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Gross, histologic, and micro-computed tomographic anatomy of the lacrimal system of snakes

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Abstract

Objective To describe the lacrimal system of snakes using contrast micro-computed tomography (micro-CT) with 3-dimensional reconstruction, fluorescein passage ('Jones') testing, histology, and gross dissection.

Animals studied One royal python and 19 snake cadavers representing 10 species.

Procedures Direct observation following injection of fluorescein into the subpectacular space, micro-CT following injection of three contrast agents into the subpectacular space, gross dissection following injection of latex into the subpectacular space, and histopathology.

Results Injection of fluorescein confirmed patency, but not course of the lacrimal duct. Barium enabled clear visualization of the lacrimal duct, whereas two iodinated contrast agents proved inadequate. Collectively, micro-CT, anatomic dissections, and histology suggest tears are produced by a single, large, serous, retrobulbar gland, released into the subpectacular space via several ductules, and drained through a single punctum originating in the ventronasal subpectacular space, and the lacrimal duct, which takes one of three routes of variable tortuosity before opening into the oral cavity in close association with the opening of the duct of the vomeronasal organ.

Conclusions The ophidian lacrimal duct has a generally tortuous course, and the details of its anatomy are species-variable. The tortuous course of the duct likely predisposes snakes to duct occlusion and must be considered when planning medical and surgical interventions in snakes with pseudobuphthalmos and subpectacular abscessation.

Key Words: Harderian gland, nasolacrimal duct, ophidia, spectacle, subpectacular space, vomeronasal organ

INTRODUCTION

Snakes and some lizards are unique among terrestrial vertebrates in that they lack mobile eyelids. This is compensated for by the possession of a spectacle (snakes) or brille (lizards), which arises from the embryonic fusion of the upper and lower eyelids over the cornea. Lying between the spectacle and the cornea is a tear-filled chamber termed the subpectacular space. In snakes, tears are produced by the accessory lacrimal (Harderian) gland, flow into the subpectacular space, and are drained through the lacrimal duct to exit medial to the vomeronasal (Jacobsen's) duct in the rostral aspect of the oral cavity.^{1–6} This unique anatomy is believed to predispose these species to a number of dis-

ease processes that involve these tissues.⁷ For example, pseudobuphthalmos and subpectacular abscessation are among the most commonly reported ocular diseases in snakes.⁷ These conditions are often attributed to obstruction of the lacrimal duct typically secondary to ascending bacterial infections from the oral cavity, as well as inflammatory or neoplastic conditions of the mouth, nose, skin, or eye itself.^{8–10} Current therapies for pseudobuphthalmos and subpectacular abscess include partial spectaculotomy/spectaculotomy, cannulation and flushing of the lacrimal duct and subpectacular space, or surgical drainage of the subpectacular space into the oral cavity.^{8–11} These techniques require detailed knowledge of cranial anatomy in general and of the lacrimal drainage system in particular.

The anatomy of the lacrimal drainage system in lizards has been assessed by gross dissection and histologically.^{3,4} However, to the authors' knowledge, detailed descriptions of the anatomy of the ophidian lacrimal system are lacking and we are aware of no reports utilizing advanced imaging techniques to delineate the course of the lacrimal duct in snakes. To address this knowledge gap, the present study was designed to determine the clinical utility of micro-computed tomography (micro-CT) with 3-dimensional (3D) reconstruction of the snake lacrimal drainage system before and after injection of three different radiographic contrast agents. Anatomic data obtained using these techniques were supplemented by fluorescein passage ('Jones') testing as well as histologic and gross anatomic descriptions. Together, this information was used to provide a detailed description of the anatomy of the lacrimal system and identification of adjacent structures along the lacrimal duct course in 10 species of snakes. The information reported here will inform discussions of the clinical implications of ophidian lacrimal system morphology, in particular pathogenesis and treatment of diseases associated with lacrimal duct obstruction.

MATERIALS AND METHODS

Animals

Twenty snakes (10 species) were used in this study. These included one live royal python (*Python regius*) and 19 cadaver specimens consisting of 8 royal pythons, 1 boa constrictor (*Boa constrictor*), 1 prairie kingsnake (*Lampropeltis calligaster*), 1 common kingsnake (*Lampropeltis getula*), 2 cornsnakes (*Pantherophis guttata*), 2 ratsnakes (*Pantherophis obsoleta*), 1 Russian sand boa (*Eryx miliaris*), 1 western hog-nose snake (*Heterodon nasicus*), 1 Kenyan sand boa (*Gongylophis colobrinus*), and 1 gopher snake (*Pituophis cantinifer*). The cadavers were obtained from a commercial provider and were stored from time of death until shipment at -20°C . Immediately prior to experiments, specimens were thawed to room temperature and examined for any gross abnormalities. Only cadaver heads without abnormalities were used. Age and sex of cadavers were not available. The anterior segment of both eyes of the live royal python was verified as normal using diffuse light and a hand-held slit-lamp biomicroscope, and images were recorded with digital slit-lamp biomicroscopy using diffuse and focal (slit) light. The live royal python weighed 862 g and was free of any abnormalities on physical examination.

