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The structure of the nasal chemosensory system in squamate reptiles. 2. Lubricatory capacity of the vomeronasal organ

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The vomeronasal organ is a poorly understood accessory olfactory organ, present in many tetrapods. In mammals, amphibians and lepidosaurian reptiles, it is an encapsulated structure with a central, fluid-filled lumen. The morphology of the lubricatory system of the vomeronasal organ (the source of this fluid) varies among classes, being either intrinsic (mammalian and caecilian amphibian vomeronasal glands) or extrinsic (anuran and urodele nasal glands). In the few squamate reptiles thus far examined, there are no submucosal vomeronasal glands. In this study, we examined the vomeronasal organs of several species of Australian squamates using histological, histochemical and ultrastructural techniques, with the goal of determining the morphology of the lubricatory system in the vomeronasal organ. Histochemically, the fluid within the vomeronasal organ of all squamates is mucoserous, though it is uncertain whether mucous and serous constituents constitute separate components. The vomeronasal organ produces few secretory granules intrinsically, implying an extrinsic source for the luminal fluid. Of three possible candidates, the Harderian gland is the most likely extrinsic source of this secretion.

1. Introduction

The vomeronasal organ is a nasal chemosensory structure found in most terrestrial vertebrates. It is embryologically derived from the olfactory placode, and is both morphologically and physiologically similar to the main olfactory organ (see Halpern 1992 for review). Both systems consist of a chemosensory epithelium whose luminal aspects are bathed in a fluid, wherein odorant chemicals must dissolve prior to neural excitation (Getchell et al 1984a, b; Takami *et al* 1995). Variable dependence on either of these chemosensory systems has been documented within squamate reptiles (Halpern 1992; Schwenk 1993a, b; Cooper 1996). Snakes are acknowledged vomeronasal specialists, based on various morphological, neuroanatomical and behavioural features (see Halpern 1992 for review). Schwenk (1993a) and Dial and Schwenk (1996) proposed that gekkotan lizards may, in contrast, be olfactory specialists. However, evidence supporting this hypothesis is based on limited morphological, neuroanatomical and behavioural observations of some gekkotan species, as well as the absence of snake-like vomeronasal behaviour (i.e., complex tongue-flicking). The morphology of the scincid lizard VNO has received some attention (Kratzing 1975; Halpern 1992). Though the vomeronasal sensory capacity in scincid lizards is unknown, none of the features indicating snake-like vomeronasal specialization are present (i.e., complex tongue-flicking behaviour: Schwenk 1993b). The structure of the gekkotan vomernasal organ is similar to that of the scincid lizards (Gabe and Saint Giron 1976; Schwenk 1993b). Thus, though varying levels of nasal chemosensory dependence has been ascribed to snakes, skinks and gekkotans, there is little data on the morphology of the gekkotan vomeronasal organ.

One aspect of the vomeronasal sense which has received little attention, is the lubricatory system. It is well accepted that the lubricatory system in the main olfactory organ consists of the submucosal Bowman's glands and sometimes the sustentacular cells (Andres 1969; Müller *et al* 1979; Getchell and Getchell 1992).The lubricatory system of the vomeronasal organ has not only received little attention but also appears to be vari

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able within tetrapods. In mammals, for example, the vomeronasal lubricatory system consists of submucosal, seromucous vomeronasal glands (see Adams 1992 for review), and the development of the vomeronasal organ is positively correlated to the presence of these glands (Cooper and Bhatnager 1976). This is not the case in squamate reptiles, in which no such glands are known (Kratzing 1975; Gabe and Saint Girons 1976). However, the absence of these glands does not seem to hinder the development of the vomeronasal organ in squamates. This suggests that there is sufficient secretion for the squamate vomeronasal organ from other sources to compensate the absence of the intrinsic vomeronasal glands.

Whether sufficient glandular material might be scattered throughout the vomeronasal organ in squamates is unknown, but seems unlikely (Bannister 1968; Altner *et al* 1970; Kratzing 1975; Gabe and Saint Girons 1976; Wang and Halpern 1980; Takami and Hirosawa 1987, 1990; Halpern 1992). However, most studies have been carried out on snake and scincid lizard species. The morphology of the vomeronasal organ in gekkotans has thus far only been reported in the survey of Gabe and Saint Girons (1976). This survey, carried out at the light microscopic level, showed some features in vomeronasal lubricatory system of gekkotans (presence of potential secretory material in the non-sensory epithelium) which were not shared with either scincid lizards or snakes. This has not since been verified with either other specimens or with ultrastructural analysis. Further examination of the gekkotan condition is thus warranted, as this potential difference in the vomeronasal lubricatory system may translate into functional differences in the vomeronasal system within squamate reptiles (akin to that potentially existing between snakes and mammals).

