A Model of the Anterior Esophagus in Snakes, with Functional and Developmental Implications

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ABSTRACT

The gross anatomy of the mouth of snakes has always been interpreted as an evolutionary response to feeding demands. In most alethinophidian species, their anatomy allows limited functional independence of right and left sides and the roof and floor of the mouth as well as wide separation of the tips of the mandibles. However, locations of the tongue and glottis in snakes suggest extraordinary rearrangement of pharyngeal structures characteristic of all vertebrates. Serial histological sections through the heads of a number of colubroid species show muscularis mucosal smooth muscle fibers appearing in the paratracheal gutter of the lower jaw at varying levels between the eye and ear regions. Incomplete muscularis externa elements appear beneath the paratracheal gutter more caudally but typically at otic levels. Both muscle layers encompass more of the gut wall at more posterior levels, encircling the gut at the level of the atlas or axis. The pattern in snakes suggests developmental dissociation of dorsal and ventral splanchnic derivatives and extensive topological rearrangements of some ventral pharyngeal arch derivatives typical of most tetrapods. When snakes swallow large prey, the effective oral cavity becomes extremely short ventrally. The palatomaxillary arches function as ratchets packing the prey almost directly into the esophagus. Our findings raise questions about germ layer origins and regulation of differentiation of gut regions and derivatives in snakes and suggest that significant aspects of the evolution of lepidosaurs may be difficult to recover from bones or molecular sequence data alone. Anat Rec, 297:586–598, 2014.   © 2014 Wiley Periodicals, Inc.

Key words: gut evolution; vertebrates; snake feeding; Squamata; mammals

INTRODUCTION

The foregut is typically thought to be that part of the gut arising anterior to the liver diverticulum (Standring, 2008; Gilbert, 2014). It includes some structures of the oral cavity plus the pharynx, esophagus, and stomach. Although the pharynx in most vertebrate embryos is relatively easy to discern, its adult form can be very different. The esophagus, however, is simply the tube connecting the pharynx to the stomach. In most tetrapods, the esophagus begins caudal to the evagination of the laryngotracheal groove or duct, which arises at the caudal end of the pharynx and forms the larynx, trachea
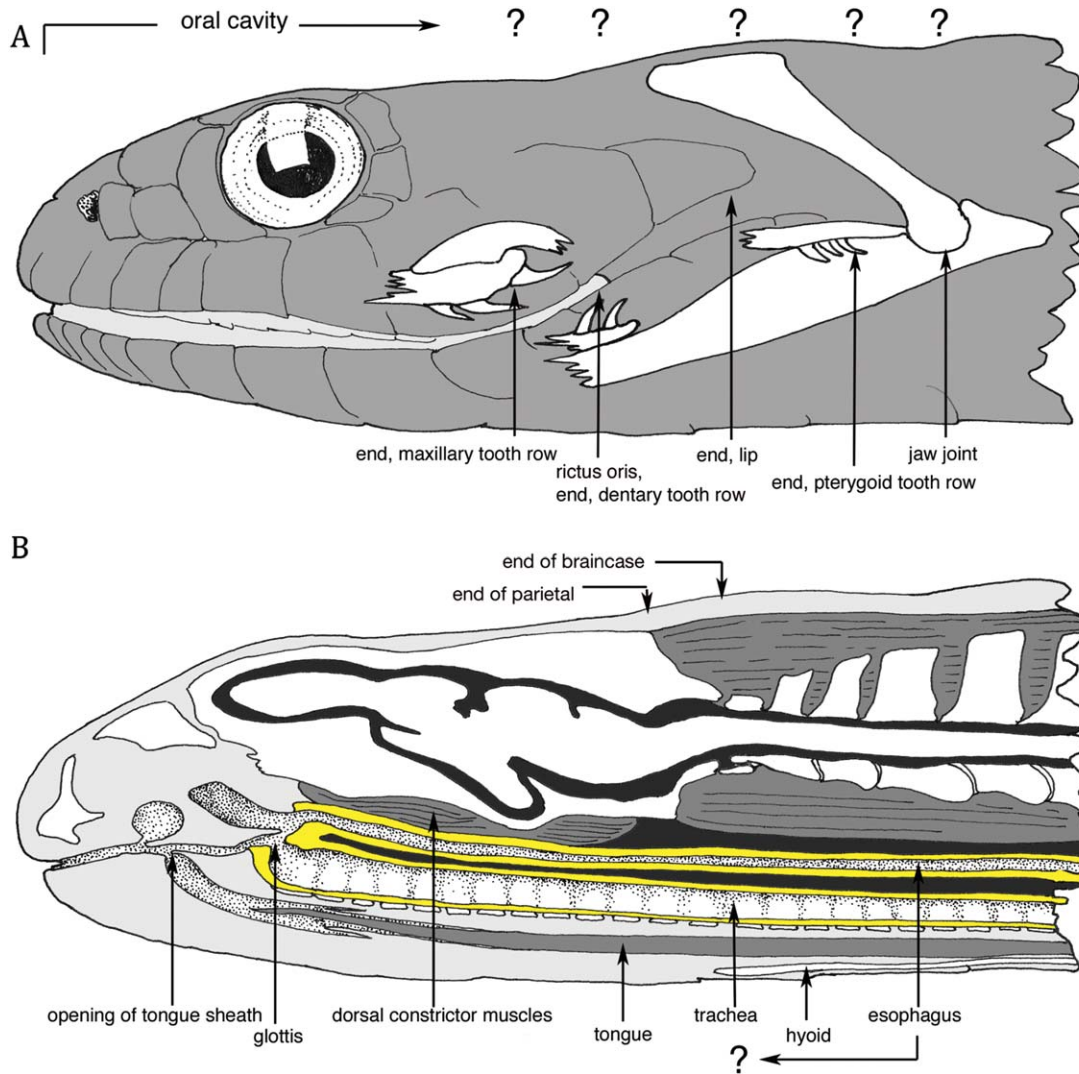
Fig. 1. (**A**) Generalized diagram of the head and anterior trunk of an alethinophidian snake (*Nerodia fasciata*, LU 2322) showing the longitudinal organization of skeletal structures associated with defining limits of the head and oral cavity. The esophageal tube has long been assumed to become oral cavity at the jaw joint. (**B**) A midsagittal view of the same head to show longitudinal relationships of the glottis, trachea, tongue, and braincase in a representative colubroid snake. Yellow indicates lining epithelia of presumed endodermal origin. Arrow marking the end of the parietal refers to the scale, not the bone, which ends slightly anterior to this point.

and lungs. The esophagus typically has transitional zones between the connective tissues and muscles of the pharynx arising from neural crest and cranial paraxial mesoderm and structures of the gut wall presumed to arise from splanchnic mesoderm (Smith, 1992; Standring, 2008; Gilbert, 2014). Developmental evidence shows that the esophagus and most of the pharynx are lined by epithelial cells of endodermal origin.

