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Anatomical and histological studies on the eyes of brown bear (*Ursus arctos horribilis*)

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Abstract: The studied animals belong to the *horribilis* subspecies, *Ursus arctos* species, Ursidae family, and Carnivora order. The eyes of 2 brown bears were used. Anatomic examinations demonstrated that the dorsoventral length of the lens was 9.03 ± 0.37 mm for the right eye and 8.54 ± 0.11 mm for the left eye. The dorsoventral length of the cornea measured 12.58 ± 0.46 mm for the right eye and 13.16 ± 0.27 mm for the left eye. In the histological examination of the tissues, the textures of the tunica fibrosa bulbi, tunica vasculosa bulbi, and tunica interna bulbi layers were evaluated. Plenty of collagen fiber bundles were observed in the sclera, and plenty of blood vessels and intense pigment cells were observed in the choroid. Various cell layers were observed in the retina. This study was designed because there are few studies on the eyes of wild animals and there was no information on the eyes of brown bears in the literature surveyed. This study may contribute to other areas of veterinary medicine such as anatomy, histology, and clinical and surgical medicine, especially ophthalmology of wild animals.

Key words: Anatomy, histology, ophthalmology, ursid, wild animal

1. Introduction

The brown bear is one of the largest land carnivores [1] and the only bear species living in Turkey [2]. It is classified under the Carnivora order, Ursidae family, *Ursus arctos* species, and *Horribilis* subspecies. It is 1–3 m high [2]. These animals have been on the (IUCN) Red List of Threatened Species, but are currently classified as a species of Least Concern [3]. The eye, which is the visual organ, has great importance in the identification of prey, especially in wild animals. This study was conducted considering this importance. The size, shape, and position of the eye are closely related to the effectiveness of the eye and the feeding habits of animals. The orbits of carnivores are not fully developed laterally, so there is no arcus orbitalis. This space is filled with the orbital ligament [4]. The bulbus oculi consists of 3 layers: the fibrous tunic (sclera and cornea), the middle vascular tunic (uvea), and the inner nervous tunic (retina). The tunica fibrosa bulbi is largely responsible for the form of the eye, and is rich in dense collagen tissue. The sclera resists intraocular pressure with elastic fibers scattered between dense collagen fibers. The tunica vasculosa bulbi has 3 layers, from posterior to anterior: choroid, ciliary body, and iris [5]. The most important feature of the uvea is its rich content of venous plexuses, capillaries, and melanin pigment. The choroid consists of loose connective tissue, which includes elastic fibers and blood vessels, and is rich in pigment cells (brown). The thickness of the Bruch's membrane in this layer varies according to species [6]. The color of the tapetum lucidum (yellow in carnivores) provides better vision in the dark, as it reflects the light falling on it as a light source [7]. In adult canines, the tapetum lucidum changes from slate to red-orange [8,9]. It is absent in the Sulawesi bear cuscus [8]. In different vertebrates, the tapetum lucidum has different structure, organization, and composition. The retinal tapetum (in crocodiles and marsupials), choroidal guanine tapetum (in gilled animals), choroidal tapetum cellulosum (in carnivores and rodents), and choroidal tapetum fibrosum (in cows, sheep, goats, and horses) have been described in different varieties [10]. The tapetum cellulosum in canines consists of several cell layers lined up like bricks. The tapetal area was found to be smaller in small canine breeds [11]. The ciliary body forms a symmetrical ring in carnivores and an asymmetrical ring in horses and ruminants. Its connective tissue contains elastic fibers, pigment cells, blood vessels, and fibers of the ciliary body muscle [12]. Among domestic animals, carnivores have the most developed ciliary body [13]. The retina has 2 parts, the pars ceca retina and the pars optica retina. The optic nerve is formed by the axons of multipolar cells in the ganglionic layer of the retina [5].

