

UC Davis

UC Davis Previously Published Works

Title

Spectral domain optical coherence tomography imaging of spectacular ecdysis in the royal python (*Python regius*)

Permalink

<https://escholarship.org/uc/item/4x2372xb>

Journal

Veterinary Ophthalmology, 18(s1)

ISSN

1463-5216

Authors

Tusler, Charlotte A
Maggs, David J
Kass, Philip H
et al.

Publication Date

2015

DOI

10.1111/vop.12176

Peer reviewed

Spectral domain optical coherence tomography imaging of spectacular ecdysis in the royal python (*Python regius*)

Charlotte A. Tusler,* David J. Maggs,* Philip H. Kass,† Joanne R. Paul-Murphy,‡ Ivan R. Schwab§ and Christopher J. Murphy*§

*Departments of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA; †Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA; ‡Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA; and §Ophthalmology and Vision Science, School of Medicine, University of California, Davis, CA, 95616, USA

Address communications to:

C.J. Murphy

Tel.: (530) 754-0216

Fax: (530) 752-3708

e-mail: cjmurphy@ucdavis.edu
and

D.J. Maggs

Tel.: (530) 752-1393

Fax: (530) 752-6042

e-mail: djmaggs@ucdavis.edu

Abstract

Objective To describe using spectral domain optical coherence tomography (SD-OCT), digital slit-lamp biomicroscopy, and external photography, changes in the ophidian cuticle, spectacle, and cornea during ecdysis.

Animals Studied Four normal royal pythons (*Python regius*).

Procedures Snakes were assessed once daily throughout a complete shed cycle using nasal, axial, and temporal SD-OCT images, digital slit-lamp biomicroscopy, and external photography.

Results Spectral domain optical coherence tomography (SD-OCT) images reliably showed the spectacular cuticle and stroma, subcuticular space (SCS), cornea, anterior chamber, iris, and Schlemm's canal. When visible, the subspectacular space (SSS) was more distended peripherally than axially. Ocular surface changes throughout ecdysis were relatively conserved among snakes at all three regions imaged. From baseline (7 days following completion of a full cycle), the spectacle gradually thickened before separating into superficial cuticular and deep, hyper-reflective stromal components, thereby creating the SCS. During spectacular separation, the stroma regained original reflectivity, and multiple hyper-reflective foci (likely fragments from the cuticular-stromal interface) were noted within the SCS. The cornea was relatively unchanged in character or thickness throughout all stages of ecdysis. Slit-lamp images did not permit observation of these changes.

Conclusions Spectral domain optical coherence tomography (SD-OCT) provided excellent high-resolution images of the snake anterior segment, and especially the cuticle, spectacle, and cornea of manually restrained normal snakes at all stages of ecdysis and warrants investigation in snakes with anterior segment disease. The peripheral spectacle may be the preferred entry point for diagnostic or therapeutic injections into the SSS and for initiating spectacular surgery.

Key Words: cornea, cuticle, ophidia, snake, spectacle, subspectacular space

INTRODUCTION

The eyes of snakes possess a number of distinctive features. Walls, in his classic treatise on comparative ocular structure and function, hypothesized that the first snakes evolved from burrowing lizards to become fossorial and that, upon emerging, they retained unique ocular anatomical features evolved during their subterranean period.¹ Notable among these unique features was the spectacle: a

modified scale that protects the cornea's single-layered epithelium and creates a tear-filled subspectacular space (SSS).² Snakes possess a tertiary-type spectacle that originates from the fusion of the upper and lower eyelid,¹ thereby providing protection against injury and desiccation of the cornea, and allowing the snake to keep its eyes continuously open to detect predators and prey.³ Like other scales, the spectacle is vascularized^{4,5} and the cuticle of the spectacle is shed with the rest of the epithelium of the

head during ecdysis. The spectacle also plays a critical optical role in refraction, almost equal to that of the lens.⁶ These distinct anatomical and physiological features of the ocular surface structures predispose snakes to the development of a collection of unique ocular disorders that can present challenges for their clinical management. Specifically, a number of ophthalmic diseases involve the spectacle and the SSS.⁷ Snakes may develop retained spectacles in association with more generalized dysecdysis secondary to integumentary disease, systemic illness, inadequate environmental humidity, tick or mite infestation, or local injury. This manifests clinically as notable opacification of the spectacle that can compromise vision, especially if bilateral. Snakes can also develop secondary diseases associated with the spectacle such as pseudobuphthalmos and subspectacular abscesses.^{7,8} Pseudobuphthalmos may occur as a result of a congenital malformation or in association with blockage of the lacrimal duct due to inflammatory processes that involve the lacrimal drainage system or compromise of drainage due to occlusive pressure from nearby space-occupying masses. Subspectacular abscessation may occur as a result of systemic disease, penetrating trauma, or via infection ascending from the oral cavity along the lacrimal duct. Impermeability of the spectacle to topically applied medications complicates therapy. Thus, intervention for ocular diseases frequently requires systemic drug administration, sometimes in conjunction with surgical manipulation of the spectacle. Surgical manipulations most commonly involving the spectacle include creating defects in the spectacle to augment delivery of topical agents into the SSS, removal of retained spectacles, and direct drainage of subspectacular fluid or exudate.^{9–11}