From a gross anatomical perspective, the esophagus of snakes has been assumed to be a tube lying caudal to the head (Ferri and Madeiros, 1975; Luppa, 1977; Cundall et al., 2012). The head in many advanced snakes, however, is a complex structure whose boundaries have been defined differently depending on the questions asked and the goals of particular studies (e.g., region anterior to the base of the skull; Bonnet et al., 2001: region anterior to the posterior end of the parietal

scales; Queral-Regil and King, 1998; Vincent et al., 2007; Hampton, 2011; see Fig. 1A). In most tetrapods, the jaws are considered part of the head, but in many snakes, the jaw joint lies well caudal to the base of the skull or the parietal scales (Fig. 1A). The roof of the gut is tightly associated with the toothed bones of the palate and the adductor muscles of the mandibles. Hence, the roof of the gut is not free of teeth and head-associated skeletal muscles until the region of the jaw joint (Fig. 1B). However, the floor of the gut in snakes is tightly associated only with the tooth rows on the mandibles and the intermandibular connective tissues and skeletal muscles anterior to the glottis and larynx (Young, 2000; see also Fig. 1B). The trachea and tongue lie in the middle of the floor of the mouth (Oldham et al., 1970; McDowell, 1972) beside which are deep grooves referred to by Cundall et al. (2012) as paratracheal gutters.

Here we show how the histology of the anterior gut in selected colubroid snake species relates to the gross anatomy of the head and test the traditional view (hypothesis) that the esophagus begins caudal to the braincase or jaw (quadrate-articular) joint. Cundall et al. (2012) commented that the anterior edge of the esophagus ought to lie at the level of the eyes. Based on our histological data, we develop a new model for the organization of the anterior gut in advanced snakes that casts a different light on how the anterior gut functions. We then explore some developmental implications of the model.

MATERIALS AND METHODS

The relationships of the gut tube to the skeleton were determined from serial sections of whole heads and anterior trunks of a small sample of colubroid snakes (Appendix A), a clade that includes the majority of living snake species (Gauthier et al., 2012; Zaher et al., 2012; Pyron et al., 2013). Data came from four juvenile watersnakes (*Nerodia sipedon*) sectioned specifically to look at esophageal relationships plus serial sections of the heads of other snakes prepared for general features of head anatomy (Cundall and Shardo, 1995). The size of snakes that could be examined histologically was limited by demands of paraffin sectioning and mounting. Hence, the majority of histological data came from small species or juveniles of larger species.

Heads and anterior trunk regions were either perfused with Ringer's solution followed by buffered formalin, or simply fixed in buffered formalin and embedded in paraffin. Sections were cut at 10 µm and three sets of slides were prepared, one stained with hematoxylin and eosin, one with a variation of Masson's trichrome, and one with either Verhoeff's elastin procedure (Humason, 1979) or with the iron gallein method of Churukian and Schenk (1976). The best trichrome method for differentiating skeletal and smooth muscle was Delafield's hematoxylin, acid fuschin, xylidine red, and fast green, with pretreatment with iron alum and differentiation of reds with phosphomolybdic acid. These methods allowed us to relate the first appearance of smooth muscle cells in the muscularis mucosa and the muscularis externa (propria) to reasonably precise points on the skull and lower jaw.

The lamina propria in the anterior esophagus of the snake species examined is extremely thin, essentially the depth of one capillary, with scattered smooth muscle fibers forming an incomplete muscularis mucosa. The submucosa was difficult to distinguish as a discrete layer until scattered smooth muscle fibers of the circular muscularis externa appeared. We noted the position and extent in the lower jaw of large spaces lined with flattened squamous epithelial cells, some or all of which are lymphatic sinuses (Chapman and Conklin, 1935; Ottaviani and Tazzi, 1977). We have so far been unable to determine the exact nature of all the epithelial-lined spaces that lack erythrocytes in the lower jaw.

To determine where the esophagus might begin anteriorly, we started by comparing our histological data for the posterior esophagus with that of Ferri and Madeiros (1975), Ferri et al. (1976), and Bessler and Secor (2012). Because the caudal end of the esophagus is clearly lined by splanchnopleuric derivatives (Standring, 2008), we followed these cranially, noting changes in tissues associated with the mucosal epithelium from the gastroesophageal junction to the anterior edges of the lower jaw and snout. Khamas and Reeves (2011) recently showed that the esophagus in the colubrid snake *Pituophis* graded into an aglandular region of the stomach. Our histological data for the posterior esophagus of *N. sipedon* showed no aglandular gastric region and matched conditions in *N. rhombifer* (Bessler and Secor, 2012). Here we deal primarily with the anterior esophagus but present limited data on the posterior esophagus to show continuity with anterior structures. These were confirmed by examining representative sections of an anterior gut (gut tube from posterior end of head to pylorus of stomach) removed from an adult *N. sipedon*, irrigated with 10% buffered formalin and then coiled before final fixation in formalin and embedding in paraffin. Longitudinal sections of this gut allowed estimation of sites of histological change between the head and stomach but did not show the anterior-most modifications of the gut wall of interest here. We assumed that regions of mucosal epithelium associated with at least one layer of smooth muscle cells underlying a lamina propria were esophageal in nature; we also assume that esophageal structure could begin at different longitudinal levels at different points of its circumference. This final criterion underlies our interpretations.

Dissections of the anterior gut and oral cavity were performed on a variety of snakes (Appendix B). These included microdissections of the muscles associated with both the roof and floor of the oral cavity and examinations of hemisected heads of a number of species to establish longitudinal relationships of structures in the anterior gut.

The behavior of the esophagus was extrapolated from video records of feeding and drinking in a number of snake species made over 30 years (for partial lists, see Cundall, 2000, 2009; Cundall and Deufel, 2006; Cundall et al., 2007). Critical information gained from ciné and video records of feeding snakes were the approximate angle of the lower jaw relative to the skull at maximum gape, whether or not the glottis was visible at the tip of the lower jaw during swallowing of large prey, and the extent of separation of the anterior tips of the mandibles.

RESULTS

Gross Morphology

The organization of major structures in the floor of the oral and pharyngeal regions of a generalized tetrapod and a colubroid snake are shown in Fig. 2A,B. Critical elements are simplified to show how the floor of the gut in alethinophidian snakes (e.g., McDowell, 1972; Groombridge, 1979; Cundall et al., 2012) compares to a generalized tetrapod (Kardong, 2012). The position of the glottis in Fig. 2A reflects the mammalian pharyngeal condition (Smith, 1992). In nonmammalian tetrapods, the glottis may lie ventral to the braincase but still caudal to the medial functional tongue surface.

