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The eye of the red-eared slider turtle: morphologic observations and reference values for selected ophthalmic diagnostic tests

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Abstract

Purpose To perform a descriptive investigation of the red-eared slider turtle (*Trachemys scripta elegans)* eye, performing selected ophthalmic diagnostic tests with the aim of establishing normal reference values for this species.

Method Thirty adult healthy red-eared slider turtles were used to establish normal ophthalmic test values in this investigation. Selected ophthalmic tests included: collection of material for bacterial culture analysis, esthesiometry, intraocular pressure (IOP), A- and B-mode ultrasonic biometry, fundus photography, and central corneal thickness (CCT).

Results and discussion Normal parameters found for the ocular diagnostic tests were: esthesiometry: 5.84 ± 0.48 cm; IOP: 5.42 ± 1.70 mmHg; CCT: 154.5 ± 0.14 μm; palpebral fissure length: 9.71 ± 0.55 mm; modified Schirmer tear test: 2.55 ± 3.4 mm; globe axial length: 7.60 ± 0.23 mm; anterior chamber depth: 0.76 ± 0.23 mm; lens axial length: 2.45 ± 0.28 mm; vitreous chamber depth: 4.31 ± 0.42 mm. An avascular retinal pattern with nerve fibers radiating from the small white circular optic disk was observed. None of the animals had a conus papillaris. The most frequent bacteria found were *Bacillus spp.* (33.33%) followed by *Proteus vulgaris* (20.69%) and *Staphylococcus aureus* (18.39%). No significant differences between left and right eyes or genders were found for any of the results. Reference data and morphologic observations obtained in this investigation might help veterinary ophthalmologists to diagnose ocular diseases in the red-eared slider turtle.

Key Words: esthesiometry, intraocular pressure, normal microbiota, *Trachemys scripta elegans*, ultrasonic pachymetry

INTRODUCTION

The genus *Trachemys* is composed of a wide variety of species in the order Testudines. The subject of the present investigation, the red-eared slider turtle (*Trachemys scripta elegans*), is a freshwater turtle, belonging to the suborder Cryptodira, family *Emydidae*. This reptile is one of the most widely distributed vertebrate species in the world, naturally occurring from the Mississippi Valley to the Gulf of Mexico, mainly inhabiting rivers, lakes and swamps, preferably in places with abundant aquatic plants and basking sites.[1–3] Its popularity as a pet has increased, due its small size, simple husbandry requirements low purchase price.[3–5]

Reptile ophthalmology publications have increased in numbers over the last few years. The available literature focuses on ocular diseases, pathophysiologic mechanisms and clinical treatments.[6–10] Some available studies with reptile species also report normal ophthalmic parameters and selected clinical tests.[11–18] Normal values for rebound tonometry[16] and tear production[17] have been reported for red-eared slider turtles. The objective of this study was to report ocular morphological observations and to perform ophthalmic tests to establish a panel of normal parameters for the red-eared turtle.

MATERIAL AND METHODS

Ophthalmic procedures on live animals

All procedures using live red-eared turtles were conducted in accordance with ARVO's Statement for the Use of Animals in Ophthalmic and Vision Research and with Federal University of Paraná's Animal Use Committee. A total of 30 adult red-eared turtles (17 males and 13 females) were

selected and used in this investigation. The 30 turtles used in this research were selected from a group of 90 animals, all residents of the Wild Animal Triage Center (Centro de Triagem de Animais Silvestres—CETAS) at Pontificia Universidade Católica, PR; PUCPR/IBAMA, located at Tijucas do Sul, Paraná, Brazil. These 90 turtles from CETAS were mostly pet turtles that were illegally acquired and subsequently confiscated by the Brazilian Institute of Environment and Renewable Natural Resources. Physical examinations, selected serum biochemical assays including globulin, glucose, uric acid, cholesterol alanine transferase, and aspartate transferase, a complete blood count including leukocyte and erythrocyte counts and measurement of total protein in plasma were performed before ocular examinations to exclude animals with indications of systemic disease. An attempt to make the sexually mature population of turtles studied as uniform as possible was made through an analysis of medical records available at CETAS and through measurements of plastron length, as suggested in the literature.[19] Sixty of these animals were excluded based on abnormalities found in these blood analyses or due to discrepancies in age, size, and weight. Ages were estimated using a combination of methods that included individual history, triage center entry records, size of the carapace, weight, and count of the annual growth lines.[19,20] The group was transported to the Veterinary Teaching Hospital of Federal University of Paraná (HV-UFPR), located in Curitiba City, Paraná State, Brazil. They were housed in 1000-L tanks, containing 15 animals each. The tanks were kept in a temperature and humidity-controlled room. A total of five tanks were used. Two tanks with a water filtration system, two tanks used only for feeding, and an empty tank, in which the animals were kept for 8 h before the tests were performed. The same group of turtles was transported back to HV-UFPR in two different occasions, 4 and 6 months later, to perform additional tests (A- and B-mode ultrasonographic biometry). Procedures and tests were split between the investigators. However, to avoid discrepancies related to different observers, the same person always performed the same ocular test on each occasion.