Despite the clinical significance of the spectacle, its relationship with the overlying cuticle, and underlying SSS and cornea, its intrinsic involvement in the process of ecdysis, and the fact that histologic changes during ecdysis of skin have been reported,¹² little is known regarding the utility of modern imaging techniques for assessing these structures or the manner in which these structures change throughout ecdysis. The ultrasonographic appearance of these structures has been described,¹³ although snakes were imaged at only one time point, precluding the possibility of identifying ecdysis-related changes. Additionally, the resolution of high-frequency ultrasound is only about 50 μm ; limiting identification of subtle alterations in the spatial relationships between the spectacle, SSS, and cornea.¹⁴ Morphologic changes occurring throughout ecdysis have been assessed using scanning electron microscopy (SEM); however, these changes were described in skin only – not the spectacle.¹⁵ The utility of modern imaging techniques in detecting ecdysis-related morphologic changes warrants investigation as slit-lamp biomicroscopy does not reliably permit clear clinical discrimination of the normal spectacle, SSS, and cornea. This limitation is exacerbated in cases where the spectacle is opacified. By

contrast, spectral domain optical coherence tomography (SD-OCT) has a resolution of about 5 μm and can be used to obtain optical cross-sections of the anterior segment *in vivo* with high scanning speed (26 kHz) and axial resolution.¹⁶

Because of its superior resolution, we hypothesized that SD-OCT would provide excellent *in vivo* images of the anterior segment of the snake eye and that knowledge of the normal anatomy of this region and its alterations throughout the ecdysis cycle would inform clinical decision making in diseases of the anterior segment as well as provide baseline information for future SD-OCT examination of snakes with anterior segment disease. Here, we describe findings for the spectacle and associated structures throughout ecdysis using the complementary imaging techniques of SD-OCT, digital slit-lamp biomicroscopy, and external photography. Dimensions of the cuticle, subcuticular space (space between the cuticle and spectacle, SCS), spectacle, SSS, and cornea at various stages during the shedding cycle were also determined.

MATERIALS AND METHODS

Four royal pythons (*Python regius*) at least 1 year of age and ranging from 1.08 to 1.28 kg were purchased from a commercial vendor. Snakes were housed in individual glass aquarium tanks with wood shavings as substrate, and provided with heat lamps and soaking dishes. Wooden hideaways were provided as a rough surface to assist in ecdysis. The substrate was changed to newspaper immediately prior to shedding of head scales to facilitate retrieval of shed material. The room was kept at 29.4 °C, with the terrarium space beneath the heat lamps at 32 °C. Humidity within the room was maintained at approximately 50%. All procedures were approved by the University of California, Davis Animal Care and Use Committee, and adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

After a 1-week acclimation period, all snakes were verified to be free of ophthalmic lesions based upon results of slit-lamp biomicroscopy and indirect ophthalmoscopy. In addition, external photographs (Canon Inc. Operations, Ohta-ku, Tokyo, Japan) and digital slit-lamp biomicroscopic images (Imaging Module 900; Haag-Streit, Koeniz, Switzerland) were obtained. Manual restraint was used for all imaging sessions. Because each snake arrived at a different stage of ecdysis, all snakes were observed daily until they had undergone a complete shed cycle. Then, beginning 1 week following a complete shed cycle and continuing for 1 complete cycle, SD-OCT (model RTVue 100-1; Optovue, Fremont, CA, USA), digital slit-lamp biomicroscopic images, and external photographs were obtained once daily from each snake. On each occasion, SD-OCT images of nasal, axial, and temporal regions of the globe surface were captured. Although an

attempt to collect images using the CAM-L (which uses a longer lens and produces an image with less magnification and wider field of view) and CAM-S (which uses a shorter lens and produces an image with greater magnification and a narrower field of view) was made at every stage, a thickened spectacle made capturing a clear CAM-S image impossible on several occasions. Consequently, only CAM-L images were used for quantitative measurements and CAM-S images were used for qualitative assessment only.

