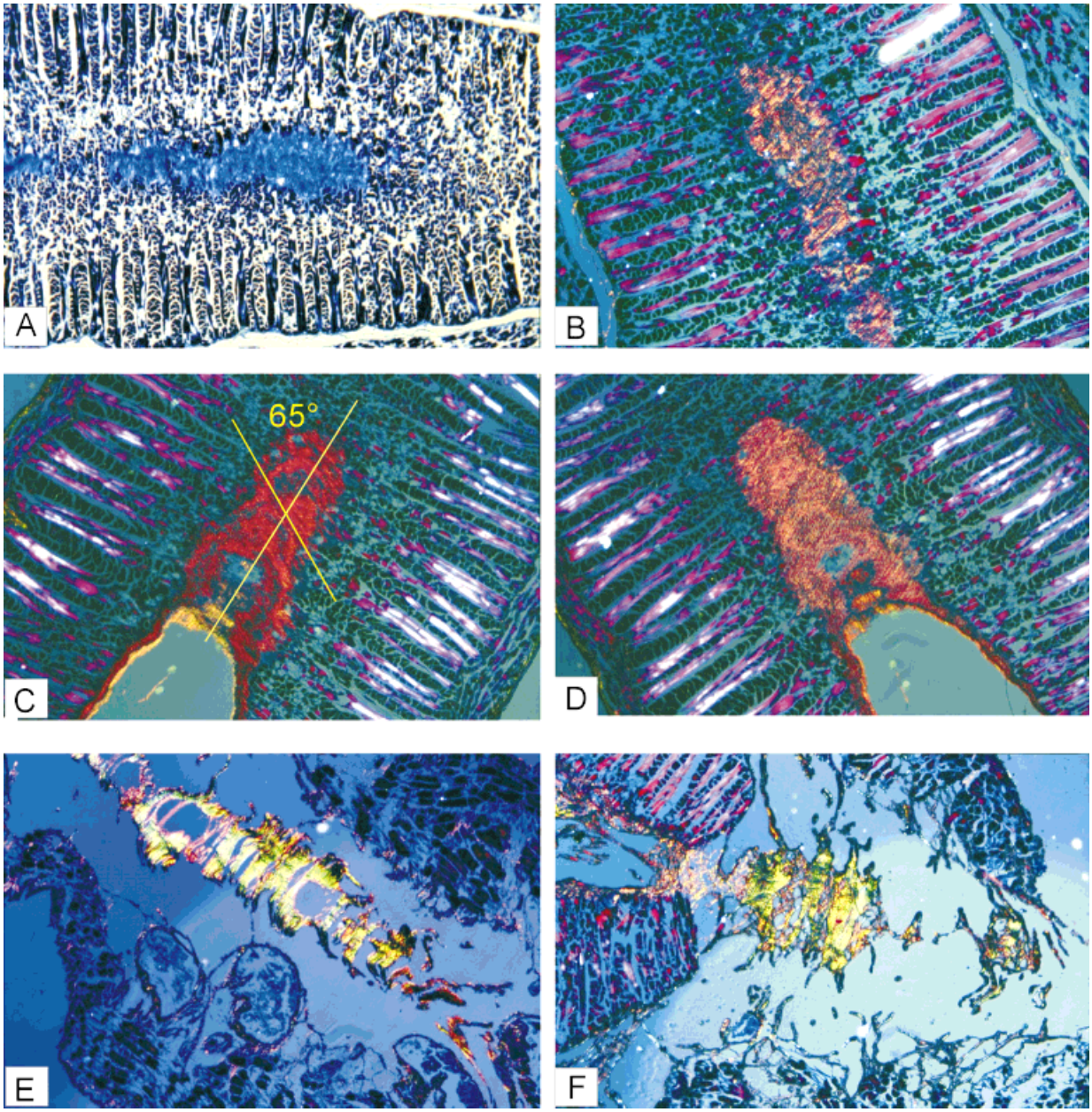

Fig. 5. **A**: Trichrome stained frontal section through the epimysial connective tissue surrounding the m. accelerator in *Chameleo jacksonii*. **B**: Same section under polarized light. Note how the orientation of the connective tissue fibers becomes apparent as a result of the birefringent properties of collagen. **C**: Frontal section through the epimysial connective tissue of the m. accelerator examined under polarized light. Note how the connective tissue fibers are oriented at an angle of 65° to the long axis of the muscle. **D**: Same section viewed with the polars oriented 90° to the previous one. **E**: Epimysial connective tissue fibers of the m. hyoglossus. Note how the fibers are oriented near perpendicular to the long axis of the muscle and will facilitate muscle elongation. **F**: Similar view of the epimysial connective tissue of the m. hyoglossus close to the insertion of the m. hyoglossus on the lateral aspect of the m. accelerator.

An analysis of the elastin-stained sections also shows the presence of elastin in the connective tissue branching off from the main sheet into the folds of the m. hyoglossus. We hypothesize that the elastin observed in the connective tissue branching off into the m. hyoglossus helps to fold the m. hyoglossus back in its retracted state after tongue projection.

As has been shown recently (Nishikawa et al., 1999; Zepnewski and Nishikawa, 1999) the orientation of epimysial connective tissue fibers can play an important role in determining the functional properties of a system and lead to distinct behavioral differences. Epimysial connective tissue fibers will tend to reorient themselves towards 54°44′ if the system is pressurized (Kier and Smith, 1985; Nish-

