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RESEARCH ARTICLE

On the median pharyngeal valve of the American alligator (*Alligator mississippiensis*)

Bruce A. Young[1] | **Hilary S. Bierman**[2]

[1]Department of Anatomy, Kirksville College of Osteopathic Medicine, A.T. Still University, Kirksville, Missouri

[2]Department of Biology, University of Maryland, College Park, Maryland

Correspondence
Bruce A. Young, Department of Anatomy, Kirksville College of Osteopathic Medicine, A.T. Still University, Kirksville MO, 63501.
Email: byoung@atsu.edu

Abstract

The middle ear cavities of crocodilians have complex connections with the pharyngeal lumen, including lateral and median components which both open into a single chamber located on the dorsal midline of the pharynx. This chamber and the surrounding soft-tissue is herein termed the median pharyngeal valve. In the American alligator (*Alligator mississippiensis*) this valve opens, for a duration of 0.3 s, approximately every 120 s; the patency of the median pharyngeal valve was not influenced by either auditory stimuli or by submersing the alligator underwater. The median pharyngeal valve has an outer capsule of dense connective tissue and fibrocartilage and an inner "plug" of loose connective tissue. These opposing surfaces are lined by respiratory epithelium and separated by a cavity that is continuous with the middle ear cavities and the pharyngeal lumen (through a central opening in the capsule termed the pore). The inner plug of the median pharyngeal valve is contacted by skeletal muscles positioned to serve as both elevators/retractors (which would open the valve) and elevators/protractors (which, in conjunction with gravity, would close the valve). Unlike other vertebrate valve systems, the median pharyngeal valve appears to function as a deformable ball check valve.

KEYWORDS

audition, crocodilian, Eustachian, middle ear

1 | INTRODUCTION

The great British anatomist, Sir Richard Owen, had a penchant for engaging in priority disputes with his colleagues and/or rivals (e.g., Camardi, 2001; Dawson, 2012). In one such example (Owen, 1850) he disparaged the earlier work of Cuvier and his colleagues (Cuvier, 1824), while describing some of the foramina in the ventral surface of the crocodilian skull. The principle subject of Owen's, 1850 contribution was the nature of the multiple, interconnected, crocodilian "Eustachian tubes." Though there are discrepancies, subsequent descriptions based on dissection of crocodilian skulls (e.g., Hasse, 1873; Miall, 1878; Hoffman, 1890; Colbert, 1946) have supported Owen's interpretation. The application of CT-technology has further clarified the complex set of chambers and bony canals that interconnect the middle ears and pharynx of crocodilians (e.g., Witmer, Ridgely, Dufeau, & Semones, 2008; Kley et al., 2010; Bona, Degrange, & Fernandez, 2012; Dufeau & Witmer, 2015).

Colbert (1946) chided the "incomplete" descriptions offered by Miall (1878) and Hoffman (1890); yet Colbert's (1946) contribution, like virtually all of the works that preceded and followed it, left out a key feature of this system. Understandably, given that most of these studies were based on dried skulls, few studies have noted that the Eustachian tube is separated from the crocodile's pharynx by what Owen (p. 523) described as a, "valvular membranous prominence." Miall (1878) repeated Owen's description, while Dufeau and Witmer (2015) describe it as a "plug of connective tissue." To date, no detailed description of this structure has been offered. For the purpose of this contribution, we will refer to this largely overlooked structure as the median pharyngeal valve (mv).

The developmental origins of the median pharyngeal valve reflect the unique nature of this structure. Owen (1850) noted that in embryonic crocodilians the middle ear cavities had extensive continuity with the pharynx. Van Beneden (1880) described the closure of this broad continuity, and the development of diverticula off of the Eustachian system. Simonetta (1956) detailed how the interconnections between the middle ear cavity and pharynx of the crocodile are a confluence of two different embryonic components. This duality was recently treated by Dufeau and Witmer (2015) who stated that (p. 5), "These two passages (pharyngotympanic and median pharyngeal) reveal two discrete, but ultimately confluent pneumatic invasions into the braincase." As Dufeau and Witmer (2015) note, the pharyngotympanic tubes do not actually reach the surface of the pharynx, but rather terminate into the median pharyngeal tube which is an embryonic remnant of the migration of Rathke's Pouch. This dual origin suggests that the median pharyngeal valve is not homologous to the Eustachian valves found in other vertebrates (e.g., Yang, 1985; Saiff, 1988; Su, 1995).

Assuming that the median pharyngeal valve forms a complete seal isolating the middle ear cavities, and all the associated canals, from the pharynx, what would be the functional role of such a valve? Owen (1850) argued that continuity between the nasal, pharyngeal, and Eustachian cavities of the crocodile would enable the crocodile to hear airborne sounds while floating underwater with only the external nares above the water surface; in this scenario the median pharyngeal valve would regulate a complex narial auditory tube. Welman (2000) misapplied Charles' Law to argue that the midline Eustachian tube served to cool the brain of early dinosaurs; in this scheme the median pharyngeal valve would have a thermoregulatory function. Colbert (1946) rejected Owen's functional hypothesis for the complex Eustachian tubes; this hypothesis was later rejected experimentally (Bierman et al., 2014). Colbert (1946) favored otic pressure regulation and noted that the anatomy would allow a "direct equalization of pressures between the ears." This is akin to what is now referred to as internally coupled ears (e.g., Carr, Christensen-Dalsgaard, & Bierman, 2016; van Hemmen, Christensen-Dalsgaard, Carr, & Narins, 2016; Vedurmudi et al., 2016). As internally coupled ears work through the propagation of pressure waves between the middle ear cavities, opening of the median pharyngeal valve could effectively "un-couple" the ears; in this way the median pharyngeal valve would function analogous to the Eustachian sphincter muscle recently described in a Ranid frog (Gridi-Papp et al., 2008). This contribution will explore the possible functional role of the median pharyngeal valve in *Alligator mississippiensis.*

2 | MATERIALS AND METHODS

2.1 | Live animals

Five live sub-adult (145–185 cm total length) American alligators (*Alligator mississippiensis)* (Daudin, 1802) were obtained from the Louisiana Department of Wildlife and Fisheries. The animals were housed communally in a 29 m^2 facility that featured three submerging ponds, natural light, and artificial lights on a 12:12 cycle. The facility was maintained at 30–33 °C, warm water rain showers were provided every 30 min which helped to maintain the facility at >75% relative humidity. The alligators were maintained on a diet of previously frozen adult rats. The husbandry and use of the live alligators followed all applicable federal guidelines, and was approved by the IACUC of A.T. Still University (Protocol #208, approved July 19, 2017).