There are several gekkotan taxa, each of which potentially vary in dependence on the vomeronasal sense. Of the three gekkotan taxa found in Australia, two (Diplodactylinae and Pygopodidae) are restricted to the Australasian region (Greer 1989). The legless pygopods possess many snake-like behavioural (i.e., oscillatory tongue-flicking) and morphological (i.e., relatively slender, slightly bifurcate tongue) characters. Both of these characters might indicate snake-like vomeronasal speciality (Schwenk 1993b). Pygopods are most closely related to the fully limbed diplodactyline geckos (Kluge 1987). Gekkoninae, is a closely related sister taxon to the Diplodactylinae/ Pygopodidae taxa, also occurs in Australia (Kluge 1987). These were then compared to the vomeronasal organ of a scincid lizard (*Morethia adelaidensis*) and a snake (*Pseudonaja textilis*). We thus aimed to determine not only whether the pygopod vomeronasal organ differed from that of geckos, but also to determine how vomeronasal organ morphology of geckos and pygopods compares to that of the scincid lizard and snake. Special attention was given to the lubricatory system.

2. Materials and methods

Adults from the following species were collected from the outskirts of Adelaide, South Australia, during spring (September–November); (Gekkota) Gekkonidae (Geckos) Gekkoninae: *Christinus marmoratus* (20), Diplodactylinae: *Strophurus intermedius* (5), Pygopodidae (flap-footed lizards): *Delma molleri* (20), (Scincomorpha) Scincidae (skinks): *M. adelaidensis* (6), Serpentes (snakes) Elapidae: *P. textilis* (18). At least one of each sex per species was examined with each of the morphological techniques. All animals were sacrificed with an intraperitoneal injection of sodium pentobarbitol (Nembutal), decapitated, and the heads placed in fixative (see below).

Either entire heads, or half heads (cut sagittally) of at least 1 specimen per species were fixed in 10% phosphate-buffered formalin for at least 1 week, decalcified in 10% aqueous EDTA, embedded in paraffin, and sectioned serially (7 μm). Alternate slides were stained with haematoxylin-eosin, in order to maximize material for the species in which only a few specimens were obtained.

Alternate slides of either full or half heads (not stained with haematoxylin-eosin) were tested histochemically for the presence of acidic mucosubstances and proteins. Neutral and acidic mucosubstances were detected by the periodic acid-Schiff (PAS), and alcian yellow (at pH 2· 5) (Ravetto 1964) methods respectively. The mercury bromo- phenol blue (BPB) test was used to detect protein (Barka and Anderson 1965), with pronase digestion for control.

For transmission electron microscopy, vomeronasal organs (at least 1 specimen per species) which had been dissected from the other side of the nasal capsule, were fixed for 4 h at room temperature in 3% formaldehyde/3% gluteraldehyde in 0· 1 M phosphate buffer at pH 7· 4, and postfixed for 1 h in 1% osmium tetroxide, then dehydrated through a series of ethyl alcohols and embedded in epoxy resin. Grids with thin sections (0· 1 μm) were stained with 2% uranyl acetate and lead citrate and examined with a PHILIPS CM 100 transmission electron microscope.

3. Results

3.1 *Histology*

The squamate vomeronasal organ is a dome-shaped, bone- and cartilage-encased structure in the rostral floor of the nasal cavity. The mushroom body, a conch-like projection from the ventrolateral aspect of the vomeronasal organ, projects into the lumen (figure 1). The vomeronasal duct connects the vomeronasal organ lumen with the mouth cavity. The vomeronasal organ appears to be in the same position and possesses roughly the same relative size in all species examined. Grossly, the only apparent difference

among species is the position of the nasolacrimal duct, which connects the anterior orbital region with the vomeronasal duct. In both gekkotan and scincid lizards, the nasolacrimal duct opens into the lateral aspect of the vomeronasal duct, hence traveling under the mushroom body. However, in the snake, *P. textilis*, the nasolacrimal duct approaches the vomeronasal duct caudally, and opens into its medial aspect.

In all species examined, there are three different epithelia lining the lumenal surfaces of the vomeronasal organ: the dorsally lining vomeronasal sensory epithelium, and two types of nonsensory epithelia lining the mushroom body and intermediate regions (figure 1). The vomeronasal sensory epithelium consists of microvillous bipolar receptor neurons, sustentacular and basal cells, in an arrangement similar to that of the olfactory epithelium. The vomeronasal sensory mucosa consists of a thick, sensory epithelium and a thin lamina propria. The snake vomeronasal sensory epithelium is much thicker than that of either the gekkotan or scincid lizards conditions. Additionally, the sensory epithelium is supported by a highly organized scaffolding, consisting of connective tissue columns (wherein run numerous blood vessels to the luminal aspects of the epithelium). No such level of columnarization is found in any of the lizards examined.

