The structure of the nasal chemosensory system in squamate reptiles. 1. The olfactory organ, with special reference to olfaction in geckos

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The luminal surface of the chemosensory epithelia of the main olfactory organ of terrestrial vertebrates is covered by a layer of fluid. The source of this fluid layer varies among vertebrates. Little is known regarding the relative development of the sources of fluid (sustentacular cells and Bowman's glands) in reptiles, especially in gekkotan lizards (despite recent assertions of olfactory speciality). This study examined the extent and morphology of the main olfactory organ in several Australian squamate reptiles, including three species of gekkotans, two species of skinks and one snake species. The olfactory mucosa of two gekkotan species (*Christinus marmoratus* and *Strophurus intermedius*) is spread over a large area of the nasal cavity. Additionally, the sustentacular cells of all three gekkotan species contained a comparatively reduced number of secretory granules, in relation to the skinks or snake examined. These observations imply that the gekkotan olfactory system may function differently from that of either skinks or snakes. Similar variation in secretory granule abundance was previously noted between mammalian and non-mammalian olfactory sustentacular cells. The observations in gekkotans suggests that the secretory capacity of the non-mammalian olfactory sustentacular cells show far more variation than initially thought.

1. Introduction

The nasal region of many tetrapods possesses two major chemosensory structures: the main olfactory organ and the vomeronasal organ. The surface of the sensory epithelia of both chemosensory systems is covered by a layer of fluid. It is in this fluid that odorant chemicals must dissolve prior to reaching the site of olfactory stimulation at the receptor cells (Getchell *et al* 1984a, b). Microchemical analysis revealed that this fluid layer consists of two distinct components: a thin, outer (superficial), watery layer, which is produced by the secretions from the submucosal Bowman's glands (Reese 1965; Andres 1969), and an inner (deeper), more viscous layer, which is produced by the sustentacular cells (Müller *et al* 1979). Whilst Bowman's glands are absent in fish and some amphibians (Kleerekoper 1969; Farbman and Gesteland 1974), they are present with little morphological variation in all other vertebrates (Getchell and Getchell 1992). Secretory granules in the olfactory sustentacular cells have been identified mainly in non-mammalian vertebrates (Getchell and Getchell 1992) and in the mouse (Frisch 1967). These secretory granules vary in their structure, secretory capacity and chemical nature (Getchell and Getchell 1992; Eisthen *et al* 1994; Jones *et al* 1994).

The olfactory organ of squamate reptiles (i.e., lizards and snakes) is relatively poorly understood. Most studies have thus far concentrated on behavioural and neuroanatomical aspects (Halpern 1992; Schwenk 1993b), with little attention being given to the structure of the main olfactory organ (Kratzing 1975; Gabe and Saint Girons 1976; Wang and Halpern 1980; Iwahori *et al* 1987; Halpern 1992 for review). In particular, little is known of the structure of the gekkotan main olfactory organ, despite evidence that they are olfactory specialists (Schwenk 1993a; Dial and Schwenk 1996). This specialized adaptation of geckos may be reflected in the morphology of their gekkotan olfactory organ. In this morphological study, we specifically targeted the lubricatory

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system of the olfactory organ as a potential indicator of specialization, because its variation within tetrapods implies some level of plasticity of this system (Getchell and Getchell 1992 for review).

To test Schwenk's (1993a) hypothesis that gekkotans may be olfactory specialists, we compared the main olfactory organ of gekkotans to that of taxa of squamates that exhibit alternate nasal chemosensory specializations. We examined snakes, which are generally considered to be vomeronasal specialists, and skinks, which have been described as being neither olfactory nor vomeronasal specialists, but may be nasal chemosensory generalists (Halpern 1992; Schwenk 1994, 1995 for review).

2. Materials and methods

2.1 *Materials*

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): *Tiliqua rugosa* (16), *Morethia adelaidensis* (9), Serpentes (snakes) Elapidae: *Pseudonaja 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), and decapitated, and their heads were placed in fixative (see below).