2.2 | Median pharyngeal valve function

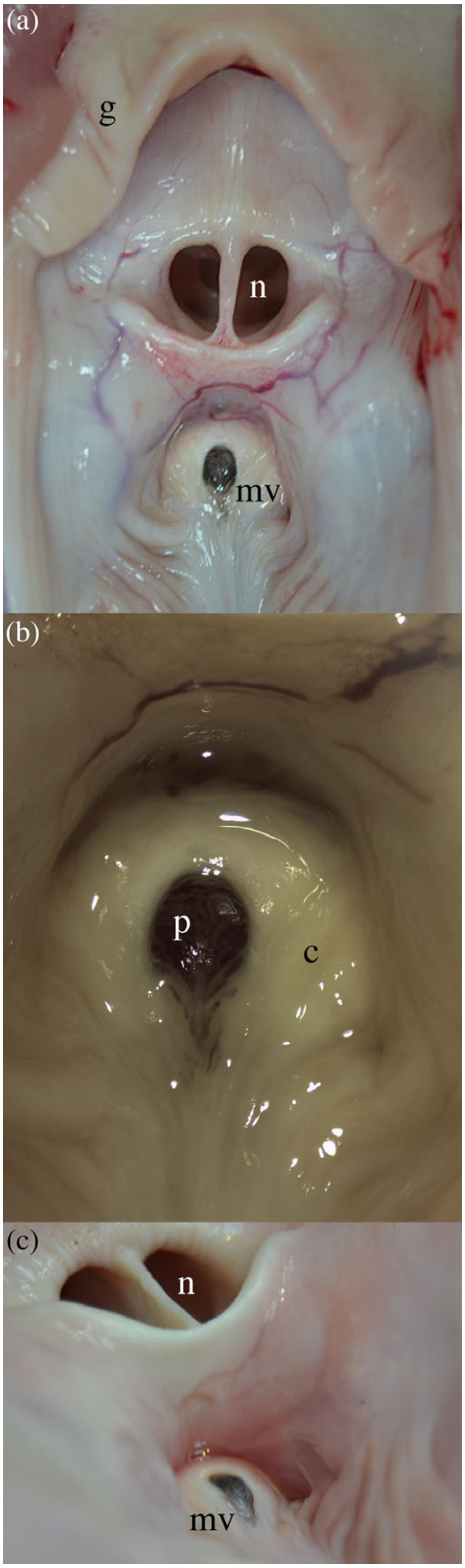
An Individual alligator was noosed and allowed to bite a 30 cm long segment of 5 cm diameter PVC pipe. The specimen's mouth was bound closed around the pipe using 5 cm wide vinyl tape; the same tape was used to hold the limbs in a retracted position against the body. The alligator was then placed on a steel plate (100 × 20 cm) and secured using 2.5 cm wide heavy duty straps (Northwest Tarp and Canvas; Bellingham, WA); the straps held the head and trunk of the alligator to the steel plate; the tail freely projected. A portable digital endoscope (NTS150RS, Teslong; Shenzhen, China) was passed through the PVC pipe, between the halves of the gular valve (Putterill and Soley, 2006), and into the pharynx adjacent to (but not in physical contact with) the median pharyngeal valve. The diameter of the endoscope (5 mm) relative to the width of the gular valve (approximately 75 mm), coupled with the smooth cylindrical shape of the endoscope, allowed for passage of the endoscope with minimal, if any, disruption of the gular valve "seal."

The steel plate, with the alligator and endoscope in place, was placed atop a 112 cm diameter × 61 cm deep tank filled with water at 27–29 °C. The tank was big enough that by displacing the alligator's tail to the side the animal could be submersed deep enough in the water to cover the ears and eyes, but leaving the external nares above the water surface. Auditory stimuli consisted of 0.5 s of pure tones at 0.2, 0.5, or 2.0 kHz, as well as 0.5 s of white noise; auditory stimuli were generated using Tone Generator software (NCH Software, Greenwood Village, CO). Three different trials were performed. (a) Airborne trials in which the stimuli were presented 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 tympanic membrane, and at a distance of 70 cm. For the airborne trials the pressure levels of the trial stimuli were held constant at 70 dB (SPL); the pressure level was verified using a digital sound meter (WS1361, Wensn, Shenzhen City, China) and a calibrated USB microphone (UMIK-1, MiniDSP, Hong Kong, China). (b) Airborne/submersed trials in which the stimuli were presented as described above but the alligator was submersed in the water. (c) Submersed trials in which the specimen was submersed in the water and the stimuli were presented using a UW-30 underwater speaker (Electro-Voice, Fairport, NY) located approximately 20 cm from the tympanum of the alligator. The same stimuli tones were presented during the submersed trials; the sounds were audible above the water tank, but the equivalent underwater pressure level of the sounds could not be quantified.

Endoscopic video was captured when the animals were first secured to the steel plate, sporadically during a subsequent 30 min acclimation period, and during the trials. Airborne trials were always performed first (to avoid complications of water retention in the meatal cavity (Higgs et al., 2002; Montefeltro, Andrade and Larsson, 2016); the order of the airborne/submersed and submersed trials was randomized. Four videos were taken during each trial, and the data were pooled across the five alligators to give 20 videos for each trial condition.

