

The Mechanism of Chemical Delivery to the Vomeronasal Organs in Squamate Reptiles: A Comparative Morphological Approach

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ABSTRACT Vomeronasal chemoreception, an important chemical sense in squamate reptiles (lizards and snakes), is mediated by paired vomeronasal organs (VNOs), which are only accessible via ducts opening through the palate anteriorly. We comparatively examined the morphology of the oral cavity in lizards with unforked tongues to elucidate the mechanism of stage I delivery (transport of chemical-laden fluid from the tongue tips to the VNO fenestrae) and to test the generality of the Gillingham and Clark (1981. *Can J Zool* 59:1651–1657) hypothesis (based on derived snakes), which suggests that the sublingual plicae act as the direct conveyors of chemicals to the VNOs. At rest, the foretongue lies within a chamber formed by the sublingual plicae ventrally and the palate dorsally, with little or no space around the anterior foretongue when the mouth is closed. There is a remarkable conformity between the shape of this chamber and the shape of the foretongue. We propose a hydraulic mechanism for stage I chemical transport in squamates: during mouth closure, the compliant tongue is compressed within this cavity and the floor of the mouth is elevated, expressing fluid from the sublingual glands within the plicae. Chemical-laden fluid covering the tongue tips is forced dorsally and posteriorly toward the VNO fenestrae. In effect, the tongue acts as a piston, pressurizing the fluid surrounding the foretongue so that chemical transport to the VNO ducts is effected almost instantaneously. Our findings falsify the Gillingham and Clark (1981. *Can J Zool* 59:1651–1657) hypothesis for lizards lacking forked, retractile tongues. *J. Exp. Zool.* 311A:20–34, 2009. © 2008 Wiley-Liss, Inc.

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The vomeronasal organs (VNOs) are paired chemosensory organs located at the base of the nasal cavity just above the palate. They are found in most tetrapod lineages including amphibians, turtles, lepidosaurians and most mammals (Bertmar, '81), but they are especially well developed in squamate reptiles (lizards and snakes). Vomeronasal chemoreception is an extremely important sensory mode in squamates, mediating nearly every important behavior, particularly those related to foraging, feeding and reproduction (see Burghardt, '70; Halpern, '92; Mason, '92; Cooper, '94; Schwenk, '95 for reviews).

In squamates, the vomeronasal chemosensory system is anatomically and functionally distinct from the main olfactory system. The paired VNOs of squamates, unlike those of other vertebrates,

have lost their connection to the main olfactory system and communicate exclusively with the oral cavity through two tiny openings (vomeronasal fenestrae) in the anterior palate (Bellairs and Boyd, '50; Parsons, '70; Halpern, '92; Schwenk, '93, '95). In addition to being structurally isolated from the main olfactory system, the vomeronasal nerves project to a separate part of the olfactory

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bulb (accessory olfactory bulb) and remain separate within the central nervous system until at least the amygdaloid nuclei (Halpern, '76; Martínez-García et al., '91; Halpern, '92; Martínez-Marcos et al., '99). An important functional difference between the two chemosensory systems is that the vomeronasal system (VNS) can be stimulated by large, nonvolatile molecules that would not typically reach the main olfactory system during inhalation. Thus, the VNS is frequently viewed as specialized for the reception of high molecular weight, nonvolatile chemicals. In contrast, the main olfactory system is seen as specialized for small, volatile chemicals that diffuse easily into the air and travel over larger distances. Although this neat dichotomy is an overgeneralization and there is overlap in the chemical domains to which each system responds, as well as the behaviors they mediate (Halpern '92; Graves, '93; Schwenk, '95; Dial and Schwenk, '96; Wyatt, 2003; Baxi et al., 2006; Eisthen and Schwenk 2008), the ability of the squamate VNS to sample both volatile and nonvolatile chemicals is clearly one of its signal attributes. This ability relates to the fact that, in squamates, vomeronasal function depends on the active, or voluntary, stimulation of the VNOs by chemicals that are collected by the tongue, as opposed to passive, or involuntary, stimulation of the main olfactory system by airborne chemicals occurring during breathing.

The mechanism of vomeronasal stimulation varies among vertebrate taxa (Wyatt, 2003), but in squamates, it is mediated by "tongue-flicking" (TF) behavior in which the tongue is used to sample environmental chemicals, either from the air or from a surface, returning them to the mouth for delivery to the VNOs (Broman, '20; Burghardt, '70; Gove, '79; Kubie and Halpern, '79; Graves and Halpern, '89; Halpern, '92; Cooper, '94; Schwenk, '95). Although it is well established that the tongue collects environmental chemicals and delivers them into the mouth where they end up within the VNOs (Halpern and Kubie, '80; Graves and Halpern, '89; Halpern, '92), surprisingly, the biomechanics of this process remain poorly understood. Schwenk ('94) pointed out that chemical *sampling* of the environment and chemical *delivery* to the VNOs are mechanically distinct tasks that need to be considered separately in order to elucidate the mechanism of chemoreception and its relationship to the evolution of tongue form in squamates. Here we argue further that chemical delivery to the VNOs must, itself, be broken down

into two mechanically distinct stages: *stage I delivery* is the transfer of chemical-laden fluid from the tongue tip surfaces to the vomeronasal fenestrae; *stage II delivery* is the movement of this fluid through the vomeronasal fenestrae and into the lumina of the VNOs, where sensory transduction occurs. Recognizing that each of these three phases of vomeronasal chemoreception (sampling, stage I and II delivery) is mechanistically distinct is critical to developing and testing biomechanical hypotheses of function.

THE BIOMECHANICS OF VOMERONASAL CHEMORECEPTION

Chemical sampling and stage II delivery

TF kinematics vary within individuals and among species, ranging from simple tongue protrusion toward the substrate (where the tongue tips of the notched or forked tongue make contact with a surface before retraction back into the mouth), to rapid extension with multiple, high-speed oscillations, with or without substrate contact (e.g., Bissinger and Simon, '79; Gove, '79; Smith and MacKay, '90; Goosse and Bels, '92; Bels et al., '94; Herrel et al., '98). It is generally held that oscillatory TFs serve to increase the volume of air sampled by the tongue, thereby increasing its exposure to volatile odorants (Gove, '79; Gove and Burghardt, '83). According to Schwenk ('96; in preparation), the actual mechanism of chemical sampling must be diffusion: during TF, scent molecules diffuse into the fluid covering the surfaces of the tongue tips. As diffusion is a notoriously slow process, oscillatory TF serves to increase its rate by disrupting the boundary layer near the tongue's surface, steepening the velocity gradient of the air moving around the tongue and introducing new molecules through turbulent mixing in order to maintain the highest possible concentration gradient between the surrounding air and the tongue's liquid surface layer. In any case, the result of TF is that environmental chemicals are collected (dissolved) within the thin fluid layer investing the tongue tips. The recognition that environmental chemicals are delivered to the VNOs dissolved within this fluid is essential in the context of stage I delivery, the focus of this study.

Skipping ahead to stage II delivery, several hypotheses have been proposed over the years regarding its biomechanics, most notable being the notion that the tongue tips are inserted into the vomeronasal fenestrae to provide direct delivery of sampled chemicals to the VNOs (Broman, '20). If true, such a mechanism would

be restricted to snakes and lizard taxa with deeply forked tongues and narrow, attenuate tongue tips, and therefore would not be possible in the vast majority of squamate species. In any case, direct and X-ray visualization of the process has falsified this hypothesis (Oelofsen and Van den Heever, '79; Gillingham and Clark, '81; Young, '90). Young's ('93) review suggests that suction generated within the lumina of the VNOs (Broman, '20) is the most likely mechanism of stage II delivery, which is supported by circumstantial evidence (Young, '93; Toubreau et al., '94). Suction is putatively generated by the elevation and relaxation of the floor of the mouth, which causes dorsad displacement of a cartilage within the VNO known as the "mushroom body" (e.g., Parsons, '70). This movement compresses the VNO lumen, forcing its contents out, then elastic recoil of the mushroom body to its resting position creates negative pressure, drawing fluids back in (Broman, '20; Young, '93).