2.2 *Histology*

Either the nasal conchae (*T. rugosa* only) or the entire heads (remaining squamates), of at least 1 specimen per species, were fixed in 10% phosphate-buffered formalin for at least one week, decalcified in 10% aqueous EDTA, embedded in paraffin, and sectioned serially (7 μm). Alternate slides were stained with haematoxylin-eosin. The remaining sections were histochemically treated for the detection of mucopolysaccharides and proteins (see below).

Nasal conchae, which had been dissected unilaterally from the nasal cavity from a minimum of 2 specimens (1 of each sex) per species, were fixed in either Bouin's solution for 48 h or 10% phosphate-buffered formalin for at least 1 week, embedded in paraffin, and used for the histochemical detection of mucins and proteins. Mucosubstances were detected by the periodic acid-Schiff (PAS) and combined alcian yellow (pH 2· 5) – alcian blue (pH 0· 5) (Ravetto 1964) methods. The mercury bromophenol blue (BPB) test was used to detect protein (Barka and Anderson 1965), with pronase digestion for control. For lipid detection, additional nasal conchae were dissected out and the 10% phosphate-buffered formalin fixed frozen sections (15 μm) were stained by the supersaturated isopropanol method (Lillie 1954).

2.3 *Ultrastructure*

For transmission electron microscopy, nasal conchae from at least 2 specimens (1 of each sex) per species were dissected from the other side of the nasal cavity, 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*

In all species investigated, the olfactory mucosa is restricted to the dorsal aspects of the nasal capsule and nasal concha (figure 1). The olfactory mucosa is distinguished from the respiratory epithelium by the presence of submucosal Bowman's glands and the absence of goblets cells. The nasal concha projects medially from the lateral aspect of the nasal wall, encompassing the body of the external nasal gland. The extent of the olfactory mucosa through the nasal capsule is thus determined by the relative development of the nasal concha. In the squamates examined, the size of the concha varies between groups (figure 1). The concha of the snake, *P. textilis*, is a small semi-circular projection attached to the lateral part of the nasal capsule. It encompasses the body of the external nasal gland, but not continuing far beyond it (figure 1A). In the skinks, the nasal concha is slightly larger. Posteriorly, the medial part of the projection is attached to the lateral wall of the nasal capsule by a cartilaginous stalk, covered by olfactory (dorsally) and respiratory (ventrally) epithelia. The posterior portion of the external nasal gland extends into this medial projection. Once the posterior part of the gland has ended, this medial projection also terminates (figure 1B). In geckos and *D. molleri*, this posterior portion does not terminate immediately after the posterior- most projection of the external nasal gland. The cresent-shaped concha of the geckos, which rostrally contains the body of the external nasal gland, expands caudally beyond this gland and fills most of the caudal part of the nasal cavity (figure 1C). Posteriorly, it thus consists only of