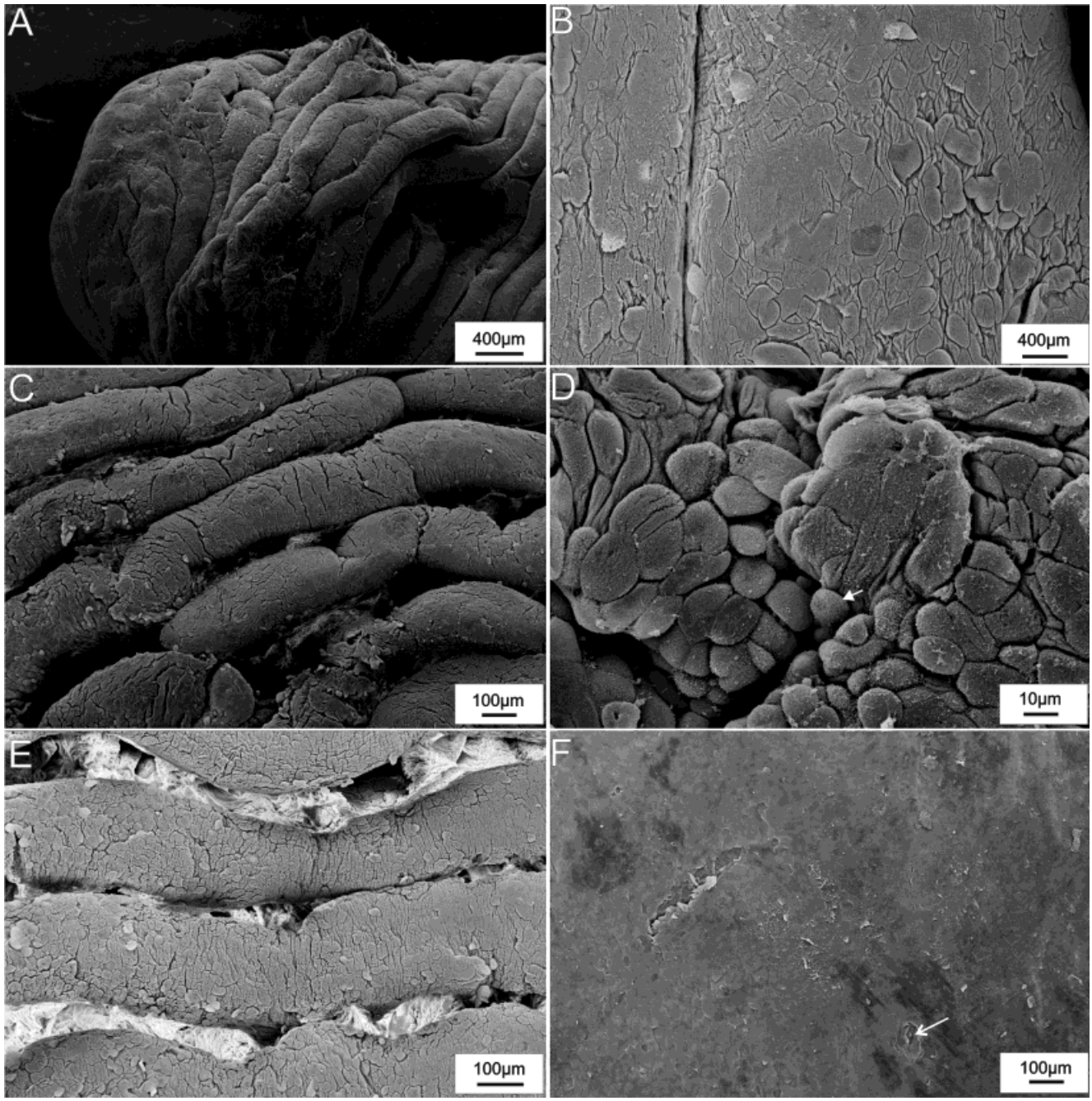

Fig. 6. SEM. **A**: Tongue tip of a *Chameleo melleri* at low magnification. Note the apparently smooth surface and lack of papillary structures. **B**: Same area at higher magnification. Note how the densely packed reticular papillae start to form. **C**: Dorsal view of the tongue pad in the pouch area. Note the transverse rows of dense cylindriform or reticular papillae. **D**: Same area at higher magnification. Note how free plume cells are observed at the edges of the papillae (arrow). **E**: Posterior tongue pad. Note how the general structure is still extremely similar to that observed on the foretongue. **F**: Posterior part of the tongue with an apparent smooth surface and an occasional taste bud (arrow).

ikawa et al., 1999). In the chameleon hyolingual system, whole muscle elongation is especially relevant for both the tongue retractor (m. hyoglossus) and protractor (m. accelerator). The tongue retractor muscle undergoes an enormous elongation during prey capture and thus it could be predicted that the connective tissue would be oriented near perpendicular to the long axis of the muscle. The analysis of the epimysial fiber orientation in the m. hyoglossus shows that fibers are indeed oriented perpendicular to the long axis of the muscle and will thus facilitate whole muscle elongation (Nishikawa et al., 1999). The epimysial connective tissue fibers of the m. accelerator are oriented at approximately 65° to the