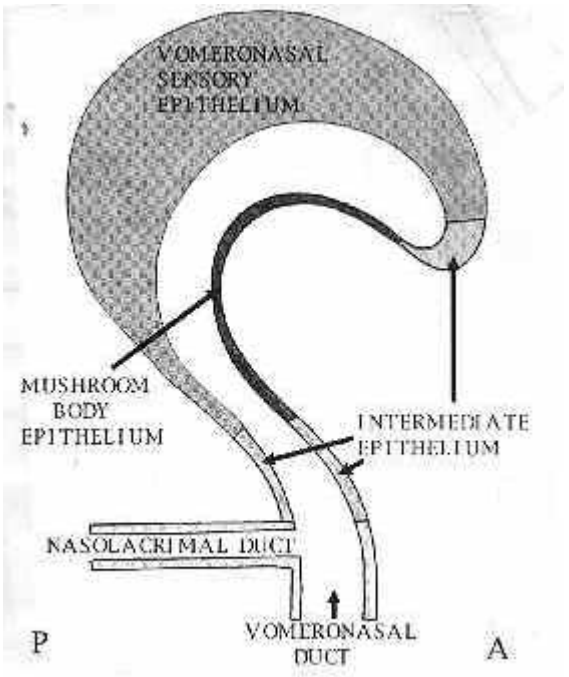

Figure 1. Diagrammatic representation of the squamate VNO, seen in the sagital plane, showing position of the epithelia.

The mushroom body is covered with a ciliated, columnar epithelium. The basic architecture of the mushroom body mucosa in all species examined was similar. The combined layers of the mushroom body mucosa do not equal the thickness of the vomeronasal sensory epithelium in any of the species.

The mushroom body epithelium is separated from the vomeronasal sensory epithelium by a zone of non-sensory epithelium displaying features intermediate between the two epithelial types. There are two types of intermediate mucosae, the thickness of which varies with respect to their relative position in the vomeronasal organ. One type, which lies between the vomeronasal sensory and mushroom body mucosae, covers the smallest area of the vomeronasal organ. Columnar secretory cells occur within the epithelium. The other type, which is much larger, lies between the vomeronasal duct and either the vomeronasal sensory or mushroom body mucosae. Herein lie cuboidal secretory cells, with an accompanying thin submucosal layer.

The lamina propria associated with each of these areas contains blood vessels, nerve fibers and connective tissue and the occasional mast cell. The lamina propria of the vomeronasal sensory epithelium contains comparatively more blood vessels and nerves, but less connective tissue, than either of the other regions. No glandular structures occurred in the lamina propria of any species examined.

3.2 *Histochemistry*

The results of the histochemical analysis are summarized in table 1. In all squamate reptiles, the luminal fluid in the vomeronasal organ stains positive with all three stains, indicating the presence of both mucous and serous secretory products.

The apical portion of the vomeronasal sensory and mushroom body epithelia in *M. adelaidensis* (skink) and *P. textilis* (snake) are weakly positive to all stains. Intensely PAS and mercury bromophenol blue positive apical granules are observed in the mushroom body epithelium of the gekkotans. This feature is weaker in *S. intermedius* (gecko) as compared to that of the pygopod *D. molleri* and the gecko *C. marmoratus*. A few columnar cells, with strongly PAS and alcian yellow (of Ravetto's method) positive apical granules, occur in the intermediate regions in all species.

3.3 *Ultrastructure*

Family level variation occurs in the presence and development of apical granules in both the vomeronasal sensory and mushroom body epithelia. In all cases, however, mucous cells occur in the intermediate epithelia.

3.3a *Vomeronasal sensory mucosa*: The apical secretory granules are the only point of variation observed in the sustentacular cells among these squamate reptiles. The sustentacular cells of the snake *P. textilis* contain a few, small (0· 3 μm diametre), apical, bipartite granules (figure 2A). Such granules are not present in any other squamate examined. Additionally, a few, smaller (0· 1 μm diametre) electron dense granules occur in the apical portion of the sustentacular cells of *P. textilis* and all gekkotans (figure 2B). Such structures are less discernible in *M. adelaidensis* (figure 2C).