2.3 | Morphology

The bony and soft tissues surrounding the median pharyngeal valve were excised from two of the specimens, and preserved in chilled neutral-buffered formalin (NBF) for 72 hr. The excised blocks were decalcified in RDO Rapid Decalcifier (Apex Engineering Products, Aurora, IL) for 24 hr prior to dehydration and paraffin embedding. Transverse and parasagittal serial sections were cut at 10 μm; mounted sections were stained with either hematoxylin and eosin, or Masson's trichrome stain (following Luna, 1968). Microscopic anatomy was documented using a DM 4000B microscope (Leica Microsystems Inc., Buffalo Grove, IL). The complete serial sections were digitally photographed, then 3-D reconstructions were performed using the BioVis3D software system (BioVis3D, Montevideo, Uruguay).



Dissections were performed on the remaining three specimens, as well as two other previously preserved specimens of hatchling *A. mississippiensis* in the private collection of BAY.

3 | RESULTS

3.1 | Morphology

The dorsal surface of the oral cavity and pharynx support three prominent surface features (Figure 1A). Rostrally the large dorsal half of the gular valve functionally separates the oral cavity from the pharynx. The dorsal surface of the pharynx is pierced by the large internal nares, which are bordered caudally by a low ridge. Caudal to the internal nares is the median pharyngeal valve (mv). Superficially the mv is formed by a central pigmented "plug" surrounded by a horse-shoe shaped rim; the incomplete or open portion of the rim is the caudal surface of the mv (Figure 1B). The mv is at a more superior plane than the internal nares, and is surrounded on its cranial and lateral surfaces by vertical walls of pharyngeal epithelium (Figure 1C). The recess in the dorsal surface of the pharynx that houses the mv roughly corresponds in position to the glottal opening on the lower jaw.

The basioccipital (caudal) and basisphenoid (cranial) articulate along a jagged suture that arches cranially (Figure 2). The ventral surface of the basioccipital supports two prominent bony crests. The basioccipital crest is located along the cranial margin of the bone and parallels the arching suture. Intersecting the basioccipital crest along the sagittal midline is the smaller basioccipital ridge which projects toward the occipital condyle for only a short distance before tapering down to the ventral surface of the bone. Where the basioccipital crest and ridge intersect there is a roughly triangular-shaped bony prominence herein termed the basioccipital eminence (Figure 2).

On the ventral surface of the skull the basisphenoid is a small wedge-shaped bone with two perpendicular surfaces. The vertical portion of the bone articulates with the arched caudal surface of the pterygoids; this articulation includes the medial surface of the bony processes of the pterygoid which extend caudally to the basisphenoid/basioccipital articulation (Figure 2). The pterygoids, which form the caudal margin of the internal nares and the caudal end of the palatal surface, are more ventral than the basioccipital. Accordingly, the small horizontal portion of the basisphenoid seems to rest between

FIGURE 1 *Alligator mississippiensis* landmarks on the caudal palate and dorsal pharynx of rostral is toward the top of the Figure. (A) Low magnification showing the relationship of the median pharyngeal valve (mv) in relation to the gular valve (g) and internal nares (n).
(B) Higher magnification of the median pharyngeal valve showing the "horse-shoe" shape of the capsule (c) and the heavily pigmented epithelium of the plug (p) visible at the pore of the capsule.
(C) Oblique view of the median pharyngeal valve (mv) and internal nares (n) showing the different frontal planes of the two landmarks

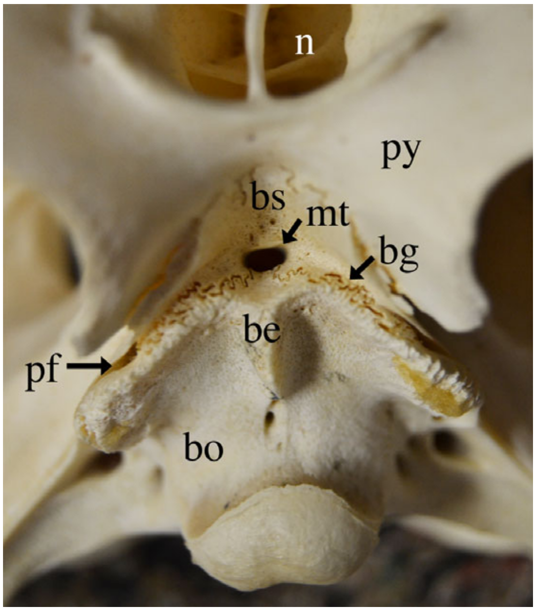


FIGURE 2 *Alligator mississippiensis* caudal surface of the skull. The pterygoid (py) forms the caudal border of the internal nares (n) as well as a vertical border with the basisphenoid (bs). The jagged suture between the basisphenoid and the basioccipital (bo) is pierced by the median pharyngeal tube (mt) at the midline, lateral the suture supports the basisphenoid groove (bg) which extends to the pharyngotympanic foramen (pf). The basioccipital supports a crest in the transverse plane and a ridge in the sagittal plane which intersect to form the basioccipital eminence (be)

two vertical bony arches, the prominent caudal surface of the pterygoids (cranially) and the basioccipital crest (caudally).

The horizontal portion of the basisphenoid incorporates three surface features. Along the sagittal midline, immediately cranial to the basisphenoid/basioccipital suture is the foramen of the median pharyngeal tube (Figure 2). Along the basisphenoid/basioccipital sutures is a shallow groove, herein termed the basisphenoid groove, which terminates at the craniolateral surface of the foramen of the median pharyngeal tube (Figure 2). At the caudolateral end of the basisphenoid groove there is a small foramen (which is effectively a gap in the basisphenoid/basioccipital suture) herein termed the pharyngotympanic foramen.

The epithelium surrounding the mv has a pleated appearance (Figure 1) and supports large lymphatic nodules forming pharyngeal tonsils (Figure 3A). When the epithelium over the mv is removed it exposes the main component of the mv which is a capsule of dense connective tissue and fibrocartilage (Figure 3A). The capsule has a roughened caudal margin, but smooth lateral and cranial surfaces (Figure 3B).