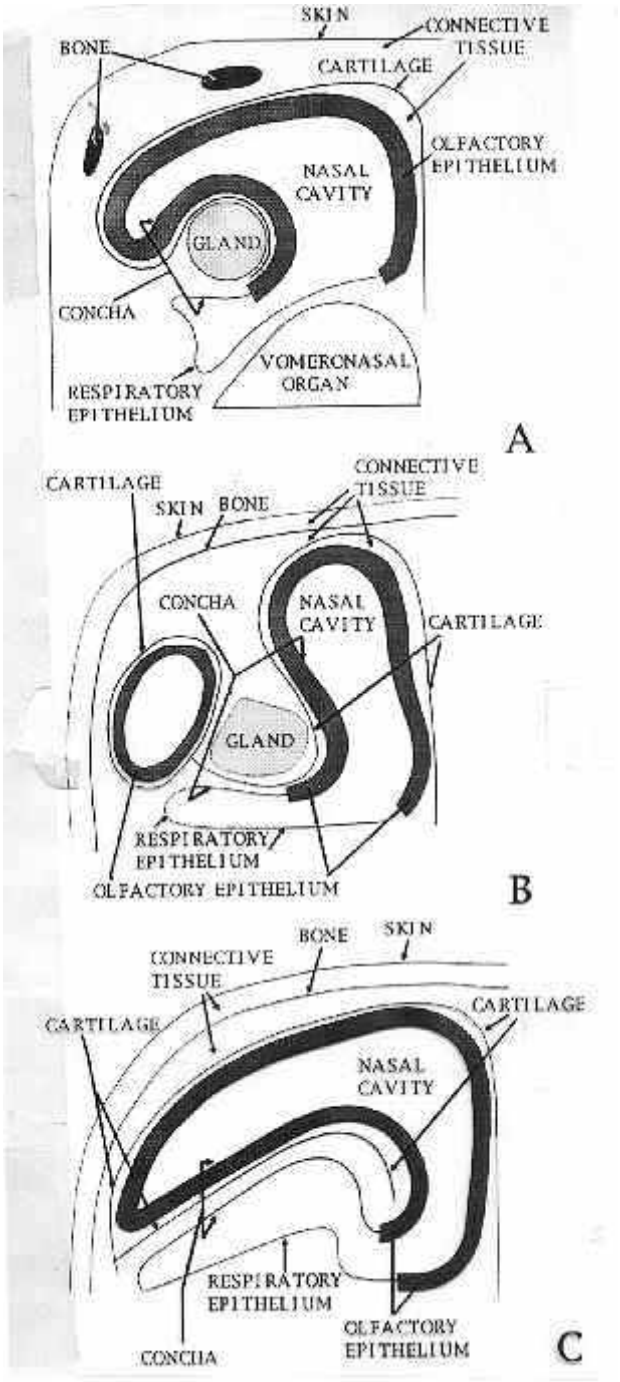

Figure 1. Diagramatic representations of a series of transverse sections through the left nasal capsules. Midline of the nose is on the right, and the dorsum is at the top of the figures. Figures are drawn midway through the nose (at the level of the vomeronasal organ), of *P. textilis* (**A**) and posterior to the vomeronasal organ in *D. molleri* (**B**) and in *C. marmoratus* (**C**) (× 40).

trally by respiratory epithelium. The increased size of the nasal concha in geckos and pygopods, therefore, provides a comparatively larger surface area for olfactory mucosa to spread out over than that in the other species examined.

The results of the histochemical study are summarized in table 1. In all species, the sustentacular cells stain positively with both PAS and alcian yellow, hence indicating the presence of acidic mucosubstances (Drury and Wallington 1980). A less intense PAS staining is observed in the gekkotan sustentacular cells. Though staining with mercury BPB is very weak in the apical region of the olfactory epithelium, no stain appears to be taken up by the secretory granules in the sustentacular cells. This indicates that granules themselves are not serous. The submucosal Bowman's glands stain with both PAS and BPB, indicating the presence of glycoproteins (Drury and Wallington 1980). The reaction of both PAS and BPB is most intense in the snake *P. textilis*. There was no evidence of lipid in either the sustentacular cells or the submucosal Bowman's glands of any squamate reptiles examined.

3.2 *Ultrastructure*

3.2a *Sustentacular cells:* The sustentacular cells have numerous apical, microvillous projections (figure 2A). Elongated mitochondria and lysosomes occur throughout the cells. In all cases, the apical portions of both the sustentacular and receptor cells are connected by a series of tight junctions and desmosomes. Tonofilaments are evident in association with the desmosomes.