Ophthalmic tests

Clinical tests were performed, while turtles were manually restrained and carefully placed on a platform, so that they were unable to move (Fig. 1a). The head was manually stabilized for taking some measurements, such as the palpebral fissure length. However, it is important to note that this restraint method was not used for intraocular pressure evaluation, as pressure on the neck might potentially result in iatrogenic IOP changes. The different procedures and measurements were performed on different occasions. The entire sequence of procedures was: (i) Day one—ocular inspection, collection of material for bacterial culture analysis, and palpebral fissure measurement; (ii) Day three—esthesiometry; (iii) Day five—tonometry and central corneal thickness (CCT). (iv) Four months later—modified Schirmer tear test and ocular ultrasonography.

Ocular examinations

A total of 60 eyes, from 30 healthy red-eared turtles (17 males and 13 females) were investigated. The anterior segment structures of the eyes of all turtles were evaluated using a slit lamp biomicroscope (Hawk Eye, Dioptrix, L'Union, France). (Fig. 1b).

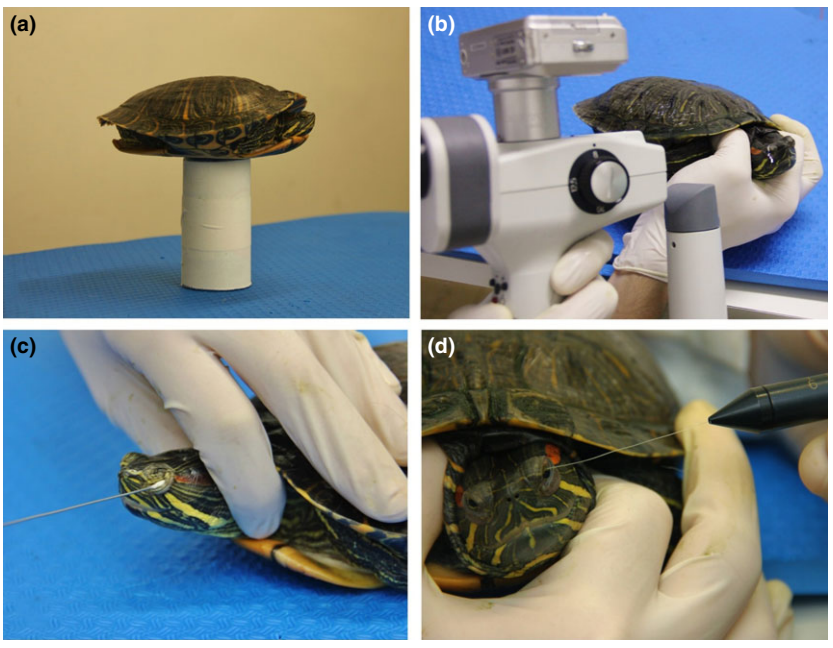

Figure 1. Photographs of selected ocular tests being performed in red-eared slider turtles (*Trachemys scripta elegans*) (a) Turtles were manually restrained and placed on a platform so that they were unable to walk. (b) Anterior ocular structures being evaluated using a slit lamp biomicroscope (Hawk Eye, Dioptrix, L'Union, France). (c) Micro-swabbing procedure being performed on the ocular surface. (d) Central corneal esthesiometry being performed.

Fundic examinations

Indirect binocular ophthalmoscopy was performed on 20 animals of the group using a Finoff transilluminator (Welch Allyn, Skaneateles Falls, NY, USA) as a source of focal light and an indirect lens (Macula Plus 5.5® Volk Optical Inc, Mentor, OH, USA). Indirect monocular ophthalmoscopy using the Panoptic® ophthalmoscope (PanOpticTM, Welch Allyn, Skaneateles Falls, NY, USA) was performed after topical administration of one drop 0.4% vecuronium bromide every 15 min for 1 h (Norcuron, Organon Inc, NJ, USA). To obtain a better view of the fundus and capture fundic images, topical endoscopy fundus imaging technique (TEFIT) as previously described[21,22] was performed on 12 animals using a 2.9-mm multipurpose rigid telescope system and a xenon lamp as a light source (Telepack Vet™ Karl Storz GmbH & Co. KG, Tuttlingen, Germany), which was connected to a 7.2 megapixel reflex digital camera (Carl Zeiss™ lens and 12x of optic zoom system, DSC-H5, Sony™, San Diego, CA, USA). The eyes were imaged after topical instillation of one drop of 0.4% vecuronium bromide (a nondepolarizing blocking agent) in each eye every 20 min for 2 h.

Microbiological analysis

For microbiological analysis, samples from 54 eyes of 27 read-eared turtles were obtained by carefully touching the ocular surface with a sterile cotton swab (Fig. 1c). Before sample collection, all the animals were kept in the same environment. The temperature was kept at 23 °C, and humidity level at 47%. No topical anesthetics were used prior to sample collection as this may interfere with growth of microorganisms. Aerobic bacterial culture was performed in BHI broth (brain heart infusion), and on 5% sheep blood agar and MacConkey plates, which were incubated at 37 °C in an aerobic environment for 48 h.