Removing the pharyngeal epithelium exposes two large skeletal muscles located adjacent to the mv. The rectus capitis ventralis courses cranially, diverging slightly laterad to course under the capsule of the mv before inserting on the basioccipital crest (Figure 4A). The rectus capitis ventralis is paralleled by the deeper (more dorsal) longissimus capitis profundus which inserts on the ventral surface of the basioccipital and the base of the basioccipital crest (Figure 4B). There is a strong connective tissue sheath separating the two muscles. Both muscles diverge slightly laterad near their insertion, so neither muscle courses under the sagittal midline of the mv. Neither the rectus capitis ventralis nor the longissimus capitis profundus insert onto the capsule of the mv.

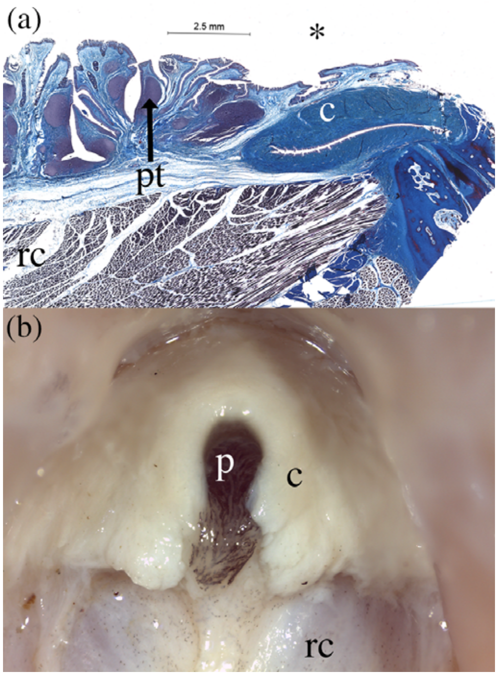


FIGURE 3 *Alligator mississippiensis* superficial features of the median pharyngeal valve. (A) Parasagittal section through the valve illustrating the pharyngeal epithelium with the pharyngeal tonsils (pt) over the rectus capitis ventralis (rc), as well as the dense connective tissue of the valve capsule (c); the pharyngeal lumen is indicated by the asterisk, rostral is to the right of the figure. (B) Ventral (pharyngeal) surface of the valve after the dissection of the pharyngeal epithelium, rostral is to the top of this image. The valve capsule (c) does not extend to the caudal midline (forming the circular pore) and exposing the pigmented epithelium of the valve plug (p); the rectus capitis ventralis (rc) courses dorsal to the valve capsule

The mv consists of two components, the capsule and the plug. The capsule is the superficial portion of the mv, consisting of dense connective tissue and fibrocartilage. The capsule anchors along the lateral portions of the basioccipital crest, and along the arching juncture between the horizontal and vertical portions of the basisphenoid. The capsule is continuous around the cranial and lateral surfaces of the mv; it covers the caudolateral surface, but not the caudomedial surface. On the ventral, superficial surface, of the mv the capsule forms the distinctive horse-shoe shape with an open central pore (Figure 3). Most of the margins of the capsule are continuous with the margins of the deeper, more central, plug of the mv (Figure 5A,B). The continuity between the superficial capsule and the deeper plug results in an enclosed space between the two; this is a real, not a potential space, as evidenced by the lining respiratory epithelium characterized by a ciliated pseudostratified epithelium with abundant goblet cells (Figure 5C). The continuity between the capsule and the plug is broken in three spots. Cranially there is a gap between the capsule and the plug at the foramen of the median pharyngeal tube (Figure 5D); a smaller gap on the lateral surface produces continuity between the basisphenoid groove and the lumen of the mv. The largest gap between the plug and the capsule occurs at the pore of the mv. The

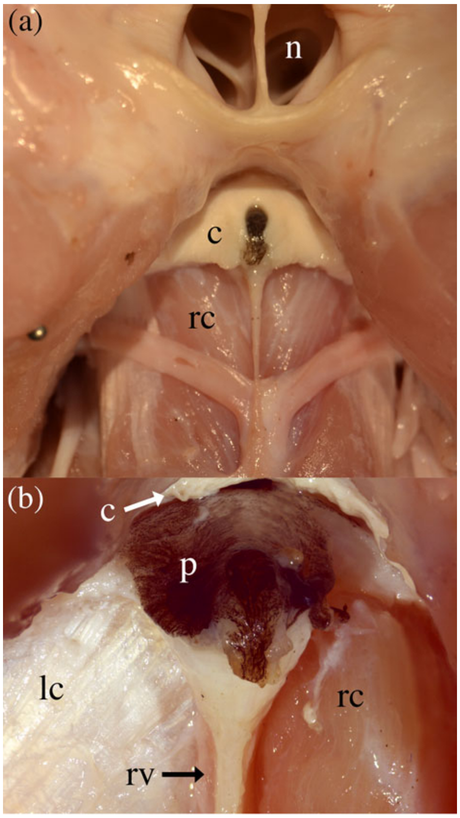


FIGURE 4 *Alligator mississippiensis* pharyngeal view of dissected median pharyngeal valve, rostral is toward the top in this figure. (A) The pharyngeal epithelium has been removed (save one strand on the midline) to expose the rectus capitis ventralis (rc) coursing craniad to the basioccipital crest; note that the muscle deflects laterad as it approaches, and courses dorsal to, the valve capsule (c). (B) Deeper dissection in which the caudal portion of the capsule has been removed to expose the plug (p) and the rectus capitis ventralis (rc) removed from one side to expose the fibrous sheath surrounding the deeper (dorsal) longissimus capitis profundus (lc). Between these paired muscles, originating primarily from the basioccipital ridge is the retractor valvula pharyngeus muscle (rv)

plug is fused to the capsule at the caudolateral margin of the pore, but over the remainder of the perimeter of the pore the two epithelial surfaces are separate. This lack of continuity means that depending on the configuration of the plug (see below) there is potential continuity between the lumen of the mv and pharynx.