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Previous ocular studies in carnivores and sloth bear are available [14,15]. However, no anatomical or histological studies have been performed on the eyes of brown bears. Knowing the normal appearance of the eyes of a particular species is important in recognizing changes, and thus diagnosing diseases that affect the eyes. Correct diagnosis is an important step in the prescription of appropriate treatment. The aim of this study was to investigate the anatomical and histological aspects of the brown bear's eyes and to compare the macroanatomic, morphometric, and histological findings of the eyes with those of previous studies on wild animals, particularly bears and other carnivores, in order to reveal similarities and differences among this animals.

2. Materials and methods

Conditional permission was obtained from the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2019-097) to conduct this study. With this conditional permission, an application was submitted to the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks. This study was approved (E.2055279/2019). The brown bears that inhabit the Sarıkamış Allahuekber Mountains National Park migrate to Artvin-Savsat in the fall to store their food. During this migration, they can become injured due to traffic accidents or poaching. Two pairs of uninjured eyes from male brown bears (*Ursus arctos horribilis*), both approximately 10 years old and 300 kg in weight, formed the sample group of this study. Both animals were brought wounded to Kafkas University Veterinary Faculty Clinics and Kafkas University Wildlife Rescue and Rehabilitation Center but could not be saved despite all interventions. They were then brought to the Anatomy Department laboratory. The eyes located in the orbita in the cranium were dissected (Figures 1A and 1B) and photographed with the Kodak Easyshare M320 Digital Camera (Eastman Kodak Company, Rochester, NY, USA). Weights were measured in grams (g) and lengths in millimeters (mm). The bulbus oculi length (dorsoventral axis) and width (temporonasal or lateromedial axis), cornea length and width, thickness of lens, and other anatomical lengths were measured with a digital caliper (stainless steel; 1–150 mm). The weight of the bulbus oculi was recorded with a digital precision scale (min: 0.0001 g, max: 220 g; code: XB220A, Precisa, Dietikon, Switzerland). A histological examination was performed after the anatomical study. For histological examinations, the tissues were fixed in a 10% formaldehyde solution, processed via routine tissue processes, and blocked in paraffin. Sections of 6-µm thickness were taken from the paraffin blocks. The tissue sections taken from paraffin blocks were stained with Crossman's modified triple staining technique to examine the general histological structure of the tissues after deparaffinization and rehydration procedures.

3. Results

3.1 Anatomical results

The lateral wall of the orbita, where the bulbus oculi is located, was open (Figures 1A and 1B). Since the arcus orbitalis was not shaped, this opening was completed with the ligamentum orbitale. Morphometric measurements of the structures observed from the outside were performed before dissecting the bulbus oculi (Figure 2A). A transversal section of the sclera was then performed, and morphometric and macroanatomic findings of the structures inside the bulbus oculi were obtained (Figures 2B and 2C). The morphometric findings are shown in the Table. The bulbus oculi was examined in 3 concentric layers (i.e. the tunica fibrosa bulbi, tunica vasculosa bulbi, and tunica interna bulbi). During the dissection, it was possible to clearly determine the boundaries between the tunics, the fibrosa bulbi seen from the outside, and the vasculosa bulbi and interna bulbi. The similarity between dorsoventral and temporonasal lengths of the bears' eyes

