

# Anatomical Basis of Dynamic Modulation of Tympanic Tension in the Water Monitor Lizard, *Varanus salvator*

DAWEI HAN AND BRUCE A. YOUNG\*

Department of Anatomy, Kirksville College of Osteopathic Medicine/Missouri School of Dentistry and Oral Health, A.T. Still University, Kirksville, Missouri

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## ABSTRACT

Amphibious vertebrates, such as the water monitor (*Varanus salvator*), require anatomical and/or neural specializations to cope with pressure changes on the tympanic membrane when transiting between air and water. *V. salvator* has internally coupled ears which are distinguished by (patent) anatomical conduits through the skull linking the middle ear cavities on both sides of the head. We describe a small skeletal muscle in *V. salvator* which inserts onto the middle ear ossicle and the tympanic membrane. Laser doppler vibrometry demonstrates that contraction of this muscle both increases the vibrational velocity of the tympanic membrane and changes the waveform pattern of the tympanic displacement. The combined anatomical and functional results suggest that *V. salvator* is capable of actively modulating the tension of the tympanic membrane. Anat Rec, 299:1270–1280, 2016. © 2016 Wiley Periodicals, Inc.

**Key words:** reptilia; auditory; middle ear

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## INTRODUCTION

Most reptiles, birds, and frogs have internally coupled ears in which the contralateral middle ear cavities are linked through expanded Eustachian tubes and the pharynx (e.g., Christensen-Dalsgaard and Manley, 2008). This anatomical continuity creates a functional linkage such that displacement of one tympanum (as by an external sound stimulus) creates an internal pressure wave that will propagate through the anatomical connection and induce displacement of the contralateral tympanum (e.g., Vossen et al., 2010). This internal coupling results in a unique suite of frequency-selective neural cues which are particularly relevant for acoustic localization (Christensen-Dalsgaard and Manley, 2005; Bierman et al., 2014; Vedurmudi et al., 2016). Recent biophysical explorations of this system (Vedurmudi et al., 2016) have shown that a key feature determining the performance of the internally coupled ear is the fundamental frequency (tension) of the tympanic membrane.

Experimental analysis has shown that the tympanic membrane of the water monitor lizard (*Varanus salvator*) has unusual dampening, at least as compared to the more extensively studied *Gekko gecko* (Vedurmudi et al.,

2016). Neither the functional basis of this dampening nor its ecological significance has been explored. As the common name—water monitor—implies, *V. salvator* is a semiaquatic lizard capable of spending considerable time submerged and foraging in the water (Stanner, 2010). Unlike other lizards which have a variety of “mental muscles” capable of reducing the exposed area of the tympanum (Wever, 1978), in *Varanus*, the same surface area of the tympanum is exposed to both air and water. Audition, and particularly sound localization, is challenging for a truly amphibious animal given the different velocities of sound in air and water (Higgs et al., 2002; Hetherington, 2008; Christensen-Dalsgaard et al., 2012). On a more fundamental level, the tympanum can be seen as a barrier between internal and external

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\*Correspondence to: Bruce A. Young; Department of Anatomy, A.T. Still University, Kirksville, MO 63501. Fax: +660-626-2346. E-mail: byoung@atsu.edu

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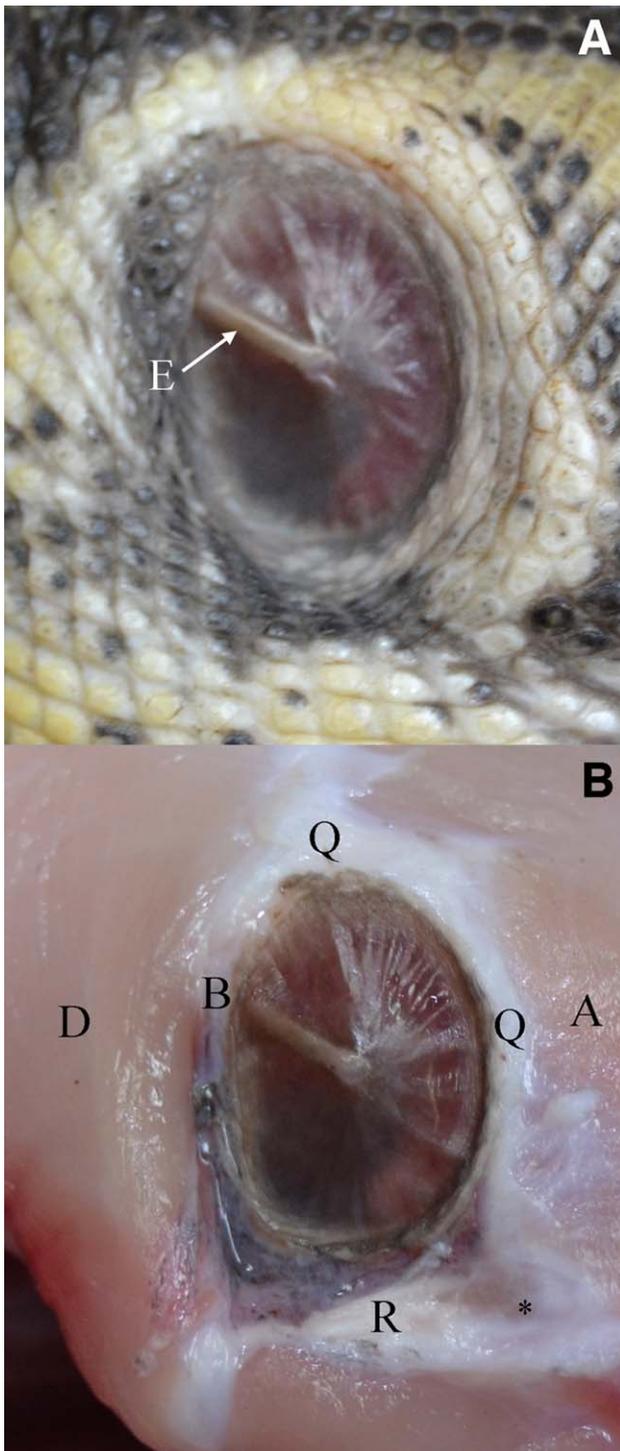


Fig. 1. The morphology of the tympanic membrane in *Varanus salvator*. (A) Superficial view showing the ovoid shape, lateral bulging of the tympanum, and the pattern of attachment of the extracolumella (E). (B) Superficial dissection. The quadrate (Q) forms the cranial and dorsal anchor for the tympanum, while the retroarticular process (R) of the mandible forms the ventral attachment. The caudal attachment is formed by the post-tympanic band (B) and the depressor mandibulae (D). (A) Jaw adductor, \* jaw joint.

pressures; in an amphibious animal like *V. salvator*, the external pressure would change every time the animal transited between land and water. This study was undertaken to explore the possible anatomical bases for modulation or regulation of tympanic tension in *V. salvator*.

The comparative anatomy of the reptilian tympanic membrane has been explored by Versluys (1898) and Wever (1978). Wever (1978) divided the diversity of lizard ears into three main groups (Iguanid, Gekkonid, and Scincid) with the explicit recognition of multiple “divergent” types (Wever, 1973, 1978). Wever considered *Varanus* to have a “standard” Iguanid-type ear, but did note some anatomical specializations which he argued were unique to this genus. In light of the fact that Wever’s (1978) text is still a benchmark reference for the lizard ear, his nomenclature will be used (as much as possible) throughout this contribution. A few lizards have a stapedial (or extracolumellar) muscle which could alter the biophysics of the tympanum and/or the middle ear (e.g., Versluys, 1898; Baird, 1970; Henson, 1974; Wever, 1978); however, previous hypotheses for the functional role of this muscle have been contradictory (Wever and Werner, 1970; see Silman et al., 1984 for a broader treatment) and this muscle has not been explored experimentally. Other anatomical features of the ear could alter the dynamics of the linkage between the contralateral tympana (e.g., Young, in press) but the prevalence and utility of these systems have not been demonstrated.