The plug of the mv supports a heavily pigmented covering of respiratory epithelium over connective tissue and skeletal muscle (Figure 6A). The plug defines the inner surface of the valve lumen, which is particularly variable along the caudal midline (the pore) where the plug is not covered by the capsule (Figure 6B). The pigmentation is darkest in the region of the pore and is lighter in the cranial portions of the plug. In the region of the mv pore, the plug has a core of loose connective tissue surrounded by a sheath of dense connective tissue. The plug is attached to the basioccipital eminence. The connective tissue sheath surrounding the plug's core of loose connective tissue serves as an attachment site for two skeletal muscles (Figure 6C,D). The retractor valvula pharyngeus (hereafter simply the retractor) forms the majority of the skeletal muscle in the plug and originates along the midline of the basioccipital and the basioccipital ridge; contraction of this muscle would deform the plug and displace the bulk of the plug caudally and dorsally relative to the capsule. The protractor valvula pharyngeus (hereafter simply the protractor) is a paired muscle that originates from the medial portions of the basioccipital crest and the horizontal surface of the basispehenoid; these muscles would deform the plug and displace it dorsally and craniodorsally relative to the capsule.

3.2 | Functional model of the median pharyngeal valve

The plug of the median pharyngeal valve (mv) has as much dorsal–ventral height as the surrounding capsule, so in non-fixed material the plug protrudes into the pore even when the specimen is inverted (Figure 1). There are likely two (transitory) seals that form in the mv; one seal forming between the plug and the margins of the pore. The second seal would form as gravity pulls the plug ventrally against the inner surface of the capsule (Figure 5A); this second seal is likely facilitated by the mucoid secretions covering the opposing surfaces (Figure 5C).

The breaks in the continuity between the plug and the capsule that occur at the median pharyngeal tube (Figure 5D) and the paired basisphenoid grooves (Figure 6B) are continuous with the capsular lumen of the mv. In this way the median pharyngeal tube and pharyngotympanic foramen are continuous at the mv, and, ultimately, continuous with the pharyngeal lumen at the pore of the mv (Figure 7).

The retractor muscle inserts primarily, but not exclusively on the loose connective tissue core of the plug (Figures 6, 8). Displacement of the plug core caudodorsally (the vector line of the retractor) would have three results: (a) the cranial margin of the plug would be pulled away from the overlying inner surface of the capsule, expanding the size of the capsular lumen (Figures 7, 8); (b) the ventral surface of the plug would be elevated and displaced relative to the pore of the capsule, this would produce continuity between the pharyngeal and capsular lumens (Figures 7, 8); and (c) the displaced portion of the plug would shift caudally along the midline where the capsule is not continuous (Figures 7, 8).

The paired protractor muscles would pull the core of the plug cranially. This displacement, coupled with the normal (ventrad) gravitational pull, would re-establish the seal at the pore and against the inner surface of the capsule.

3.3 | Functional analysis

When viewed endoscopically in non-anesthetized alligators, the opening of the mv was easily recognized. During the opening of the mv, the darkly pigmented portion of the plug visible at the pore shifted caudally (Figure 9A). This caudal displacement caused a slight bulging in the caudal portion of the mv (and the adjacent pharyngeal epithelium), exposed a lighter plug epithelium at the pore (Figure 9B), and shifted the plug dorsad relative to the capsule of the mv (Figure 9C). The dorsad shift in the plug (relative to the capsule) more clearly defined the margins of the pore and produced continuity between the lumen of the capsule and the pharyngeal lumen. These motions were all reversed during the closing of the valve (Figure 9D). In the trials

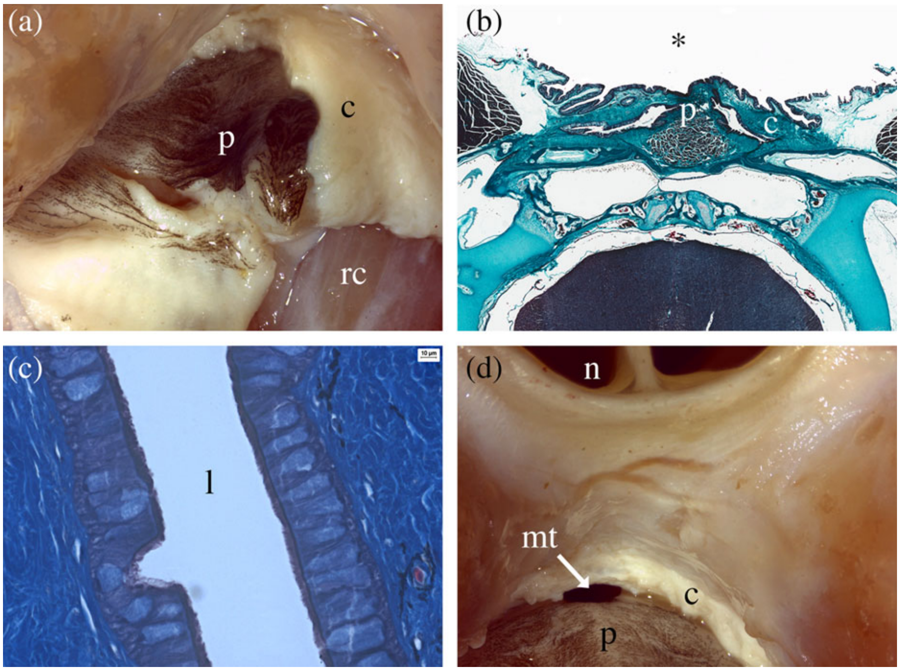


FIGURE 5 *Alligator mississippiensis* morphology of the capsule of the median pharyngeal valve. (A) A portion of the capsule has been excised and reflected to demonstrate the relationship between the superficial (ventral) capsule (c) and the deeper (dorsal) plug (p) of the valve; both structures are superficial (ventral) to the rectus capitis (rc) muscle. (B) Transverse section near the caudal margin of the valve (the pharyngeal lumen is indicated by the asterisk) showing the continuity between the superficial capsule (c) and the deep plug (p) which are fused superficially at this level of the valve. The clear valve lumen can be seen between the capsule and plug, as can the abundance of skeletal muscle within the plug. (C) The lumen (l) of the median pharyngeal valve is lined on both the capsule and plug surfaces by respiratory epithelium. (D) A gap between the capsule (c) and the plug (p) of the median pharyngeal valve allows for continuity between the lumen of the valve and the median pharyngeal tube (mt)