Like some other vertebrates with elongated tongues (Reiss, 2001), the hyoid apparatus in snakes has unusual forms and positions (Langebartel, 1968). The primary intrinsic tongue muscle (hyoglossus) arises from the caudal ends of elongated hyoid cornua (Smith and MacKay, 1990) that typically lie somewhere between the
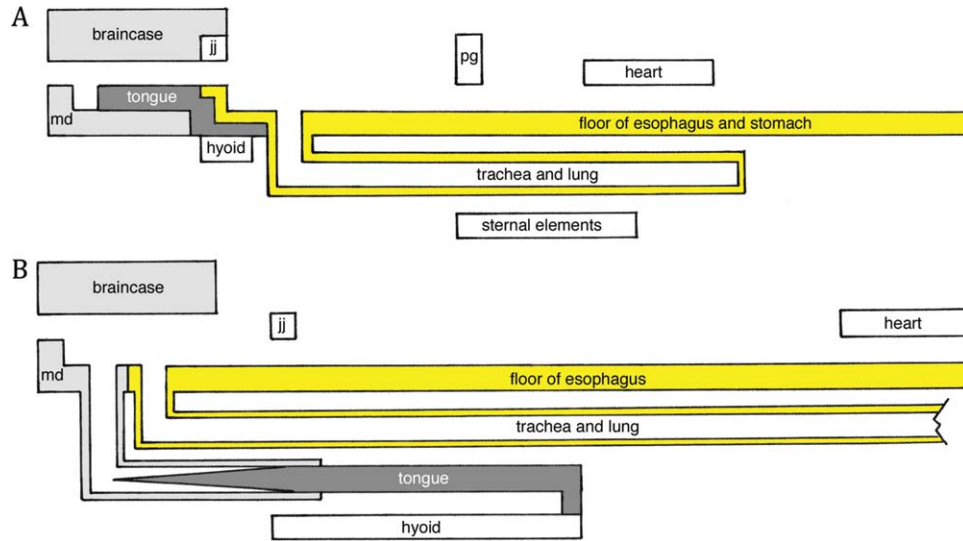
Fig. 2. Organization of the floor of the anterior gut in a representative amniote tetrapod (**A**) and in a snake (**B**). Although considerable liberty has been taken with anatomy, in almost all tetrapods except snakes, the trunk-cervical boundary is marked by pectoral elements and skeletal connections (ribs and costal cartilages) between the dorsal and ventral axial components (vertebrae [not shown but in line with the braincase] and sternum). The cranial-cervical boundary is typically marked by the jaw joint, base of the tongue and hyoid, and the glottis. Yellow indicates presumed distribution of epithelial cells of endodermal origin lining the floor of the gut. Abbreviations: jj, jaw joint, and hence location of posterior end of mandible; md, anterior end of mandible; pg, pectoral girdle.

head and the heart (Fig. 2B). As a result, the tongue base is topologically displaced well caudal to the head. The remarkable aspect of tongue organization, however, is that in its resting condition, it lies ventral to the larynx and trachea and caudal to the glottis, unlike the condition in most other tetrapods (Figs. 1B, 2). If the esophagus of snakes were to begin caudal to the tongue, then it would begin at the rear ends of the hyoglossus muscles and hyoid cornua, which vary considerably in position among different species of snakes (reviewed in Langebartel, 1968), lying somewhere between 2 and 25 vertebrae behind the head. The relationship of the tongue to the glottis is therefore unlikely to be a measure of esophageal relationships.

If the esophagus of snakes were to begin immediately caudal to the separation of the respiratory tract from the digestive tract, then the esophagus of snakes would begin at the anterior edge of the orbits, a position just caudal to the glottis. However, if one uses the degree of attachment of the digestive tract to surrounding structures, the situation becomes more complex. Whereas the gut lining is closely attached to the roof of the oral cavity and to the palatal bones (maxillae, palatines, and pterygoids), the gut lining on the floor of the oral cavity lateral to the trachea and caudal to the glottis is folded and separated to varying degrees from underlying soft tissues by spaces.

Histology

Progressing anteriorly from the stomach, the esophagus shows gradual decreases in the thickness of all layers outside the mucosal epithelium, gradual increases in numbers of ciliated and goblet cells, and decreases in mucous and "columnar" cells (Ferri et al., 1976; Khamas and Reeves, 2011). Immediately anterior to the stomach,

the mucosa consists entirely of mucous cells with small basophilic secretory vesicles (Figs. 3, 4A,B) like those illustrated by Khamas and Reeves (2011). The gastro-esophageal junction in *Nerodia sipedon* appears as in *N. rhombifer* (Bessler and Secor, 2012) and lacks the submucosal mucous glands that are present in some other species of snakes. The muscularis mucosa tends to be continuous or nearly so and composed of three to four layers of smooth muscle cells with irregular orientations. The lamina propria is very thin, about the width of a capillary or less. The submucosa varies considerably in thickness and shows scattered lymphoid nodules similar to those described by Jacobson and Collins (1980). The muscularis externa appears to consist primarily of 10–15
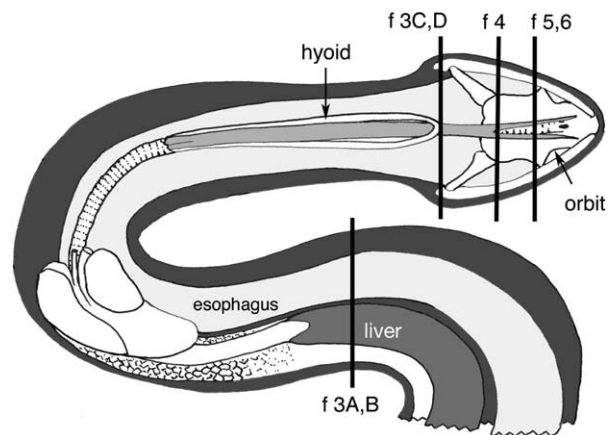

Fig. 3. Abstract diagram of general longitudinal relationships of the head and anterior trunk in a generalized snake to show levels for sections in Figs 4–7.
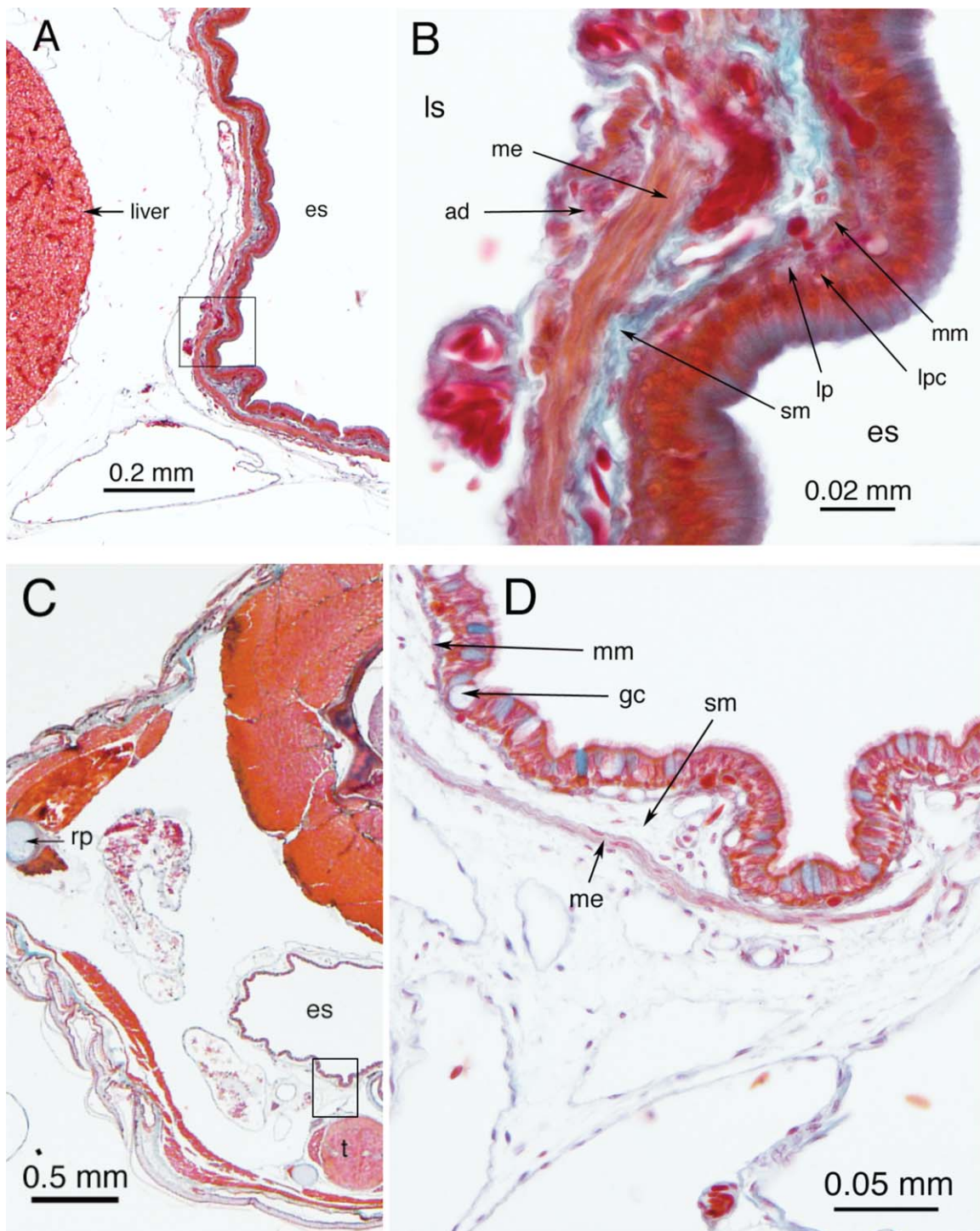
Fig. 4. Micrographs of the esophagus and its histology in juvenile *Nerodia sipedon* at (**A**,**B**) the anterior end of the liver, and (**C**,**D**) the anterior trunk at the level of the axis. In C, the end of the retroarticular process of the mandible is visible at left. Rectangles in A and C mark areas shown at right in B and D. Abbreviations: es, esophagus; gc, goblet cell; lp, lamina propria; lpc, capillary in lamina propria; ls, lymph sinus; me, muscularis externa; mm, muscularis mucosa; rp, retroarticular process of mandible; sm, submucosa; t, tongue.