Secretory granules are mainly restricted to the supranuclear region of the cells in all species studied. The secretory granules vary among species in both abundance and ultrastructure. In all gekkotans, the maximum diameter of the granules is 0·5 µm. The granules of both skinksand *P. textilis* are at most 1 µm in diameter. The relative abundance of secretory granules varies among the squamates, with least in *C. marmoratus* and *S. intermedius* (only a few scattered granules in the apex of the cell: figure 2A), an intermediate amount in *D. molleri* (figure 2B), and the most in *P. textilis* (figure 2C) and the skinks (secretory granules filling the entire supranuclear region). In general, the granules are of a homogeneous consistency (figure 2A, B). However, the granules of both the gecko *C. marmoratus* and the snake *P. textilis* exhibited internal compartmentalization (figure 2C).

3.2b *Bowman s glands*: The secretory cells of the Bowman's glands are polarised cells, with basal rough endoplasmic reticulum and nuclei, and apical secretory granules. Mitochondria are found among the secretory granules. Golgi complexes were not observed. Junctional complexes, including desmosomes, occur in the apical portion of these cells (figure 2D). All these features are typical of serous secreting cells (Junquiera *et al* 1989).

There is no discernible interspecific variation in the abundance and distribution of either the Bowman's glands or the granules in their secretory cells. In all species, the granules are at most 1·8 μm in diametre. Ultrastructural variations in the serous granules in the Bowman's glands were noted. In *S. intermedius* (gecko) *M. adelaidensis* (skink) and in some *D. molleri* specimens (pygopods), the granules were spherical and homogeneous. In *M. adelaidensis* (skink) these granules are non-spherical and homogeneous (figure 3A). In the snake *P. textilis* (figure 2D), other specimens of *D. molleri*, *T. rugosa* (skink) and *C. marmoratus* (gecko) (figure 3B) the secretory granules were spherical and exhibited internal compartmentalization.

4. Discussion

Previous studies showed variation in the development of the lubricatory system (e.g., absence of secretory granules in the sustentacular cells of most mammals) of the main olfactory organ within tetrapods (see Getchell and Getchell 1992 for review). Due to the paucity of information regarding the squamate main olfactory organ, we specifically examined a comparative series of squamates. This study supports previous suggestions that the non-mammalian main olfactory organ possesses two sources of secretion for the fluid layer overlying the sensory epithelium: sustentacular cells and submucosal Bowman's glands (Andres 1969; Müller *et al* 1979). Our observations of gekkotans not only support previous findings about the spread of their olfactory mucosa (Gabe and Saint Girons 1976), but also suggests that they differ from other squamates in the structure of the intrinsic lubricatory system within the main olfactory organ. These observations are consistent with the suggestion that gekkotans differ from most other squamates in olfactory function (Schwenk 1993a; Dial and Schwenk 1996). The implications of both these differences, in reference to squamate phylogeny, is addressed below.

4.1 *Lubricatory system*

The lubricatory system of the main olfactory organ, consisting of sustentacular cells and submucosal Bowman's glands, shows variation in secretory capacity among the squamates examined. Both histochemical and ultrastructural analyses reveal that the number of secretory granules produced by the olfactory sustentacular cells can be roughly subdivided into two extreme groups, and a series of intermediates. At one extreme, the sustentacular cells of skinks and the snake, *P. textilis* contain many supranuclear, and sometimes sub-nuclear granules (Kratzing 1975). The opposite extreme, with only a few supranuclear granules, were observed in the sustentacular cells of the gecko *C. marmoratus*. In the remaininggekkotans (*D. molleri* and *S. intermedius*), the number of secretory granules present in the sustentacular cells varied between the two extremes.

Table 1. Summary of histochemical analysis of squamate olfactory mucosae.

	Sustentacular cells				Bowman's glands			
	PAS	BPB	AY/AB	SSIM	PAS	BPB	AY/AB	SSIM
Gekkota								
Gekkonidae								
Gekkoninae:								
C. marmoratus	+	+/–	Y+	–	+	+	–	–
Diplodactylinae:								
S. intermedius	+	+/–	Y+	–	+	+	–	–
Pygopodidae:								
D. molleri	+	+/–	Y+	–	+	+	–	–
Scincomorpha								
Scincidae:								
T. rugosa	++	+/–	Y+	–	+	+	–	–
M. adelaidensis	++	+/–	Y+	–	+	+	–	–
Serpentes								
Elapidae:								
P. textilis	++	+/–	Y+	–	++	++	–	–

Abbreviations: SC, Sustentacular cells; BG, Bowman's glands; PAS, periodic acid-schiffs; BPB, mercury bromophenol blue; AY + AB, combined alcian yellow and alcian blue method; SSIM, supersaturated isopropanol method; "–" , no reaction; "+", positive reaction; "++", very positive reaction; "Y+", positive reaction to alcian yellow.