3.3b *Mushroom body mucosa*: The mushroom body epithelium consists of two cellular layers, and overlies a thick submucosal area (figure 3A). The upper cell layer consists of columnar cells attaching basally to the lamina propria, with apical protrusions into the vomeronasal lumen. Nuclei are centrally located, whereas elongate mitochondria, rough endoplasmic reticulum, Golgi complexes and lysosomes are present throughout the cell. Apical desmosomes and tight junctions are replaced by basal interdigitations between cells in the nuclear and sub-nuclear levels. At the apex of the cell, both cilia and microvilli occur. The cells in the lower layer were attached solely to the lamina propria, and barely reach past the mid point of the epithelium. Mitochondria and rough endoplasmic reticulum surround the nuclei of these cells.

The only source of variation among species is the presence and size of apical granular formations in the upper columnar cells. No discernible apical granules occur in either the snake *P. textilis* or the skink *M. adelaidensis*, (figure 3B). A few small (0· 7 μm diameter), electron-dense granules occur in both the gecko *C. Marmoratus* (figure 3C) and the pygopod *D. molleri* (figure 3D). In the gecko *S. intermedius*, however, these electron-dense granules are both larger (0· 4 μm diameter) and more numerous (figure 3E) than in the other Gekkotan species.

3.3c *Intermediate mucosa*: The cuboidal secretory cells in the larger transitional zone (see figure 1) have central nuclei and apical microvilli (figure 4A). Few granules are found in the apical portion of some cells. The size and shape of these granules shows some interspecific variation. In *P. textilis*, *D. molleri* and *M. adelaidensis*, they rarely surpass 0· 7 μm in diameter and are generally homogeneous in nature (figure 4B, D). In the geckos, these granules are both bipartite (showing two distinct internal compartments) and relatively large (up to 1 μm diameter) (figure 4C). Mitochondria, Golgi complex and a few lysosomes are spread throughout the cell cytoplasm. The sides of the cells adhere to each other by desmosomes and interdigitating cell walls. Tight junctions are found in the apex of the cell.

The columnar secretory cells, in the shorter transitional zones, possess small luminal microvilli and basal nuclei. Abundant apical secretory granules (more than in the cuboidal cells), some over

Table 1. Summary of the histochemical results on the VNO of the squamates examined.

	VNE			MBE			IE			Fluid layer		
	PAS	BPB	R	PAS	BPB	R	PAS	BPB	R	PAS	BPB	R
Gekkota												
Gekkoninae:												
C. marmoratus	–	–	–	+1/2*	+1/2*	–	+	–	+	+	+	+
Diplodactylinae:												
S. intermedius	–	–	–	++*	++*	–	+	–	+	+	+	+
Pygopodidae:												
D. molleri	–	–	–	+1/2*	+1/2*	–	+	–	+	+	+	+
Scincomorpha												
Scincidae:												
M. adelaidensis	+	+	+	–	–	–	+	–	+	+	+	+
Serpentes												
Elapidae:												
P. textilis	+	+	+	–	–	–	+	–	+	+	+	+

Since the results were fairly uniform within the squamates examined (with exception of gekkotans), only the general observations are listed. "–", No reaction; "+", slightly positive reaction; "++", very positive reaction; PAS, periodic acid-Schiffs; BPB, mercury bromophenol blue; VNE, vomeronasal epithelium; MBE, mushroom body epithelium; IE, intermediate epithelium; Y, yellow stain with Ravetto's methods (acidic mucopolysaccherides). *, "++" in diplodactyline geckos and "+1/2" in gekkonine geckos and pygopods.

2 µm in diameter, are their most prominent feature (figure 4E). The granules are homogenous, but vary in electron density among species.

4. Discussion

At the anatomical level, the vomeronasal organ of all the squamates studied exhibits some morphological variation. This includes the route of the nasolacrimal duct and the columnarization of the vomeronasal sensory epithelium (both of which differentiates the snakes from the lizards) and the structure of the lubricatory system. The last of these, the structure of the lubricatory system, shows much variation. Thus, it is much harder to make generalization. Each facet of the vomeronasal lubricatory system will thus be discussed individually.