The time required to undergo a full ecdysis cycle varied among snakes from 5 to 9 days. Therefore, external photographs, digital slit lamp images were retrospectively categorized into five grossly apparent stages of ecdysis (S0–S4) defined as follows:

- (1) S0 [7 days after completion of a full shed cycle]
- (2) S1 [first clinical evidence of spectacular opacity]
- (3) S2 [50% completion of a full ecdysis cycle determined retrospectively as half the total number of days for the snake to go from the start of ecdysis (S0) to 1 day after end of shed was clinically evident (S4)]
- (4) S3 [1 day prior to the first clinical evidence of shedding]
- (5) S4 [1 day after shedding was clinically complete]

Optimal images were selected from each stage for analysis.

For all snakes, SD-OCT images at each stage were retrieved from the Optovue software (6.0) and exported as jpeg files before being viewed at 300% of the original image size using NIH ImageJ software (US National Institutes of Health USA, Bethesda, MD, USA). Before assessment of each image, the automatically embedded 250- μm scale was calibrated according to the manufacturer's recommendations using the commercial software. At each of the five stages and for each of the three regions (nasal, axial, and temporal), spectacular thickness, SSS width, and corneal thickness were measured. When the cuticle was present, its thickness and the width of the SCS also were measured. Images in which margins between structures were unclear were omitted from data analysis. Finally, in all images, the cuticle, spectacle, and cornea were evaluated qualitatively for patterns of hyper- or hyporeflectivity.

RESULTS

Images taken at S0 (1 week after completion of a full ecdysis cycle) consistently showed a distinct spectacle, cornea, anterior chamber, and iris (Fig. 1). The cornea was sharply arched and thicker peripherally than axially. Schlemm's canal was apparent in a number of images obtained. The SSS was often completely collapsed, but when observed, was typically more distended peripherally than axially. At S0, median (range) thickness of the nasal, axial, and temporal regions of the spectacle was 83.3

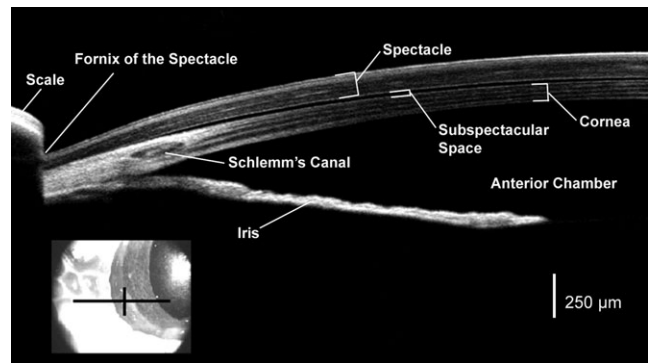


Figure 1. Spectral domain optical coherence tomography (SD-OCT) CAM-L image of the nasal region of the anterior segment of the left eye of an adult royal python (*Python regius*) at S0 (1 week after completion of a full ecdysis cycle). The inset is an external photograph generated by the SD-OCT showing the region of the eye from which the image was obtained.

(72.5–112.4), 75.3 (55.5–97.9), and 92.6 (62.7–127) μm , respectively. Median (range) thickness of the nasal, axial, and temporal regions of the cornea was 145.0 (125.0–160.0), 132.0 (123.5–148.8), and 140.5 (94.8–172.4) μm , respectively. Although duration of the shed cycle varied (5–9 days), analysis of SD-OCT data demonstrated a predictable series of changes in the spectacle and associated structures during each cycle for all animals (Fig. 2). At S1 (the first clinical evidence of spectacular opacity), distinct separation of the cuticle from the underlying stroma of the spectacle was evident (Fig. 2a). The height of separation was greatest axially. Additionally, at this time (Fig 2a), the most superficial aspect of the underlying stroma of the spectacle was notably hyperreflective compared with the same area of the stroma imaged at S0 (Fig. 1). At S2 (when the ecdysis cycle was 50% complete), the shedding cuticle had separated further from the underlying spectacle, which remained hyperreflective (Fig. 2b). Multiple, small, hyper-reflective foci were frequently evident in the SCS when imaged by SD-OCT. At S3 (1 day prior to the first clinical evidence of shedding), the cuticle was further separated from the underlying stroma of the spectacle, which had now become less hyper-reflective than during S0, S1, or S2. The reflective foci in the SCS were less apparent leaving the SCS as a more uniform black zone (Fig. 2c). At S4 (1 day after shedding was clinically complete), the cuticle was no longer apparent and the spectacle and cornea had returned to a relatively uniform reflectivity (Fig. 2d) approximating that seen at S0 (Fig. 1). Slit-lamp images did not permit accurate differentiation of the spectacular cuticle or stroma, cornea, SCS, or SSS at any stage.