The reduced secretory capacity of the sustentacular cells in geckos implies that fewer mucins are found in the fluid layer overlying the olfactory epithelium in geckos. This, in turn, indicates that the composition of the mucus layer may be different between the geckos and the other squamates (skinks and *P. textilis*). If the composition of the mucus layer varies among squamate species, then perhaps there are functional differences in the main olfactory organ as well.

Similar variation in lubricatory system morphology of the main olfactory organ occurs within tetrapoda. Tetrapod main olfactory organ types can be subdivided into two groups: those whose sustentacular cells are secretory (non-mammals) and those in which they are not (mammals) (see Getchell and Getchell 1992, for review). Once again, this implies that there may be variation in the composition of the mucus layer between mammalian and non-mammalian olfactory systems, based on the secretory capacity of the sustentacular cells. The results of this study show that similar variations occur within Squamata, hence calling into question the aforementioned tetrapod dichotomy. Thus, generalizations about the non-mammalian olfactory organ morphology need to be reconsidered. In any case, further comparative biochemical analyses of the olfactory mucus layer are required before the true nature of this morphological variation can be determined.

Additionally, the results of both this study and the aforementioned studies, fail to take into account the possibility that the secretory state of the cells may vary temporally in an individual. Such variations include the physiological state of the sustentacular cell, and time of day and season in which the animal was sacrificed. These variations may be a confounding factor or a source of noise.Future studies thus require a larger sample size in an attempt