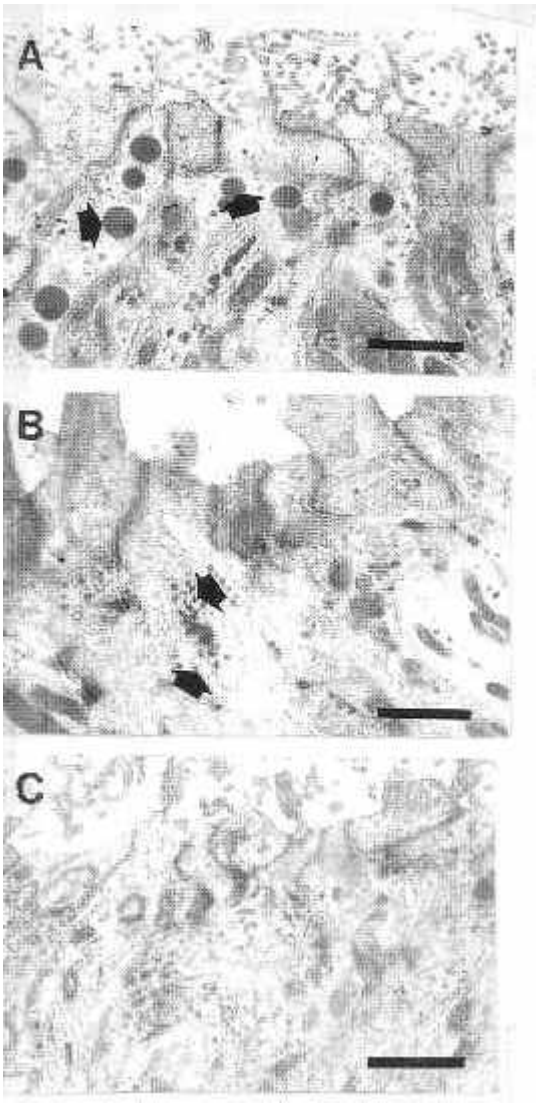

Figure 2. The apices of the vomeronasal sensory epithelia of *P. textilis* (**A**), *S. intermedius* (**B**) and *M. adelaidensis* (**C**). Note the presence of large granules in *P. textilis* (arrow heads), with smaller ones present in both *P. textilis* and *S. intermedius* (arrow heads). (Bar: 1 µm.)

Histochemically, both acidic mucopolysaccharides (based on reactivity to PAS and alcian yellow stains: Drury and Wallington 1980) and proteins (based on reactivity to mercury bromophenol blue: Barka and Anderson 1965) occur in the squamate luminal fluid. It is uncertain whether the two types of secretion form separate layers, are mixed together, or a combination of the two. A heterogeneous fluid layer, with two chemically distinct lamina, covers the sensory epithelia of the main olfactory organ of terrestrial vertebrates (Andres 1969; Müller *et al* 1979; Getchell and Getchell 1992). There is some evidence for such layering of fluid in the mammalian vomeronasal organ (Takami *et al* 1995). Though this layering appears to be important in the function of the main olfactory organ and the vomeronasal organ, the precise function of the fluid components is speculative. The most likely function for the fluid is as a medium for chemicals to dissolve before they can stimulate the neural components of the vomeronasal sensory epithelium (Getchell and Getchell 1992; Getchell *et al* 1993). Additionally, the fluid may provide sustenance for the epithelia. The fluid may also contain stimulus binding proteins which transport the stimulus to the vomeronasal sensory epithelium, or enzymes which break down the complex chemicals to smaller units which would then bind to the vomeronasal receptor neurons. Further microchemical analyses of this fluid are required before either the laminous nature or the function of the vomeronasal fluid components can be ascertained.

The fluid filling the lumen of the vomeronasal organ is produced by a vomeronasal lubricatory system. An intrinsic lubricatory system (i.e., secretory structures found either within or in close proximity to the chemosensory mucosae) is less well developed in squamate reptiles than in other tetrapods. Since a lubricatory system is needed to produce the luminal fluid, the possibility that there is an external source needs to be explored. In the remainder of this paper, evidence for intrinsic versus extrinsic sources of vomeronasal fluid is examined.

4.1 *Intrinsic sources of luminal fluid*

Intrinsic secretory structure for the vomeronasal organ may come in the form of distinct glandular masses (vomeronasal glands) or many scattered secretory cells within or in close proximity to the chemosensory mucosa. The composition of the intrinsic sources for the vomeronasal luminal fluid varies within tetrapods. The mammalian and caecilian amphibian vomeronasal organs contain well developed vomeronasal glands (Cooper and Bhatnager 1976; Badenhorst 1978; Adams 1992). In

anuran and urodele amphibians, however, the nasal glands in the adjoining nasal capsule are thought to be the source of the fluid (Dawley and Bass 1988; Døving *et al* 1993). In squamates, however, the vomeronasal organ has comparatively few intrinsic secretory structures, with no evidence of any glandular material development. Additionally, since the squamate vomeronasal duct lacks a connection to the nasal cavity (and thus cannot directly receive fluid from the nasal gland), unlike the condition in other tetrapods, the nasal glands in squamates are an unlikely source of fluid for the vomeronasal organ.