Subspectacular injection technique

All injections into the subspectacular space were performed in the same manner. The snake's head was placed in lateral recumbency under an operating microscope, and a 30-gauge needle with 1 cc syringe attached was introduced into the peripheral aspect of the subspectacular space at the ventrottemporal region. This area was chosen because the subspectacular space is widest at this

point.^{8,10,12} Each agent was injected using manual pressure until the injected material was observed at the rostral aspect of the lacrimal duct, close to midline of the roof of the mouth or until distension of the spectacle was noted. Using this technique, the total injected volumes varied, likely due to viscosity of the agent injected, as well as differences in snake size, spectacle and lacrimal drainage system integrity, species, and lacrimal drainage system volume. The needle was then removed, gentle pressure was applied to the injection site, and excess injectate was dried using a cotton-tipped applicator. The injection site was then sealed with a small amount of cyanoacrylate adhesive (Duro quick gel[®] Loctite Brand Consumer Products, Henkel Corporation, Westlake, OH, USA).

Fluorescein injections

One live royal python received a subspectacular injection of fluorescein while under general anesthesia. General anesthesia was induced using an intramuscular injection of 0.8 mg/kg dexmedetomidine (Dexdomitor, Pfizer, New York, NY, USA) and 5 mg/kg ketamine (Ketavet, Vedco Inc., Saint Joseph, MO, USA). The snake was then intubated, and anesthesia was maintained with 1.4% isoflurane (Isoflurane, VetOne, Boise, ID, USA) in 0.8 L/min oxygen (Airgas, Woodland, CA, USA) and periodic manual ventilation. A stock solution of 10% Fluorescein (AK-Fluor[®], Akorn Inc., Lake Forest, IL, USA) was diluted 1:6 with balanced salt solution (BSS, Akorn Inc., Lake Forest, IL, USA), and 0.5 mL was injected into the left subspectacular space as described. Immediately after injection, the oral cavity was examined in a darkened room using a cobalt blue-filtered light source from a table-mounted slit lamp (HAAG-STREIT, Koeniz, Switzerland). Because fluorescein in BSS did not allow visualization of the lacrimal duct, after approximately 20 min, 0.4 mL of 10% fluorescein diluted 1:6 with 1.8% sodium hyaluronate viscoelastic material (Visco Supreme, C.L.R. Medicals Int'l. Inc., Pomona, CA, USA) was similarly injected into the same eye and the mouth was reexamined as before using cobalt blue-filtered light.

Micro-computed tomography

For micro-CT, the subspectacular space of seven eyes of five royal python cadavers was injected with barium or one of two non-ionic iodinated contrast agents in various forms. Iopamidol (Isovue 370[®], Bracco Diagnostics Inc., Princeton, NJ, USA) was injected at stock concentration (370 mg/mL) in one cadaver eye or diluted 1:1 ($n = 1$), 1:3 ($n = 1$), or 1:5 ($n = 2$) with rubber latex (Carolina Biological Latex rubber Supply Company, Burlington, NC, USA). Volumes injected ranged from 0.05 to 0.25 mL. A commercial, low-viscosity, isotonic, iodinated contrast agent (Fenestra[™] VC, Advanced Research Technologies Inc., Montreal, Canada) was injected at stock concentration (50 mgI/mL) into the right subspectacular space of one royal python. The volume injected was 0.15 mL. Barium sulfate suspension 105% w/v (Liquid Polibar Plus[®], E-Z-EM Canada Inc., Lake Success,

NY, USA) was diluted 10:1 with rubber latex, and 0.1 mL was injected into the left subseptacular space of a royal python immediately after it had been euthanized (156 mg/kg intracardiac Beuthanasia-D, Schering-Plough Animal Health, Union, NJ 07083). In all cases, micro-CT was performed over a 60-min period using a Siemens multimodal model scanner #5001 with the Inveon Acquisition Workplace program (Siemens Corporation, Washington DC, USA) and reconstructed by Cobra software (Exxim Corp., Pleasanton, CA, United States). Pixel size was set at 20.9 microns, with 360-degree rotation and 720 steps. All 3D reconstructions were performed using commercial imaging software (OsiriX v.4.1.2, Pixmeo SARL, Switzerland).