layers of circular smooth muscle cells surrounded by scattered bundles of longitudinal fibers.

At the level of the atlas and axis, gaps in the muscularis externa become more extensive and the externa itself is reduced to three to five layers of smooth muscle cells arranged predominantly circularly. Collagen in the submucosa appears more scattered and the muscularis mucosa is reduced to one to two layers of smooth muscle
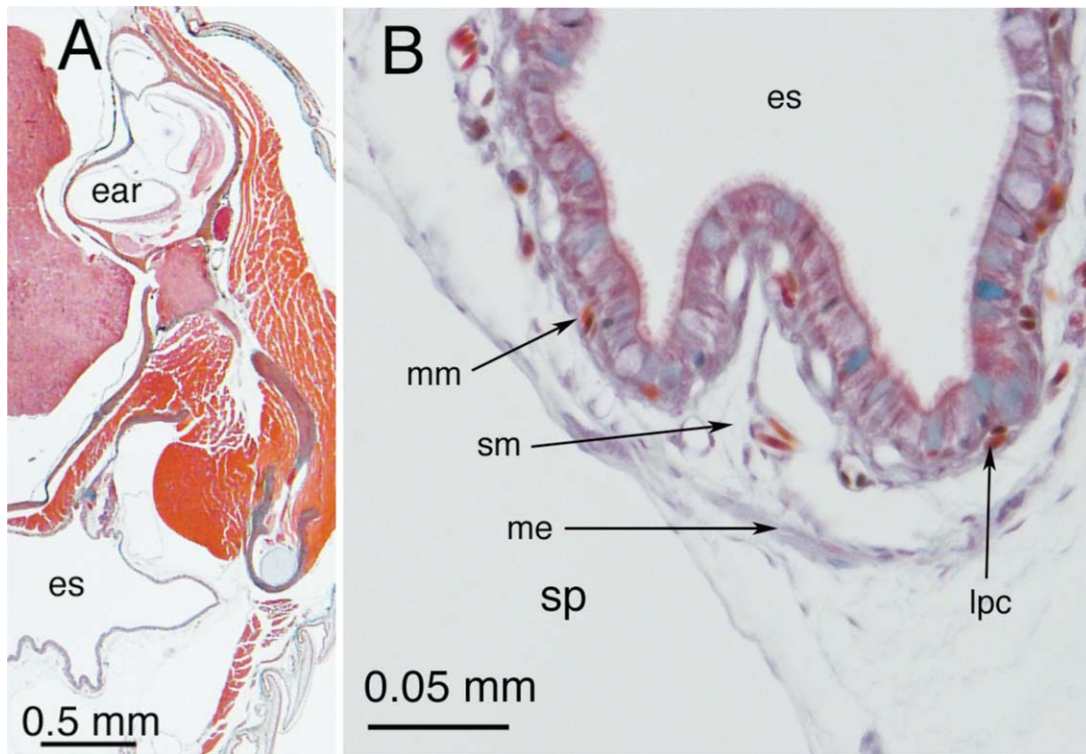
Fig. 5. Micrographs showing the gut of *Nerodia sipedon* at the approximate middle of the otic region at the level of the entry of the mandibular nerve into the compound bone. Abbreviations: es, esophagus; lpc, capillary in lamina propria; me, muscularis externa; mm, muscularis mucosa; sm, submucosa; sp, space, presumably lymphatic sinus but possibly coelomic space.

fibers. The lamina propria contains abundant capillaries (Fig. 4C,D) but is otherwise similar to its appearance at more caudal levels. The esophagus roof is attached to the overlying rectus capitis anterior muscles by collagenous connective tissues and whereas much of the lateral surfaces appear to be covered by a serosa-like layer of loose connective tissue surmounted by simple squamous epithelium (mesothelium?), the ventral wall of the esophagus is associated with an adventitial connective tissue in which lie the paired carotid arteries, anterior jugular veins, trachea and tongue and a number of lymph sinuses (Fig. 4C,D).

Anterior to the atlas, the muscularis externa disappears in the roof of the gut at the rear edge of the protractor quadrati, which arises from connective tissues overlying the basioccipital anterior to the occipital condyle. Smooth muscle cells of the muscularis externa remain on the lateral and ventral gut wall but show gaps of increasing size and progressive reduction of the lateral extent of the layer in more anterior sections. The last remnants of a muscularis externa are typically seen at mid-otic levels. In juvenile *Nerodia*, this level on the lower jaw lies where the mandibular nerve enters the mandible (Fig. 5A,B) at the anterior edge of the mandibular fossa of the compound bone (Cundall and Irish, 2008). At this point, however, a muscularis mucosa consisting of a single layer of smooth muscle persists around most of the ventral and lateral gut wall but has disappeared in the roof of the gut, which is bound closely to the pterygoid and the protractor pterygoideus.