Stage I chemical delivery

Like sampling, stage I delivery has received little formal attention and is poorly understood. Broman's ('20) discredited direct-insertion hypothesis combined stage I and II delivery into a single step, but there is no evidence to support any single stage mechanism. The only formal hypothesis for stage I delivery, widely accepted in the literature, is that of Gillingham and Clark ('81), who observed snakes during unusual, possibly aberrant, open-mouthed TFs. They proposed that chemicals collected on the tongue tips are wiped across raised pads on the floor of the mouth known as sublingual plicae ("anterior processes") during tongue retraction. After this transfer, the tongue is further retracted and the pads are elevated so that they contact the palate directly, transferring chemicals, once again, to the vomeronasal fenestrae where, presumably, they are sucked into the VNOs (stage II delivery; see above).

Despite its wide acceptance in the literature, the generality of the Gillingham and Clark ('81) hypothesis for stage I delivery has never been tested. As noted, their hypothesis is based exclusively on the observations of snakes (during potentially aberrant TFs). However, the form of the tongue and the oral cavity in snakes is highly derived and specialized in comparison with lizards (McDowell '72; Schwenk '88; Filoramo and Schwenk '98; Filoramo and Schwenk, in preparation; see below). The snake tongue is slender,

deeply forked and fully retractable within a lingual sheath lying beneath the larynx (McDowell, '72), which is situated very far anterior in the floor of the mouth compared with other squamates (McDowell, '72; Buchtova et al., 2007). Therefore, following retraction of the tongue into the mouth after TF, the tongue can be withdrawn from the oral cavity so that nothing lies between the sublingual plicae and the palate (vomeronasal fenestrae). In contrast, the tongue in most lizards is broad and neither forked nor retractile (Gnanamuthu, '37; Schwenk, '88, '95, 2000). After retraction following TF, the tongue remains within the oral cavity, lying between the sublingual plicae and the vomeronasal fenestrae. Finally, the floor of the mouth and the palate are uniquely modified in snakes, differing substantially in form from lizards, including species with forked tongues (Iwasaki et al., '96; Filoramo and Schwenk, '98; Filoramo and Schwenk, in preparation).

In this study we consider the mechanism of stage I chemical delivery through a comparative analysis of oral and lingual morphology in a phylogenetically diverse sample of nonophidian squamate reptiles (Table 1). We focus here on taxa with generalized tongues possessing only notched, rather than forked, tongue tips in order to ascertain the biomechanics of stage I delivery in species lacking snakes' derived modifications. We are thus able to test the generality of the Gillingham and Clark ('81) hypothesis for squamates, as a whole. This is critical for clarifying

TABLE 1. A list of the species included in this study

<i>Iguania</i>	
Iguanidae	<i>Urosaurus graciosus</i> (LM) <i>Phrynosoma mcallii</i> (LM) <i>Phrynosoma</i> sp (Macro photos of preserved specimen) <i>Anolis gemmosus</i> (LM) <i>Chamaelinorops barbouri</i> (LM) <i>Dipsosaurus dorsalis</i> (LM)
Agamidae	<i>Physignathus cocincinus</i> (LM) <i>Phrynocephalus</i> sp. (LM) <i>Leiolepis</i> sp. (LM) <i>Uromastyx ocellatus</i> (LM, SEM) <i>Stellio (Agama) stellio</i> (Macro photos of preserved specimen)
<i>Gekkota</i>	
Gekkonidae	<i>Coleonyx variegates</i> (LM, SEM) <i>Gekko gecko</i> (SEM)
<i>Scincomorpha</i>	
Xantusiidae	<i>Xantusia vigilis</i> (LM)

LM, serially sectioned and examined using light microscopy. SEM, imaged using a scanning electron microscope.

patterns of phenotypic evolution of the VNS within Squamata (we consider fork-tongued species in a separate paper [Filoramo and Schwenk, in preparation]). Our data, in conjunction with biomechanical theory, permit us to make robust functional inferences and to propose a novel hypothesis for the mechanism of stage I chemical delivery in squamate reptiles.

MATERIALS AND METHODS

We used gross anatomy, scanning electron microscopy (SEM) and light microscopy (LM) of serially sectioned squamate heads to examine the features of the tongue, palate, floor of the mouth and the three-dimensional relationships among these. Table 1 lists the species included in this study and the techniques (SEM or LM) applied to each. Specimens were either borrowed from museum collections, collected in the field, donated or purchased. Live animals were sacrificed humanely by means of overdose with pentobarbital sodium administered through intraperitoneal injection.

Species were chosen to represent a phylogenetically broad, morphologically diverse cross section of those squamate taxa lacking forked tongues (see Fig. 1). In the following descriptions we focus on species for which we have the most complete data. However, we have partial data for many more species that are not described here. Schwenk ('88 and unpublished data) found relatively little morphological variation in tongue form within nominal families. Therefore, focal species are representative of the family-level lineages (and even larger taxa) to which they belong.

Scanning electron microscopy

The snout and lower jaw were removed from each lizard after sacrifice and fixed in aldehyde (1.5% gluteraldehyde+1.5% paraformaldehyde+3mM MgCl₂ in 0.1M HEPES buffer) and 4% osmium tetroxide in accordance with standard electron microscopy procedures. Most specimens were soaked in 8N HCl in a 60°C oven for 10–15 min after fixation and before dehydration in order to remove any mucus covering the surfaces (Iwasaki et al., '96). After dehydration in a graded ethanol series and critical point drying, each specimen was mounted on a stub and sputter coated with a mixture of 60% gold (Au) and 40% palladium (Pd) for 1–2 min. SEM was carried out using a LEO/Zeiss DSM982 field emission SEM (Oberkochen, Germany) operated at 2.0 kV.

Light microscopy

Immediately after sacrifice, the head from each specimen was removed and fixed by immersion in 10% formalin. The heads were then decalcified in 20% formic acid A solution (Presnell and Schreiberman, '97), dehydrated in a series of ethanol baths, cleared in toluene, and infiltrated and embedded in 100% paraffin using standard procedures (Presnell and Schreiberman, '97). The embedded specimens (whole head, snout only or lower jaw with tongue only) were then serially sectioned at a thickness of 10 µm. The sections were mounted on glass slides, and stained either with hematoxylin and eosin, or Weigert iron hematoxylin and picroponceau (Presnell and Schreiberman, '97).

RESULTS

General description of the tongue, palate and floor of the mouth

In most squamates, with the notable exception of snakes and varanid lizards, the tongue is a large, muscular body that virtually fills the oral cavity at rest. As such, head and snout shape are good predictors of tongue size and shape. This is not true for the highly derived snakes and varanids, in which the tongue has been reduced to a slender, muscular shaft that is specialized for hydrostatic elongation and is retractile within a lingual sheath. These taxa also possess the most deeply forked tongues of any squamates and are dealt with in a separate paper (Filoramo and Schwenk, in preparation) (Fig. 2). On the ventral surfaces of the foretongue lie a pair of thickened, heavily keratinized pads or ventral pallets (McDowell, '72) (Fig. 3).

Regardless of lingual form, the tongue at rest lies within the mouth with the ventral pallets lying on top of elevated regions of the mouth floor known as sublingual plicae (Figs. 3, 4). The sublingual plicae are the external manifestation of the underlying sublingual glands. Their ducts drain to the oral cavity directly beneath and along the margins of the foretongue. The sublingual glands and plicae usually form a U-shaped prominence lateral to the tongue margin, following the arc of the mandible. The tongue lies within a depression or concavity bounded by the plicae and the ventral surface of the foretongue rests on their dorsomedial surfaces anteriorly (Figs. 4, 7).