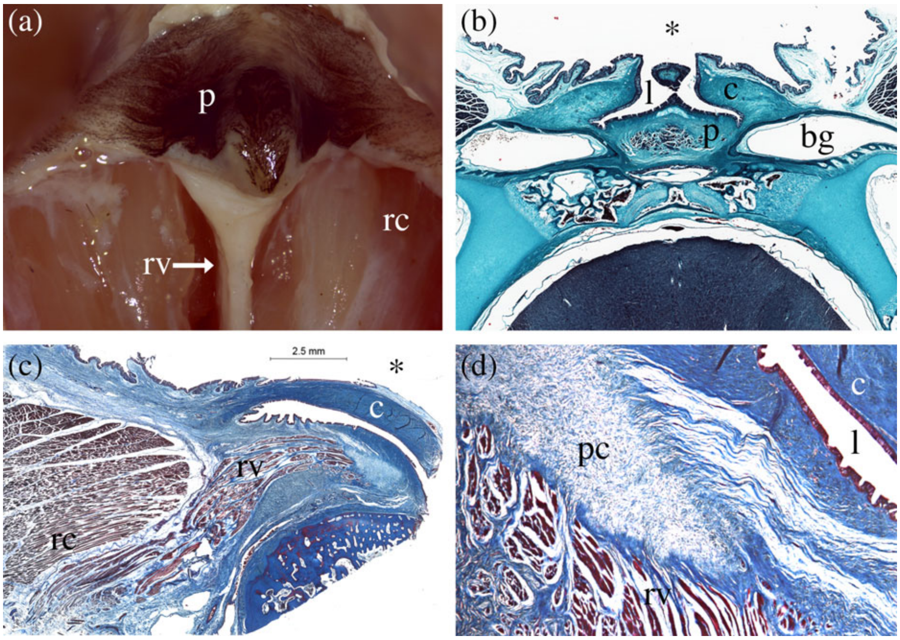


FIGURE 6 *Alligator mississippiensis* morphology of the plug of the median pharyngeal valve. (A) Dissection showing the contours and shape of the plug (p) of the valve. (B) Transverse section through the pore of the valve; histological contraction has caused the plug to move away from the capsule (c) showing continuity between the lumen (l) of the valve and the pharyngeal lumen (*). Near this level the basisphenoid groove (bg) will open into the lumen of the valve. (C) Parasagiital section showing the large rectus capitis ventralis (rc) and the smaller retractor valvula pharyngeus (rv) muscle, the latter coursing into the plug to insert onto the core (pc) of loose connective (asterisk is the pharyngeal lumen). (D) Higher magnification showing the core (pc) of loose connective tissue, the inserting retractor valvula pharyngeus muscles, and the dense connective tissue underlying the respiratory epithelium bordering the valve lumen (l)

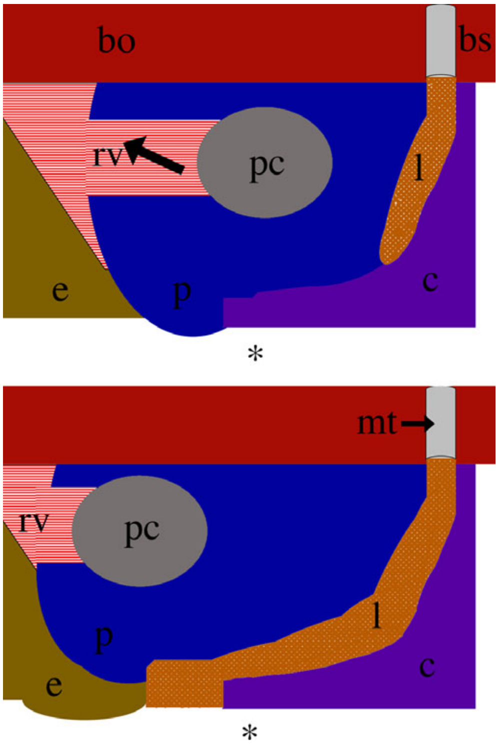


FIGURE 7 Simplified schematic model for the function of the median pharyngeal valve, based on a sagittal section through the valve at the pore. (A) At rest the contours of the plug (p) combined with gravity, ensure a seal forms between the mucoid epithelium of the plug and the mucoid epithelium of the capsule (c) at the pore. The seal prevents the continuity between the median pharyngeal tube (mt) and the lumen (l) of the valve from extending to the pharyngeal lumen (*). Contraction of the retractor valvula pharyngeus (rv) muscle along the line of action (arrow) will open the median pharyngeal valve. (B) The contraction of the retractor valvula pharyngeus (rv) muscle has shifted and deformed the loose connective tissue of the plug core (pc) causing the plug to displace the more caudal pharyngeal epithelium (e) and to shift dorsally away from the pore

conducted the durations between the onset of valve opening to the completion of valve closing were approximately 0.3 s, though this was not examined using high-speed imaging.