In the floor of the mouth, smooth muscle cells that appear to constitute a muscularis mucosa extend to different levels in different snake species. In the pitviper, *Agkistrodon piscivorus*, the muscularis mucosa extends rostrally to a point immediately caudal to the glottis. In other snake species (*Nerodia sipedon, Pantherophis spiloides, Thamnophis sauritus, T. sirtalis*), it appears not to extend as far rostrally as in *Agkistrodon*, ending just caudal to the orbit (Figs. 6A,B, 7). In *Regina*, it disappears just rostral to the point at which the muscularis externa disappears at the rear edge of the otic capsule. In all species examined, the smooth muscle cells appear as a single layer, the fibers apparently varying in orientation, some of them passing over endothelial cells lining capillaries under the mucosa (Fig. 7). In all species examined, the level to which a muscularis mucosa extends has one or more large spaces lined by simple squamous epithelium (Figs. 5, 6B, 7). Between this squamous epithelium and the muscularis mucosa is loose collagenous tissue. In most species, the point at which the muscularis externa disappears lies near the point at which the hyotrachealis muscle becomes associated with the loose connective tissue underlying the paratracheal gutter.

The organization of tissues in the lower jaw at the level where the muscularis externa first appears when progressing caudally can vary considerably but a number of skeletal muscles (genioglossus, geniotrachealis) typically lie between the mucosa of the paratracheal gutter and the tongue sheath or trachea. However, these
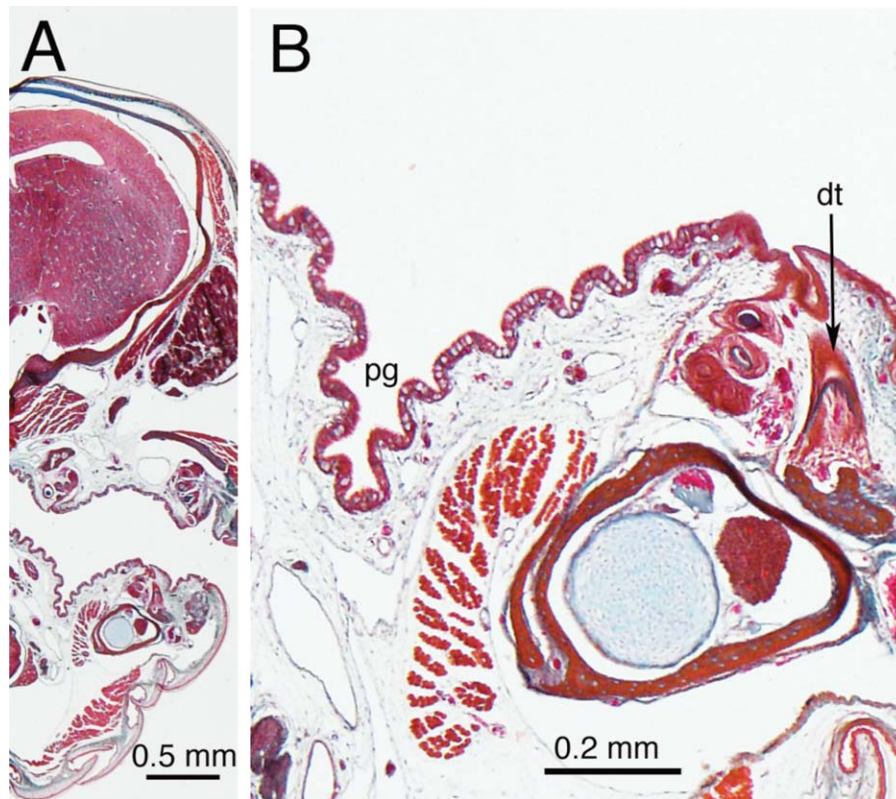
Fig. 6. Cross sections of the head of a juvenile *Nerodia sipedon* just anterior to the corner of the mouth. The caudal ends of the maxilla (with overlying ectopterygoid) and the dentary and their associated teeth are seen on the right in (**A**). The Harderian gland lies lateral to the brain. (**B**) shows a magnified view of the edge of the lower jaw with dentary tooth (dt) and paratracheal gutter (pg).

muscles are not associated with the mucosal lining of the mouth or with the loose connective tissue most closely associated with that lining. As noted above, there are large lymphatic or coelomic spaces lined by simple squamous epithelium in these regions. As a result, part of the gut wall could be viewed as having a serosa although the developmental origin of the squamous epithelium forming the edge remains undetermined. The more anterior spaces between the gut wall and the trachea are probably lymphatic sinuses but our techniques do not provide unequivocal demonstration of this. The critical point is that the gut is not surrounded by a single epithelial-lined cavity but by a number of cavities, some of which are interconnected and some of which match descriptions of lymphatic sinuses described by Ottaviani and Tazzi (1977).

The mucosal lining of the lower jaw and the roof of the mouth in all species examined have similar patterns of epithelial distribution. Stratified, noncornified squamous epithelium lies along all tooth rows (Fig. 6B) and in narrow strips along the inner (mesial) edges of the labial scales. In the roof of the mouth, a narrow strip of stratified, noncornified squamous epithelium also underlies the middle of the snout (vomers and septomaxillae medial to the vomeronasal cupolae) to the internal nares. In the floor of the mouth, stratified, noncornified squamous epithelium occupies the darker areas shown in Fig. 8. The rest of the mucosa is a simple or bilayered columnar epithelium with abundant ciliated and goblet cells (Fig. 7).

With respect to the potential boundaries of the esophagus, the histological data support a traditional post-cranial beginning for the roof of the esophagus (Fig. 9A,B) but the floor of the esophagus is more complex. Muscularis externa regions extend rostrally to the level of the otic region in all colubroids examined and muscularis mucosal elements extend to levels between the orbits and otic capsules in the paratracheal gutter regions of the mucosa (Fig. 9B). Importantly, the mucosa of the paratracheal gutters is underlain not only by a thin lamina propria and muscularis mucosa but simple squamous-lined lymphatic or coelomic spaces become increasingly prominent in the loose connective tissues separating the gut mucosa from the trachea and mandibles. This histological organization in conjunction with folding of the mucosal epithelium in the paratracheal gutter provides potential for considerable lateral extension of tissues between the trachea (and tongue) and the mandibles. Hence, we propose that the esophagus in snakes has an irregular anterior border, its floor extending well anterior to the rear edge of the braincase on either side of the trachea to end, in the resting state, somewhere between the otic and orbital regions.
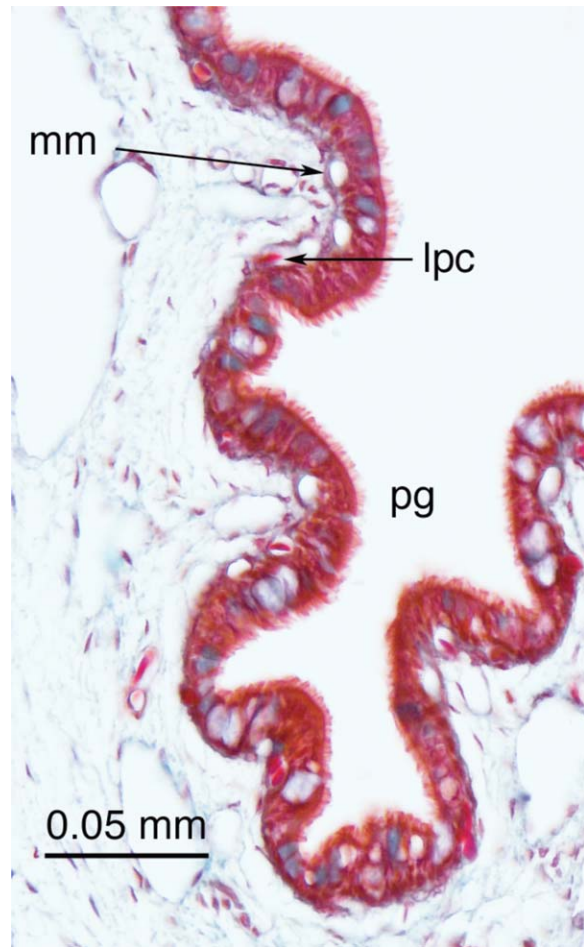
Fig. 7. Magnified view of the mucosal region of the paratracheal gutter (pg) shown in Fig. 6. A single layer of scattered smooth muscle fibers constitutes the muscularis mucosa (mm). Capillaries of the lamina propria (lpc) lie against the basement membrane of the mucosal epithelium. Larger spaces lined by squamous epithelium to the left of the loose connective tissue underlying the muscularis mucosa appear to be lymph sinuses.