Above the foretongue are the vomeronasal fenestrae leading through the palate to the VNOs within the snout. In most species, the anterior

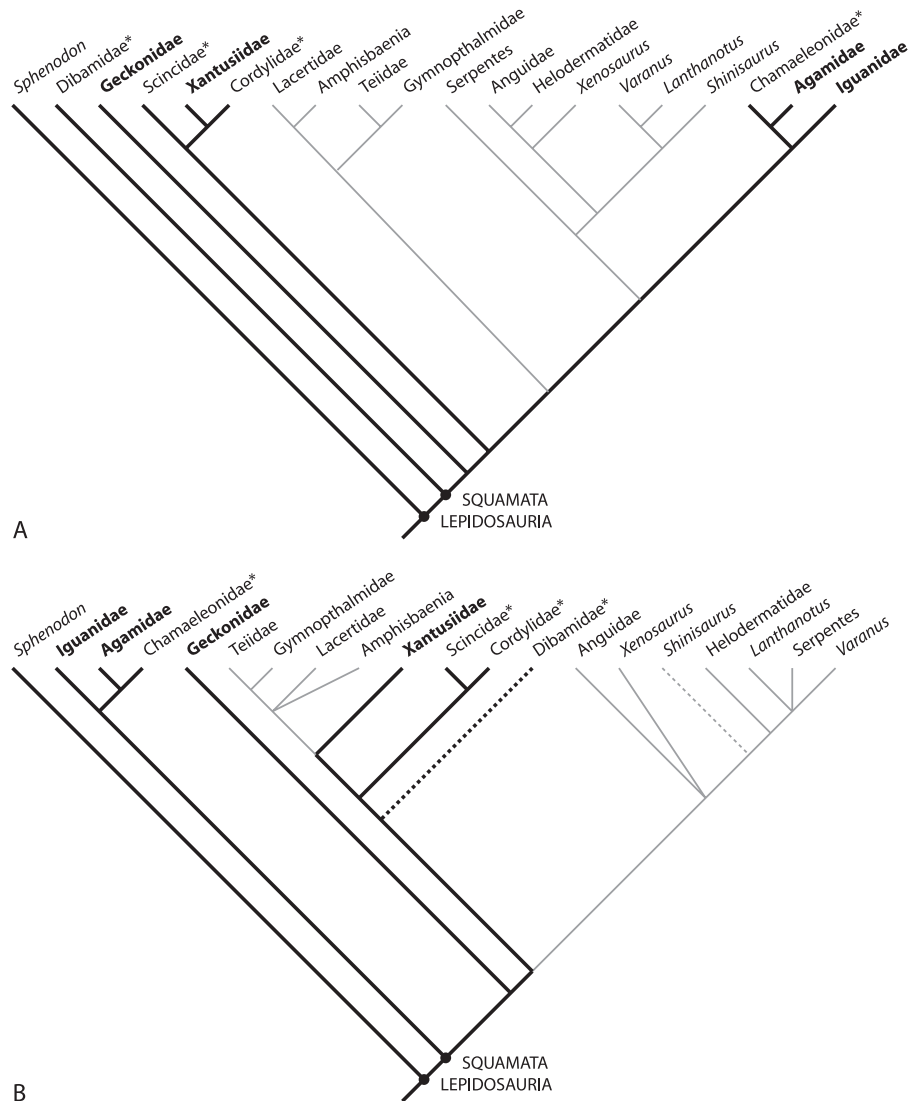


Fig. 1. Two phylogenetic hypotheses for squamate reptiles. In both trees, taxa represented in this study are shown in bold. Taxa for which we have some, but incomplete, data are indicated by an asterisk (although not described in this article, data from these taxa are consistent with what are reported here). Thinner, gray lines indicate lineages with tongues that are forked to varying degrees. Owing to important mechanistic issues pertaining specifically to fork-tongued species, we treat them in a separate paper (Filoramo and Schwenk, in preparation). (A) A nuclear gene-based tree from Townsend et al. (2004). (B) A morphological tree based primarily on Estes et al. ('88).

palate shows a depression that conforms to the shape of the foretongue. The choanae, or internal nares, open within the posterior part of this depression and the vomeronasal fenestrae lie anterior to the choanae, usually within tiny crevices (Fig. 4).

Iguanidae

The palate in iguanids is smooth and lined with typical squamous epithelium. Anterior to the VNOs the palate is without any major ridges or

depressions and ranges from flat to slightly convex. At the point of entry of the vomeronasal ducts the palate becomes more domed, reflecting the shape of the tongue (Fig. 5). In *Phrynosoma mcallii* (Fig. 5) the palate also mirrors the topography of the sublingual plicae such that there is a groove that runs along the lateral margins of the palate that correspond to the crest of the sublingual plicae lateral to the tongue.

The floor of the mouth is also lined with typical squamous epithelium. The sublingual plicae form a “U”-shaped depression on the floor of the mouth