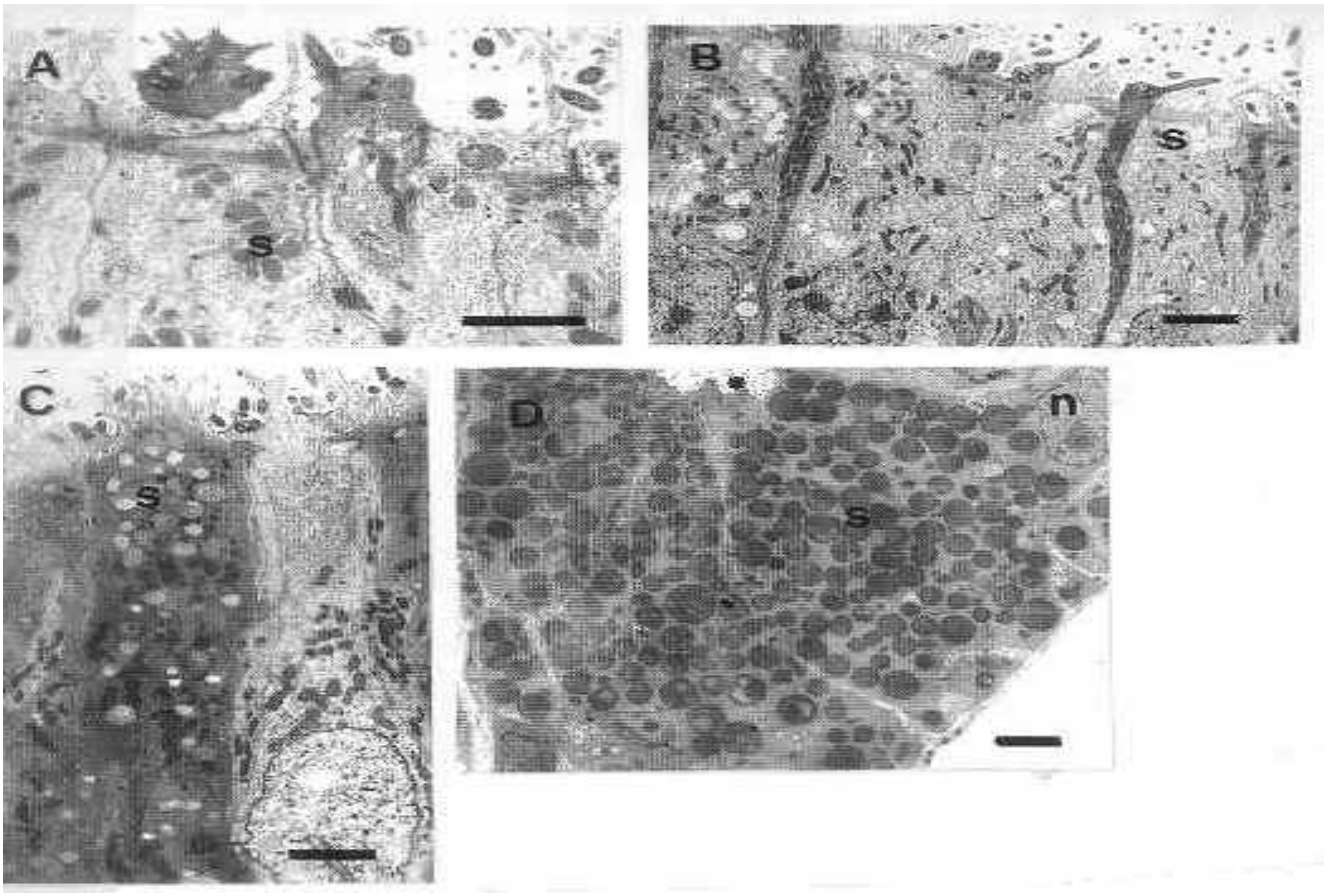

Figure 2. Secretory granules in the sustentacular cells of *S. intermedius* (**A**), *D. molleri* (**B**) and *P. textilis* (**C**). Note the varying abundance of secretory granules in sustentacular cells. Low magnification TEM of the Bowman's gland of *P. textilis* (**D**) shows the overall structure. n, Nuclei; s, secretory granules; asterisk indicates lumen (Bar: **A**, 1 μm; **B D**, 2 μm).

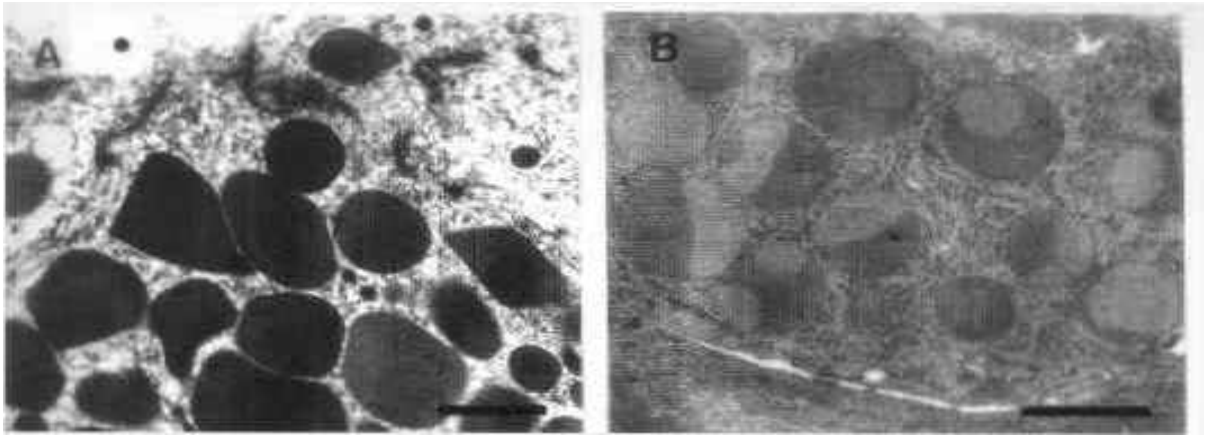

Figure 3. Secretory granules in Bowman's glands of *M. adelaidensis* (**A**) and *C. marmoratus* (**B**). Note the variation in the ultrastructure of the secretory granules. n, Nuclei; s, secretory granules; asterisk indicates lumen (Bar: 1 μm).

to determine the true level of variation in the morphology of the olfactory mucosae.