Behavior

When snakes feed on prey with a relatively large cross-sectional area (Cundall and Deufel, 2006; Close and Cundall, 2012), the prey fills the oral cavity and the esophagus. To fill the oral cavity, the mandibles are depressed (Fig. 10A) and spread laterally as the prey fills the space between them. In many snake species, the tips of the mandibles can also be separated widely (Fig. 10B). This movement typically carries the origins of the geniotrachealis and genioglossus muscles laterally and potentially pulls both the glottis and tongue closer to the edge of the lower jaw. In some species the glottis (but not the tongue) is often protruded below the prey when transporting very large prey (Fig. 10B).

Because what has been defined as the oral cavity at rest appears to be a flat, slit-like space (see Fig. 2 in Cundall et al., 2012), the difference that may be achieved in the circumference of the gut lining is remarkable, and significantly greater than can occur in animals with mandibular symphyses and mandibular rotations limited by

Fig. 8. Approximate distribution of ciliated epithelium in the floor of the lower jaw of snakes. Ciliated epithelial area is light gray, stratified squamous epithelium is dark gray. White represents sectioned muscle, bone, and skin.

quadrate-articular or temporal-mandibular joint limitations. As the mandible is depressed, the effective length of the oral cavity decreases. In some snakes, the mandibles may be depressed to the point at which they are essentially vertical to the long axis of the braincase (Fig. 9A). At that point, it is debatable whether an oral cavity exists.

The behavior of the anterior gut is relatively simple. The mucosal lining of the roof of the mouth is deformed by movements of the palatomaxillary arches during ingestion, but these movements are limited in extent (Cundall and Greene, 2000). It is the mucosal lining of the floor of the mouth that is stretched to surround the prey as the anterior end of the lower jaw is carried caudally. When eating prey of relatively large diameter, the
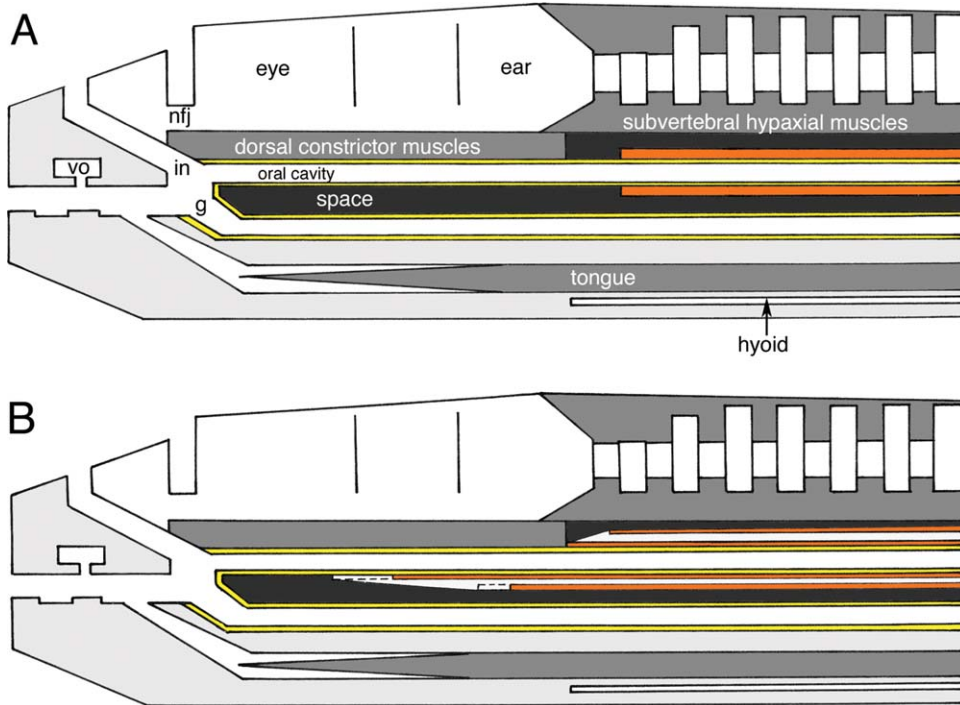
Fig. 9. Summary diagram showing what we suspect to be (A) the traditional model of the longitudinal relationships of the esophagus in colubroid snakes and (B) our suggested revision of the model. The space shown in both models ventral to the gut are assumed to be lymphatic sinuses. Red indicates muscle, divided in **B** into muscularis mucosa (against the gut epithelial layer in yellow) and a muscularis externa. Abbreviations: g, glottis; in, internal naris; nfj, nasofrontal joint; vo, vomeronasal organ.

tips of the mandibles may come to lie immediately ventral to the rear edge of the braincase. In this case, the parts of the mucosa associated with a lamina propria and muscularis mucosa are behaving as an esophagus and, in fact, simply plotting these regions on a lateral view of a snake in this condition suggests that the gut tube behind the glottis is esophagus (Fig. 11).

DISCUSSION

The histological organization of the floor of the gut in snakes allows lateral extension of the mucosal lining during feeding while also providing a novel structural solution for drinking (Cundall et al., 2012). The fact that the muscularis externa and muscularis mucosa extend further rostrally in the floor of the mouth of snakes than
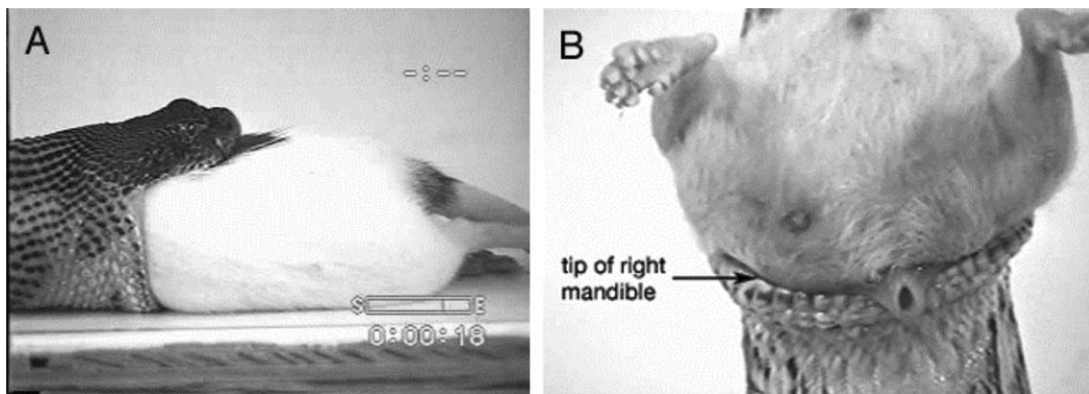