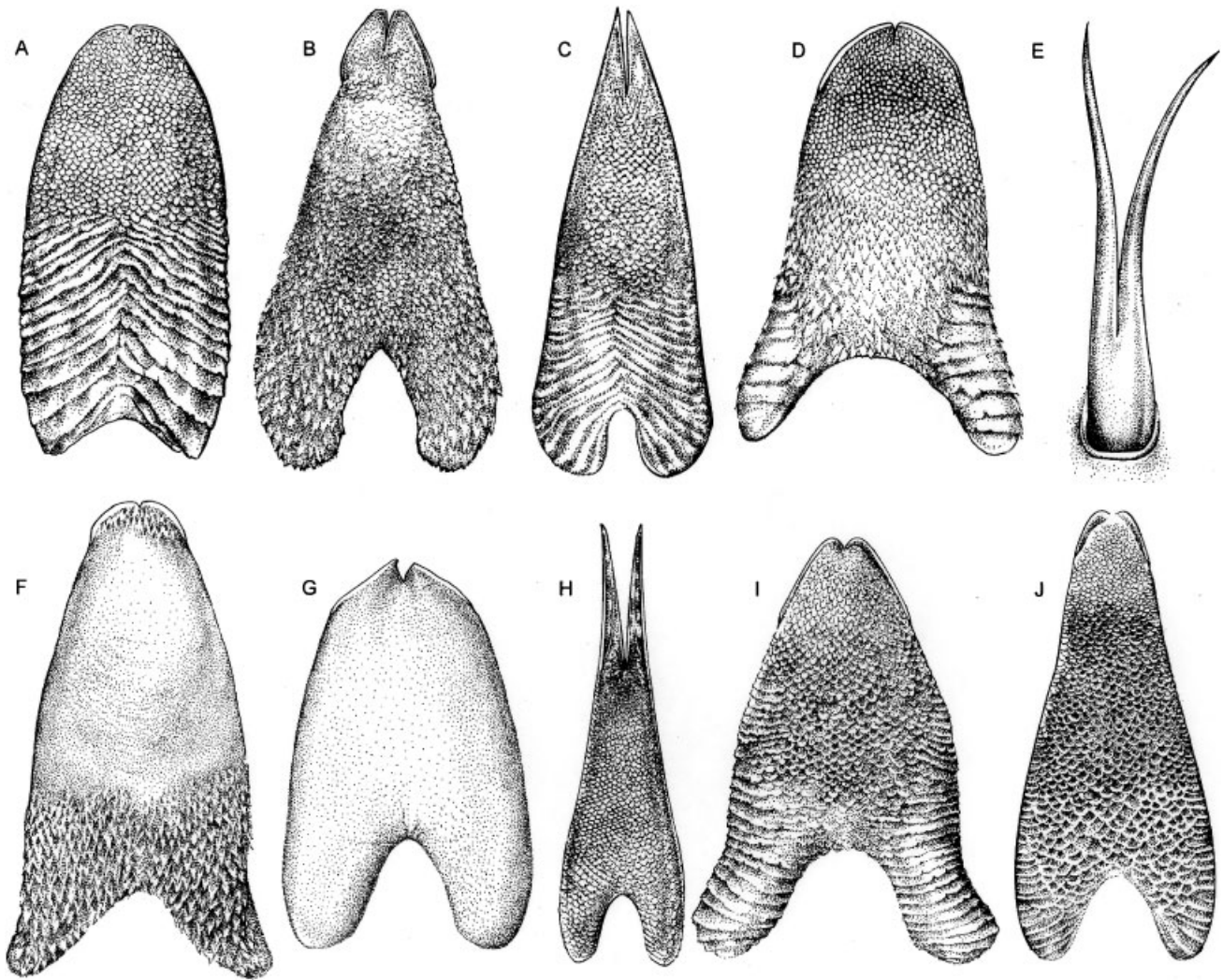


Fig. 2. Variation in tongue form in squamate lizards. (A) *Xantusia* (Xantusiidae). (B) *Abronia* (Anguinae). (C) *Podarcis* (Lacertidae). (D) *Coleonyx* (Gekkonidae). (E) *Varanus* (Varanidae). (F) *Gonocephalus* (Agamidae). (G) *Crotophytus* (Iguanidae). (H) *Cnemidophorus* (Teiidae). (I) *Cordylus* (Cordylidae). (J) *Dasia* (Scincidae). (From Schwenk, '95; Drawing by M. J. Spring.)

in which the tongue rests. The dorsomedial surfaces of the sublingual plicae are moderately to steeply sloped depending on the contours of the tongue, such that the tongue rests upon these surfaces. The crests of the sublingual plicae are widely spaced, becoming more so posteriorly to accommodate the tongue as it becomes wider. The sublingual glands are diffused in their distribution, filling nearly all the space between the surfaces of the sublingual plicae and the mandible. The midline of the floor of the mouth is composed primarily of connective tissue. Some iguanids (e.g., *Anolis*, Fig. 5) possess a small fleshy projection from the midline of the mouth floor that corresponds to a groove in the ventral surface of the tongue tip. In some species (*Urosaurus graciosus*

and *Chamaelinorops barbouri*) there is a narrow trough running between the medial surfaces of the sublingual plicae and the midline of the floor of the mouth, whereas in others (*P. mcallii* and *Anolis gemmosus*; Fig. 5) the transition between the sublingual plicae and the midline of the floor of the mouth is seamless.

Agamidae

Agaminae: The palate is lined with typical squamous epithelium. The region of the palate anterior to the position of the VNOs is flat to slightly convex, and there are no prominent ridges or depressions. However, the region of the palate into which the vomeronasal fenestrae open is

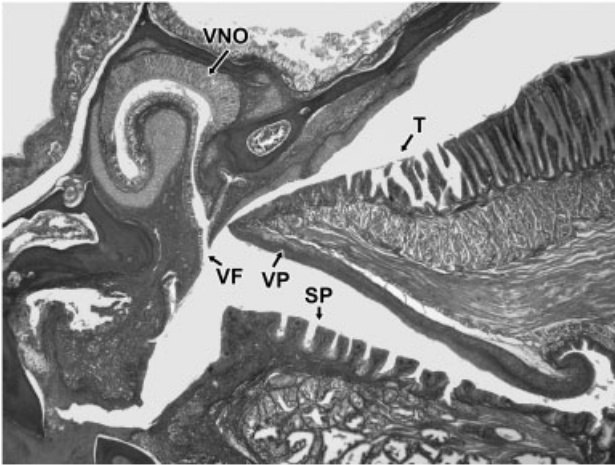


Fig. 3. Parasagittal section of the head of *Dipsosaurus dorsalis* (Iguanidae). Anterior is to the left. VF = vomeronasal fenestra; VNO=vomeronasal Organ; VP = ventral pallet; SP = sublingual plica; T=Tongue.

deeply convex, forming a domed chamber in which the tongue lies at rest (Fig. 6).

The floor of the mouth is also lined with typical squamous epithelium. The crests of the sublingual plicae are widely spaced and become progressively more widely separated posteriorly. The medial surfaces of the sublingual plicae are moderately to steeply sloped (Fig. 6). In *Physignathus cocincinus*, there is a distinct break between the sublingual plicae and the midline of the floor of the mouth with a relatively deep and wide trough separating them. In addition, the fleshy midline ridge is recessed relative to the sublingual plicae (Fig. 6). In *Phrynocephalus* there is also a trough between each plica and the midline of the floor of the mouth (Fig. 6). However, it is not very pronounced anteriorly and even posteriorly these troughs are neither as deep nor as pronounced as they are in *P. cocincinus*. The fleshy, midline ridge in this lizard is also recessed relative to the position of the sublingual plicae. The quality of the *P. cocincinus* specimen did not allow for an accurate description of the distribution of the sublingual glands. In *Phrynocephalus* the sublingual glands are compact in their distribution (in cross section they look like a narrow oval tipped toward the lateral margins of the lower jaw), and do not fill the entire space between the surfaces of the plicae and the mandibles as they do in the iguanids.

Leiolepineae: The palate of these lizards is lined with typical squamous epithelium. However, unlike the other iguanians described thus far, these lizards have a prominent midline ridge (Fig. 7).

This ridge runs from just anterior to, to just between the openings of the vomeronasal fenestrae. There are no other prominent ridges or depressions in the palate of leiolepine lizards. At the point at which the vomeronasal fenestrae open into the oral cavity, the palate is moderately domed. Owing to relatively poor fixation of the *Leiolepis* specimen, the remaining descriptions of the palate are based solely on *Uromastix*. Each side of the domed palate has two prominences—a lateral one just medial to the teeth and a more medial one ventral to the VNOs. Between the two medial prominences is a depression with a small, medial ridge (Fig. 7). When the mouth is closed, the lateral prominence would come into apposition with the crests of the sublingual plicae below and the midline ridge would pass through the notched tip of the tongue, connecting with the fleshy midline ridge found on the floor of the mouth.

The floor of the mouth is also lined with typical squamous epithelium. There is a deep trough between each plica and the midline of the floor of the mouth, and the fleshy midline ridge is recessed relative to the sublingual plicae (Fig. 7). Once again, the remaining descriptions apply only to *Uromastix ocellatus*. The medial surfaces of the sublingual plicae are very steeply sloped (Fig. 7). The crests of the sublingual plicae become more widely spaced posteriorly to accommodate the widening tongue. Scanning electron micrographs (Fig. 7) also show that the medial surfaces of the sublingual plicae are covered with a series of transverse slits $\sim 30\mu\text{m}$ in width. The sublingual glands are relatively compact, "hugging" the medial surfaces of the sublingual plicae; they do not fill the space between the surfaces of the sublingual plicae and the mandible as they do in the iguanid lizards.