## MATERIALS AND METHODS

**Anatomy:** The middle ear and interaural connections were dissected on four juvenile/adult specimens of *V. salvator* which had been previously preserved in 10% neutral-buffered formalin and stored in 70% ethanol. The middle ear regions of two specimens were excised with a radial saw and examined under a TM3000 scanning electron microscope (Hitachi High-Technologies Corp., Northridge, CA).

Blocks of tissue were excised from the otic region of previously preserved specimens of *V. salvator*. Tissue blocks were decalcified (RDO rapid decalcifier, Apex Engineering Products, Aurora, IL), dehydrated through an ethanol series, and embedded in paraffin prior to sectioning at 10  $\mu\text{m}$ . Mounted sections were stained with Masson’s trichrome, hematoxylin and eosin, an orcein elastin stain, or Verhoeff’s elastin stain. Complete (dorsal–ventral) serial sections were digitally photographed, and then a 3-D reconstruction was performed using the BioVis3D software system (BioVis3D, Montevideo, Uruguay).

The middle ear regions of two additional (previously preserved) specimens of *V. salvator* were excised, then cleared and stained following the procedure of Taylor and van Dyke (1985).

**Functional Analysis:** The mechanics of this system were explored using four juvenile/adult (total lengths = 46–156 cm) specimens of *V. salvator* that were obtained commercially. The lizards were housed in individual (60  $\times$  120  $\times$  35 cm tall) terraria within a special reptile holding facility with a 12:12 light cycle, water *ad libitum*, and a temperature range of 28°C–32°C. The animals were maintained on a diet of previously frozen

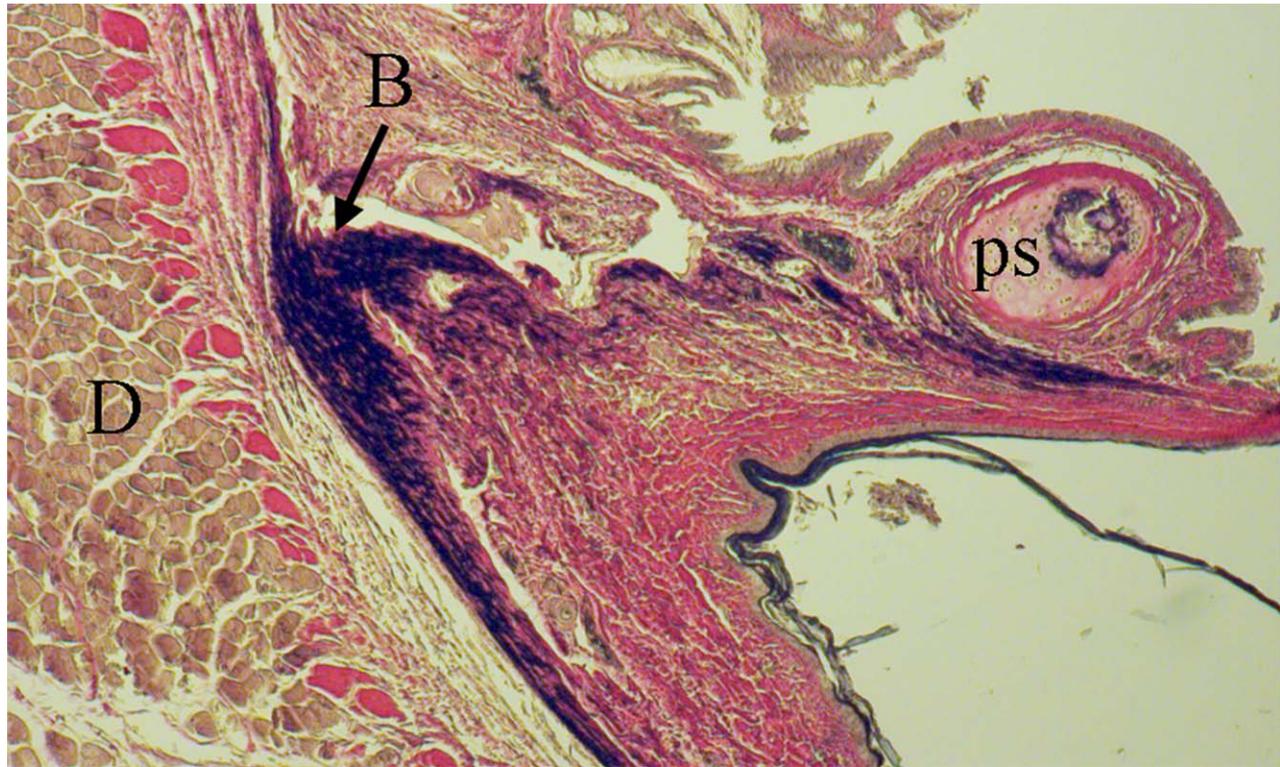


Fig. 2. Frontal section through the caudal attachment of the extracolumella showing the caudal tip of the pars superior (ps) of the extracolumella attached to the cranial border of the post-tympnic band (B), which is integrated into the perimysium of the depressor mandibulae (D). This section is stained with Orcein and shows the positive response for elastin (arrow) within the post-tympnic band.

rodents (Colorado Rocky Mountain Rodents, Paonia, CO) and frogs. The housing, care, and use of the live *V. salvator* followed all applicable national guidelines and were approved by the Institutional Animal Care and Use Committee of A.T. Still University (Protocol #186 approved 10/15/2014).

Individual specimens were anesthetized with isoflurane, then placed on a vibration isolation table (Micro-G; Backer-Loring Corp.; Peabody, MA) within a custom-built acoustic isolation chamber. A laser doppler vibrometer (OFV 518 and 2802; Polytec Inc., Irvine, CA) was positioned to laser the tympanic membrane slightly cranioventral to the distal end of the extracolumella. Pure 500 Hz tones of 500 ms duration were generated using Tone Generator software (NCH Software, Greenwood Village, CO) and presented to the animal using a 1208-8-SPS BWX speaker (Peavey Electronics Corp., Meridian, MS) positioned perpendicular to the long axis of the head, at the same horizontal level as the tympanum, and at a distance of 95 cm. The tones were all presented at the same intensity (50 dB SPL), which was standardized using a digital sound-level meter (WS1361, Wensn) and a calibrated USB microphone (UMIK-1, MiniDSP). This relatively low level of stimulation was chosen to avoid swamping out the influence of the tympanus muscle and because an earlier series of experiments demonstrated a clear tympanic response to the 50 dB stimulus. A custom-built bipolar-stimulating probe was placed on the posterior margin of the tympanic membrane, such

that the leads of the probe were on opposite (dorsal/ventral) sides of the extracolumella. A GRASS S-88 dual-channel stimulator (Natus Neurology Inc., Warwick, RI) was used to stimulate the tympanic musculature. The output from the laser doppler system (tympanic velocity) was recorded (at 10 kHz) using an MiDAS (Xcitex Inc., Woburn, MA) data acquisition system; synchronized output from the stimulator and the tone generator were also recorded. A minimum of 10 stimulus responses were recorded from each of three trial conditions (stimulus tone alone, muscle stimulation alone, muscle stimulation, and stimulus tone). With this system, it was possible to document physical displacement of the tympanic membrane during stimulation of the tympanum, as well as any change in sensitivity of the ear caused by stimulation of the tympanic musculature.