Corneal esthesiometry

Corneal sensibility analysis was performed in all 30 animals using a *Cochet-Bonnet* esthesiometer (Luneau Ophtalmologie, Chartres Cedex, France) (Fig. 1d). This instrument uses an adjustable nylon monofilament with defined diameter, which is extended to different lengths and then touched to the center of the cornea, with shorter lengths producing a more noxious stimulus. The turtles' reactions to the monofilament reaching a threshold length (and thus force) included eyelid closure and marked retraction of the globe.

Intraocular pressure

Intraocular pressure (IOP) was measured in 60 eyes from 30 animals, using a veterinary rebound tonometer (Tonovet®, Veterinary Division of S&V Technologies AG, Henningsdorf, Germany) with the P setting, which was a preset for other animals except dogs and horses. Six measurements were taken and averaged by the tonometer′s internal software, while the turtles were on the restraint platform (Fig. 2a,b).

Central corneal thickness

Central corneal thickness (CCT) measurements were taken after instillation of sterile topical anesthetic (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA). CCT was measured in all 30 turtles using an ultrasonic pachymeter (Fig. 2c).

Modified Schirmer Tear Test

The Modified Schirmer Tear Test (mSTT) strip was created by using a scalpel to transect a 35-mm-length/5-mm-wide commercial STT strip, while still in the package (Teste de Schirmer, Ophthalmos, São Paulo, Brasil) into two 35-mm-long/2.5-mm-wide strips. Only the unnotched half was used. The mSTT1 was performed similarly to

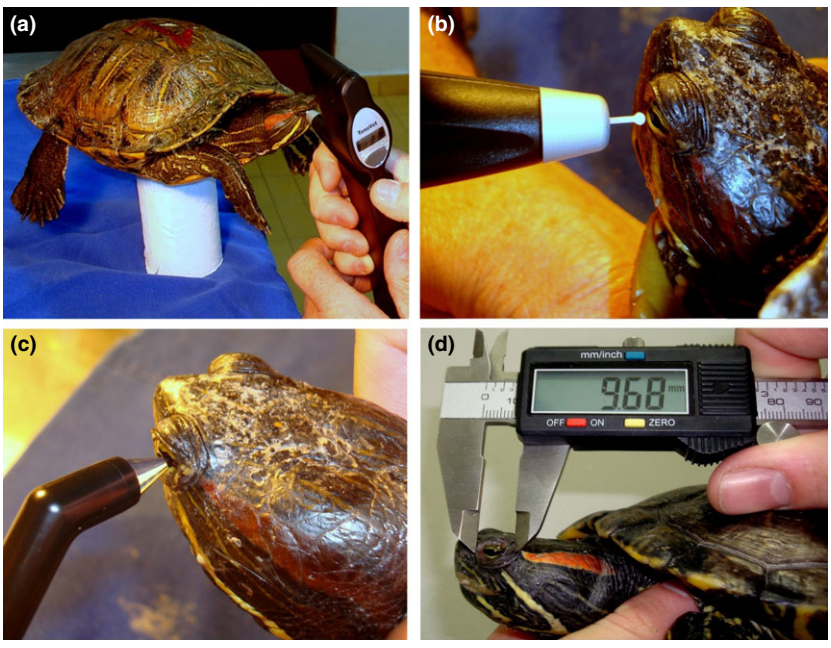

Figure 2. Photographs of additional selected ocular tests being performed in red-eared slider turtles (Trachemys scripta elegans) (a) Animals did not have the head immobilized during the IOP evaluation. (b) Rebound tonometry in a turtle's left eye. (c) Central corneal pachymetry (d) Palpebral fissure length measurement.

the standard STT1, in which the length of the moistened area was measured and recorded in millimeters after 1 min. (Fig. 3).

B-mode ultrasonographic biometry

Ocular B-mode ultrasonographic imaging was performed in 60 eyes of 30 turtles, after instillation of sterile topical anesthetic (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA), using a B-mode ultrasound system (MyLab 30 - Esaote, Genova, Italy) equipped with a 12-MHz linear ultrasound transducer. All ultrasound examinations were performed by the same two people, one of whom restrained the turtle by the neck, while the other performed the imaging. Ultrasound gel was applied and the transducer was gently positioned with minimal pressure on the ocular surface (Fig. 4a). The long axis of the transducer was held horizontally between the lateral and medial canthus with the marker pointing nasally. The biometric measurements were obtained according to the following sequence: axial globe length, anterior chamber depth, lens thickness and vitreous chamber depth (Fig. 4b).

A-mode ultrasonographic biometry

To validate the B-mode ultrasonic biometry data, additional A-mode ultrasonographic examinations were performed in 12 eyes of six turtles after instillation of sterile topical anesthetic (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA), using an ultrasound system (Model 200P+; Micropach_ Sonomed, Lake Success, NY, USA) with the A-mode ultrasound probe included with the system. Care was taken during probe placement to avoid corneal indentation and to find optimal positioning to register the highest and clearest possible spikes for each ocular interface difference along the pupillary axis, namely: probe/cornea, aqueous/anterior lens capsule, posterior lens capsule/anterior vitreous, and posterior vitreous/retinal surface. The biometric measurements between the spikes were registered according to the following sequence: anterior chamber depth, lens thickness, vitreous chamber depth, and axial globe length.