Gekkonidae

The palate is lined with typical squamous epithelium. As in the iguanians, the contours of the palate reflect the shape of the tongue. The region of the palate anterior to the position of the VNOs is very broad, smooth and flat, with no prominent ridges or depressions. At the point at which the ducts of the VNOs open into the oral cavity the palate mirrors the contours of the floor of the mouth and the tongue such that two lateral prominences just medial to the tooth row come into apposition with the slightly elevated outer margins of the sublingual plicae during mouth closure (Fig. 8). These would effectively seal the

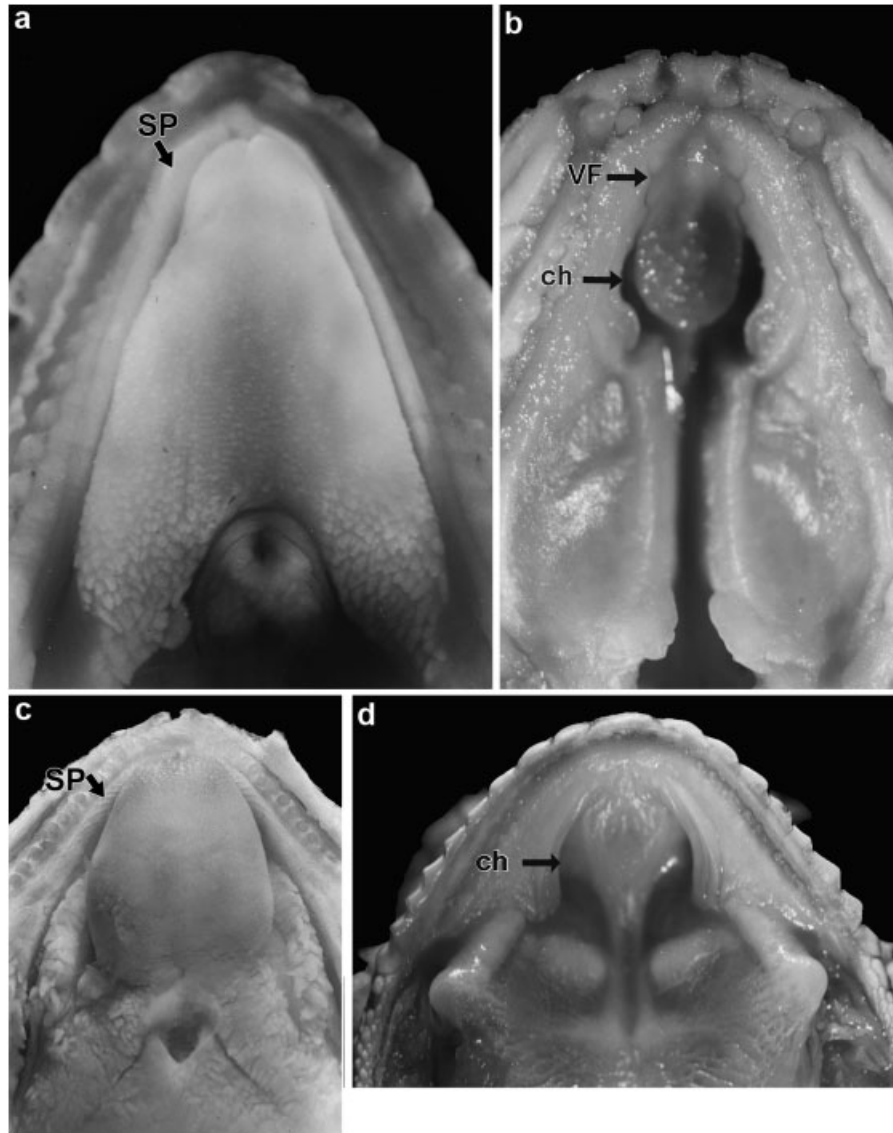


Fig. 4. Photographs of the floor of the mouth, tongue and palate of two iguanian lizards, *Stellio* (*Agama*) *stellio* (Agamidae) (a, b) and *Phrynosoma* sp. (Iguanidae) (c, d). Note that in both taxa the sublingual plicae form ridges that lie alongside the tongue so that the foretongue lies in a trough. The tongue tips rest on the dorsomedial surfaces of the sublingual plicae anteriorly. In (b) and (d), palatal prominences are evident that conform to the shape of the foretongue. These prominences abut the sublingual plicae when the mouth is closed, forming a tight chamber around the anterior end of the tongue. The vomeronasal fenestrae lie anteriorly within this chamber (evident in [b]). The chamber opens posteriorly into the choanae (internal nostrils). (a) *Stellio stellio*, tongue and floor of the mouth. (b) *Stellio stellio*, palate. (c) *Phrynosoma douglassi*, tongue and floor of the mouth. (d) *Phrynosoma* sp., palate. ch = choana; SP = sublingual plica; VF = vomeronasal fenestra.

foretongue within a closed chamber. The palate contains numerous palatine glands and is punctuated liberally with the openings of their ducts.

The floor of the mouth is also lined with typical squamous epithelium. The crests of the sublingual plicae are barely elevated from the floor of the mouth and the medial surfaces are very shallowly sloped. The sublingual glands are diffusely and

broadly distributed beneath the floor of the mouth. The glands largely filled the area between the surfaces of the sublingual plicae and the mandible (Fig. 8). The medial surfaces of the sublingual plicae are not covered with transverse slits, as seen in iguanians. Rather, the ducts of the sublingual glands are scattered across the mouth floor (Fig. 8). There is no gap between the plicae

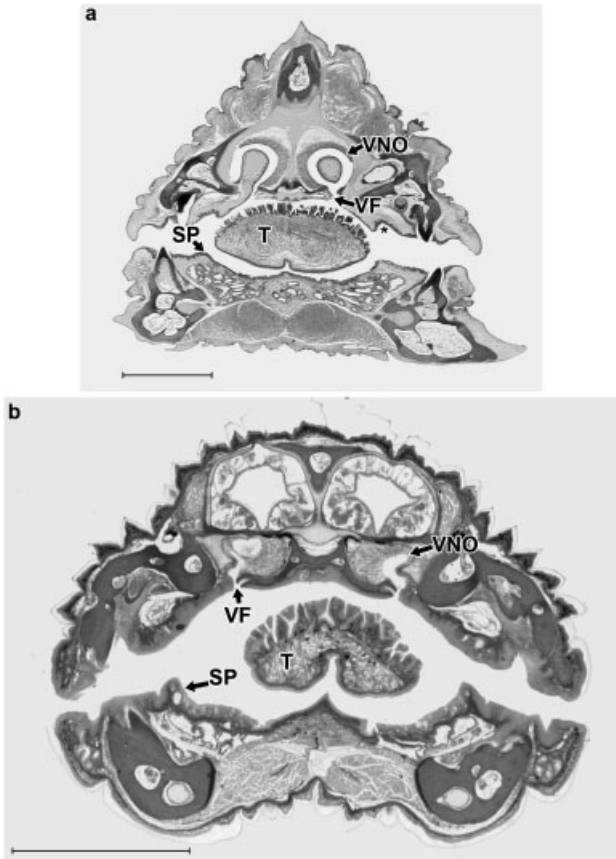


Fig. 5. Transverse sections through the anterior snouts of iguanid lizards at the level of the VNOs. (a) *Phrynosoma mcallii*. (b) *Anolis gemmosus*. SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ; * = palatal groove into which the corresponding sublingual plica would fit during mouth closure. Scale bar = 1 mm.

and the midline of the floor of the mouth. There is a small, fleshy, midline ridge that would fit into the ventral groove between each half of the bifurcated tongue tip.

Xantusiidae

There is some distortion in the *Xantusia* specimen owing to the displacement of the tongue during preservation (Fig. 9). However, many facets of the morphology of the palate and the floor of the mouth can still be accurately described. The palate is lined with typical squamous epithelium. As in gekkonids, the palate anterior to the position of the VNOs is broad, smooth and flat with no prominent ridges or depressions. However, in the region of the vomeronasal fenestrae there is a depression just medial to each fenestra with a midline prominence between them (Fig. 9).