## RESULTS

**Anatomy:** The tympanic membrane of *V. salvator* is nearly oval (Fig. 1A), with the long (dorsal–ventral) axis 138% the length of the short (cranial–caudal) axis (s.d. = 2.4%, N = 8). The long axis is tilted 20° (s.d. = 1.2°, N = 8) caudad relative to the dorsum of the head. The attachment point of the extracolumella is clearly visible on the inner surface of the tympanum (Fig. 1A). The cranial portion of this attachment, the pars inferior, occurs near the center of the tympanum (mean 46% of short axis length from the caudal and 48% of long axis length

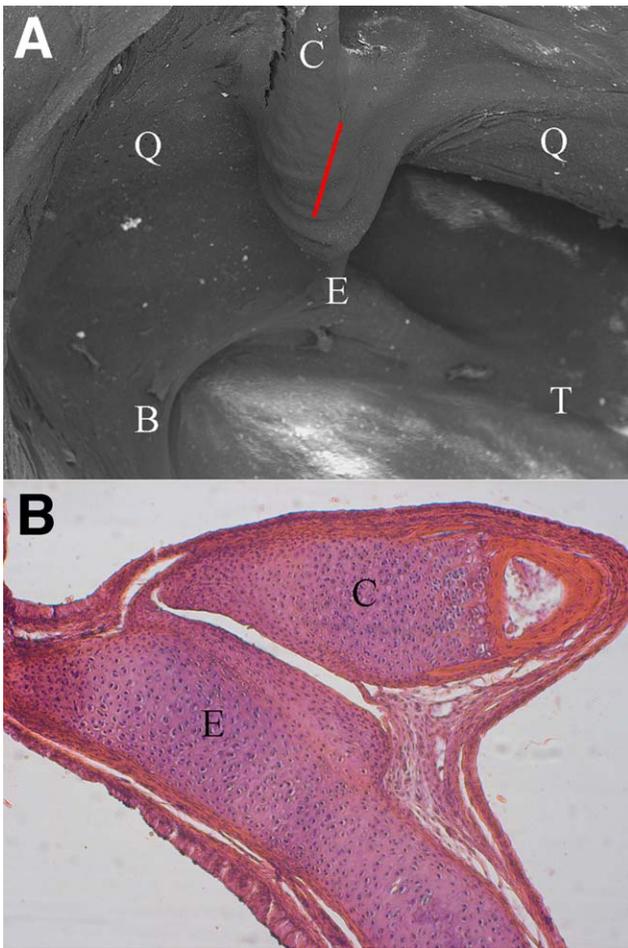


Fig. 3. The junction between the proximal columella and the distal extracolumella in *Varanus salvator*. (A) Scanning electron micrograph of the interior of the middle ear showing the columella (C) and extracolumella (E) coursing over (and bound to) the surface of the quadrate (Q). The distal end of the extracolumella expands to contact the tympanic membrane (T) and become integrated into the post-tympanic band (B). The location and orientation of the columella/extracolumella joint is indicated by the red line. (B) Frontal section through the joint between the columella (C) and the extracolumella (E); note the presence of a distinct joint cavity and the suggestion of a synovial membrane.

from the ventral). The remainder of the extracolumellar attachment extends caudad from this central point deflecting some 20° dorsally (relative to the dorsum of the head). Along the length of the extracolumellar interaction with the inner surface of the tympanum, the varying forms of contact (see below) are evident by differences in color and texture; the two ends of the contact (the pars inferior cranially and the pars superior caudally) are generally distinct from the middle segment (Fig. 1A).

In conscious and anesthetized *V. salvator*, the contact point between pars inferior and the inner surface of the tympanum extends laterally further than any other point of the membrane, creating a “bulge” in the center of the tympanum and radiating corrugations on the surface of the membrane (Fig. 1A,B). Looking internally through the tympanum, the smooth arching surface of

the quadrate can be seen on the cranial and dorsal surfaces (Fig. 1A,B). The quadrate forms the bony support for the cranial, and most of the dorsal surface of the tympanum. The cranioventral portion of the tympanum contacts the lower jaw joint, and the ventral portion of the tympanum is supported by the retroarticular process of the mandible (Fig. 1B). The dorsocaudal portion of the tympanum approaches the squamosal–quadrate joint, while the caudal surface is supported by a connective tissue band (termed the post-tympanic band by Wever, 1978) which integrates with the connective tissue surrounding the depressor mandibulae muscle. Histological analysis of the post-tympanic band revealed it to be composed predominantly of elastin fibers (Fig. 2).

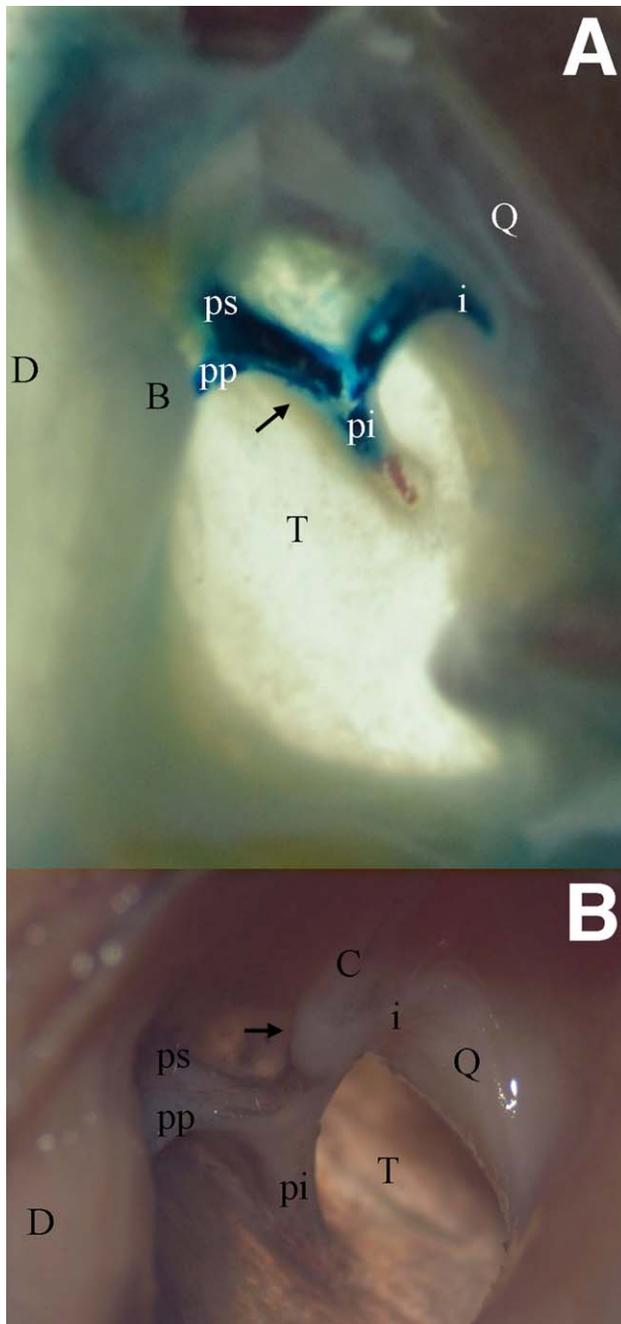
The middle ear of *V. salvator* includes two skeletal elements, the columella and the extracolumella. Proximally, the columella is osseous with an expanded medial footplate which fills the oval window. The distal end of the columella parallels the proximal end of the extracolumella, where they overlap the columella is caudal to the extracolumella, and the two are wrapped by a capsule of connective tissue (Fig. 3A). The distal end of the columella is capped with hyaline cartilage which is more globose than the shaft. The cranial surface of this globose head supports an articular facet (Fig. 3B); this facet is oriented obliquely such that the distal end is more caudal than the proximal end. There is no direct contact between the columella and the (purely cartilaginous) extracolumella in *V. salvator*. The opposing articular surfaces are parallel, with no obvious features on either which would limit joint mobility. The connective tissue surrounding the joint is fairly thin, being more robust on the ventral and dorsal surfaces (Fig. 3B). The inner lining of this joint capsule is a vascularized membrane of loose connective tissue which projects into the joint cavity, resembling a synovial joint (Fig. 3B).