The periodicity of valve opening, or the duration between successive valve openings, was quantified under four conditions (control, in air with airborne stimuli, tympana submerged with airborne stimuli, and tympana submerged with aquatic stimuli). The results were quite consistent (Figure 10). During these trials *A. mississippiensis* vented the mv approximately once in every 2 min. The mv was never maintained in a patent state (longer than the patency of the "typical" venting cycle), nor was the mv ever observed to remain closed for longer than 140 s (Figure 10). Using ANOVA the periodicity of mv venting was compared between the three different combinations of environment and stimulus source, no significant differences were found ($F = 0.22$, $df = 2$, $p = .8027$). There was a significant difference in the periodicity of mv venting by auditory stimulus type ($F = 9.35$, $df = 3$, $p < .001$); the periodicity during presentation of the 2.0 kHz tones (Mean 118 s) was longer than that of the other tones (the means of which ranged from 110–116 s). Tukey's post hoc analysis did not support a significant difference in the "response" to the 2.0 kHz auditory stimuli, nor did it support any significant differences between the periodicities of mv venting during presentation of the auditory stimuli and the periodicity recorded without auditory stimuli (Figure 10). Additional informal

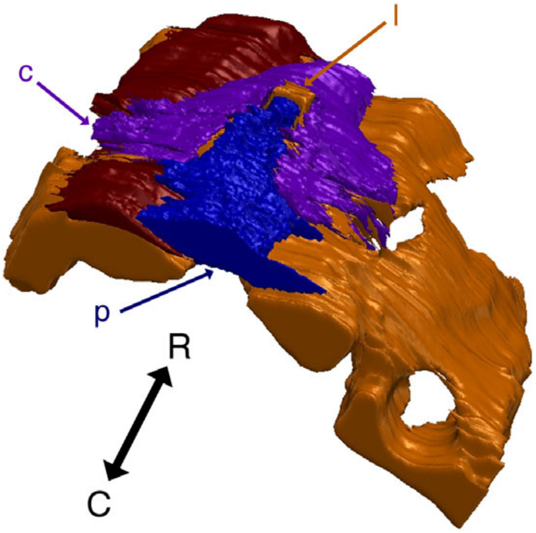


FIGURE 8 3-D reconstruction of the median pharyngeal valve based on serial transverse sections; this is a oblique view of the superficial (ventral) surface (R—Rostral, C—Caudal). In this reconstruction the pharyngeal epithelium superficial to the valve has been omitted, and the relevant bones (basioccipital and basisphenoid, red) only rendered on one side. The distinctive horse-shoe shape of the capsule (c, purple) is evident, as is the plug (p, blue) resting deep to the capsule but extending to the surface at the pore. The lumen of the valve (l, brown) extends between the capsule and the plug, and can be traced dorsally and laterally through the skull bones into the middle ear cavities as the true Eustachian system

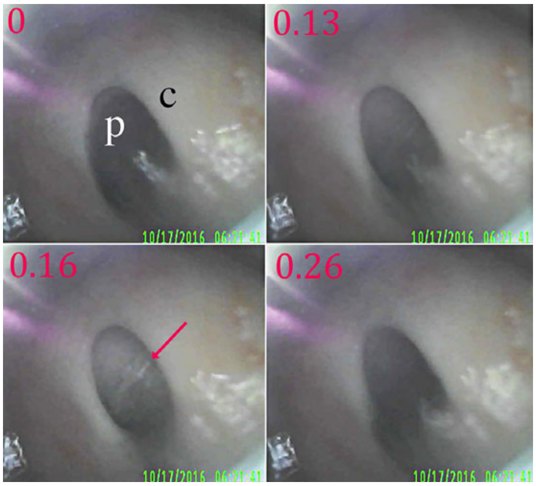


FIGURE 9 Isolated frames from the endoscopic video. These are pharyngeal views of the median pharyngeal valve in which rostral is to the upper left; the horse-shoe shaped capsule (c) is distinct from the darkly pigmented plug (p). The numbers listed are the duration (in seconds) from the first frame. The pigmented epithelium of the plug can be seen shifting (caudally, toward the lower right) forming a ridge (arrow) which both deforms the pharyngeal epithelium and exposes the borders of the pore (and in doing so creates luminal continuity). The last frame (0.26 s from the onset) shows the plug epithelium returning to the closed condition

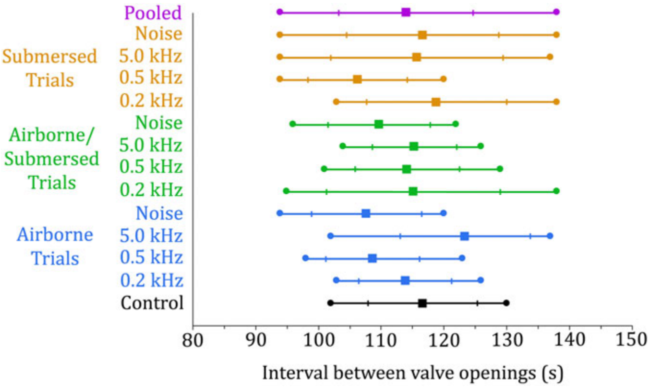


FIGURE 10 Temporal pattern of the median pharyngeal valve openings. For each of the trial conditions (Y axis) the median (square), standard deviation (vertical lines) and range (circles) of the duration between successive openings of the valve (X axis) is given. Twenty videos (evenly distributed from the five specimens) were pooled for each trial. Note the relative similarity between the temporal patterns from all of the trials, and the consistently "long" intervals (minimum of 94 s) which are difficult to reconcile with an auditory function

trials were performed using publicly available calls of *A. missssissippiensis* as stimuli; none of these trials yielded positive results (defined as change in the temporal pattern or frequency of median pharyngeal valve patency).

4 | DISCUSSION

The cranial passages associated with the middle ear or tympanic cavity have inspired a long history of, often conflicting, studies (e.g., Cuvier, 1824; Owen, 1850; Hasse, 1873; Miall, 1878; Van Beneden, 1880; Colbert, 1946; Simonetta, 1956; Dufeau and Witmer, 2015). Most of the material examined in these studies consisted of dried skulls, so it is not surprising that few descriptions of the median pharyngeal valve have been provided. Owen (1850) and Miall (1878) described the mv as "membranous", while Dufeau and Witmer (2015) refer to it as connective tissue. Van Beneden (1880) offered perhaps the most detailed description, noting the horse-shoe shape, the black pigmentation, and the continuity of the valve lumen with the "Eustachian" system. None of these earlier studies reported the skeletal muscle component of the valve, the continuity between the plug and capsule of the valve, or the core of loose connective tissue within the plug.