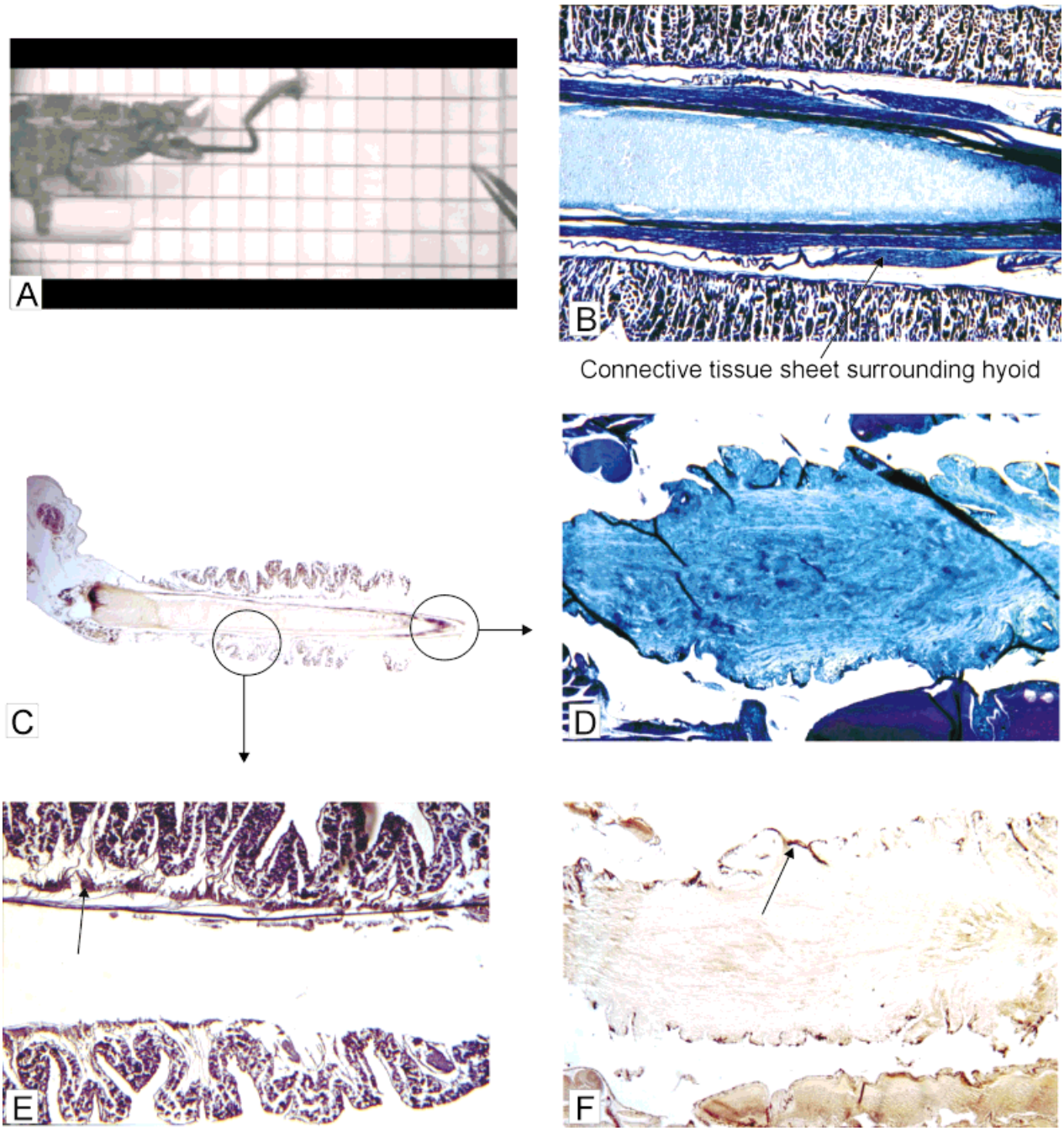

Fig. 7. **A**: Image taken from a video recording of a *Chameleo jacksonii* capturing a cricket. Note how the tongue is traveling back faster than the animal can pull it back. Eventually the tongue will hit the animal in the face, before being reeled in. **B**: Frontal section through the hyoid of a *C. jacksonii*. Note the thick connective tissue sheet surrounding the entoglosssal process that is folded back onto itself at rest. **C**: Sagittal section through the hyoid and m. hyoglossus of a *C. oustaleti* stained for the presence of elastin. **D**: Trichrome-stained sagittal section through the connective tissue sheet after removal of the hyoid. **E**: Higher magnification of **C**, showing the presence of elastin in the connective tissue branching of into the folds of the m. hyoglossus. **F**: Same section as **D**, but stained for elastin. Note the presence of an elastin layer (black stain) at the periphery of the connective tissue sheet.

long axis of the muscle. Upon activation of the muscle during the early stages of tongue projection (see Wainwright and Bennett, 1992a), the muscle will elongate until the fibers reach their optimal angle of 54°44′. In order to function as proposed by Wainwright and Bennett (1992a,b), the amount of elongation enabled by the epimysial connective tissue must be directly related to the amount of elongation needed for the muscle to reach the tapered part of the entoglossal process. Preliminary analysis of the m. accelerator and its position on the entoglossal process in the chameleons examined in this study

indicate that such a tight correlation is indeed observed.