In the same parasagittal plane, the extracolumella gives rise to the large, cranially directed, internal process which approaches the medial surface of the quadrate (Fig. 4A). More distally the shaft of the extracolumella essentially bifurcates. The cranially directed portion extends as the pars inferior ultimately contacting the tympanum near its midpoint (Fig. 4A,B). The caudally directed portion extends for a short distance before it bifurcates; the second bifurcation produces the more dorsal pars superior (Fig. 4A,B) and the more ventral posterior process both of which approach the caudal margin of the tympanum.

The internal process of the extracolumella is bound by a short connective tissue band to the adjacent surface of the quadrate. There is a second connective tissue band anchoring the internal process; Wever (1978) termed this the internal ligament, but it lacks the histological features of a true ligament. This second connective tissue band courses caudally, passing ventral to the columella to span the joint between the quadrate and the squamosal and anchor on the paraoccipital process (Fig. 5). The pars superior of the extracolumella supports the largest connective tissue attachment; this band extends dorsally along the surface of the quadrate, crosses the joint between the quadrate and the squamosal to anchor onto the paraoccipital process at a point adjacent to the band from the internal process (Fig. 5). The connective tissue anchoring the pars superior is much closer in structure (histologically) to a true ligament, and is

roughly 5× the size of the band from the internal process.

The distal portions of the extracolumella contact, or are integrated into, the tympanic membrane. The medial surface of the tympanum extends along and attaches to the pars inferior. There is a prominent dense connective tissue pad located between the lateral surface of the pars inferior and the outer (lateral) surface of the tympanic membrane (Fig. 6). This dense connective pad covers the entire lateral surface of the pars inferior, but is not present in any other portion of the tympanic membrane.



A small (roughly 1.5 mm diameter and 7 mm in length in a 1.5 meter lizard) skeletal muscle, the tympanus, originates near the quadrate/squamosal joint, and courses medially over the depressor mandibulae and the post-tympanic band (Fig. 7A,B). This muscle inserts, in part, onto the caudal surface of the pars superior of the extracolumella (Fig. 7B), but also, by way of a thickened tendon, along the lateral surface of the pars inferior of the extracolumella (Fig. 6).

No clear morphological variation was noted within the middle ear structures of the specimens examined.

**Biomechanical model:** A 3-D reconstruction of the middle ear of *V. salvator* reflects the close contacts between the extracolumella and the quadrate, columella, and even the tympanic membrane (Fig. 8A). Portions of the distal end of the extracolumella are not as evident in the reconstruction because of the close contact between the processes and the tympanic membrane, and, particularly, the lateral bulge in the tympanum which obscures much of the pars inferior (Fig. 8A).

The origin and course of the tympanus is such that contraction of this muscle should cause a medial and caudal displacement of the distal portion of the extracolumella (Fig. 8B). Since the distal portion of the extracolumella is intimately bound to the tympanic membrane, a displacement of the former should alter the tension of the latter. We hypothesize that contraction of the tympanus will produce (1) an increase in tension of the (elastic) post-temporal band, caused by the caudal displacement of the extracolumella; (2) a physical displacement of the tympanic membrane (or at least the central bulge in the membrane) medially; and (3) closer apposition at the columella/extracolumella joint, caused by rotation of the extracolumella about its contact point on the quadrate.

In fresh or preserved specimens of *V. salvator*, tension applied to the tympanus with forceps readily displaces the extracolumella.

**Functional analysis:** There was a delay (mean 3.5 ms) between the presentation of the tone stimuli and the vibrational response of the tympanum due to the propagation of the sound over the 95 cm between the source and the lizard's head, and the inertia of the tympanum. The membrane then exhibited an increase in velocity over the resting level (Fig. 9). The fundamental frequency ( $f_0$ ) of the tympanum of *Varanus* is 0.5 kHz

**Fig. 4.** Morphology of the extracolumella in *Varanus salvator*. **(A)** Cleared and stained preparation. The extracolumella first supports a proximal internal process (i), which attaches to the quadrate (Q), then extends laterally before bifurcating to form the pars inferior (pi) and, through a second bifurcation, the pars superior (ps) and pars posterior (pp). The pars inferior and pars superior form the attachment to the tympanic membrane (T), with a pad of connective tissue spanning the gap between them (arrow). The caudal tips of the pars superior and pars posterior integrate into the post-tympanic band (B) which, in this preparation, appears as a dense cranial margin to the depressor mandibulae (D). **(B)** Interior view of a dissection of the middle ear. The columella (C) courses over the medial surface of the quadrate (Q) before forming a joint with the extracolumella (arrow). The extracolumella gives rise to four discrete processes: the internal process (i), which is bound to the quadrate; the pars inferior (pi) which attaches near the middle of the tympanic membrane (T); and the pars superior (ps) and pars posterior (pp) which both integrate into the post-tympanic band located along the cranial margin of the depressor mandibulae (D).

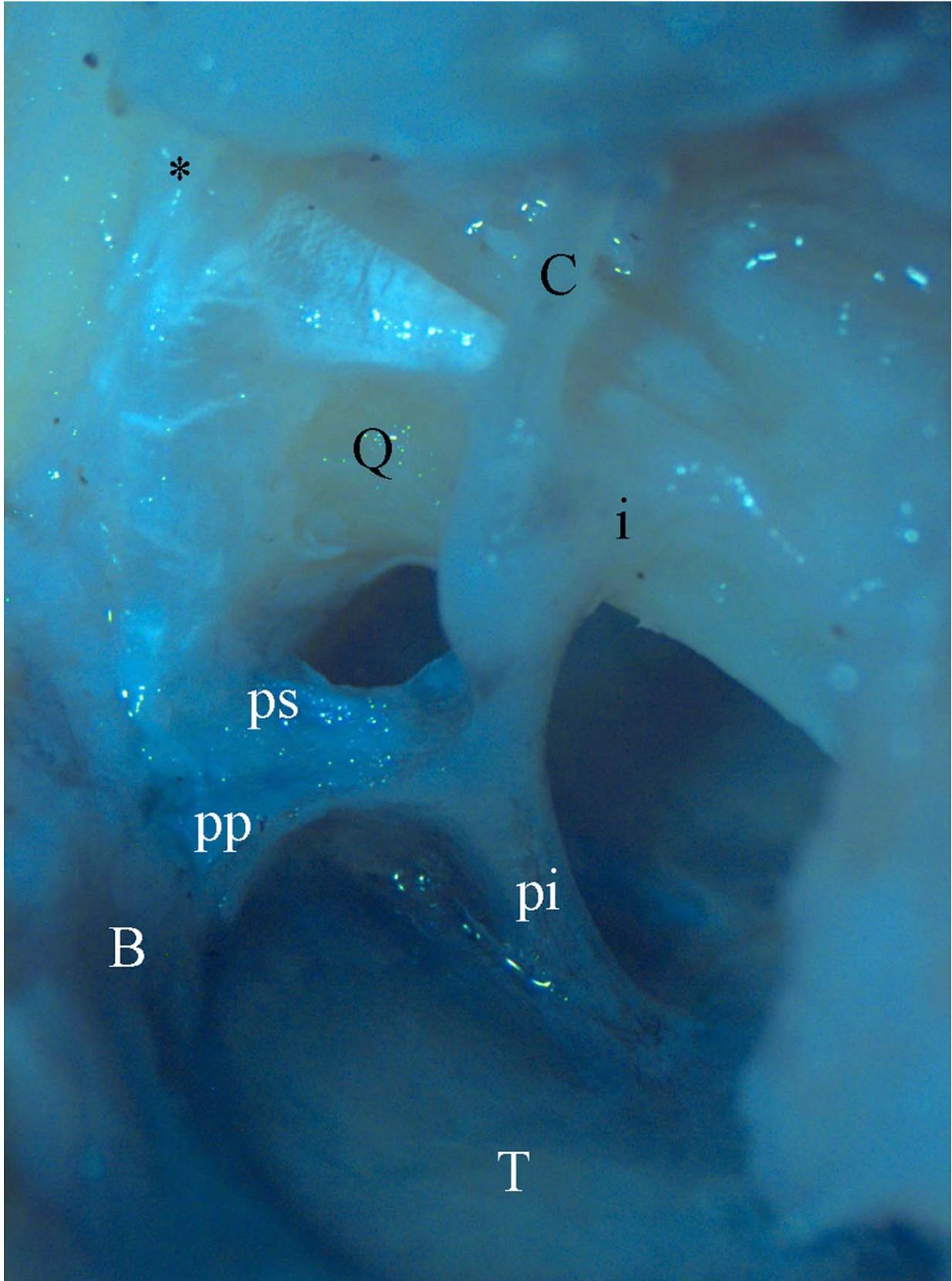


Fig. 5. Dissection of the middle ear of *Varanus salvator* showing the connective tissue attachments of the extracolumella. The internal process (i) gives rise to a connective tissue band that courses under the columella (C) along the surface of the quadrate (Q) toward the