Palpebral fissure length

A stainless steel caliper with an LCD display and an accuracy of ± 0.02 mm (Neiko Tools, Klamath Falls, OR, USA) was used to measure palpebral fissure length in all 30 turtles (Fig. 2d). The animals were manually restrained to measure palpebral fissure length of both eyes.

Gross evaluation of two eyeballs

Two 10% neutral-buffered formalin-fixed eyeballs from a single animal that died at CETAS-PUCPR were donated to the UFPR's Comparative Ophthalmology Laboratory. Optic disk diameter, horizontal and vertical corneal diameters, and horizontal and axial longitudinal globe lengths were measured. The globes were sectioned and the posterior segment exposed for gross analysis to validate the data obtained on ophthalmoscopic evaluations.

Statistical analyses

Descriptive statistical analyses were performed on the data. Unpaired *t*-tests were used for data comparison between, right and left eyes and males and females. Paired *t*-tests were used for data comparison between the two ultrasonographic modalities applied: A-mode versus B-mode. Pearson's correlation tests were used to explore possible correlations for globe size, carapace length, and circumference. *P*-values < 0.05 were deemed significant. JMP (SAS Institute, Inc., Cary, NC, USA) software was used to perform both descriptive and inferential statistical analyses.

RESULTS

General observations on live animals

The male-to-female ratio in the population studied was 57% male and 43% female (17 males and 13 females). The age range of the group was 7 years. Maximum age was 12 years and minimum age was five. According to the records of the Triage Center, 14 of the 30 turtles had a known age, ranging between eight and 12 years of age. The precise mean age could not be exactly deter-

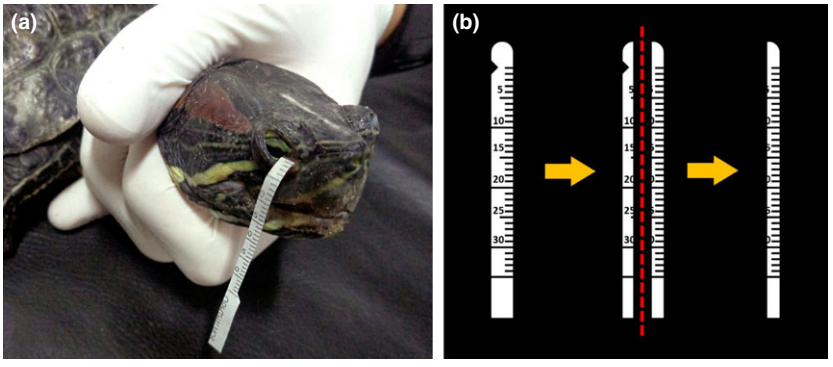

Figure 3. (a) Manually trimed STT strip in the right eye of a slider turtle while gentle physical restrained was made by the neck. (b) Schematic drawing demonstrating a standart Schirmer tear test strip after been transected with a scalpel blade into two 35 mm-long/2.5 mm-wide strips.

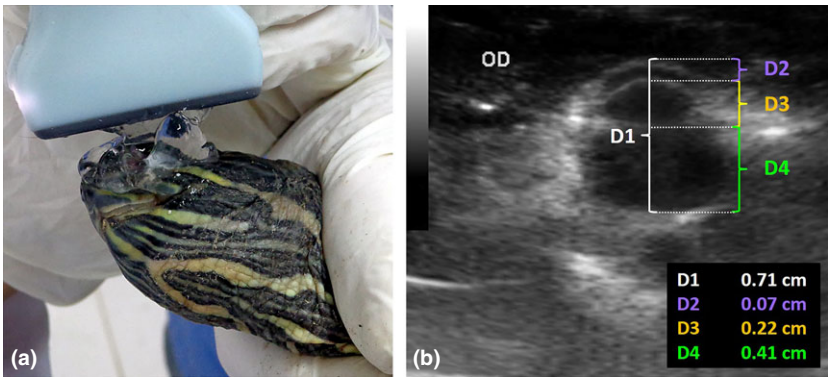

Figure 4. (a) Ultrasound gel was applied and the transducer was gently positioned with minimal pressure on the eye surface (b) Representative B-scan ultrasonogram after optimal positioning. The four principal landmarks (cornea, anterior lens surface, posterior lens surface and retinal surface) along the globe axis are all perpendicular. Four measurements were performed on the B-scan image: D1: Axial globe length (anterior cornea to the chorioretinal surface) D2: Anterior chamber depth (posterior cornea to anterior lens capsule); D3: Lens thickness (anterior lens capsule to the posterior lens capsule); D4: Vitreous chamber depth (posterior lens capsule to the chorioretinal surface).

mined because some turtles had limited information in their medical records. The age of these remaining turtles was estimated based on the similar size of those with an already known age and using the method of counting the carapace growth lines. Thus, the age estimation of these remaining turtles ranged from 5 to 10 years. The mean ± standard deviation (SD) weight was 1.47 kg ± 33 g for males and 1.46 kg ± 27 g for females. The mean (± SD) size for males was 39.69 ± 2.85 cm body circumference around the carapace and plastron and the carapace was 22.27 ± 1.56 cm in length. For females, the measures were 39.44 ± 2.50 cm around the carapace and plastron and the carapace length was 22.54 ± 1.84 cm.