Anatomic dissections

For gross dissections, pigmented latex (Carolina Biological Supply, Burlington, NC) was injected into the subseptacular space of both eyes of 16 cadavers (five royal pythons, two cornsnakes, two ratsnakes, and one specimen of each of the following species: boa constrictor, Russian sand boa, hognose snake, Kenyan sand boa, gopher snake, prairie kingsnake, and common kingsnake). Injected volumes ranged from 0.03 to 0.8 mL. Injected latex was allowed to set for 15 min prior to immersion fixation of the cadaver heads in 10% neutral buffered formalin (VWR International, Westchester, PA, USA) for approximately 3–4 days. Two skulls were then sectioned sagittally ($n = 1$) or transversely ($n = 1$) using a diamond-tip saw. The remaining latex-injected cadavers ($n = 14$) were examined by careful manual dissection to expose the latex-injected lacrimal duct. The course of the lacrimal duct was photo-documented using a digital camera with ring flash (Canon Inc. Operations, Ohta-ku, Tokyo, Japan).

Histology

The royal python cadaver whose subseptacular space had been injected with barium sulfate suspension diluted 10:1 with rubber latex for micro-computed tomography was subsequently decapitated and the head fixed in 10% neu-

tral buffered formalin for 3 days before being decalcified in 15% formic acid solution (Fisher Chemical, Fairlawn, NJ) diluted from 88% in deionized water until sufficiently soft for sectioning (7 days). The head was sectioned transversely at three locations: 1) immediately caudal to the most rostral teeth, 2) at the approximate midpoint of the nasal bone, and 3) immediately rostral to the globe. The resulting tissue samples were paraffin-embedded, step-sectioned, and stained with hematoxylin and eosin so as to identify essential elements of the lacrimal generation and drainage systems.

RESULTS

Fluorescein injections

Following injection of fluorescein diluted in balanced salt solution or sodium hyaluronate into the subseptacular space of the live royal python, diffuse fluorescein staining of the rostral palate of the oral cavity was noted; however, neither the lacrimal duct course nor a distinct oral punctum was observed.

Micro-computed tomography

Both iodinated contrast agents at all dilutions tested failed to provide images that permitted distinct delineation of the lacrimal duct in any royal python imaged (Fig. 1). In contrast to iodinated contrast media, barium diluted 10:1 with rubber latex clearly delineated the subseptacular space and lacrimal duct on micro-CT images (Fig. 2). On 3D reconstruction, many small tributaries were also identified that extended from the subseptacular space to the retrobulbar area. These small tributaries were interpreted to probably represent emissary ductules emerging from the Harderian gland and filled with barium in a retrograde fashion from the subseptacular space. It is also possible that they represent caudal (retrobulbar) extensions of the subseptacular space, although a functional reason for this could not be identified. In contrast to the iodinated contrast agents, leakage of barium outside of the lacrimal

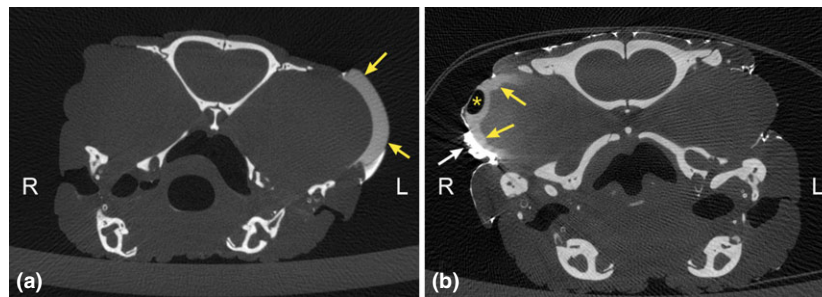


Figure 1. Transverse micro-CT images of the head of a royal python at the level of the orbit after administration of (a) iopamidol (Isovue 370[®], Bracco Diagnostics Inc., Princeton, NJ, USA) into the subseptacular space (yellow arrows) of the left eye or (b) a commercial, low-viscosity, isotonic, iodinated contrast agent (Fenestra[™] VC, Advanced Research Technologies Inc., Montreal, Canada) into the subseptacular space of the right eye. The white arrow in (b) indicates glue placed over the injection port. Iodinated contrast media diffused from the subseptacular space into the cornea as seen in (b). *Indicates an air bubble in the subseptacular space that was introduced during the injection process.