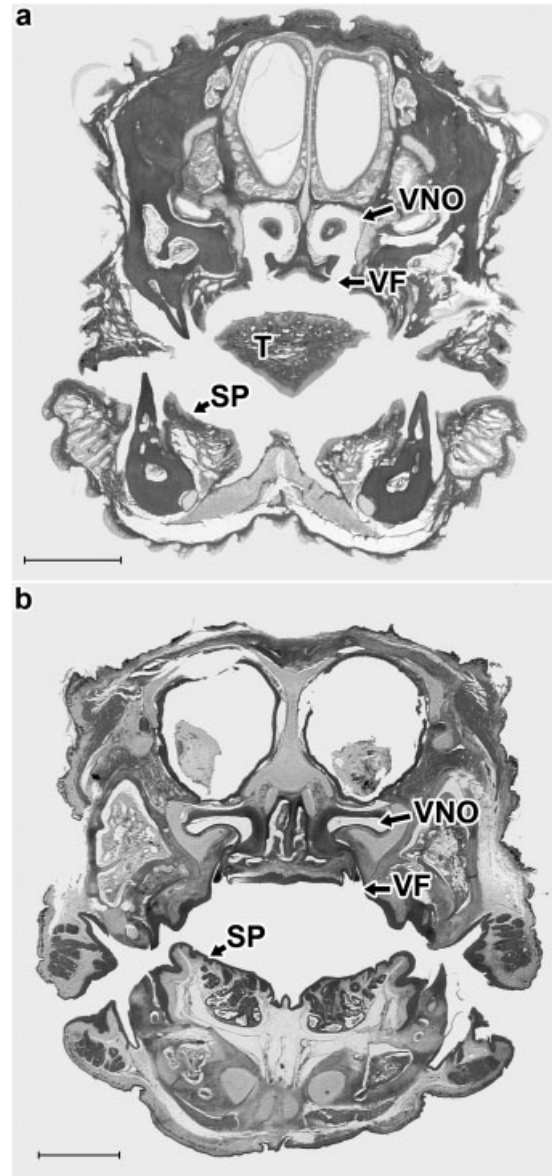


Fig. 6. Transverse sections through the anterior snouts of agamine lizards at the level of the VNOs. (a) *Physignathus cocincinus*. (b) *Phrynocephalus* sp. SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ. Scale bar = 1 mm.

There are no other prominent ridges or depressions lateral to these medial palatal depressions.

The floor of the mouth is lined with typical squamous epithelium. The sublingual plicae are poorly developed (as in gekkonids) and form gentle prominences that correspond to concavities on the ventral surface of the foretongue (Fig. 9). The sublingual glands are very compact and ovoid to nearly spherical in cross section, and are located close to the midline.

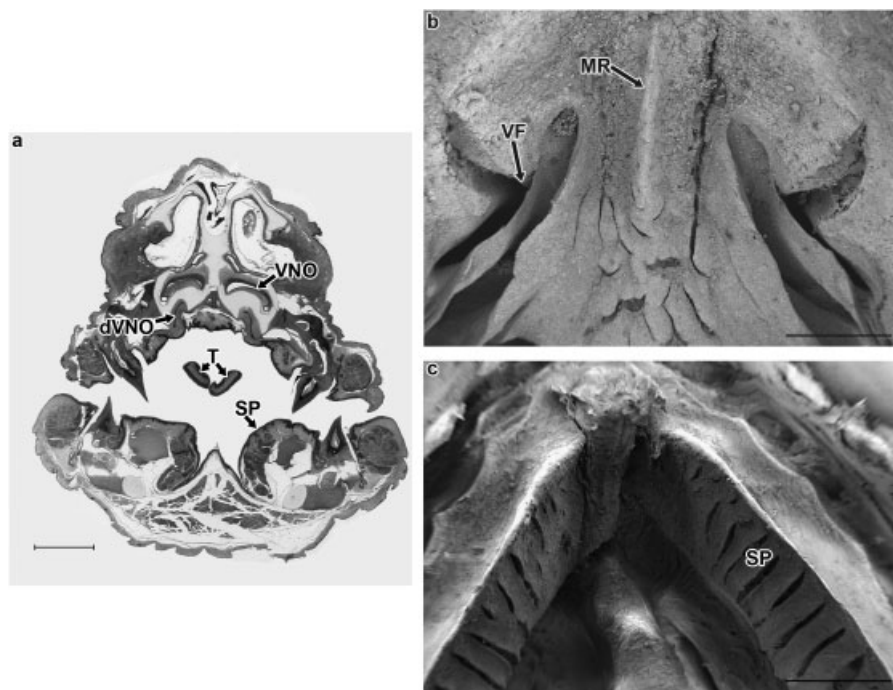


Fig. 7. *Uromastix ocellatus* (Leiolepineae, Agamidae). (a) Transverse section through the anterior snout at the level of the VNOs; Scale bar=1 mm. (b, c) Scanning electron micrographs of the palate (b) and floor of the mouth (c); Scale bar=500 μ m. dVNO = duct of the VNO (opens through palate at VF); MR = midline ridge; SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ.

Relationship of the tongue and oral cavity

Despite significant variation in the precise contours of the palate and the floor of the mouth in the lizards examined, a salient characteristic shared by all is the correspondence of the shape of the foretongue with the palate and the floor of the mouth, and often, between the palate and the floor of the mouth lateral to the tongue such that a tight fit would appear to be created around the tongue tips when the mouth is closed (Figs. 10, 11). In essence, when the mouth is closed, a chamber is created within the oral cavity that mirrors the shape of the tongue, and into which the foretongue fits snugly. In most cases (with the exception of *Xantusia*) the lateral margins of this chamber would be created by the connection between the crests of the sublingual plicae and the palate. In some, the palate has a groove or depression that corresponds with the sublingual plicae, thus ensuring a tight seal. In addition, most species have a prominent, fleshy ridge emerging from the midline of the mouth floor that would fill the notch at the tongue's tip. One group (Leiolepineae) has, in addition, a prominent fleshy ridge along the midline of the palate that would

also fit into the notched region of the foretongue. All of these features would seem to ensure that a seal could be created around the anterior part of foretongue. One must also bear in mind that the tongue and the oral tissues are all compliant so that when the mouth is closed surfaces in apposition will conform to each other's shape.

DISCUSSION

A test of the Gillingham and Clark ('81) hypothesis for stage I delivery

Based on their observations of open mouth TF in snakes, Gillingham and Clark ('81) proposed that chemicals on the tongue tips are deposited on the sublingual plicae as the tongue is retracted into its sheath within the oral cavity, and that the plicae then act as direct conveyors of chemicals to the VNO fenestrae to effect stage I delivery. Gillingham and Clark ('81) incorrectly refer to the sublingual plicae as the "anterior processes" of the sublingual plicae. However, the structures to which they refer are not projections emerging from the sublingual plicae, but rather are highly modified versions of the sublingual plicae found in