Microbiological analysis

Bacterial growth was observed in 100% of the eyes. A single bacterial colony was isolated in twenty eyes (37.0%). In some eyes, more than one type of bacterial colony was isolated: Thirty-two (59.3%) showed growth of two different bacterial colonies and two (3.7%) showed growth of three bacterial colony types. Bacterial organisms were identified in microbiological samples from 54 eyes of 27 turtles. Eighty-seven (N) isolates were obtained. Forty-five (63.2%) isolates were Gram-positive and included five different species of bacteria. Thirty-two (36.8%) isolates were Gram-negative representing eight different species. The most frequent bacteria found were *Bacillus spp.* (33.3%) followed by *Proteus vulgaris* (20.7%) and *Staphylococcus aureus* (18.4%). *Corynebacterium spp.* (1.2%), *Providencia rettgeri* (1.2%), *Enterobacter cloacae* (1.2%), *Enterobacter sazakii* (1.2%), *Enterobacter aglomerans* (1.2%), *Enterobacter spp.* (2.3%), *Citrobacter freundii* (3.5%), *Staphylococcus spp.* (4.6%), *Streptococcus spp.* (5.8%), and *Proteus miriabillis* (5.8%) were also found in fewer eyes. Results of the microbiological analyses can be seen in Table 1.

Table 1. Results obtained from the microbiological bacterial analyses in 54 eyes of 27 red-eared slider turtles (*Trachemys scripta elegans)*

Bacterial type	*N* (%)
Gram-positive	
Corynebacterium spp.	1 (1.2)
Staphylococcus spp.	4 (4.6)
Streptococcus spp.	5 (5.8)
Staphylococcus aureus	16 (18.4)
Bacillus spp.	29 (33.3)
Gram-negative	
Providencia rettgeri	1 (1.2)
Enterobacter cloacae	1 (1.2)
Enterobacter sakazakii	1 (1.2)
Enterobacter agglomerans	1 (1.2)
Enterobacter spp.	2 (2.3)
Citrobacter freundii	3 (3.5)
Proteus miriabillis	5 (5.8)
Proteus vulgaris	18 (20.7)
Total samples with growth	87 (100)

Ocular morphological observations on live animals

The eyes are positioned laterally on the skull, similarly to a prey animal, however, the eyeball mobility was very efficient and clearly able to produce at least a modest binocular field of vision. The eyelids are somewhat thin and pigmented with a dark green-masked pattern. Inferior palpebral mobility is more efficient than the superior one, and the third eyelid is extremely mobile. Iris surface color ranges from green to yellow with a characteristic horizontal dark band running horizontally in the center. The pupil has a circular shape. The bulbar conjunctiva usually has a greenish appearance and has a specific pattern of pigmentation and follows the pattern of the iris (Fig. 5). Another important feature is that this animal has a great capacity to retract the globe as part of the palpebral reflex when the ocular surface is touched. In normal examination, room lighting pupils measure about 2 mm in

Figure 5. External appearance of the red-eared slider turtle's right eye. Note, in this representative example, the bulbar conjunctival pattern of color that exactly follows the dark horizontal band present on the nasal and temporal sides of the iris.

diameter. Pharmacological dilation of the pupils for fundic examination was not completely achieved with one drop of 0.4% vecuronium bromide (a nondepolarizing blocking agent) applied to the corneal surface every 15 min for 1 h. A slightly better pupil dilation of approximately 3.5 mm in diameter was obtained with one drop of topical 0.4% vecuronium bromide every 20 min for 2 h. Pupillary response to a light stimulus also was suppressed with this protocol. During fundus examination using indirect ophthalmoscopy, it was only possible to partially examine small portions of the fundus; however, better and wider fundus images were obtained using TEFIT. It was possible to fully observe the typical avascular retinal pattern and nerve fibers radiating from the small white circular optic disk (Fig. 6a). A large choroidal blood vessel running from the nasal to the temporal aspect was observed (Fig. 6a). None of the animals had a conus papillaris. All ophthalmic test results can be seen in Table 2.

Corneal esthesiometry

The mean ± SD central corneal sensitivity was 5.7 ± 0.18 cm for males and 5.8 ± 0.08 for females. There were no significant differences between males and females ($P = 0.2567$) and no significant differences between left and right eyes ($P = 0.6919$).

Intraocular pressure

The mean ± SD value for IOP was 5.5 ± 0.3 mmHg for males and 5.3 ± 0.3 mmHg for females. There was no significant difference in IOP between males and females ($P = 0.56$) and no significant differences between left and right eyes ($P = 0.82$).