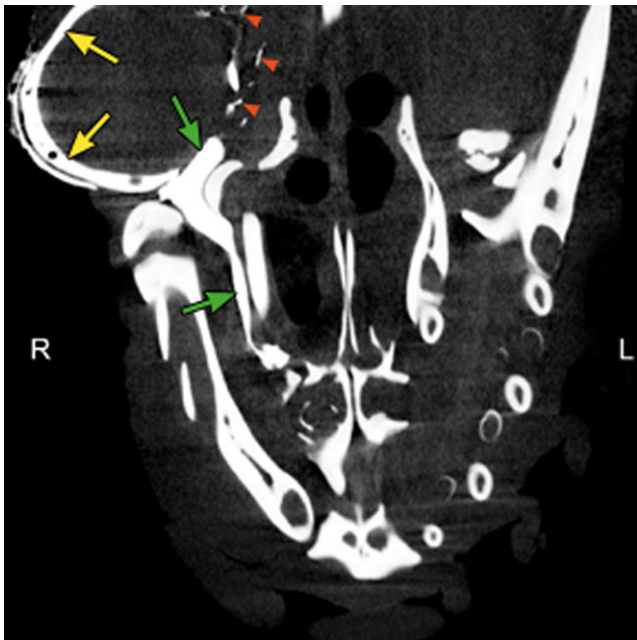


Figure 2. Dorsal plane micro-CT image of a royal python head at the level of the orbit following injection of barium diluted 10:1 with rubber latex into the subpectacular space (yellow arrows) of the right eye. The lacrimal duct (green arrows) was clearly delineated as it passed from the subpectacular space and exits into the roof of the rostral aspect of the oral cavity. Orange arrowheads indicate the small communicating tributaries of the lacrimal system in the retrobulbar space.

system was not observed. Following 3D reconstruction, the 10:1 barium/latex mixture outlined numerous fine ductules converging at a bullous sac-like structure in the ventronasal orbit and continuous with the subpectacular space (Fig. 3a). The lacrimal duct exited the ventronasal

region of the subpectacular space, passed through the osseous prefrontal (lacrimal) foramen, and then descended in a rostral and medial direction between the caudal aspect of the maxillary and palatine bones (Fig. 3b). It then coursed between the vomer bone and the hypochoanal cartilage to a point dorsal to the rostral aspect of the palatine bone where it made a nearly 90-degree turn medially and traversed to the medial aspect of the rostral portion of the palatine ridge. At this point, it completed another near 90-degree turn to travel rostrally and medially before exiting into the oral cavity through the soft palate medial to the orifice of the vomeronasal duct. On the ventro-dorsal view, the opening of the duct overlaid the caudal aspect of the premaxillary bone just lateral to the midline.

Anatomic dissections

In all species studied, the lacrimal duct originated as a single punctum in the ventronasal region of the subpectacular space (Fig. 4a) and emerged into the oral cavity in close proximity to the vomeronasal duct (Fig. 4b). The angle of descent and exact path taken between the origin and termination of the lacrimal duct varied among the species examined. Considering all specimens dissected (16 snake cadavers from 10 species), three broad patterns of lacrimal duct anatomy were observed (Fig. 5).

Group 1 This group included the royal python and cornsnake. In these species, the lacrimal duct passed through the osseous lacrimal foramen, then coursed rostrally and medially between the caudal aspect of the maxillary and palatine bones before passing between the vomer bone and the hypochoanal cartilage and ending in an opening into the oral cavity in close proximity to the opening of the vomeronasal duct.

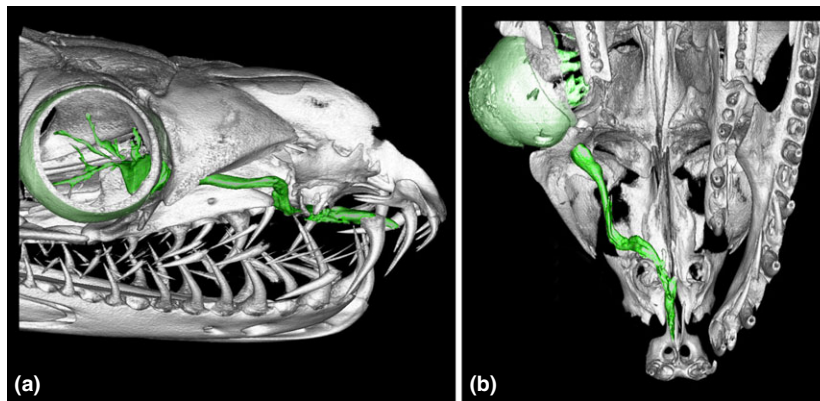


Figure 3. Three-dimensional reconstruction of micro-CT images acquired from a royal python following injection of barium diluted 10:1 with rubber latex into the subpectacular space of the right eye. Threshold Hounsfield values for barium were utilized to selectively color the regions of the subpectacular space and lacrimal system containing barium, green. (a) Right lateral view of the lacrimal duct from its origin in the ventronasal aspect of the subpectacular space to its exit into the roof of the rostral aspect of the oral cavity. Portions of the bony elements of the orbital rim and maxilla have been selectively digitally removed to visualize the lacrimal duct. The more superficial aspect of the barium-laden subpectacular space has also been digitally removed to visualize the retrobulbar emissary ductules from the Harderian gland emptying into the ampulla. As detailed in the histologic description, the ampulla communicates with the subpectacular space and is in close proximity to the opening of the lacrimal duct. (b) Ventral view of the lacrimal duct. Portions of the mandible, maxilla, palatine, and vomer bones have been selectively digitally removed from the image to visualize the lacrimal duct in its entirety.