Cuticular thickness could be assessed using SD-OCT during S1, S2, and S3 and was relatively constant at these stages (Fig. 3a), whereas width of the SCS tended to increase from S1 to S3 (Fig. 3b). Relative to S0 and S4,

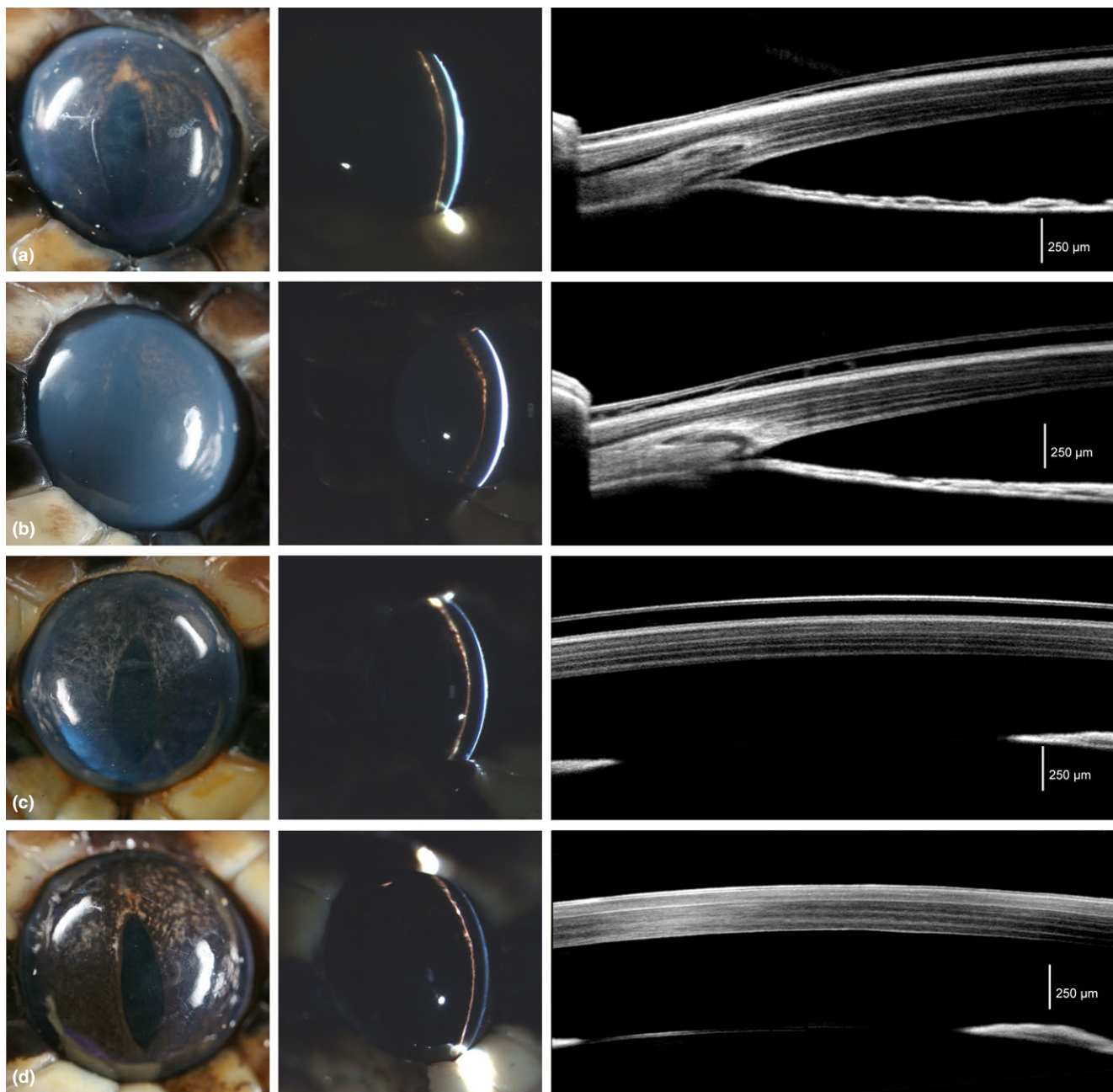


Figure 2. Representative external (left), slit-lamp biomicroscopy (middle), and spectral domain optical coherence tomography (SD-OCT) CAM-L (right) images of the anterior segment of eyes of adult royal pythons (*Python regius*) at four stages of a complete shed cycle. (a) S1 (the first clinical evidence of spectacular opacity). The cuticular zone of the spectacle overlies the now hyper-reflective stromal layer of the spectacle (b) S2 (when ecdysis was 50% complete). Multiple, small, reflective foci are visible in the subcuticular space. (c) S3 (1 day prior to the first clinical evidence of shedding). The spectacular cuticle is further separated from the now less hyper-reflective spectacular stroma. (d) S4 (1 day after clinical evidence of shedding was complete). The spectacle and cornea are now of relatively uniform reflectivity and the cuticle is no longer apparent.