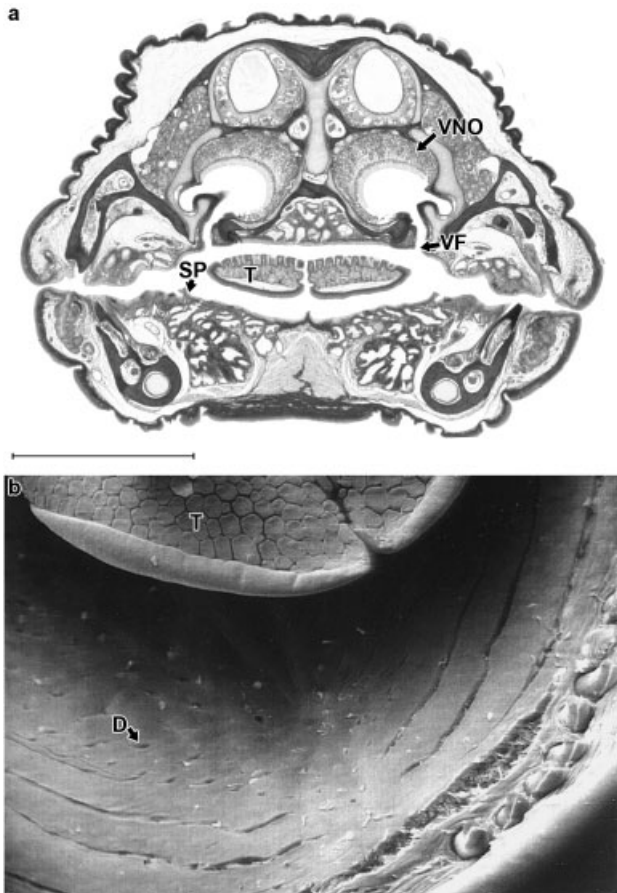


Fig. 8. *Coleonyx variegatus* (Geckonidae). (a) Transverse section through the anterior snout at the level of the VNOs. Note that although sublingual plicae are virtually absent (the floor of the mouth is flat), there are small prominences lateral to the tongue that would abut with corresponding palatal prominences when the mouth is closed. Furthermore, a tiny midline ridge at the anterior end of the mouth floor even fills the notch between the tongue tips (not evident in B, probably owing to shrinkage). (b) SEM of the floor of the mouth. Note that during preparation for electron microscopy, the tip of the tongue receded posteriorly owing to shrinkage during drying, exposing more of the mouth floor than would be the case in life. D = duct of a sublingual gland; SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ. Scale bar = 1 mm.

lizards (Filoramo and Schwenk, in preparation). Therefore, we will refer to these structures simply as sublingual plicae sensu McDowell ('72).

As noted above, the sublingual plicae of snakes are highly derived as compared with those of lizards, and this study demonstrates the high degree of variability in the form of these structures among lizards. Indeed, some species (notably geckos) virtually lack sublingual plicae. Instead, the sublingual glands of geckos are diffusely

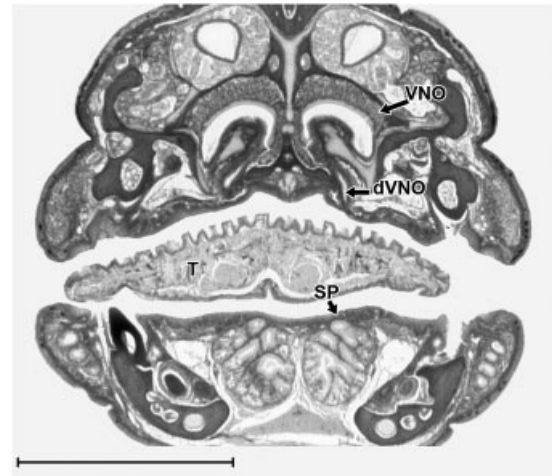


Fig. 9. Transverse section through the anterior snout of *Xantusia vigilis* (Xantusiidae) at the level of the VNOs. Note that lateral protrusion of the tongue beyond the tooth row on the left side of the micrograph is an artifact that occurred during fixation of the specimen. In life, the tongue at rest lies entirely within the oral chamber medial to the teeth. dVNO = duct of the vomeronasal organ; SP = sublingual plicae; T = tongue; VNO = vomeronasal organ. Scale bar = 1 mm.

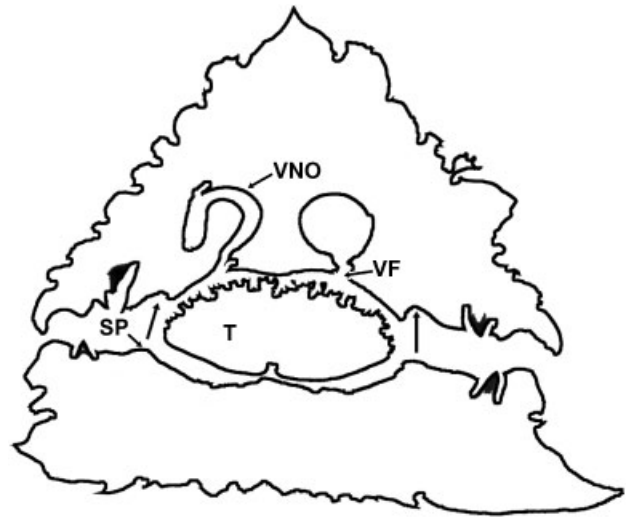


Fig. 10. Outline made from a transverse section of the anterior snout of *Phrynosoma mcallii* (Iguanidae) at the level of the VNOs highlighting the correspondence between the cross-sectional shapes of the tongue and the oral chamber. Vertical arrows indicate points of contact between the palate and the sublingual plicae during mouth closure, such that a sealed chamber is formed filled almost entirely by the tongue. SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ.

distributed in the floor of the mouth and only slight lateral prominences are evident. Thus, a general mechanism of stage I delivery must be

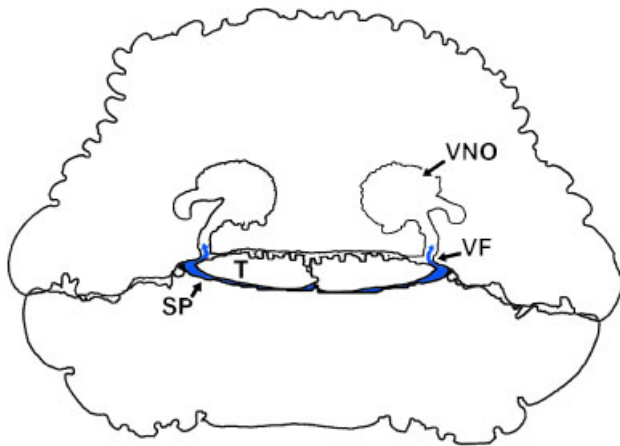


Fig. 11. Diagram illustrating our proposed hydraulic mechanism of stage I chemical delivery in squamates with unforked tongues (*Coleonyx variegatus*, Gekkonidae, illustrated). When the floor of the mouth is elevated, a seal around the tongue is formed. The tongue constitutes a compliant piston within this chamber. Anterior tongue movement and oral compression generates pressure that simultaneously expresses fluid from the sublingual glands and forces chemical-laden fluid on the tongue tip surfaces dorsally and posteriorly around the margins of the tongue to the vomeronasal fenestrae where it is positioned for stage II transport. For illustrative purposes, we have left a larger space around the foretongue than would actually exist. SP = sublingual plicae; T = tongue; VF = vomeronasal fenestra; VNO = vomeronasal organ.

compatible with the extraordinary variation in sublingual plica form. Furthermore, our study shows that in the vast majority of lizards the foretongue intervenes between the dorsomedial surfaces of the sublingual plicae and the vomeronasal fenestrae. Even if the tongue were to be pulled posteriorly, exposing the sublingual plicae, the crests of the sublingual plicae are well lateral to the fenestrae and the dorsomedial surfaces are unlikely to be elevated sufficiently to come into direct contact with the VNO fenestrae, thus preventing the plicae from transporting chemicals directly to the fenestrae. As such, elevation of the sublingual plicae can only serve to press the tongue into the palate and the vomeronasal fenestrae (see below).

Based on the considerations above, we suggest that the Gillingham and Clark ('81) hypothesis for stage I chemical delivery in squamate reptiles is falsified, at least in application to lizards with generalized tongues. Ongoing studies are considering the case of snakes and derived lizards with forked tongues (Filoramo and Schwenk, in preparation).

Relationships among the tongue, sublingual plicae, vomeronasal fenestrae and the oral cavity

At rest the ventral pallets of the tongue tips lie directly upon the dorsomedial surfaces of the sublingual plicae, which contain the openings of many ducts from the sublingual glands. Thus, the tongue is constantly bathed in the seromucous secretions from these glands (see also Dornesco and Andrei, '66; Gabe and Saint Girons, '69; Kochva, '78; Schwenk, '88). Fluids on the tongue surface might also be provided by lingual glands (refer to above), but these are reduced or lost in the foretongues of many squamates and are lacking completely from the tips of all species (Schwenk, '88). Thus, the sublingual glands are the most likely source of the fluid surrounding the tongue tip surfaces.