Central corneal thickness

The mean ± SD CCT was 152.1 ± 0.1 μm for males and 157.6 ± 0.1 μm for females. There was no significant difference in CCT between males and females ($P = 0.74$) and no significant differences between left and right eyes ($P = 0.52$).

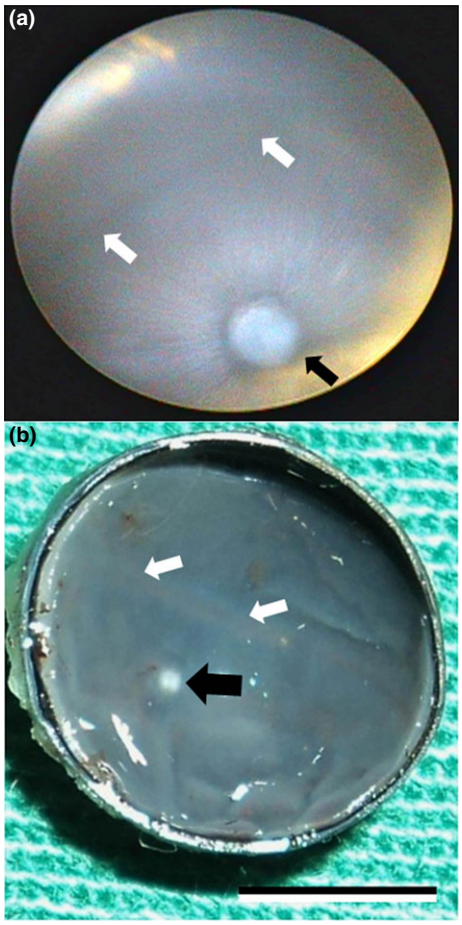

Figure 6. (a) Fundoscopic appearance captured using TEFIT. Note the typical avascular retinal pattern and from the small white circular optic disc (black arrow) readily visible nerve fibers seem to radiate to the periphery (a). Note the faint image of the large choroidal blood vessel that runs from the nasal to the temporal aspect (white arrows) on the midline above the optic nerve (b) Transverse section of a 10% formalin-fixed red-eared turtle globe showing the fundus. Note the optic disc (black arrow) and another view of the same choroidal blood vessel (white arrows) described in (a). Bar 0.4 cm.

Modified Schirmer Tear Test

Mean ± SD value for mSTT was 2.55 ± 3.4 mm. There was no significant difference in mSTT between males and females ($P = 0.31$) and no significant differences between left and right eyes ($P = 0.32$).

B-mode ultrasonographic biometry

The mean ± SD for axial length of the eyeball was 7.60 ± 0.23 mm for males and 7.58 ± 0.29 mm for females. Considering the axial length, there was no significant difference between males and females ($P = 0.82$) and between left and right eyes ($P = 0.09$). Also, there was no significant correlation between globe axial length and carapace length ($P = 0.06$) or body circumference length ($P = 0.12$), with low correlation coefficients, Pearson's $R = 0.25$ and $R = 0.20$, respectively. The mean ± SD value for the anterior chamber depth was 0.76 ± 0.23 mm. The

Table 2. Results obtained for select ophthalmic diagnostic tests for 30 red-eared slider turtles (*Trachemys scripta elegans*)

Ophthalmic test	Mean	Median	SD	CI 95%	CV*
Esthesiometry (cm)	5.8	6	0.5	4.8–6.8	0.1
Intraocular pressure (mmHg)	5.4	5	1.7	3.4–8.8	0.3
Central corneal thickness (μm)	154.5	155	0.1	154.3–154.7	0.1
Modified Schirmer tear test (mm)	2.55	1	3.4	0–9.35	12
Palpebral fissure length (mm)	9.7	9.7	0.6	8.5–10.9	0.6

*Coefficient of variation.

mean ± SD value for the lens length was 2.45 ± 0.28 mm. The mean ± SD value for the vitreous chamber length was 4.31 ± 0.42 mm. A conus papillaris was not observed in any of the ocular ultrasonograms of the animals studied.

A- mode ultrasonographic biometry

The mean ± SD was 7.45 ± 0.26 mm for axial length, 0.76 ± 0.07 mm for the anterior chamber depth, 2.28 ± 0.25 mm for the lens thickness, and 4.24 ± 0.26 mm for the vitreous chamber depth. Mean echobiometric values from and the results of the comparison between each method are summarized in Table 3.

No significant differences were found comparing the mean values of each biometric parameter evaluated using A- and B-mode ultrasonography (Table 3).

Palpebral fissure length

For palpebral fissure length, mean ± SD value was 9.9 ± 0.1 for males and 9.5 ± 0.1 for females. Males had a significantly bigger fissure length than females ($P = 0.0100$), although there was no difference between left and right eyes ($P = 0.4657$) and no correlation of palpebral fissure length with carapace length ($P = 0.55$) (Pearson's $R = 0.07$) or body circumference ($P = 0.26$) (Pearson's $R = 0.14$).

Gross evaluation of two formalin-fixed eyeballs

The following measures were taken: horizontal and vertical globe diameters were 0.8 cm. The horizontal corneal diameter was 0.5 cm and the vertical diameter was 0.4 cm.