thickness of the spectacular stroma approximately doubled during the middle stages of ecdysis (S1, S2, and S3; Fig. 3c). By contrast, corneal thickness changed little throughout ecdysis (Fig. 3d). These general trends in thickness of the cuticle, spectacle, and cornea, and width of the SCS were noted in each snake whether measurements were taken nasally, axially, or temporally. Likewise,

thickness of the cuticle, spectacle, and cornea, and width of the SCS at each region measured varied little among snakes at each stage (Fig. 4). The SSS was identified inconsistently throughout the shed cycle; however, it was observable more frequently at S0 than at other stages and tended to be collapsed axially and more pronounced peripherally.

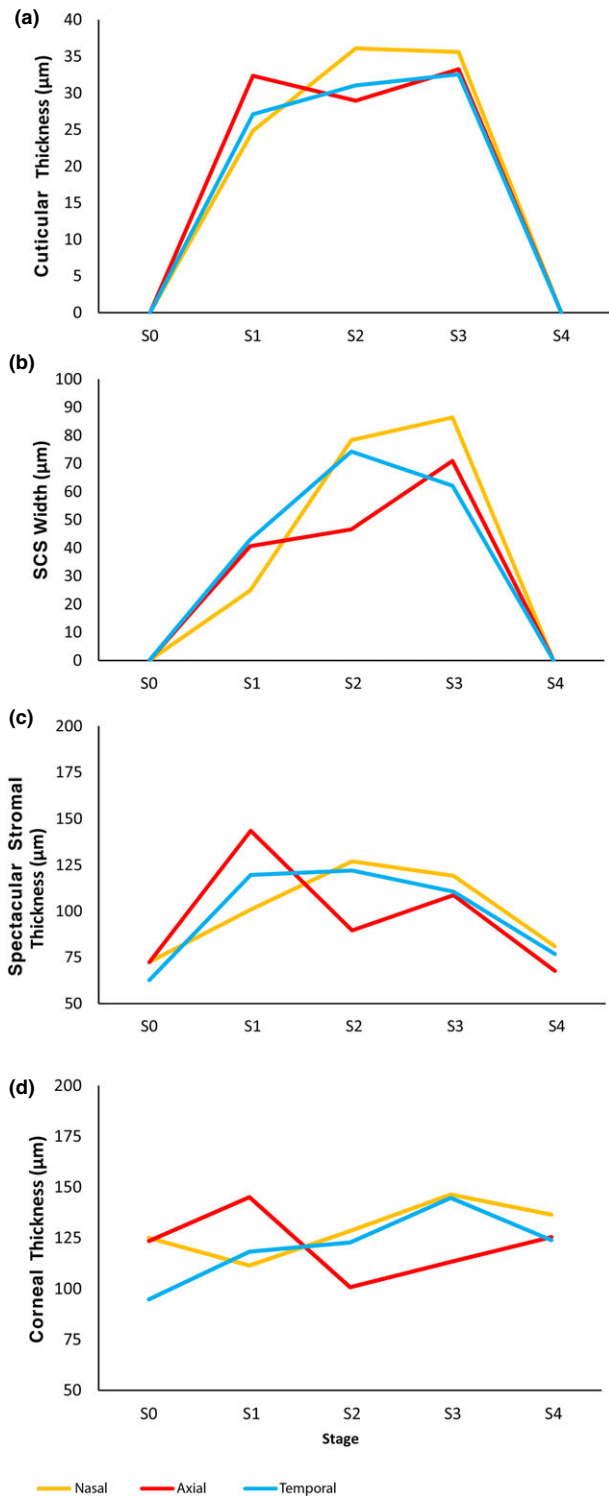


Figure 3. Thickness of the spectacular cuticle (a), width of the subcuticular space (SCS) (b), and thickness of the spectacular stroma (c) and cornea (d) as measured from spectral domain optical coherence tomography (SD-OCT) imaging of nasal, axial, and temporal sites for one royal python (*Python regius*; Snake 4) over time. Other snakes showed the same general trends. See Figs 1 and 2 for definition of stages S0–S4.