Figure 4. (a) Lateral view of the left eye of a boa constrictor and (b) intra-oral view of the hard palate of a royal python which had blue (a) or pink (b) pigmented latex injected into the subpectacular space of both eyes followed by careful dissection of the lacrimal duct over its course from its origin at a single lacrimal punctum in the ventronasal subpectacular space (arrow, a) to its exit (indicated with forceps) in the rostral hard palate in close proximity to the vomeronasal duct.

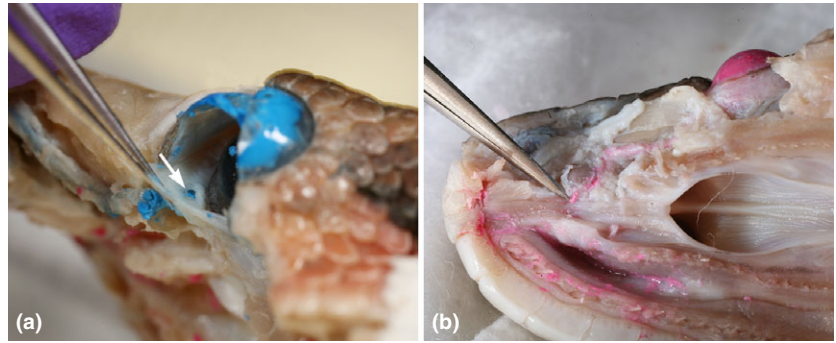
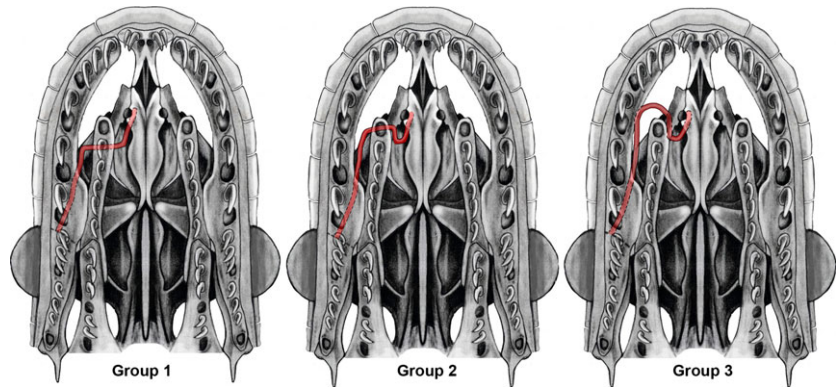


Figure 5. Artist rendition of the three broad groupings of lacrimal duct anatomy found in the 10 snake species assessed. (Drawings do not represent species variation in skull morphology). Group 1 included the royal python and cornsnake. Group 2 included the boa constrictor, Russian sand boa, Kenyan sand boa, gopher snake, ratsnake, and the common kingsnake. Group 3 included only the prairie kingsnake. (Illustration by Sara DeRemer.)



Group 2 The second anatomic group included the boa constrictor, ratsnake, Russian sand boa, Kenyan sand boa, gopher snake, and the common kingsnake. In this group, the lacrimal duct followed a more tortuous course in both the dorsoventral and lateral planes than that seen in Group 1 (see Fig 5). After leaving the subpectacular space, the lacrimal duct passed ventrorostrally through the lacrimal foramen and, further rostrally, underwent a series of complex turns. The first was an approximately 90-degree turn medially at a point more rostral than that seen in the python followed by a caudal and medial ‘U-turn’. It then proceeded along a similar course and to a similar oral exit point as that described in the royal python. Lacrimal duct anatomy of the sand boa varied slightly from this general scheme. In this species, the duct did not pass through a bony prefrontal foramen but, instead, turned ventrally and passed under the prefrontal bone before continuing rostrally and ultimately turning medially as for the rest of the group. Following the medial deviation, it coursed caudally for a short distance before rejoining the pathway common to this group. The lacrimal duct deviated less in the dorsal-ventral plane along its course in the common kingsnake than in the remainder of the group.