Fig. 10. (**A**) Lateral view of a *Crotalus horridus* (timber rattlesnake) during oral transport of a rat with a body mass approximately 0.48 that of the snake. (**B**) Anteroventral view of a *Broghammerus* (*Python*) *reticulatus* (*Reticulated python*) during protrusion of the glottis during oral transport of a rat (prey mass:-snake mass = 0.24).
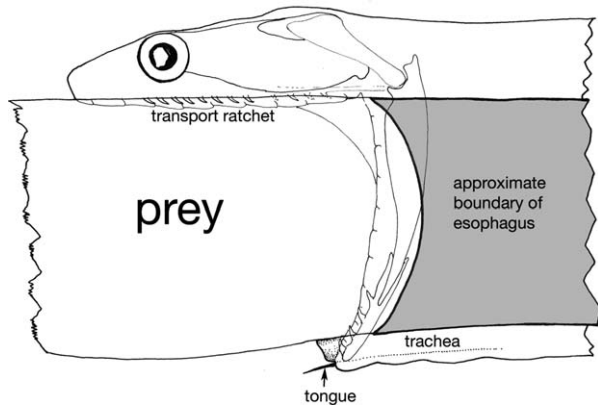
Fig. 11. Lateral diagrammatic view of the head and anterior trunk of a macrostomate snake to show how the floor of the mouth is arranged during ingestion of large prey. The net effect is that the mandibles surround the entry to the esophagus, not the oral cavity, while the palato-maxillary arches rachet the snake's head over the prey. The tongue is normally not visible during transport, possibly because the tongue sheath is compressed beneath the prey.

in the roof correlates with their feeding mechanism and its dependence on structural modifications of the lower jaw for extraordinary degrees of extension, allowing lateral separation of the two mandibles. Much of the extension appears to be achieved by unfolding. In this light, only the muscularis externa can function in restoring mucosal folding in the paratracheal gutter. The functional relevance of the anterior extension of the muscularis mucosa remains unclear. However, it is possible that all of the histological reorganization in the lower jaw is associated with the anterior migration of the glottis. This latter feature appears functionally related to feeding on large prey that frequently cover the entire lower jaw for extended periods of time, potentially occluding a glottis that could not be protracted beyond the rim of the lower jaw (Figs. 10B, 11).

The developmental implications of our findings relate to the histological differences between the roof and floor of the anterior gut in snakes. Although the mucosal lining of the esophagus and much of the pharynx is derived from endoderm, the origin of esophageal muscle and connective tissue has been established for remarkably few species (e.g., Roberts, 2000; Sherwood et al., 2009). In most tetrapods, the esophagus gradually acquires some mesodermal derivatives a relatively short distance caudal to the glottis and pharynx but the full complement of gut wall structures (lamina propria, muscularis mucosa, submucosa, muscularis externa, and adventitia or serosa) may appear sequentially (Patt and Patt, 1969; Andrew and Hickman, 1974). Their organization in snakes is less clear (Ferri et al., 1974, 1976; Ferri and Madeiros, 1975; Khamas and Reeves, 2011). However, all past studies on the histology of the gut caudal to the head show that mesodermally derived tissues in the wall of the esophagus decrease in thickness anteriorly. No studies actually defined a border between the esophagus and the pharynx, and in fact, we know of no anatomical descriptions of the pharynx in adult snakes. It is in this light that definitions of heads, mouths, oral cavities, pharynges, and necks become significant because embryonic snakes clearly have a pharynx.

Recent studies of early development of the heads of pythons suggest that the floor of the pharynx shows unusual relationships in tongue and laryngotracheal duct development (Boughner et al., 2007). Combining these findings with those of Boback et al. (2012) for a colubroid species shows that as the head straightens, all of the structures lying medial to the initial appearance of Meckel's cartilage (Fig. 12A–C) end up lying nearer its anterior end following rapid elongation of the lower jaw and rearward migration of the quadrate-articular joint (Fig. 12D,F) between stages 3 and 7. The end result leaves the laryngeal cartilages, presumably derived from neural crest associated with arches five and possibly six in squamates, lying far anterior to the hyoid apparatus, presumed to derive from neural crest cells in arches two, three or four. The distribution of endoderm in early snake embryos remains unclear—we assume that the laryngotracheal duct arises from endoderm and hence we show the boundary between endoderm and ectoderm lying at the caudal edge of the developing tongue in early snake embryos (Fig. 12B,C). Boughner et al. (2007) could not define this boundary based on their material–this remains a question of critical importance. Given the distribution of ciliated cells in the mucosal epithelium of floor of the mouth (Fig. 8) and the roof (see above), we think it unlikely that either reflects germ layer origins as accurately as do ciliated and nonciliated cells in the mammalian middle ear (Thompson and Tucker, 2013).

Similarly, the spaces lined by simple squamous epithelium in the lower jaws of snakes raise questions about the limits of major body regions in snakes, such as cephalic, cervical, and dorsal or trunk regions, all of which have been traditionally defined by skeletal features (Hoffstetter and Gasc, 1969; Caldwell, 2000). More recent efforts to define the neck of snakes have been based on different assumptions and data (Cohn and Tickle, 1999; Tsuihiji et al., 2012; Woltering, 2013). Our efforts relate loosely to this issue inasmuch as the esophagus is typically considered to be a cervical and anterior trunk (thoracic in mammals) structure (Kardong, 2012; Standring, 2008) lying partly in the mediastinum, the median "space" in the mammalian thorax between the paired pleural cavities (Standring, 2008). The fate of the anterior embryonic coelom in snakes and the origin of the cells lining different cavities in the bodies of snakes have not been explored with cell lineage tracing techniques and hence Cundall and Greene's (2000) statement that the pleuroperitoneal (coelomic) cavity extends to the head in snakes remains undemonstrated.

The histological reorganization of tissues in the anterior gut of snakes, in combination with the position of the glottis, suggests that major changes have occurred in the regulation of gut development and regionalization. If our interpretation of esophageal boundaries is any reflection of endodermal/splanchnic mesodermal relationships, other developmental relationships may have experienced similar changes, as reflected in heterochronic lower jaw and tongue growth relative to braincase growth in early development (Boughner et al., 2007; Buchtova et al., 2007; Boback et al., 2012).