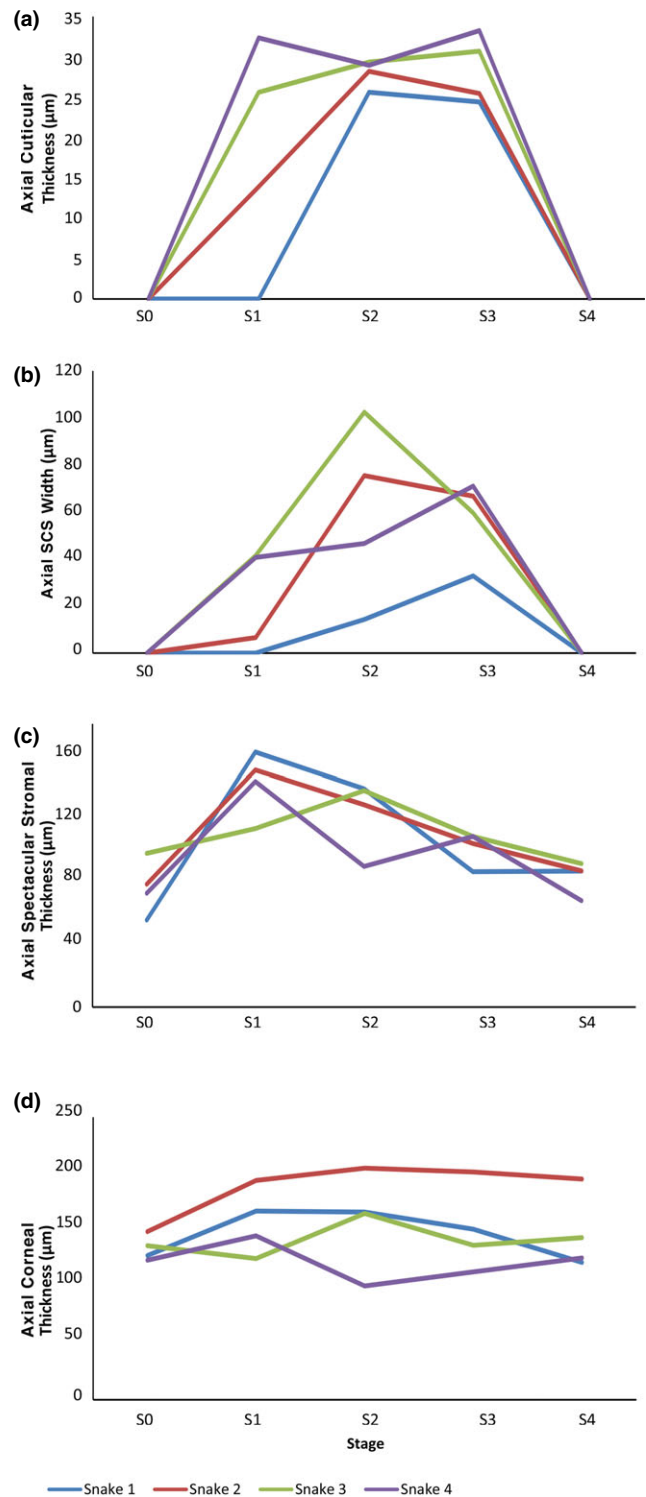


Figure 4. Thickness of the axial cuticle of the spectacle (a), width of the axial subcuticular space (SCS) (b), and thickness of the axial spectacular stroma (c) and axial cornea (d) as measured from spectral domain optical coherence tomography (SD-OCT) imaging varied little among four royal pythons (*Python regius*) throughout ecdysis. Data from imaging of the nasal and temporal sites were similar. A cuticle was not detected in Snake 1 until S2 (a and b). See Figs 1 and 2 for definition of stages S0–S4.

DISCUSSION

To the authors' knowledge, this is the first report of SD-OCT imaging of the anterior segment of the snake eye. This advanced clinical imaging modality routinely provided excellent quality images of the iris, anterior chamber, iridocorneal angle, Schlemm's canal, and particularly, the cornea-spectacle-cuticle complex in normal snakes. The SD-OCT images were easily obtained using only manual restraint and permitted accurate qualitative and quantitative analysis of changes in the cuticular and stromal layers of the spectacle, the cornea, and the SCS throughout ecdysis. By contrast, the SSS was not as reliably assessed in normal snakes of this report; however, when seen, it was wider peripherally than axially. Considering values obtained from all snakes and all (nasal axial, and temporal) sites on the globe at S0 (1 week after completion of a full ecdysis cycle), median (range) spectacular thickness was 80 (55–127) μm and median (range) corneal thickness was 140 (95–172) μm . These differ somewhat from measurements obtained using high-frequency ultrasonography in which spectacular thickness was 188–220 μm and corneal thickness was 110–125 μm .¹³ This is likely due to differences in the resolution of the two methods and potentially the stage of ecdysis at which images were collected as this was not reported in the study utilizing ultrasonography.¹³ Compared with slit-lamp biomicroscopy in the present study, SD-OCT images provided much better visualization and permitted accurate measurement of these ocular surface structures in normal snakes. Although not directly compared in the present study, SD-OCT images of the normal serpentine anterior segment also provided better tissue resolution than did those obtained with high-frequency (50 MHz) ultrasonography; however, ultrasonography did allow visualization of the SSS and structures within the posterior segment.¹³ Together, the present study and the study reporting ultrasound data¹² provide valuable pilot data and images against which assessments of snakes with disease processes involving the spectacle, SSS, and cornea can be made.