Group 3 The third anatomic variation was observed only in the prairie kingsnake in which the duct ran further rostrally than the other two groups prior to sharply deviating medially, passed deep and rostral to the palatine ridge and completed 2 ‘U-turns’ prior to exiting adjacent to the vomeronasal duct as in the other species (see Fig. 5).

Histology

Histologic examination of the decalcified royal python head, in which the subpectacular space had been injected with barium, revealed the accessory lacrimal (Harderian) gland deep within the orbit nasal to the globe and slightly dorsal to the posterior pole (Fig. 6a). As previously described,^{13–15} the Harderian gland was composed of acini of predominantly serous glands and scattered mucus cells with several ductules communicating with a dilated ampulla at the ventral aspect of the gland. The ductules and ampulla were dilated with barium. In adjacent sections, the ventral-most aspect of the ampulla communicated with the subpectacular space, although a distinct punctum was not seen in the sections examined (Fig. 6b). Near its junction with the Harderian gland, the ampulla’s lining was a simple squamous to cuboidal epithelium with scattered goblet cells as previously described.¹⁵ Near its junction with the subpectacular space, it was lined by a bilayer of cuboidal epithelial cells, typical of a duct. The subpectacular space was also distended with barium. A single punctum was identified that denoted the origin of the lacrimal duct. A sac-like dilation of the duct in the vicinity of the orbital rim (characteristic of many mammalian species) was not identified in the sections examined. Sections taken near the midpoint of the nasal bone revealed the lacrimal duct’s passage through dense connective tissue ventral to the nasal bone and dorsal to the mucosal lining of the oral cavity. In some sections in this region, the duct was sectioned twice (Fig. 6c) consistent with the tortuous path seen in reconstructed CT images (see Fig. 3) and in gross