4.2 *Olfactory mucosa expansion*

The extent of olfactory mucosa is directly related to the architecture of the nasal cavity. Since the olfactory mucosa is restricted to the dorsum of the main nasal cavity, an increase in the olfactory mucosa can only be accomplished by increasing the surface area available for the mucosa. This can be done either by directly increasing the size of the nasal cavity or by adding a series of evaginations or invaginations to the internal nasal cavity. In squamates, an evagination of the lateral nasal wall, the nasal concha, provides such an expansion. It is covered dorsally by olfactory mucosa. Thus variations in nasal concha size (and thus olfactory mucosal spread) are likely to be correlated with olfactory acuity. In comparison to *P. textilis*, both geckos examined possess an enlarged nasal cavities and a distended nasal concha. The nasal concha occupies the entire nasal cavity, whereas in the other species (pygopod, skinks and *P. textilis*) it is much smaller. This confirms the previous observations of the olfactory mucosal extent in squamates (Stebbins 1948; Gabe and Saint Girons 1976), and supports Schwenk's (1993a) hypothesis that geckos have greater olfactory sensitivity that other squamates. In contrast, in snakes, which are thought to have relatively poor olfactory acuity (Schwenk 1993a), the olfactory mucosa is spread over a smaller area. The intermediate condition in pygopods and skinks indicates greater olfactory acuity than the snake, but less than that of the geckos. These findings once again suggest that there are differences in relative use of the main nasal olfactory organ among squamates.

5. Conclusions

This study indicates that there is variation in the morphology and extent of the olfactory mucosa within Squamata. In geckos, the unique combination of a large nasal concha (and hence greater surface area of olfactory mucosa) and few granules in the sustentacular cells, in addition to the other features noted by Schwenk (1993a) and Gabe and Saint Girons (1976), support the notion of geckos as olfactory specialists (Schwenk 1993a; Dial and Schwenk 1996). In addition, since the gecko condition is reminiscent of mammals (Getchell and Getchell 1992), perhaps the main olfactory organ in geckos functions in a similar manner. Further comparisons between gekkotan lizards and mammals are indicated, as geckos possess an olfactory mucosa exhibiting intermediate features between those of mammalian and non-mammalian tetrapods.

Additionally, the intrinsic lubricatory capacity and the extent of the main olfactory organ in squamates shows some variation among, and in the case of the gekkotans, within, families. Previous reports showed little variation in the architecture of the main olfactory organ of squamates, describing it only as typically non-mammalian in structure. The results of this study, with respect to the gekkotan condition, suggest that this may not be the case. The greatly reduced secretory capacity of the gekkotan sustentacular cells suggests that these cells, unlike those of the skinks and the

snake, may not function to produce most of the overlying fluid layer. Variation within Squamata not only suggests differences in olfactory sensitivity, but also implies differences in olfactory function (i.e., role of the lubricatory system in the olfactory organ).