Motor Endplates

In general, three types of motor endplate distributions were observed in the hyolingual musculature of chameleons. The first type consists of aggregations of motor endplates near the origin and insertion of the muscle, the second type consists of an apparently random distribution of endplates, and a third one consists of a series of oblique bands running across the muscle surface. The motor endplates of the muscles examined in this study all seem to be of the en-plaque type, indicating the absence of tonic fibers. Noticeable are the large numbers of motor endplates, which in combination with relatively short muscle fibers implies potentially high levels of control in all muscles. Given these observations, hypotheses of muscle function including differential activation of different areas within the same muscle are plausible. The m. hyoglossus would be the most likely candidate for such an activation pattern due to its length, and previous observations that suggested differential activation of different locations within this muscle (pers. obs. based on preliminary emg data).

Histochemistry

In the chameleon species examined here, about half of the muscles consist exclusively of fast glycolytic fibers: two of the hyoid protractors (mm. mandibulohyoidei 1 and 3), the posterior m. genioglossus, and the pouch retractor. The hyoid retractors (m. sternohyoideus and the m. sternothyroideus), the intermandibularis musculature (both anterior and posterior), the m. mandibulohyoideus 2, and the m. hyoglossus consist of both fast glycolytic and fast oxidative glycolytic fibers. Only the m. accelerator and the m. genioglossus anterior consist solely of fast oxidative fibers and, surprisingly, none of the hyolingual muscles examined show slow oxidative or tonic fibers. To judge the significance of these findings in a comparative framework is nearly impossible, as no data are available on the histochemical properties of the hyolingual system in other lizards. Recent data on the histochemical characteristics of some hyolingual muscles in frogs (Peters, 1999) show that tonic fibers are absent here, too. Whereas both the genioglossus and hyoglossus in frogs show both FOG and FG fibers, only the m. genioglossus shows SO fibers. Apparently the constraint on tongue retraction to pull the prey back into the mouth quickly dictates the functional properties in lingual feeding organisms.

Our data partly contradict the assumption by Van Leeuwen (1997), who predicted that all the fibers in the accelerator would be of one, very fast type. Although the fiber distribution in the m. hyoglossus in chameleons is uniform, the fiber type observed is not a fast glycolytic one. The electromyographic data in Wainwright and Bennett (1992a) indicate a long activity duration in the m. accelerator, which would require at least some oxidative properties, as are indeed observed. Interestingly, the m. hyoglossus shows spatial differences in the distribution of functional fiber types. Whereas most of the muscle is composed of only fast glycolytic fibers, the anteriormost part shows two distinct fiber types (FG and FOG). This difference in fiber type distribution might point to functional differences between different areas of the m. hyoglossus. Unfortunately, electromyographic data are only available for the more posterior region (Wainwright and Bennett, 1992a). As most of the m. hyoglossus is extremely folded when the tongue is withdrawn into the oral cavity, the posterior area is likely only functional during prey capture (which is largely a one-time event, given the accuracy with which chameleons capture prey; Bell, 1990). The anterior region, however, might function during tongue retraction during intra-oral transport (So et al., 1992), and thus require a larger oxidative capacity. Still, the hyoid retractors presumably take over much of the function of the m. hyoglossus during food transport in chameleons, which might explain their larger oxidative capacity. Whereas hyoid retraction is very distinct, hyoid protraction seems to be rather limited in chameleon intra-oral transport (So et al., 1992). As the genioglossus muscles in chameleons do not insert onto the tongue, the m. accelerator might be taking over their function during intra-oral transport. Given that the oxidative capacity in the m. accelerator is larger than in the hyoid protractors, we also suggest an important role for this muscle during tongue protraction in intraoral transport cycles. The genioglossus muscles might be aiding the accelerator by pulling the mouth floor, and the tongue with it, upward and forward during transport. Unfortunately no electromyographic data of prey transport are available for chameleons so all the above-stated hypotheses remain speculative.