Table 3. Descriptive statistics of A- and B- mode echobiometric values (mm) for the red-eared slider turtles (*Trachemys scripta elegans*)

	B-mode		A-mode		Paired *t*-test
Measure	Mean (mm)	SD (mm)	Mean (mm)	SD (mm)	(*P* values)
Globe axial lenght	7.59	0.25	7.45	0.26	0.06
Anterior chamber depth	0.76	0.23	0.76	0.07	0.9
Lens thickness	2.45	0.28	2.28	0.25	0.2
Vitreous chamber depth	4.31	0.42	4.24	0.26	0.62

The optic disk was 0.75 mm in diameter. Gross morphological evaluation after sectioning the globe showed an avascular retina, absence of a conus papillaris, and presence of a large choroidal blood vessel running from the nasal to the temporal aspect near the horizontal midline of the posterior segment above the optic disk (Fig. 6b).

DISCUSSION

Among all reptiles, turtles are the longest lived. Some species have been known to live over 100 years and weigh up to 263 kg.[23] Because they are ectothermic, environmental temperature greatly influences their sex determination, basal metabolic rate and growth rate.[23,24] In this context, it is clear that determining the exact age of these animals can be a very difficult task. Even using methods described in the literature to estimate individual age,[19,20] the individuals examined in this study might not be the exact age estimated. The population of animals studied in this investigation can be considered as young adult, as the longevity expected for this turtles under a natural environment approximately is 30 years.[25]

Reptile bacterial microbiota studies available in the literature typically focus on the zoonotic potential that these animals have to transmit salmonellosis to humans.[26–29] The most common microorganism isolated in our investigation was *Bacillus spp.* This bacteria seems to be common in reptiles, as it was also found in conjunctival samples from green iguanas,[30] from samples of the tympanic cavity of Eastern box turtles[31] and from oral samples of Geoffroy's toadhead turtle *(Phrynops geoffroanus)*.[32] *Proteus spp.* was the second most prevalent bacteria in our study, followed by *Staphylococcus aureus*, which was the most prevalent microorganism isolated in conjunctival samples from green iguanas[30] However, as it is true for most ocular bacterial microbiota studies, the possibility that these microorganisms may be transient inhabitants from the environment cannot be ruled out. The results obtained here demonstrated that the conjunctival bacterial microbiota of this group of freshwater turtles is composed of a wide spectrum of microorganisms, including some potential reptile pathogens such as *Citrobacter freundii* which has been associated with the development of a cutaneous ulcerative disease in wild box turtle,[31] and *Enterobacter agglomerans* that has been associated with development of stomatitis and dermatitis in alligators[33] despite the fact that De Morais *et al.* (2011) has demonstrated that the family enterobacteriacae are a normal part of the bacterial microbiota of the mouth and cloaca of two species of Brazilian freshwater turtles.[34]

Even so, we believe that additional studies to try to determine whether or not this bacteria is a normal inhabitant of slider turtle eye should be performed. It is important to mention that in this investigation, the possible seasonal variations in bacterial microbiota of the conjunctiva as reported to occur in horses for instance[35] were not explored.

One of the most interesting findings noted during the external evaluation of the eye was the remarkable ability that these animals have to retract the eyeball. This is probably due to a prominent retractor muscle complex, as has been observed in other species of testudines.[36]

Fundus examination using indirect ophthalmoscopy was very difficult to perform, even after topical administration of vecuronium bromide to induce mydriasis because of the small size of the globe and consequently the small pupil aperture. TEFIT allowed for a better interpretation of the fundus appearance and proved to be an effective alternative technique in these animals, as already demonstrated for other species.[21,22] The observed avascular fundoscopic pattern resembles previously described patterns in other chelonians.[37,38]

The conus papillaris, which is a collection of vessels protruding from the optic disk responsible for inner retinal nutrition of several species of reptiles,[37] was absent in all animals examined. Duke-Elder also noticed the absence of a conus papillaris in several chelonians.[38] This absence may be due to a true lack of conus papillaris, or to a natural age-related regression of this structure, which has been reported in other adult testudines.[37,38] To investigate this possibility, further research with immature red-eared slider turtles should be performed in the future.

Duke-Elder also has observed the presence of nerve fibers radiating from the optic disk to the periphery of the retina.[38] The presence of a large choroidal blood vessel running from the nasal to the temporal aspect was observed in other reptile species.[39]