paraoccipital process (\*). A larger ligament runs perpendicular to the first spanning from the paraoccipital process to the pars superior (ps) of the extracolumella. Abbreviations: B, post-tympanic band; pi, pars inferior; pp, posterior process; T, tympanic membrane.

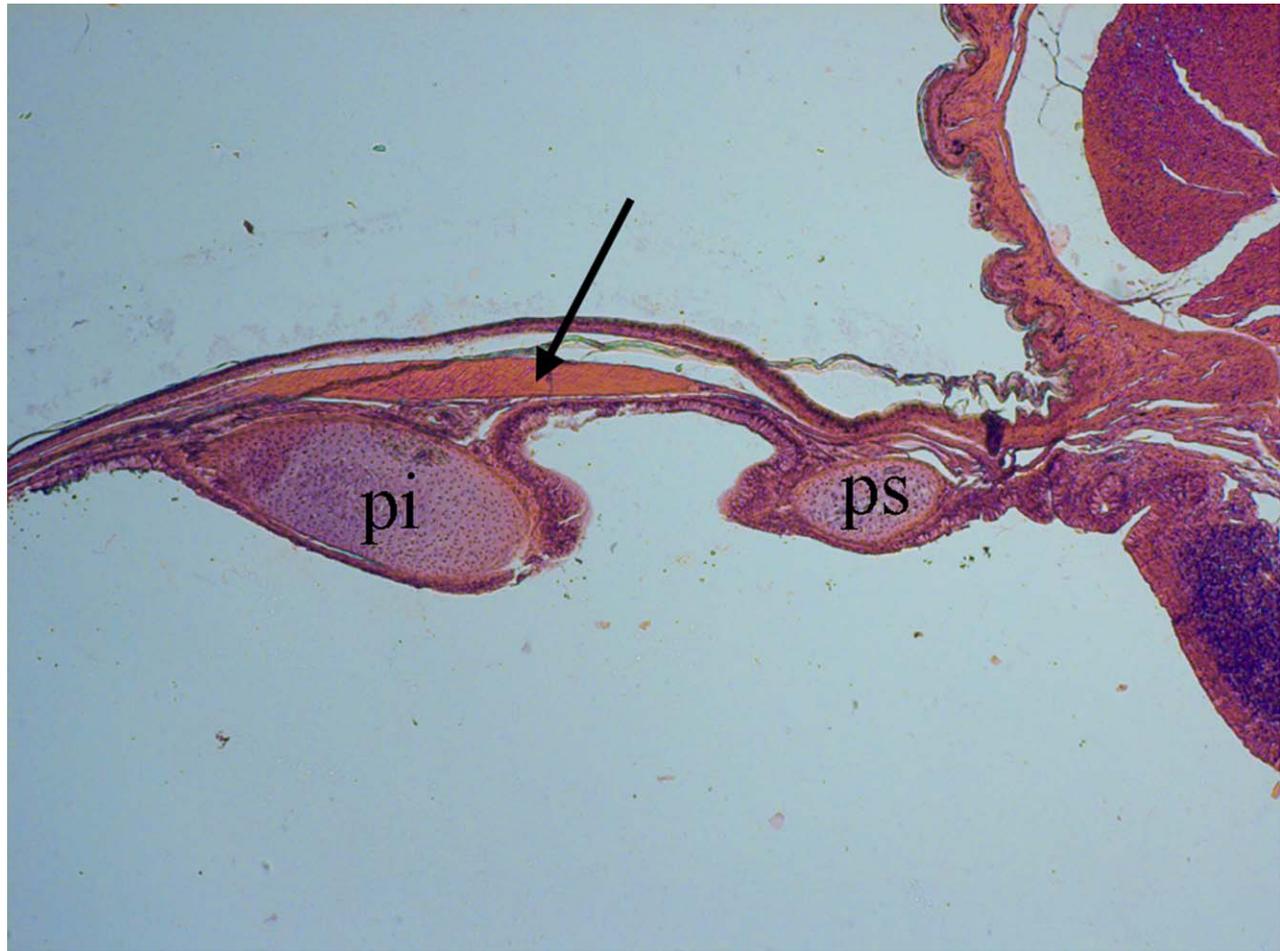


Fig. 6. Frontal section through the caudal half of the tympanic membrane of *Varanus salvator*. Two portions of the extracolumella, the caudal pars superior (ps) and the (larger) cranial pars inferior (pi) are integrated into the tympanum as evident by the inner epithelial surface covering these two cartilage bodies. The tympanum is thickened in this region by a band of connective tissue (arrow) spanning between the two processes of the extracolumella.

(Vedurmudi et al., 2016), so the trial tone produced harmonics and a complex pattern of movement within the tympanum (Fig. 10).

The application of an electrical stimulation to the tympanus (by way of the caudal surface of the tympanic membrane) caused a physical displacement of the tympanum; this displacement is evident in the laser doppler data by a shift in the Y-axis “baseline,” as opposed to the velocity reading which is given by the peak-to-peak height of the signal (Fig. 11). The displacement of the tympanum following stimulation reached a peak in approximately 20 ms, the tympanum then quickly returned to its resting position (Fig. 11). Cessation of the electrical stimulus was associated with a second, smaller shift in membrane position; this second shift was in the opposite direction from the first (Fig. 11).

While the tympanus was contracting, the tympanic membrane produced amplified and irregular velocities in response to the trial tone; these displacements decreased poststimulation (Fig. 12). Quantification of tympanic velocity reveals that presentation of the trial tone was always associated with a significant increase in

tympanic response (ANOVA  $F = 473.9$ ,  $df = 2$ ,  $P < 0.001$ ), and that the contraction of the tympanus significantly increased the membrane’s response to the same trial tone (Tukey’s HSD = 0.49, critical value at  $P = 0.05$  is 0.042) Stimulation of the tympanus results in marked changes in the tympanic membrane waveform, even though the same trial tone was presented at the same intensity and distance (Fig. 10).