The paucity of intrinsic secretory structures is a striking feature of the squamate vomeronasal lubricatory system (Kratzing 1975; Gabe and Saint Girons 1976; Halpern 1992). Secretory granules are few and limited to mucous secretory cells in the intermediate region in all squamates examined and also to sustentacular (mucous) and mushroom epithelial (serous) cells in the snake and gek

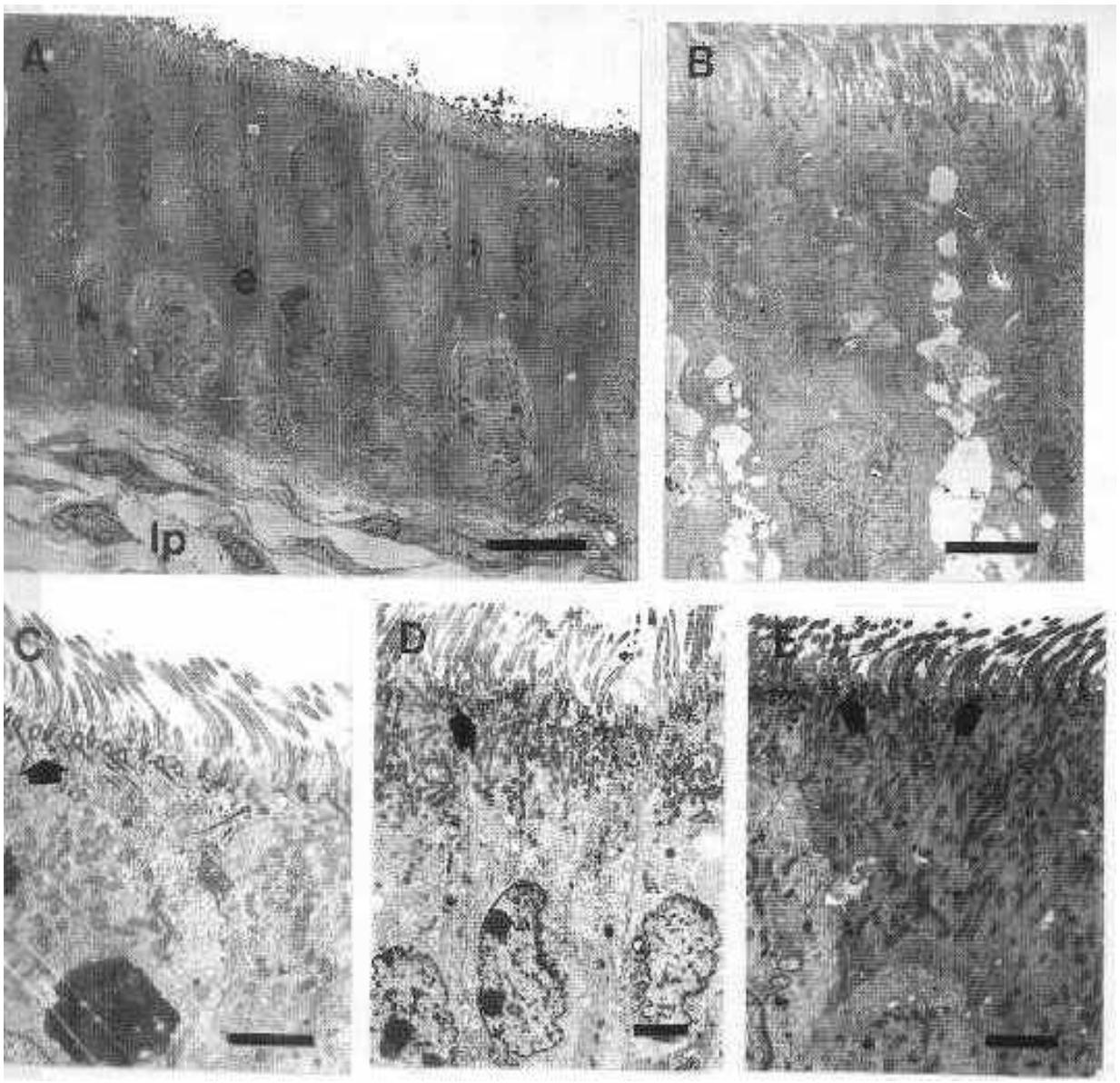

Figure 3. The mushroom body epithelia of *P. textilis* (**A**), *M. adelaidensis* (**B**), *C. marmoratus* (**C**), *D. molleri* (**D**) and *S. intermedius* (**E**). Arrows point to apical e-dense granules. e, Epithelium, lp, lamina propria. (Bar: **A** = 5 μm; **B E** = 2 μm).

kotans, respectively. Apical mucous secretory granules occur in the sustentacular cells of several other squamate species (Bannister 1968; Altner *et al* 1970; Gabe and Saint Girons 1976; Wang and Halpern 1980; Takami and Hirosawa 1990). Thus, the presence of mucous granules in the sustentacular cells is not unique to snakes and is unlikely to be associated with snake vomeronasal specialization.