Intraocular pressure in wild animals has previously been evaluated using indentation tonometry[40] and applanation tonometry[12,14,15,41–44] and rebound tonometry.[13,16,41] Few studies about IOP in reptiles, especially testudine species, were reported in the literature.[12–15] Specifically, in red-eared slider turtles, there is only one study reporting this parameter.[16] This study showed higher IOP values compared to our investigation. This might be explained by a number of possibilities: (i) IOP variations due to different environmental, dietary, and husbandry conditions; (ii) Variations in size, weight and age of the population studied; (iii) Variations caused by the calibration of the tonometer, as in the previously mentioned study the Tonovet® was used on the setting 'D' which was the setting for dogs and cats, while in our investigation, the setting used was 'P,' for other species except dogs and horses. The IOP found in our study (5.5 ± 0.3 mmHg for males and 5.3 ± 0.3 mmHg for females) were similar to the IOP found in loggerhead sea turtle (*Caretta caretta)* (5 mmHg: range 4–9 mmHg).[12] In both studies, the animal positions used to evaluate IOP were the same; however, our values were measured with a different types of tonometer. Other studies using the applanation tonometer in different species of turtles found higher IOP values than those observed in our red-eared slider turtles. In Hermann's tortoises (*Testudo hermanni*), the mean IOP 15.7 ± 0.2 mmHg[13] were similar to the values observed in red-footed tortoises (*Geochelone carbonaria)* range between 14.2 ± 8.2 to 15.7 ± 9.3 mmHg[14] and yellow-footed tortoises (*Geochelone denticulata*) with mean 14.2 ± 1.2 mmHg.[15] This may suggest that aquatic or semi-aquatic species of turtles may have lower IOP as they are animals that are subjected to higher pressure levels when they are submerged. To answer that question, a detailed study comparing IOP from terrestrial and aquatic testudines might be performed in the future.

CCT in red-eared turtles measured using an ultrasonic pachymeter (154.5 ± 0.1 μm) is considerably thinner than that reported for the leatherback sea turtle (300 μm),[36] which was measured on histologic sections so a direct comparison cannot be made. CCT using an ultrasonic pachymeter has been determined in several other exotic mammals[42–45] and the values obtained for red-eared turtles are somewhere between CCT values obtained in rats (156 ± 30 μm)[45] and chinchillas (340 ± 30 μm).[44]

The measurement of tear production in aquatic or semi-aquatic species such as the slider turtle, for obvious reasons, should be always performed in a controlled environment, as in the natural habitat the high humidity levels can cause changes in the test interpretation.

The use of mSTT in this research proved to be an effective method to measure the aqueous fraction of the tears but it did require special restraint skills to be performed. Restraint of the turtles in this study was very difficult due the aggressive behavior, the extreme neck musculature strength that these turtles had. Because of this, it was very hard to perform the mSTT in both eyes simultaneously, resulting in an increase in the restraint time and consequently an increase in the stress levels. One important consideration is that when performing mSTT is that manually trimming the regular STT trips *per se* might directly affect the results if the strips are unevenly bisected, which they commonly are. This might add an important variable to the test, given that even small differences in the way the strip was cut might affect the results. For these reasons, the authors suggest, as an alternative, the measurement proposed using standardized endodontic paper point test (EPPT) that gives readings with a smaller variation. Lacrimal production data for red-eared slider turtles using endodontic absorbent paper point test has been previously published.[17]

Echobiometric evaluation using B-mode imaging techniques has proved to be a useful tool in ocular examination for wild species, including avian[46,47] mammal[44] and reptile species.[18,48,49] The ocular ultrasonographic evaluation of the slider turtle suggests that the general eye appearance is analogous to other turtles species.[29,47] The fact that the ultrasound examination was carried out without the use of chemical restraint should be considered, because even after instillation of anesthetic eye drops, animals could move their eyelids, so the investigator had to wait for the exact moments in which the eyelids were open to capture desired images, unlike what was reported in

Yacare Caiman.[49] Despite the difficulty in obtaining the images, due to low resolution of the ultrasound equipment and the extremely small eye size of these turtles, the researchers believe that B-mode ultrasonographic measurements presented in this study are useful for clinical evaluation and can provide reliable information about the intraocular anatomy of the slider turtle eye.

Corneal sensitivity, using a Cochet-Bonnet esthesiometer, has already been determined in various domestic animals species such as dogs,[50] cats,[51] alpacas,[52] and horses,[53] as well in exotic species such as chinchillas[44] and guinea-pigs;[54] however, according to our knowledge, there are no other reports of this test in reptiles. Corneal touch threshold values obtained in our study (5.8 ± 0.5 mm) were higher than those reported in the aforementioned studies, indicating a high corneal sensitivity in freshwater turtles.

Measuring palpebral fissure length is not a routine practice in the animal ophthalmic examination. However, we believe that this is an important anatomic parameter to be described when thoroughly characterizing the morphology of the eye of an exotic species. Additionally, knowledge of this anatomic feature is important when considering a surgical procedure of the eyelid and even to diagnose some congenital disorder of ocular adnexa, such as microphthalmia, which has been described as the most frequent congenital disorder of reptile ocular adnexa.[55] Furthermore, this measure has proven to be an important parameter in exotic animal ophthalmology to decide what type of tear production test will be used, as a regular Schirmer tear test strip measures 0.5 cm making it difficult if not impossible to use in species with smaller eyes.[44,45,56] We also believe that this parameter can also be considered in assessing the integrity of the eyelids as is frequently observed in hypovitaminosis A in freshwater turtles.[57,58]

Finally, we think a detailed study comparing normal values for ophthalmic tests and biometric data, including histology, from two or more species of terrestrial and aquatic testudines to evaluate the possible natural selection and adaptative reasons for the potential differences would be interesting for future studies.

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