## DISCUSSION

Wever (1978) described the ear of *Varanus*, which he considered to be an example of an “Iguanid type” ear. While there is a general agreement between the anatomical description provided herein and that given by Wever (1978), there are several important distinctions. Wever (1978) did not specify the nature of the columella/extracolumella joint in *Varanus*, but did note that in most reptiles, the columella is fused to the extracolumella. In *V. salvator*, there is a clear mobile joint between the two elements (Fig. 3B), the architecture of which suggests

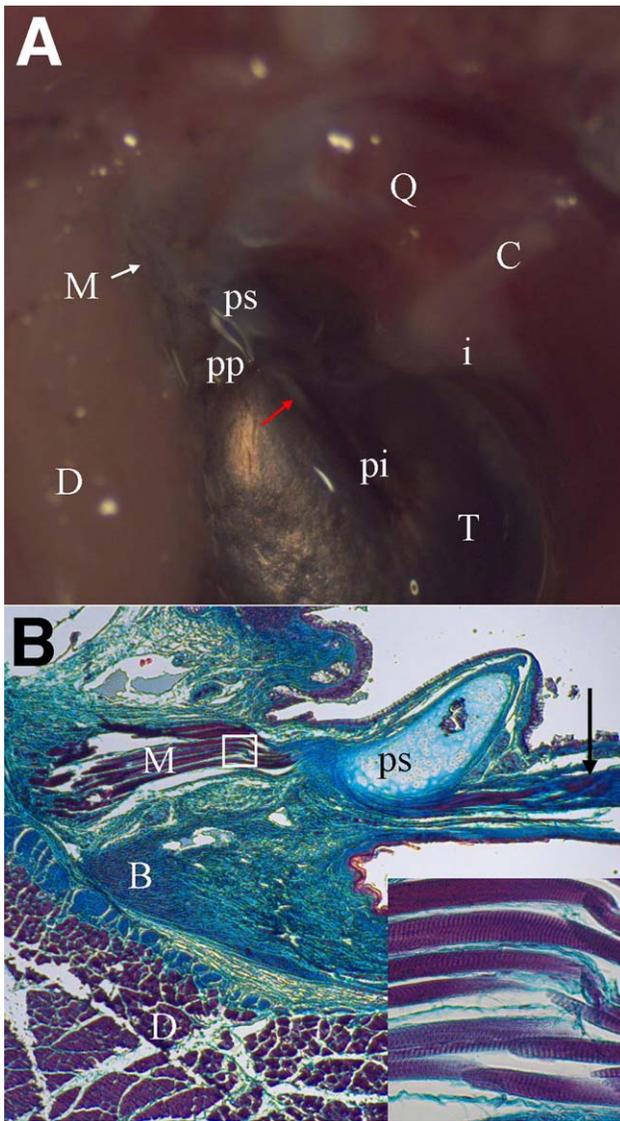


Fig. 7. The tympanus muscle of *Varanus salvator*. (A) Dissection of the middle ear showing the tympanus (M) coursing medial to the depressor mandibulae (D) to insert onto the pars superior (ps) of the extracolumella. The tympanus also gives rise to a tendon which courses on the lateral surface of the pars superior and pars inferior (pi) thickening the tympanic membrane in this region (arrow). (B) Frontal section showing the tympanus (M) deep to the depressor mandibulae (D) and post-tympanic band (B), as well as the attachment to the pars superior (ps) and the tendonous band (arrow). Insert: the staining characteristics and striations clearly identify the tympanus as a skeletal muscle. Abbreviations: C, columella; i, internal process; Q, quadrate; pi, pars inferior; pp, posterior process; T, tympanum.

that the extracolumella can displace in the frontal plane relative to the columella.

Wever (1978) used the term “post-tympanic band” for the tissue that binds the caudal surface of the tympanum to the cranial margin of the depressor mandibulae. Wever (1978) described the composition of this band as “connective tissue,” but did not offer any further detail. In *V. salvator*, this band has a positive response with elastin-specific stains (Fig. 2) and branched elastin fibers

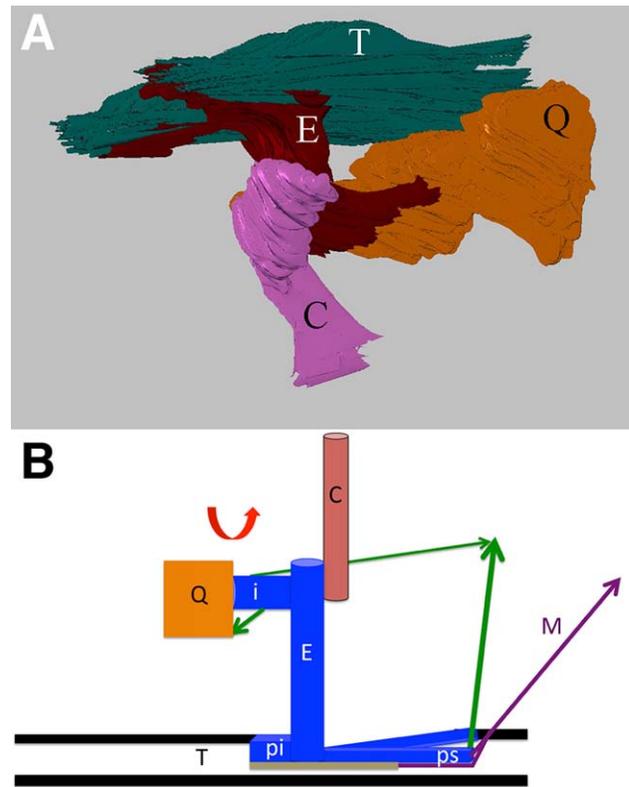


Fig. 8. Biomechanics of the middle ear. (A) 3-D reconstruction (from serial histological sections) of the extracolumella (E) in relation to the tympanic membrane (T), the quadrate (Q) and the columella (C); note that only portions of the tympanic membrane and quadrate are shown. (B) Simplified biomechanical diagram of the extracolumella. Contraction of the tympanus (M) should result in caudal and medial displacement of the extracolumella, as well as rotary displacement (red arrow) of the extracolumella about its internal process (i) attachment to the quadrate (Q). Abbreviations: C, columella; E, extracolumella; pi, pars inferior; ps, pars superior; T, tympanum.

can be clearly identified both on the long axis of the band and perpendicular, forming the interface between the band and the caudal margin of the tympanum. It is unclear how the presence of elastin might influence the vibratory response of the tympanum or transmit tensile forces arising from contraction of the depressor mandibulae.

Versluys (1898) described skeletal muscle within the middle ear of some reptiles, but not *Varanus*. Baird (1970), Henson (1974) and Wever (1978) added notes on other reptilian middle ear muscles (though, again, not in *Varanus*), and Wever and Werner (1970) noted that the functional role of these muscles had never been established. The present contribution represents not only the first description of a middle ear muscle in *Varanus*, but also the first experimental analysis of the function of the middle ear muscle of any reptile.

The course of the tympanus passes deep, and nearly perpendicular to the elastic post-tympanic band. The insertion of tympanus on the caudal and lateral surfaces of the extracolumella is such that contraction of this muscle would induce rotation of the extracolumella in the frontal plane with the distal end moving caudally

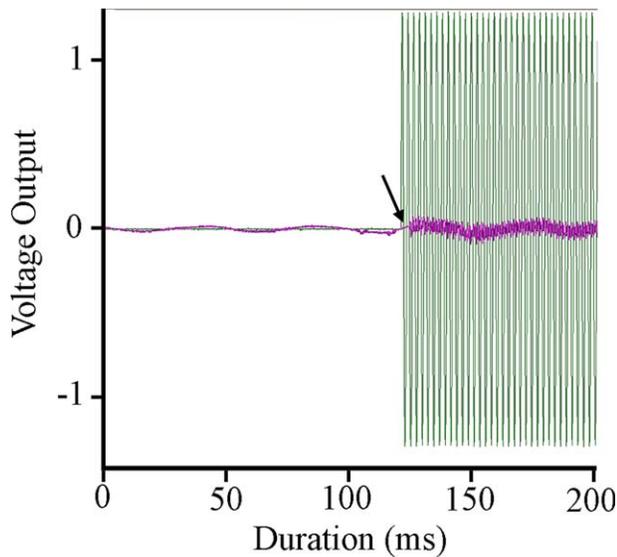


Fig. 9. Raw laser doppler vibrometry data showing the impact of a 500 Hz trial tone (green trace) on tympanic membrane vibrational velocity (magenta trace). After a short (mean 3.5 ms) latency period (arrow), the presented sound waves induce a significant increase in tympanic velocity.

(Fig. 8). This rotation presumably occurs about a pivot point formed by the contact between the internal process of the extracolumella and the quadrate. This rotation would cause the superior and posterior processes of the extracolumella to displace the post-tympanic band caudally, presumably stretching those elastin fibers oriented along the long axis of the band.