Like ultrasonography, SD-OCT imaging permitted longitudinal observation of ophidian ocular surface structures in a minimally invasive manner throughout all stages of ecdysis. Despite variation in duration of ecdysis in individual snakes within the present study, we were able to describe transitions in the cuticle-spectacle-cornea complex that were relatively conserved among snakes and independent of whether SD-OCT images were collected from nasal, axial, or temporal sites on the globe. From the point we defined as baseline (S0, or 1 week after completion of a full ecdysis cycle), the process began with an initial thickening of the spectacle followed by its separation into superficial cuticular and deep, hyper-reflective stromal components. This corresponded to the time when the first signs of spectacular opacity were grossly visible (S1). This spectacular separation led to the development and gradual expansion of the

SCS, which achieved maximal width about 1 day prior to the first clinical evidence of shedding (S3). During this process of spectacular separation, the spectacular stroma regained original reflectivity, and multiple hyper-reflective foci were noted within the SCS. These were considered most likely to be spectacular fragments shed from the interface of the cuticular and stromal zones. The cornea was unchanged in character or thickness throughout all stages of ecdysis. By contrast to SD-OCT, slit-lamp images did not permit observation of these findings.

Given that the spectacle is believed to have formed phylogenetically by the fusion of the upper and lower eyelids, it is perhaps not surprising that the ocular surface transitions we noted using SD-OCT throughout ecdysis appear similar to the histologic changes in snake epidermis during ecdysis as described by Maderson.¹² He reduced these to six histologic stages, five of which can be detected grossly. He designated the first stage (or 'resting condition') as the period immediately following shed when the snake's skin colors are bright and the spectacle is clear. Histologically, Maderson described the epidermis at this time as consisting of only the outer epidermal generation and a basal stratum germinativum. This would be equivalent to S4 in the present study and appears consistent with our SD-OCT images, which revealed a spectacle of relatively uniform reflectivity with no overlying cuticle evident. Maderson's second histologically distinct stage could only be identified retrospectively through daily biopsies as it had no clinically distinct features making comparison between the two studies difficult. However, at this stage, the stratum germinativum underwent intense proliferation and formed a new presumptive inner epidermal generation. This would have approximately coincided with S0 in the present study. Maderson's third stage was characterized clinically by a slight cloudiness of the spectacle and overall dulling of the snake's coloration and corresponds with S1 in the present study. Histologically at Maderson's third stage, the inner epidermal generation continued to differentiate and began to keratinize. Using SD-OCT, we noted a distinct separating cuticular layer at this stage overlying a hyper-reflective spectacular stroma, which may represent the keratinization described histologically by Maderson. At Maderson's fourth stage, the spectacle was nearly opaque and the animal's colors were at their duller. Histologically, he described a new layer between the inner and outer epidermal generations in the region of the stratum intermedium. This appears to approximate the stage we describe in the present study as S2 (the retrospectively determined half-way point of ecdysis). At this time point, SD-OCT data supported Maderson's inner and outer epidermal generations (representing the spectacular stroma and cuticle, respectively), but failed to identify the new layer of the spectacle that he described between them. Maderson assigned changes seen about 5–8 days before shedding to the fifth stage. At this time, the snake's colors suddenly became bright again and the spec-

tacle was clear, presumably as a result of breakdown of the lacunar tissue of the stratum intermedium, which he observed histologically. In our subjects, the spectacle became clear again in only two snakes and only 1 day prior to shed (= S3). This distinct clinical stage was not noted in the remaining two snakes in the present study. Clinically detectable sloughing of the skin defined Maderson's final (sixth) stage when he noted complete histologic breakdown between the outer and inner epidermal generations within the stratum intermedium, with the old keratin totally shed within 24 h.¹² Images obtained using SD-OCT in the present study during S3 and S4 show very similar changes to those observed histologically by Maderson.¹² Thus, it appears that, not surprisingly, the spectacle undergoes ecdysis in a very similar manner to that Maderson described for the rest of the epidermis and that SD-OCT is an *in vivo* imaging modality that closely reflects the changes previously only observable through histologic examination of samples collected by biopsy or at postmortem.¹²