One unresolved problem is the nature of the cavities surrounding the esophagus that must facilitate the extraordinary distension seen in many snakes. The functional problem goes beyond simply describing the histology of the esophageal wall and extends to relationships between the esophagus and surrounding tissues. More
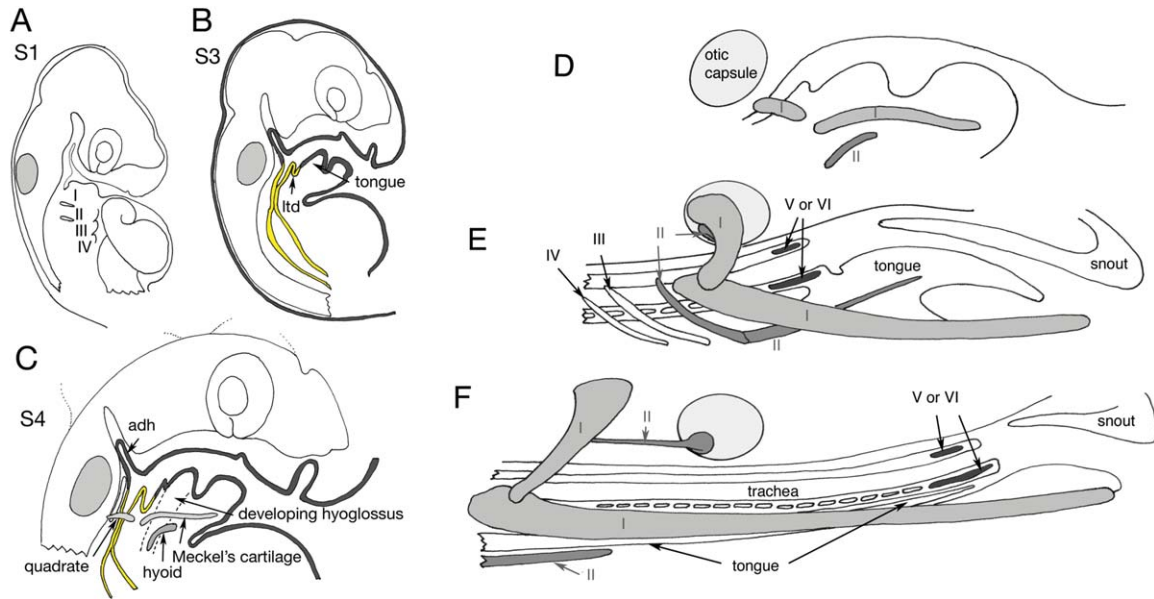
Fig. 12. Associations of pharygeal structures in snakes in early development (based on recent studies of python (Boughner et al., 2007) and colubroid (Boback et al., 2012) embryos): shaded ovals in all figures are the otocyst or otic capsule to provide a topographical reference. (**A**) A Stage 1 snake embryo showing pharyngeal arches I–III, arch IV just beginning to develop. (**B**) Stage 3 embryo, showing the appearance of the laryngotracheal duct and tongue. (**C**) A Stage 4 embryo showing cartilage development in the mandibular and hyoid arches. (**D**) repeats Stage 4 to show the topographical changes that occur in the derivatives of pharyngeal arches I and II versus V or VI in adult nonsnake tetrapods (**E**) and in snakes (**F**). The model shows the degree of dissociation of arch derivatives in snakes. Abbreviations: I-VI-pharyngeal arches 1–6, adh-adenohypophysis (Rathke's pouch), ltd-laryngotracheal duct.

detailed study of tongue, hyoid and laryngeal/tracheal relationships as well as esophageal histology in anguimorph lizards might reveal some of the elements of the anatomical route by which snakes evolved. Whether this would relate to phylogenetic history and provide clues to the origin of snakes awaits future discoveries.

Whereas macrostomy in snakes is reflected in skeletal features alone, there had to be a variety of soft tissue modifications to permit engulfing and storing of very large prey. Modifications of the gut for increasing its circumference appear to be associated with many of the skeletal and muscular features documented for snakes (Cundall and Greene, 2000). The existence of additional specializations seems likely, particularly modifications for extreme extension at cellular levels (Close and Cundall, 2013).

Given the complexity of soft tissues in the heads of squamates and the diversity of tongue forms and lower jaw arrangements (McDowell, 1972; Schwenk, 1988), the organization of the gut may be more variable than has been appreciated. Varanid lizards approach snakes in tongue form and in having the glottis lying relatively far anteriorly without having many of the critical synapomorphies defining macrostomatan snakes (e.g., independence of left and right palatal elements, loss of the mandibular symphysis, posteriorly angled quadrate, solidly enclosed braincase). It seems likely that many of the features of the gut of macrostomatan snakes are foreshadowed in other squamate lineages, their appearance driven by selection for properties unrelated to macrostomy. Without anatomical and developmental evidence, the evolution of the gut cannot be analyzed, and neither bones nor DNA sequences will tell the story.

ACKNOWLEDGEMENTS

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APPENDIX A: HISTOLOGICAL MATERIALS EXAMINED

Serial Sections

Colubridae, Colubrinae: *Lampropeltis getula* (LU series 121, 122) serial sections posterior head and anterior trunk, *Pantherophis guttatus* (LU series 125, 126) serial sections posterior head and anterior trunk. Natricinae: *Nerodia sipedon* juveniles (LU series 179, 501, 503, 505, 508, 511, 514) head and anterior trunk; adult,

representative sections of esophagus only (LU series 516–518); *Nerodia fasciata* (LU series 314) serial sections of head, *Regina septemvittata* (LU series 176, 206, 207) serial sections of posterior head, *Thamnophis brachystoma* (LU series 296) serial sections (poor) posterior head, *Thamnophis sauritus* (LU series 168) serial sections of posterior head; *Thamnophis sirtalis* juveniles, serial sections of posterior head (LU series 131, 156, 163, 172). Dipsadinae: *Carphophis amoenus* (LU series 341, 342) serial sections (poor) whole head, *Diadophis punctatus* (LU series 343) serial sections (poor) whole head, *Heterodon platirhinos* (LU series 332, 335) serial section (poor) posterior head. Viperidae, Crotaline: *Agkistrodon piscivorus* (LU series 290) whole head of late embryo, (LU series 339) whole head of juvenile.

APPENDIX B: SPECIES DISSECTED

Cylindrophiidae: *Cylindrophis ruffus* (LU 1353, 1411) dissected head. Xenopeltidae: *Xenopeltis unicolor* (LU 1527, 2384) dissections of the lower jaw, (LU 2400) sagittal section of whole head. Pythonidae: *Python bivittatus* (LU 1093) sagittal section of whole head. Boidae: *Boa constrictor* (LU 2523) dissection of the lower jaw. Viperidae: *Agkistrodon contortrix* (LU 2578) dissected head, *Agkistrodon piscivorus* (LU 2320, 2321, 2529) hemisected head, (LU 2515, 2523, 2524) dissected head, *Crotalus atrox* (LU 2524) sagittal section of head, *Crotalus horridus* (LU 2318) dissected lower jaw.

Colubridae, Colubrinae: *Opheodrys aestivus* (LU 1377, 2546) microdissection of whole head, *Opheodrys vernalis* (LU 2537, 2544, 2545) microdissections of whole head, *Pantherophis spiloides* (LU 2525- 2528, 2530) dissections of whole head. Natricinae: *Nerodia fasciata* (LU 2322) midsagittal section of whole head, *Nerodia rhombifer* (LU 2338–2341, 2476) dissections of whole head, *Nerodia sipedon* (LU 2519) dissection of whole head, *Nerodia taxispilota* (2517) thick sections of head and anterior trunk, *Thamnophis validus*: (LU 2579) midsagittal section of whole head. Dipsadinae: *Farancia abacura* (LU 2330) thick sections of the anterior trunk, *Farancia erytrogramma* (AMNH 2249) dissection of lower jaw, *Heterodon platirhinos* (LU 2457, 2458, 2459, 2461) dissection of head.