The rotation of the extracolumella about the contact between the internal process and the quadrate is distinct from the center of rotation of the extracolumella produced by vibrational displacement of the tympanic membrane. Wever and Werner (1970) determined that vibrational displacement in *Crotaphytus* acts about an axis of rotation located near the caudal tip of the pars superior. The rotation of the extracolumella on the quadrate would be limited by both the short connective tissue band spanning this joint, as well as the much longer internal ligament (Fig. 5). The largest connective tissue band in the middle ear, and the only true ligament, nearly parallels the course of the shorter tympanus muscle. This ligament presumably functions to limit excessive lateral displacement of the tympanic membrane as well as excessive stretching of the tympanus.

Given the small scale of displacement likely to result from contraction of the tympanus, laser doppler vibrometry was used to explore this system. Previous descriptions of the tympanic membrane of reptiles (e.g., Versluys, 1898; Wever, 1978) have never described muscle tissue (smooth or striated) within the membrane, nor were any intrinsic muscle fibers observed in *V. salvator*. The stimulating electrodes were placed on the superior and inferior surfaces of the caudal end of the extracolumella. In this position, the probe was adjacent to the tympanus, but separated from the depressor mandibulae by the post-tympanic band. Electrical stimulation resulted in displacement of the tympanic membrane (Fig. 12). The pattern of displacement is suggestive of a

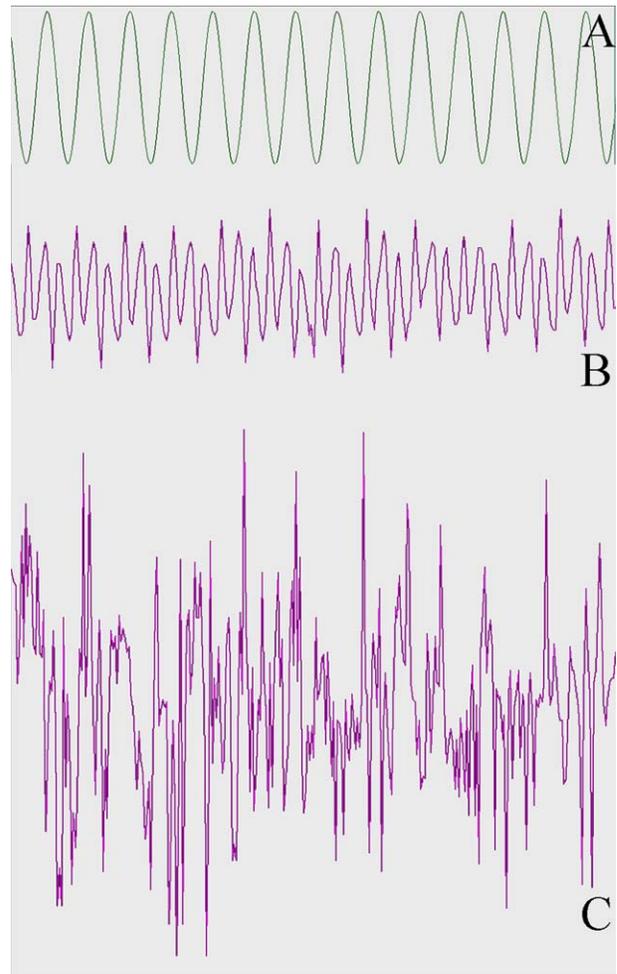


Fig. 10. Raw laser doppler vibrometry data traces showing the waveform of tympanic velocity. (A) The 500 Hz trial tone, this was a steady-state tone with no amplitude or frequency modulation. (B) The waveform of tympanic response without muscle stimulation, note the presence of multiple harmonics within the waveform. (C) The waveform of tympanic response during muscle stimulation. The waveform becomes more chaotic, and is amplified. All three traces have a duration of 30 ms, and the scale of the Y-axes are the same in the B and C traces; the increased peak-to-peak height in trace C reflects an increase in tympanic vibrational velocity.

combination of muscle tension and elastic recoil (Fig. 12), but there is no direct evidence of the latter.

Contraction of the tympanus has a significant influence on the vibratory response of the tympanic membrane. Presentation of a 500 Hz tone coupled with stimulation of the tympanus results in a significant increase in tympanic vibration compared to the trial tone alone. Furthermore, the waveform pattern of tympanic vibration changes following contraction of the tympanus (Fig. 12).

Since these functional changes in the tympanus resulted from the contraction of the tympanus, which is a skeletal muscle (Fig. 7), this is interpreted as an active form of tympanic modulation. Though other possible mechanisms exist (Young, in press), this is the first report of a tuning system in reptiles which is based on modulation of the middle ear. Gridi-Papp et al. (2008)

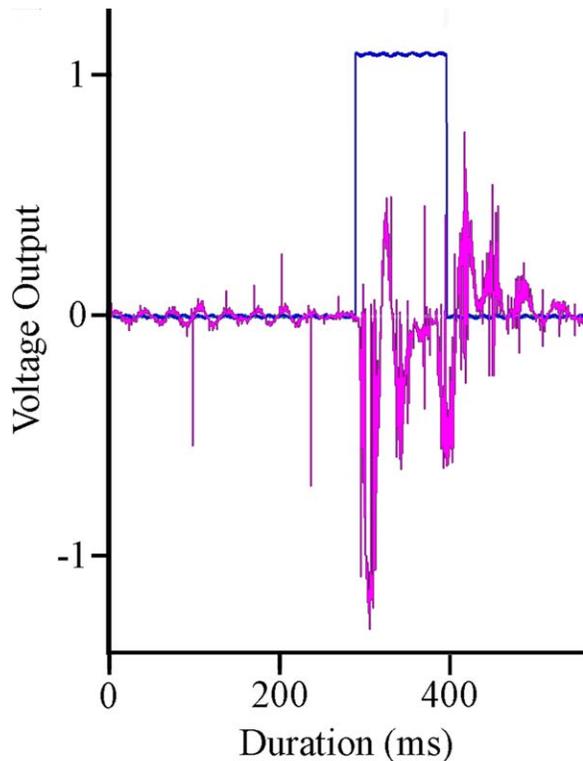


Fig. 11. Raw laser doppler vibrometry data showing the influence of muscle stimulation (blue trace) on tympanic membrane vibrational velocity (magenta trace). These traces were recorded without the trial stimulus tone, and demonstrate that stimulation of the tympanus results in physical displacement of the tympanic membrane. The repeated movements seen during a single stimulus, and their opposite direction, are suggestive of elastic recoil from the post-tympanic band.

described an anuran system in which a skeletal muscle “sphincter” was capable of actively modulating the patency of the Eustachian tube, thereby regulating the performance of the ear.

In *Varanus*, the contralateral tympana are anatomically linked through the skull, forming what is called an internally coupled ear. A recent biophysical model of internally coupled ears (Vedurmudi et al., 2016) demonstrated that these systems produce unique temporal and amplitude cues which are frequency-dependent. More precisely, the relative magnitude of these cues depends on the relationship of the stimulus frequency to the fundamental frequency of the tympanum (Vedurmudi et al., 2016). The fundamental frequency of the tympanum is directly related to tympanic tension; contraction of the tympanus would alter tympanic tension and, in this way, shift the relationship between the stimulus frequency and the resulting neural cues.