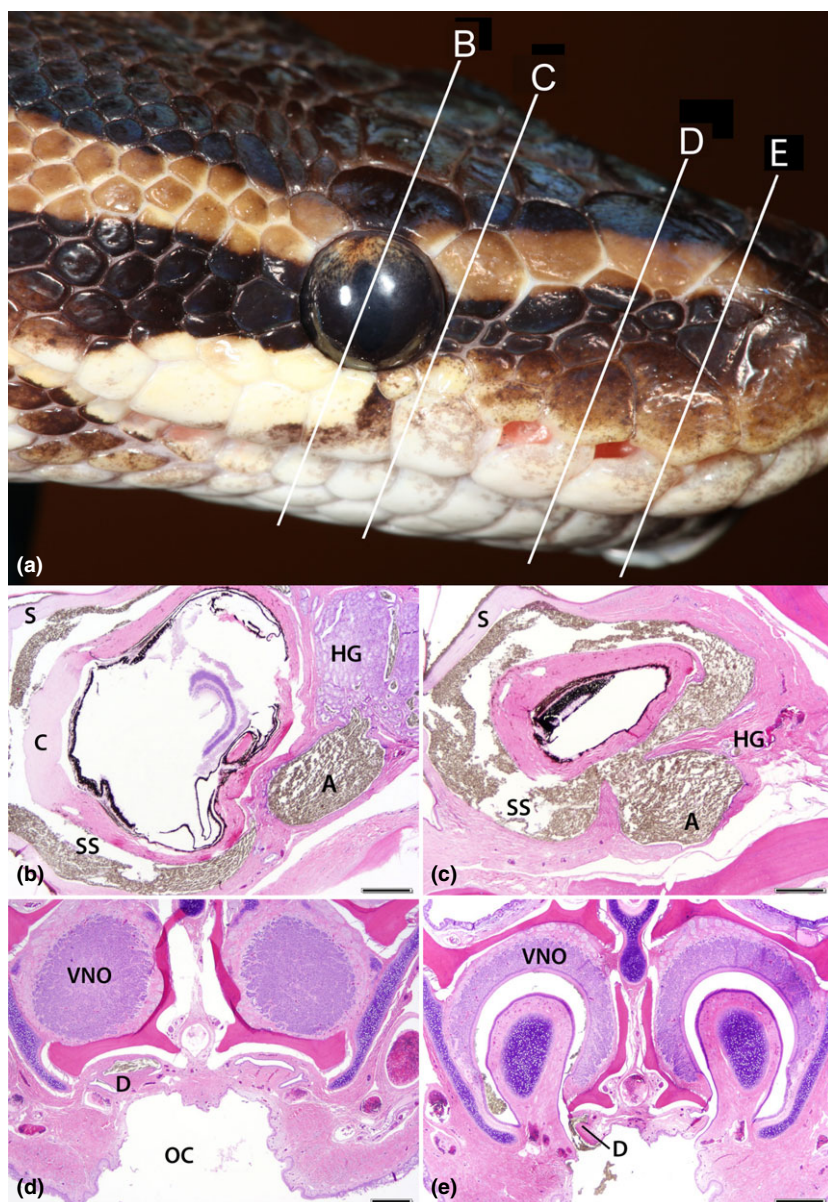


Figure 6. (a) Photograph of the head of a royal python demonstrating the location of the four transverse planes from which histologic sections were obtained (b–e). Histologic sections were collected following injection of 105% w/v barium sulfate suspension diluted 10:1 with rubber latex into the subspectacular space of one eye only, formalin fixation, decalcification, and staining with H&E. 2× magnification. (b) Histologic section taken centrally through the eyes. Note barium/latex filling the subspectacular space (SS) between the cornea (C) and spectacle (S), and the ampulla (A) ventral to the Harderian gland (HG). (c) Histologic section taken at the rostral edge of the eye (several hundred microns anterior to the site from which (b) was collected). Note the communication between the barium-filled SS and ampulla (A). A few peripheral lobules of the HG are visible adjacent to the ampulla (A). (d) Mid frontal histologic section. Note the dual lacrimal duct profiles (D) on each side corresponding to the tortuous path of the duct between the ganglia of the vomeronasal organ (VNO) and oral cavity (OC). (e) Rostral-most histologic section. Note the junction of the lacrimal duct (D) with the duct of the vomeronasal organ (VNO) just as the two enter the oral cavity. Barium is also evident within the lumen of the VNO.

dissections (see Fig. 5). The lacrimal duct passed ventral to the vomeronasal organ before merging with the medial aspect of the vomeronasal duct just prior to their entering the oral cavity through a common orifice in the roof of the mouth (Fig. 6d). The lacrimal components of the contralateral noninjected eye were anatomically and histologically similar to those on the injected side.

DISCUSSION

To the authors' knowledge, this represents the most comprehensive description to date of the anatomy of the ophidian lacrimal system. We also believe that it represents the first assessment of numerous imaging techniques for these structures in snakes. Taken together, our data gathered from prosection, numerous imaging modalities, and histologic assessment suggest that a single, large, serous,

retrobulbar gland in the dorsonasal orbit delivers tears via several ductules that empty into an ampulla that communicates with the ventral aspect of the subspectacular space. The precorneal tear film drains through a single punctum in the ventronasal quadrant of the subspectacular space that forms the ostium of the lacrimal duct. The pathway of the lacrimal duct follows one of three general routes of varying tortuosity and ends in a single opening into the oral cavity in close association with the duct of the vomeronasal organ. The relationship of the lacrimal ductules, ampulla, and subspectacular space is particularly interesting in that tears apparently empty into the ampulla and ventral subspectacular space near the origin of the lacrimal duct. Neither histology nor any imaging modality revealed any ductules emptying into the dorsal subspectacular space. While it is possible that small ductules in the dorsal SSS may have been missed using the methodology employed in the present

study, our data suggest that the SSS is a closed system that receives tears from the posterior and ventral aspect and drains from the inferonasal aspect. This anatomic pattern would seem to promote delivery of a majority of the tears directly to the mouth and a minority to the subspectacular space, which is consistent with the hypothesis that lacrimal secretions may aid in lubrication and swallowing of food in Ophidia.¹⁶ In the present study, we confirmed a number of other unique anatomic and functional features in the snake which support this hypothesis. These features include the large Harderian gland of the snake (especially considering that tear film evaporation would be reduced by presence of a spectacle), and the observation that the lacrimal duct exits into the mouth rather than the nose (as is common in mammals).

The degree of lacrimal duct tortuosity noted in the present study, especially in the boa constrictor, ratsnake, Russian sand boa, Kenyan sand boa, gopher snake, and the common and prairie kingsnakes examined in this study, is striking. The tortuosity of the duct likely plays a central role in the pathogenesis of many of the common ocular diseases in snakes that involve the lacrimal drainage system, especially pseudobuphthalmos and subspectacular abscess formation. A 26-year retrospective study of the prevalence of ocular disease in 67 snakes compared with a reference population of 441 snakes reported pseudobuphthalmos and subspectacular abscessation developed more frequently in Colubrid than in non-Colubrid snakes.⁷ Therefore, it is interesting to note that four of the five species of the Colubridae we examined were assigned to the groups characterized by possessing ducts with relatively greater tortuosity (Groups 2 and 3), and the only snake assigned to the group with the most tortuous pathway (Group 3) was a member of the Colubridae family (the prairie kingsnake). The lacrimal duct of mammals prone to dacryocystitis and epiphora, such as rabbits and brachycephalic cats, has also been reported to have a tortuous course.¹⁷⁻¹⁹ The evolutionary reason for such a tortuous pathway is unclear; however, it appears that shortening of the muzzle is an important factor.¹⁸ Although there is much interindividual variation in skull shape within an ophidian genera, the snakes examined here tended to increase in snout length from Group 1 to Group 3 (personal communication Ryan S. De Voe, DVM, MSpVM, Dipl. ACZM, Dipl. ABVP (Avian), Dipl. ABVP (Reptile/Amphibian)). However, in the present study, we examined 20 snakes representing only 10 different species. Given that there are approximately 3460 known species of snakes,²⁰ the three broad groupings suggested by our data should be interpreted cautiously as there may be notable individual variation and many more patterns likely exist.