The valves that occur in the vertebrate body are of four general types. Flap valves are attached on one side and the mobile portion of the valve (the flap or cusp) move as a "stiff" unit to form occlusion (e.g., atrioventricular, Thesbian, Tympanopharyngeal, valve of Hasner, uretovesical; see Williams and Jew, 2004). Semilunar valves are attached along one surface and the mobile portion of the valve (the valvules or cusp) deforms to form occlusion with other valvules (e.g., pulmonary, aortic, venous valves, lymphatic valves; see Cliff and Nicoll, 1970). Sphincters are bodies of smooth muscle, skeletal muscle, or vasculature which can alter the patency of a duct or orifice by applying external force (e.g., pyloric sphincter, sphincter of Oddi, ileocaecal valve, iris sphincter; see Bharucha and Fletcher, 2007). Spiral or ridge valves are a series of (generally static) internal partitions within a vessel which, while not occluding the vessel, function to physically redirect and slow the passing material (e.g., cystic valves, horizontal folds of the rectum, spiral intestine of Selachians; see Taylor and Carpenter, 1975). The morphology of the median pharyngeal valve cannot be reconciled with a flap or semilunar valve, there is no body of muscle (smooth or skeletal) surrounding the valve or the pore that is suggestive of a sphincter, and the mobile mv lacks the internal partitioning characteristic of a spiral valve.

Hasse (1873) and Van Beneden (1880) both described the median pharyngeal valve as a semilunar valve. Neither the morphology, the kinematics, nor the physics of gaseous pressure balance support a semilunar designation for this valve. The median pharyngeal valve (mv) resembles a deformable ball check valve. Like a typical ball check valve the mv plug forms a seal against the pore of the capsule, producing a seal that holds air pressure within the lumen of the mv. Unlike a typical ball check valve, the plug of the mv does not move away from the pore as a solid unit, but rather deforms and partially displaces with the ventral portion of the plug moving while the remaining (fixed) parts undergo little deformation or displacement (Figures 7–8). We are unaware of a similar valve system being described from any organ system of any vertebrate.

The endoscopic observations of the median pharyngeal valve are consistent with a deforming ball check valve. No valve flap was observed, however the plug was displaced relative to the pore, and appeared to shift caudally (Figure 9).

As noted above, previous treatments of the "Eustachian" system of crocodilians have centered on the ducts, chambers, and passages between the contralateral middle ear cavities. Within this context, even when the authors have speculated about the functional significance of the anatomy, it is not clear what functional role they propose for the valve. Given that disclaimer, previous functional speculation about this system can be divided into three general groups: nasal audition, intertympanic audition, and pressure regulation.

Presumably the close physical proximity between the internal nares and the mv led Owen (1850) to speculate that when a crocodilian was partially submerged airborne sounds could travel through the nasal passageway, then enter the auditory system through the mv. This hypothesis was repeated by Miall (1878), discredited by Colbert (1946), and could not be supported experimentally (Bierman et al., 2014). The stimulus tones presented during the endoscopic trials span the frequency range both of sounds most commonly produced by crocodilians (Vergne, Pritz and Mathevon, 2009; Young, Mathevon and Tang, 2014), and of the crocodilian aquatic and airborne auditory sensitivities (Higgs et al., 2002; Bierman and Carr, 2015). When live alligators were positioned as described by Owen (1850) and presented with airborne stimuli (the submersed/airborne trials of Figure 10), the mv was neither held open to ensure transfer of the stimuli, nor opened in direct response to the stimuli. The temporal pattern of mv opening under these conditions was no different from the control condition.

Van Beneden (1880) provided a clear expression of the intertympanic auditory hypothesis by postulating that the crocodilian Eustachian system was a specialization to induce resonance when the animal encountered underwater sounds. The resonance waves would then propagate through the Eustachian system to reach the two ears.

The ability of an auditory pressure wave to propagate through the skull between the two tympana is at the heart of what is now commonly referred to as internally couple ears (e.g., Carr et al., 2016; van Hemmen et al., 2016; Vedurmudi et al., 2016). Internally coupled ears are normally treated as morphologically "static" with the frequency response of the system dependent on the dimensions of the internal coupling (e.g., Vedurmudi, Young and van Hemmen, 2016). A patent mv could prevent the pressure coupling between the two tympana, much as an occluded Eustachian tube has been shown to do in a Ranid frog (Gridi-Papp et al., 2008). If the mv functions to regulate the acoustic coupling between the tympana, then the patency of the mv should respond to acoustic stimuli, or should differ when the animal is submersed (as the greater speed of sound underwater alters the performance of the internally coupled ear). In the trials conducted there were no significant responses of the mv to auditory stimuli, and submersing the head had no influence on the temporal pattern of mv patency (Figure 10).

Pressure regulation of the middle ear cavity is necessary since the cavity is a closed chamber the volume and/or pressure of which can be altered through displacements of the tympanic membrane or gasses discharged from the lining structures (e.g., Gaihede et al., 2010). Valves, located within the Eustachian (tympanopharyngeal) tubes can alter the pressure within the middle ear cavity even if they are only patent for a short period (Siedentop, Tardy and Hamilton, 1968). Studies of the avian middle ear have shown regular patterns of middle ear venting ranging from approximately 20–180 s (Larsen, Dooling and Ryals, 1997; Larsen, Christensen-Dalsgaard and Jensen, 2016). A recent study of the avian middle ear (Caes et al., 2018) found that the Eustachian valves opened approximately every 60 s in the domestic chicken (*Gallus Gallus domesticus*) and approximately every 180 s in the Mallard (*Anas platyrhynchos*). The results of the current study of *A. mississippiensis* are in good agreement with the findings from the avian middle ear and lend strong support to interpreting the median pharyngeal valve as functioning primarily, if not exclusively, in pressure regulation.

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ORCID

Bruce A. Young https://orcid.org/0000-0002-0988-7731

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