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References

Andres K H 1969 Der olfaktorische Saum der Katze; *Z. Zellforsch. Mikrosk. Anat*. **96** 250–274

Barka T and Anderson P J 1965 *Histochemistry: Theory, practice and bibliography* (New York: Harper and Row)

Dial B E and Schwenk K 1996 Olfaction and predator detection in *Coleonyx brevis* (Squamata: Eublepharidae), with comments on the functional significance of bucca; pulsing in geckos; *J. Exp. Zool*. **276** 415–424

Drury R A B and Wallington E A 1980 *Carleton s histological technique* (Oxford: Oxford University Press)

Eisthen H L, Seneglaub D R, Schroeder D M and Alberts J R 1994 Anatomy and forebrain projections of the olfactory and vomeronasal organs in axolotls (*Ambystoma mexicanum*); *Brain Behav. Evol*. **44** 108–124

Farbman A I and Gesteland R C 1974 Fine structure of olfactory epithelium in the mud puppy, *Necturus maculosus*; *Am. J. Anat*. **139** 227–244

Frisch D 1967 Ultrastructure of mouse olfactory mucosa; *Am. J. Anat*. **121** 87–120

Gabe M and Saint Girons H 1976 Contribution a la morphologie compareé des fosses nasales et de leur annexes chez les Lépidosauriens; *Mém. Mus. Nat. Hist. Nat*. **A98** 1–87

Getchell M L and Getchell T V 1992 Fine structural aspects of secretion and extrinsic innervation in the olfactory mucosa; *Micros. Res. Tech*. **23** 111–127

Getchell M L, Rafols J A and Getchell T V 1984a Histological and histochemical components of the salamander olfactory mucosa: effects of Isoproterenol and olfactory nerve section; *Anat. Rec*. **208** 553–565

Getchell T V, Margolis F L and Getchell M L 1984b Perireceptor and receptor events in vertebrate olfaction; *Prog. Neurobiol*. **23** 317–345

Halpern M 1992 Nasal chemical senses in reptiles: Structure and function; in *Biology of the Reptilia*. Vol. 18, *Physiology E* (eds) C Gans and D Crews (Chicago: University of Chicago) pp 424–532

Iwahori N, Kiyota E and Nakamura K 1987 Olfactory and respiratory epithelia in the snake, *Elaphe quadrivirgata*; *Okaj. Fol. Anat. Jpn*. **64** 183–192

Jones F M, Pfeiffer C J and Asashima A 1994 Ultrastructure of the olfactory organ of the newt, *Cynops pyrrhogaster*; *Ann. Anat*. **176** 269–275

Junquiera L C, Carneiro J and Kelley R O 1989 *Basic histology* (New Jersey: Prentice-Hall)

Kleerekoper H 1969 *Olfaction in fishes* (Bloomington: Indiana University Press)

Kratzing J E 1975 The fine structure of the olfactory and vomeronasal organs of a lizard (*Tiliqua scincoides scincoides*); *Cell Tissue Res*. **156** 239–252

Lillie R D 1954 *Histopathologic technic and practical histochemistry* (New York: Blakiston Co.)

Müller H, Drenkhahn D and Haase E 1979 Vergleichend quantitative und ultrastrukturelle Untersuchungen am Geruchsorgan von Haustaubenrassen; *Z. Mikrosk. Anat. Forsch*. **93** 888–900

Ravetto C 1964 Alcian blue-Alcian yellow: A new method for the identification of different acid groups; *J. Histochem. Cytochem*. **12** 44–45

Reese T S 1965 Olfactory cilia in the frog; *J. Cell Biol*. **25** 209–230

Schwenk K 1993a Are geckoes olfactory specialists?; *J. Zool. London* **229** 289–302

Schwenk K 1993b The evolution of chemoreception in squamate reptiles: a phylogenetic approach; *Brain Behav. Evol*. **41** 124–137

Schwenk K 1994 Why snakes have forked tongues; *Science* **263** 1573–1577

Schwenk K 1995 Of tongues and noses: Chemoreception in lizards and snakes; *Tree* **10** 7–12

Stebbins R C 1948 Nasal structure in lizards with reference to olfaction and conditioning of the inspired air; *Am. J. Anat.* **83** 183–221

Wang R T and Halpern M 1980 Scanning electron microscopic studies of the surface morphology of the vomeronasal epithelium and olfactory epithelium of garter snakes; *Am. J. Anat*. **157** 399–428

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