Data from the present study can be used to inform surgical interventions in snakes with spectacular, subspectacular, lacrimal, and oronasal disease. For example, subspectacular abscessation and pseudobuphthalmos are sometimes treated using a wedge spectaculotomy.^{9,10,21-23} Although many have performed or recommended that this

is best performed in a dependent (ventral) portion of the spectacle,^{9,10,21} our data suggest that the ventronasal quadrant should be avoided so as to limit the chance of iatrogenic damage to the lacrimal punctum located there in snakes of all species examined in the present study. It would seem preferable to perform the resection at the ventrolateral quadrant. Likewise, our data suggest that techniques used in other species for treatment of obstructions of the lacrimal drainage apparatus such as cannulation, irrigation, conjunctivorhinostomy, and conjunctivalostomy would be more challenging in snakes and should be guided by knowledge from the present study showing the tortuosity of the lacrimal duct and anatomic variability of its course among species. Pre-operative imaging of the normal side in unilaterally affected species is recommended.

To the authors' knowledge, this study also represents the broadest assessment of imaging techniques of potential utility in anatomically describing and clinically assessing the ophidian lacrimal system. Of the methods assessed, only fluorescein and the two iodinated contrast agents are likely to be safe and practical in living animals; barium and latex were utilized only to aid anatomic descriptions. Unfortunately, these two clinically viable solutions failed to outline anatomic detail of the lacrimal apparatus sufficiently clearly, even when the viscosity of these solutions was increased through their combination with other compounds. Fluorescein staining has been used to gauge lacrimal duct patency and transit time in dogs and cats.²⁴ Fluorescein has also been used to outline the lacrimal duct course along the roof of the mouth in a blood python,²³ however, this was unsuccessful in the present study presumably because the duct was obscured by overlying tissue. Fluorescein is not successful for this purpose in other species because the duct lies under densely vascular mucosa for much of its course. Additionally, fluorescein typically is not useful for specific identification of the distal lacrimal duct punctum in many species for a number of reasons including inability to visualize the anatomic site, supernumerary or accessory openings, or the opening being located sufficiently caudally within the nose that stain is visible only within the mouth.²⁵ Additionally, in the present study, fluorescein did not permit specific identification of the oral punctum because the dye rapidly became dispersed over a broad area within the mouth. Given that lacrimal secretions are alleged to assist with lubrication and swallowing of food, it is possible that the exit of the lacrimal duct promotes wide dispersion of fluid across the hard palate. In summary, our data show that fluorescein, delivered into the subspectacular space, can be used to assess patency but, in the species examined, did not allow visualization of the course of the lacrimal duct.

Data from this study showed that, although micro-CT with 3D reconstruction provides an excellent method for delineating many cranial structures of snakes, the two iodinated contrast agents used at various viscosities did not reliably outline the lacrimal duct. Barium mixed with latex

provided very clear micro-CT images and excellent anatomic detail but is not a clinically viable method. The iodinated contrast media at various concentrations tended to extend into neighboring tissues including the cornea of cadaver snakes. Iodinated molecules used in contrast media are small, extravasation is expected when given via vascular routes, and diffusion of contrast media through endothelium and into the interstitial space is enhanced when permeability is increased. These factors along with post-mortem autolysis may explain extension of the contrast media into surrounding tissues including the cornea. It is possible that antemortem use of these agents would result in less extension into the surrounding tissues and better anatomic depiction of the region. To the authors' knowledge, the only other description of contrast injected into the subspectacular space involved the diagnosis of microphthalmos in a northern Californian kingsnake; however, in that study, the lacrimal duct was not outlined.²³ Regardless of injectate, the subspectacular injection technique used in this study was practical and could likely be carried out safely provided that it was carried out by an operator with some experience in microsurgical techniques, the snake was under general anesthesia, and an operating microscope was used. Injection pressure must be carefully controlled, as excessive pressure did elevate the spectacle. This might be more likely in animals with lacrimal duct obstruction.

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