Taken collectively, findings in the present study prove the utility of SD-OCT for imaging anterior segment structures of the globe and, in particular, the cornea-spectacle-cuticle complex of manually restrained normal snakes at all stages of ecdysis. Findings described here provide normative data regarding the *in vivo* cuticular, spectacular, and corneal thicknesses, and *in vivo* width of the SCS and SSS. Additionally, they provide an essential foundation for future examinations of snakes with anterior segment pathology, in particular retained spectacle, subspectacular abscessation, and pseudobuphthalmos. Our finding that the SSS is wider peripherally than axially suggests that the peripheral spectacle represents the preferred entry point for injections of therapeutic or diagnostic agents into the SSS, and for initiating spectacular incisions necessary for treatment of subspectacular abscesses.¹⁰

ACKNOWLEDGMENTS

The authors thank Monica Motta, Sarah DeRemer, Rebecca Seraphin, and Theresa Aller for their assistance with animal husbandry and handling, Dr. Shinae Park for overseeing the snake's veterinary care, and John Doval for graphic design and assistance with figures.

REFERENCES

1. Walls G. Adaptations to media and substrates. In: *The Vertebrate Eye and its Adaptive Radiation*. (ed. Walls G). Hafner Publishing Company, New York, 1963; 368–461.
2. Duke-Elder SS. The ophidian eye. In: *The Eye in Evolution*. (ed. Duke-Elder SS) Henry Kimpton Publishers, London, 1958; 383–395.
3. Neher EM. The origin of the brille in *Crotalus confluentus lutosus* (great basin rattlesnake). *Transactions of the American Ophthalmological Society* 1935; **33**: 533–545.
4. van Doorn K, Sivak JG. Blood flow dynamics in the snake spectacle. *Journal of Experimental Biology* 2013; **216**(Pt 22): 4190–4195.
5. Quekett J. Observations on the vascularity of the capsule of the crystalline lens, especially that of certain reptilia. *Transactions of the Microscopical Society of London* 1852; **3**: 9–13.
6. Sivak J. The role of the spectacle in the visual optics of the snake eye. *Vision Research* 1977; **17**: 293–298.
7. Hausmann JCHS, Hawkins MG, Kass PH *et al*. Distribution and outcome of ocular lesions in snakes examined at a veterinary teaching hospital: 67 cases (1985–2010). *Journal of the American Veterinary Medical Association* 2013; **243**: 252–260.
8. Millichamp N, Jacobson E, Wolf E. Diseases of the eye and ocular adnexae in reptiles. *Journal of the American Veterinary Medical Association* 1983; **183**: 1205.
9. Kern TJ. Exotic animal ophthalmology. In: *Veterinary Ophthalmology*, 3rd edn. (ed. Gelatt KN). Lippincott Williams and Wilkins, Philadelphia, 1999; 1279–1284.
10. Maas AKD, Paul-Murphy J, Kumaresan-Lampman S *et al*. Spectacle wound healing in the royal python, *python regius*. *Journal of Herpetological Medicine and Surgery* 2010; **20**: 1–8.
11. Millichamp N, Jacobson E, Dziezyc J. Conjunctivalostomy for treatment of an occluded lacrimal duct in a blood python. *Journal of the American Veterinary Medical Association* 1986; **189**: 1136.
12. Maderson PFA. Histological changes in the epidermis of snakes during the sloughing cycle. *Proceedings of the Zoological Society of London* 1965; **146**: 98–113.
13. Hollingsworth SR, Holmberg BJ, Strunk A *et al*. Comparison of ophthalmic measurements obtained via high-frequency ultrasound imaging in four species of snakes. *American Journal of Veterinary Research* 2007; **68**: 1111–1114.
14. Foster FS, Pavlin CJ, Harasiewicz KA *et al*. Advances in ultrasound biomicroscopy. *Ultrasound in Medicine and Biology* 2000; **26**: 1–27.
15. Irish FJ, Williams EE, Seling E. Scanning electron microscopy of changes in the epidermal structure occurring during the shedding cycle in squamate reptiles. *Journal of Morphology* 1988; **197**: 105–126.
16. Nork TM, Rasmussen CA, Christian BJ *et al*. Emerging imaging technologies for assessing ocular toxicity in laboratory animals. In: *Assessing Ocular Toxicology in Laboratory Animals* (eds Weir AB, Collins M) Humana Press, New York, 2013; 53–121.