The sublingual plicae range from barely perceptible elevations in the floor of the mouth (e.g., gekkonids and xantusiids) to sharp elevations that form a deep cavity within which the tongue lies at rest (e.g., Agamidae). They lie along the lateral margins of the tongue (except in *Xantusia*, in which the slight elevations of the sublingual glands correspond to slight concavities on the ventral surface of the tongue). Beneath the free, anterior end of the tongue there is often a midline elevation that inserts neatly into the notch at the tongue's tip. In sum, the floor of the mouth conforms closely to the contours of the ventral surface of the foretongue and tongue tips and in most species forms a depression or cavity within which the resting tongue sits.

As for the palate, anteriorly it conforms closely to the shape of the tongue tips and anterior foretongue. Posteriorly, along the margins of the choanae, there are often slight elevations that correspond to the shape of the foretongue. Anterior to the choanae lie the VNO fenestrae. The fenestrae usually lie in grooves or crevices that open posteriorly into the choanae. In some cases there is a slight midline prominence anterior to the fenestrae that would lie between the notched tips when the mouth is closed and the palate contacts the dorsal surface of the tongue (e.g., in the agamid, *Uromastyx*, Fig. 7). Laterally on the palate, medial to the upper tooth rows, lie prominences containing the palatine glands, which are sometimes found within a groove. Like the sublingual plicae, these form elevations that lie along the lateral margins of the foretongue.

When the mouth is closed the sublingual plicae and the palatine glands would come into apposi-

tion along the lateral margins of the foretongue, effectively sealing it within the oral cavity. In *Xantusia*, narrow epithelial ridges take the place of the sublingual plicae in meeting the palatine glands. With the mouth closed there is very little space around the tongue. Given the compliant nature of the tongue and the surrounding tissues of the mouth floor and the palate, one can reasonably infer that with slight elevation of the sublingual plicae and the floor of the mouth, the small space remaining around tongue would be compressed and eliminated. However, it is important to note that the extremely tight fit between the oral cavity and the tongue described here applies only to the anteriormost part of the foretongue. Posterior to the vomeronasal fenestrae, the palate becomes vault-like where the choanae open into the nasal cavities. Thus, the (virtual) space surrounding the anterior foretongue would be sealed anteriorly and laterally, but would open posteriorly into the relatively open space of the oral cavity and pharynx.

A hydraulic hypothesis for stage I chemical delivery

During TFs directed into the air, chemicals are concentrated in the fluids covering the tongue tips. However, during substrate-directed TFs, which are most common in basal squamate clades such as iguanians (e.g., Gove, '79; Herrel et al., '98), chemicals are likely to be concentrated in the fluid covering the ventral pallets, the lingual structures that contact the surface. Thus, any proposed mechanism of chemical delivery must be able to explain how the chemical-laden fluid on the tongue tips is moved from the ventral side of the tongue to the vomeronasal fenestrae in the palate. Furthermore, this transfer must occur quickly, as the time between TFs is often very short (seconds or fractions of a second).

We propose that the transfer of chemicals from the tongue to the vomeronasal fenestrae (stage I delivery) occurs hydraulically when the foretongue is compressed by elevation of the floor of the mouth. As noted above, elevation of the floor of the mouth would cause the soft tissues of the tongue, palate and sublingual plicae to "mold" together, eliminating space around the tongue and pressurizing the fluid on the tongue tip surfaces. As the oral cavity is sealed around the anterior end of the tongue, but open posterodorsally toward the choanae in the palate, the incompressible fluid would be forced to flow in this direction, and thus,

directly toward the vomeronasal fenestrae. In addition, elevation of the sublingual plicae would serve to express additional fluid from the sublingual glands, washing the ventral and lateral surfaces of the tongue as the fluid is squeezed dorsally and posteriorly. The transverse slits seen in the sublingual plicae of many species may help to channel the fluid around the lateral margins of the foretongue. It is important to remember that the chamber formed around the tongue by the sublingual plicae and the palate confines the fluid within a chamber so that it is directed toward the vomeronasal fenestrae rather than laterally or anteriorly to the margins of the oral cavity. In effect, the tongue serves as a piston within a sequestered compartment of the anterior oral cavity and together with the sublingual plicae, serves to pressurize the fluid on the surfaces of the tongue tips so that it (and its payload of dissolved chemicals) is delivered almost instantly to the vomeronasal fenestrae in the palate (Fig. 10). It is possible that elevation of the floor of the mouth and the sublingual plicae is not necessary for hydraulic delivery to occur. Anterior movement of the tongue in its oral chamber might be sufficient. However, elevation of the mouth floor is often observed after a tongue flick (Gillingham and Clark, '81; Young, '90; Toubeau et al., '94; personal observation). Such elevation would have the advantage of simultaneously effecting stage I delivery and priming the VNOs for suction by elevating the mushroom bodies (Broman, '20; Young, '93). Relaxation and return of the mouth floor to the resting state would cause elastic recoil of the cartilaginous mushroom body, generating suction for stage II delivery synchronized with hydraulic stage I delivery.

The hydraulic hypothesis of stage I delivery is applicable to all squamates regardless of tongue form. It does not depend on the specialized features of the sublingual plicae, nor does it require that the tongue be retracted out of the way of the plicae. Hydraulic delivery only requires that the anterior end of the tongue be enclosed within a chamber that is sealed anteriorly and laterally, but open posteriorly. Our study demonstrates that these criteria are met in all squamates examined.

The problem of forked tongues

The focus of this study has been on lizards with broad tongues that are only slightly notched at their tips. This condition of the foretongue

represents the ancestral condition in squamates (Schwenk, '88) and is the necessary starting point for determining the mechanistic basis of vomeronasal chemoreception. Vomeronasal chemoreception is a shared trait among all squamates, along with TF behavior, bifurcation of the tongue tip and loss of a VNO–nasal connection (Schwenk, '93). Thus, the biomechanical basis for chemical sampling and delivery must be applicable to all squamates, even if it turns out to have been subsequently modified in derived taxa. The question of whether the hydraulic mechanism we propose here can be generalized to all squamates, including those with deeply forked tongues with attenuate tips, remains. A deeply forked tongue is an adaptation for tropotaxis (the ability to sample and sense relative signal strength from each side of the body separately and simultaneously) (Schwenk, '94), and as such, there is an additional requirement for the process of stage I chemical delivery in such species—it must assure that the chemicals concentrated in the fluids from the left and right tines of the forked tongue are delivered to the ipsilateral VNO without mixing. This is because tropotaxis requires that the concentration and composition of chemicals sampled by the left and right forks can be differentiated in order to determine the direction in which the chemical signal is stronger. In squamates with tongues that are not deeply forked, this is not an issue because they lack the ability to sample two disparate points with their tongues.

If our current hypothesis of a hydraulic mechanism for stage I chemical delivery is to be applied to squamates with deeply bifurcated tongues, several predictions about their oral morphology can be made. First, as in the lizards examined in this study, features of the palate and the floor of the mouth should reflect and conform to the shape of the bifurcated tongue tip. Second, there should be features of their oral morphology that serve to keep the chemicals on each fork separate during hydraulic stage I delivery, i.e., to prevent mixing of chemical-laden fluid from each tine. In essence, we would predict that instead of a single chamber formed around the foretongue, the oral morphology would be modified so that each fork of the tongue is housed in its own chamber and that the elevation of the floor of the mouth would cause fluids to flow within each of these separate chambers to the ipsilateral vomeronasal fenestra. We are currently testing these predictions in a companion study examining oral morphology and the process of chemical delivery in forked-tongued

squamates (Filoramo and Schwenk, in preparation).

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