Wever and Werner (1970) discussed the factors influencing impedance matching in the reptilian ear (impedance matching is a particular challenge for an amphibious reptile given the marked differences between water and air). Their analysis centered on two anatomical factors, the effective area of the tympanic membrane and the transformer function. The transformer function arises by the difference in area between

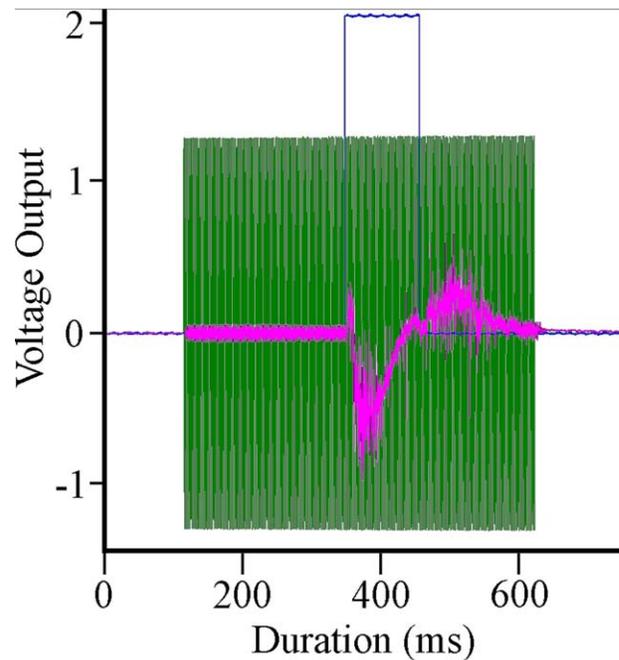


Fig. 12. Raw laser doppler vibrometry data showing the influence of muscle stimulation (blue trace) on tympanic membrane vibrational velocity (magenta trace) in the presence of the trial 500 Hz tone (green trace). Stimulation of the tympanus results in a significant increase in the velocity of the tympanic membrane.

the tympanic membrane and the columellar footplate (see also Müller and Tsuji, 2007), but also must include the efficiency of vibration transmission between the two ends. Since the margins of the tympanum are fixed, there will always be a pattern of differential vibratory response at different spots of the tympanum; the effective area excludes the (relatively) nonvibratory portions. Contraction of the tympanus could potentially alter both factors contributing to impedance matching. By shifting the extracolumella, and with it the attached portions of the tympanum, the tympanus is likely altering the effective area of the tympanic membrane. This displacement of the extracolumella is also hypothesized to increase the apposition at the columella/extracolumella joint, potentially altering the efficacy of vibration transfer across this joint. In this way, the tympanus may be a specialization that allows *V. salvator* to modulate the impedance matching of its middle ear to maximize the utility of the auditory system in both the terrestrial and aquatic environments.

Monitor lizards can voluntarily expand their pharyngeal space ventrally forming a “gular pouch.” Expansion of the gular is used as a visual display for both inter- and intraspecific encounters (Murphy and Mitchell, 1974; Murphy and Lamoreaux, 1978), and it can also be used as an additional means of ventilator airflow (Owerkovicz et al., 1999). Alterations in the size of the pharyngeal cavity, which are under active control, are associated with changes in intrapharyngeal pressure (Owerkovicz et al., 2001). Since *Varanus* has internally coupled ears, any change in pharyngeal pressure would alter the pressure balance at the tympanic membrane.

The tympanus muscle could function in at least three, nonmutually exclusive ways: to actively regulate auditory tuning, to control impedance (pressure) matching during transitions between land and water, or to compensate for changes in intrapharyngeal pressure. Direct experiment evidence of the contractile state of this muscle (e.g., through sonomicrometry or electromyography) will be difficult to obtain given its anatomical location and small size. Other experimental approaches may be needed to test these functional hypotheses.

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### LITERATURE CITED

- Baird IL. 1970. The anatomy of the reptile ear. In: Gans C, Parsons TS, editors. *Biology of the reptilia* (Vol. 2). New York: Academic Press. p 193–275.
- Bierman HS, Thornton JL, Jones HC, Koka K, Young BA, Brandt C, Christensen-Dalsgaard J, Carr CE, Tollin DJ. 2014. Biophysics of directional hearing in the American alligator (*Alligator mississippiensis*). *J Exp Biol* 217:1094–1107.
- Christensen-Dalsgaard J, Manley GA. 2005. Directionality of the lizard ear. *J Exp Biol* 208:1209–1217.
- Christensen-Dalsgaard J, Manley GA. 2008. Acoustical coupling of the lizard eardrum. *J Assoc Res Otolaryngol* 9:407–416.
- Christensen-Dalsgaard J, Brandt C, Willis KL, Christensen CB, Ketten D, Edds-Walton P, Fay RR, Madsen PT, Carr CE. 2012. Specialization for underwater hearing by the tympanic middle ear of the turtle, *Trachemys scripta elegans*. *Proc Roy Soc B* 279: 2816–2824.
- Gridi-Papp M, Feng AS, Shen J, Yu ZL, Rosowski J, Narins P. 2008. Active control of ultrasonic hearing in frogs. *Proc Nat Acad Sci (USA)* 105:11014–11019.
- Henson OW. 1974. Comparative anatomy of the middle ear. In: Keidel WD, Neff WD, editors. *Handbook of sensory physiology*, Vol. 1. New York: Springer. p 39–110.
- Hetherington T. 2008. Comparative anatomy and function of hearing in aquatic amphibians, reptiles, and birds. In: Thewissen JGM, Nummela S, editors. *Sensory evolution on the threshold: adaptations in secondarily aquatic vertebrates*. Berkeley: University of California Press. p 183–209.
- Higgs DM, Brittan-Powell E, Soares D, Souza M, Carr CE, Dooling RJ, Popper AN. 2002. Amphibious auditory responses of the American alligator (*Alligator mississippiensis*). *J Comp Physiol* a 188:217–223.
- Müller J, Tsuji L. 2007. Impedance-matching hearing in Paleozoic reptiles: evidence of advanced sensory perception at an early stage of amniote evolution. *PLOS One* DOI: 10.1371/journal.pone.0000889.
- Murphy J, Lamoreaux W. 1978. Threatening behavior in Merten's water monitor *Varanus mertensi* (Sauria: Varanidae). *Herpetologica* 34:202–205.
- Murphy J, Mitchell L. 1974. Ritualized combat behavior of the pygmy mulga monitor lizard, *Varanus gilleni* (Sauria: Varanidae). *Herpetologica* 30:90–97.
- Owerkowicz T, Farmer C, Hicks J, Brainerd E. 1999. Contribution of gular pumping to lung ventilation in monitor lizards. *Science* 284:1661–1663.
- Owerkowicz T, Brainerd E, Carrier D. 2001. Electromyographic pattern of the gular pump in monitor lizards. *Bull Mus Comp Zool* 156:237–248.
- Silman S, Counter E, Rosler G. 1984. Theories of middle-ear muscle function. In: Silman S, editor. *The acoustic reflex: basic principles and clinical applications*. New York: Academic Press. p 63–99.
- Stanner M. 2010. Mammal-like feeding behavior of *Varanus salvator* and its conservational implications. *Biawak* 4:128–131.
- Taylor W, van Dyke G. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* 9:107–119.
- Vedurmudi A, Goulet J, Christensen-Dalsgaard J, Young BA, Williams R, van Hemmen JL. 2016. How internally coupled ears generate temporal and amplitude cues for sound localization. *Phys Rev Lett* 116:028101.
- Versluys J. 1898. Die mittlere und äussere Ohrsphäre der Lacertilia und Rhynchocephalia. *Zool Jahrb Abt Anat* 12:161–406.
- Vossen C, Christensen-Dalsgaard J, van Hemmen JL. 2010. Analytical model of internally coupled ears. *J Acoust Soc Am* 128:909–918.
- Wever EG. 1973. The function of the middle ear in lizards: divergent types. *J Exp Zool* 184:97–126.
- Wever EG. 1978. *The reptile ear: its structure and function*. Princeton: Princeton University Press.
- Wever EG, Werner YL. 1970. The function of the middle ear in lizards: *Crotaphytus collaris* (Iguanidae). *J Exp Zool* 175:327–342.
- Young BA. Anatomical influences on internally-coupled ears in reptiles. *Biol Cyber* (in press).