In addition to the geckos, iguanids and some other squamate reptiles also possess a few apical secretory granules in the mushroom body epithelium (Gabe and Saint Girons 1976). Whether there is enough serous secretion produced to contribute significantly to the serous component of the luminal fluid is unknown. It is thus apparent that there is little (gekkotan) or no (snake and skinks) intrinsic source of serous secretion in the squamate VNO. Therefore, the source of the mucous and serous fluid in the squamate VNO lumen is unlikely to be intrinsic.

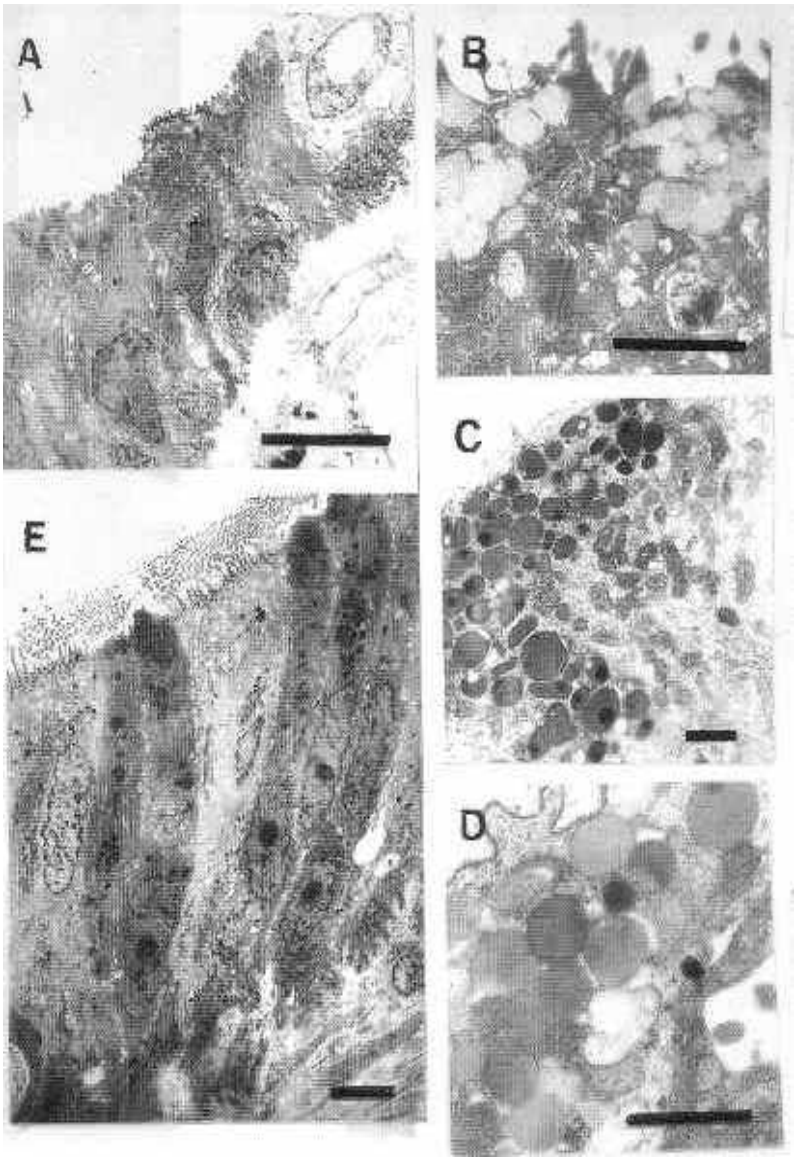

Figure 4. The intermediate epithelia of *P. textilis* (**A** and **D**), *D. molleri* (**B**) and *S. intermedius* (**C** and **E**). **A D** show the cuboidal epithelium, with higher magnifications in **B D** indicating structural diversity of the secretory granules. **E** shows the columnar epithelium (Bar: **A** and **E** = 5 μm; **B D** = 1 μm).