Tongue Pad

Scanning electron microscopy of the tongue pad of *Chameleo melleri* revealed few taste buds, as was expected given the highly visual nature of chameleons (Evans, 1961, 1967; Madison, 1977; Ott and Schaeffel, 1995). The only other data available on the presence and distribution of taste buds in chameleons are for a juvenile *C. jacksonii* (Schwenk, 1985). Both for *C. melleri* (present study) and *C. jacksonii* (Schwenk, 1985), taste buds were observed on or near the tongue tip. This can be correlated with the fact that chameleons use substrate touches, during which only the tongue tips are extended and brought in contact with the substrate (Parcher,

1974; pers. obs.). Presumably, the animals thus gather chemical deposits from the substrate to evaluate the presence of conspecifics, predators, or other unknown properties. The only differences observed between *C. jacksonii* and *C. melleri* are that in the former species taste buds were also observed on the foretongue (Schwenk, 1985). In the latter species, taste buds are confined to the tongue tips and the hindtongue. Whereas the relevance of this difference in taste bud distribution remains obscure, it is known that chameleons do discriminate between prey on taste (Larsen, 1992). Likely this is mediated by gustatory receptors on the tongue surface and oral epithelia. Nevertheless, when compared to most agamid and iguanid lizards chameleons are distinctive by virtue of the relatively smaller number of taste buds present. The reduced functionality of the chameleon vomeronasal and gustatory systems are apparently compensated by a highly intricate and complex visual system (Harkness, 1977; Ott and Schaeffel, 1995; Ott et al., 1998).

The papillary structure of lizard tongues has previously been invoked in various functional attributes such as prey capture and transport (Schwenk, 1988, 2000; Herrel et al., 1998). Whereas plumose papillae are assumed to play an important role in the interlocking of the tongue onto prey surface irregularities (Schwenk, 2000), densely packed reticular papillae with prominent microstructure are assumed to play an important role during prey transport (Herrel et al., 1998). Surprisingly, in the chameleon species examined here very little spatial variation in papillary structure was observed. Moreover, very few plumose papillae were observed in the so-called pouch or dimple, as would have been expected given that this is the area of the tongue contacting the prey during capture. In iguanid and agamid lizards, which typically do rely on interlocking for prey prehension, the area of the tongue (= foretongue; see Schwenk, 1985) that contacts the prey is characterized by large numbers of plumose papillae (e.g., see fig. 3C in Herrel et al., 1998; Delheusy et al., 1994). The absence of such papillae points to the significance of the observations made by Herrel et al. (2000), who demonstrated that chameleons use a completely novel prey prehension mechanism. Schwenk (2000) reports large numbers of these plumose papillae in the pouch of a chameleon. However, the species on which the observations were made, and the techniques used, were not specified. Possibly interspecific differences may be large and need to be explicitly addressed in future studies. The results for *C. melleri* are unequivocal, and in clear contrast with data presented previously for most iguanid and agamid lizards. The structure of the papillae on the tongue of *C. melleri* corresponds more to the papillary structure of the hindtongue in *Uromastix acanthinurus*, where such papillae have been invoked to play an important role in transport.

The results presented here show how a detailed analysis of the morphological and functional properties of the hyolingual system in chameleons may help explain observed patterns of muscle recruitment and hyolingual movements. Clearly, the data gathered raise many more questions than can be answered and demonstrate the need for more, carefully planned experiments where specific hypothesis are tested by experimental manipulation of the system. Nerve transection experiments combined with multiple electrode emg recording from the hyolingual muscles might be especially insightful in understanding how the chameleon hyolingual system functions and is controlled during prey capture and transport.

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