4.2 *Extrinsic sources of luminal fluid*

The absence of any associated internal or nearby glands, or diffuse secretory structures, suggests that either the squamate vomeronasal organ does not require a well developed lubricatory system or that there are alternative sources of fluid. The presence of mucous and serous components in the overlying fluid indicates that the fluid is necessary and that the squamate vomeronasal organ functions in a manner similar to that of other tetrapods. If this is the case, then the absence of the intrinsic glandular structures, which is essential in the mammalian vomeronasal sense (Cooper and Bhatnager 1976; Takami *et al* 1995), implies that there is an extrinsic source for the fluid in the squamate VNO (Kratzing 1975). There are two criteria which a potential external source of fluid for the squamate vomeronasal luminal fluid must meet. First, the fluids must have ready access to the vomeronasal organ. Second, since the histochemical results indicate the presence of protein in the luminal fluid, the external source must be capable of producing serous secretions. Three extrinsic sources for the squamate vomeronasal organ have been suggested, including the salivary glands, secretions of the nasolacrimal duct, and the Harderian gland (Kratzing 1975; Halpern 1992; Rehorek 1997b).

4.2a *Salivary glands*: Although, in squamates, saliva is copiously produced by the salivary glands, and secreted into the mouth cavity, and it is possible for the fluids to flow into the vomeronasal organ via the vomeronasal duct, there is no experimental evidence linking the saliva in the mouth to the fluid layer of the VNO. Furthermore, the salivary glands of squamates produce both serous and mucous fluids (Saint Girons 1988). Since there is currently no evidence to either support or refute the role of the salivary glands in the vomeronasal sense, further studies are warranted.

4.2b *Nasolacrimal duct*: The nasolacrimal duct opens directly into the duct of the vomeronasal organ, or in the vicinity thereof, in all squamates with vomeronasal organs (Bellairs and Boyd 1950; Rehorek 1997a). Even when the vomeronasal organ is absent, the nasolacrimal duct still opens in the same relative region (Bellairs and Boyd 1950; Slaby 1984). Thus, Kratzing (1975) proposed that the nasolacrimal duct may be a source of lubricant for the squamate vomeronasal organ. However, the nasolacrimal duct appears to possess few secretory granules (Saint Girons 1982; Rehorek 1997a). Thus, the nasolacrimal duct itself fails to meet one of the criteria. It is therefore unlikely that the nasolacrimal duct itself is a source of secretion for the vomeronasal organ.

4.2c *Harderian gland*: The Harderian gland is an enigmatic, ubiquitous, serous secreting structure, whose ducts open to the anterior portion of the orbit in squamates (Saint Girons 1982; Chieffi *et al* 1992; Rehorek 1997a, b). These ducts are closely associated with the proximal part of the nasolacrimal duct (Bellairs and Boyd 1947; Saint Girons 1982; Rehorek 1997b). Despite some minor variations, the nasolacrimal duct and Harderian gland are associated directly or indirectly with the VNO in all squamate reptiles thus far examined (Bellairs and Boyd 1947; Saint Girons 1982; Rehorek 1992, 1997a, b). Thus, the Harderian gland meets both criteria (ready access to vomeronasal organ and serous secretory) of an external source for the vomeronasal luminal fluids.

Of the three candidates, the Harderian gland is the most likely source of serous secretion for the fluid in the squamate vomeronasal organ. Tracing studies have confirmed both the route and the presence of Harderian gland secretions (via the nasolacrimal duct) in the lumen of the squamate vomeronasal organ (Rehorek *et al* 1999). What the functional role of these secretions is, or even whether they are the sole contributors to the vomeronasal luminal fluid, is unknown, and further research is warranted.

5. Conclusions

The squamate vomeronasal organ has fewer intrinsic secretory structures than that of either amphibians or mammals. The fluid in the vomeronasal organ, particularly its serous component, is unlikely to derive solely from an intrinsic source, and would thus have to be derived, at least in part, from extrinsic sources.Much remains to be determined with respect to the lubricatory system in the squamate vomeronasal organ. This includes not only the source of the fluid in the vomeronasal organ, but also the role of the secretory granules in the vomeronasal epithelia.

This study supports previous morphological and tracing studies suggesting that the Harderian gland plays a role in the vomeronasal sense of squamate reptiles. The significance of this observation can be appreciated on several levels. If the Harderian gland functions in the squamate vomeronasal sense, then a 300 year old mystery may finally be solved. Therefore, examination of the squamate Harderian gland could lead to insights into the function of the relatively understudied vomeronasal organ (physiological examinations of this structure are currently limited by its inaccessibility). Further studies of the VNO and Harderian gland interaction need to be carried out at both the comparative and molecular levels. Examination of this system in a variety of squamate and non-squamate tetrapods would establish the evolutionary history of this unusual system (i.e., why or how an orbital gland came to be associated with a nasal chemosensory organ). At the molecular level, the precise role of the fluid in the squamate vomeronasal organ, and the relative contribution of the Harderian gland, would lead to a better understanding of the vomeronasal sense.

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