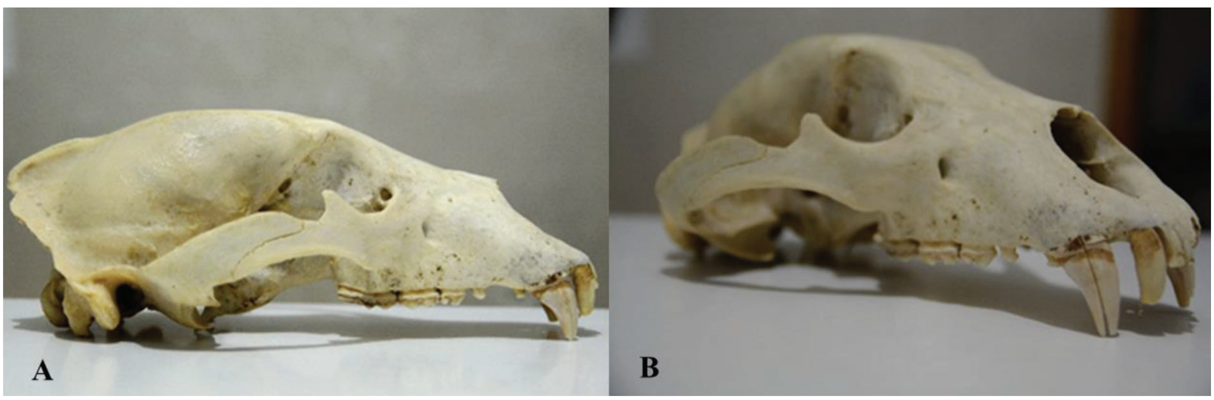

Figure 1. A and B) Photograph showing lateral and craniolateral view of brown bear skull and shape of orbit.

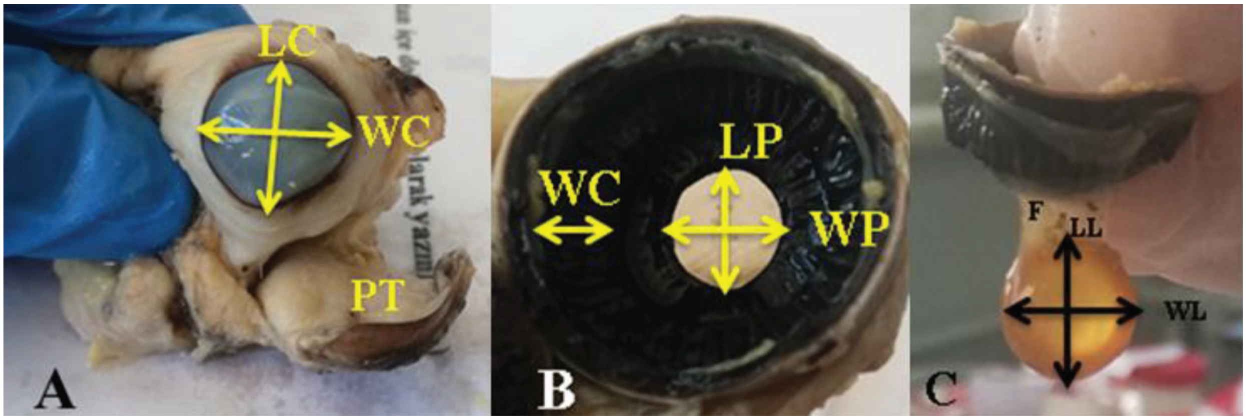

Figure 2. Anatomical structures of brown bear's eye. A) LC: Dorsoventral length of cornea, WC: Temporonasal length of cornea, PT: Palpebra tertia; B) LP: Dorsoventral length of pupilla, WP: Temporonasal length of pupilla, WC: Width of ciliary body; C) LL: Dorsoventral length of lens, WL: Temporonasal length of lens, F: Fibra zonulares.

Table. Measurement values of some ocular parameters of two brown bears eyes [Weights were measured in gram (g) and lengths in milimeter (mm)].

Parameters	Right mean ± SD	Left mean ± SD	General mean ± SD	Minimum	Maximum
Eyes weight	11.50 ± 2.12	12.00 ± 2.83	11.75 ± 2.06	10	14
Length of axis bulbi externus	17.62 ± 1.09	19.67 ± 1.37	18.65 ± 1.56	16.85	20.64
Length of equator	17.51 ± 0.16	19.05 ± 1.37	18.28 ± 0.96	17.40	19.50
Dorsoventral length of cornea	12.58 ± 0.46	13.16 ± 0.27	12.87 ± 0.45	12.26	13.35
Temporonasal length of cornea	13.55 ± 0.78	13.72 ± 0.39	13.64 ± 0.51	13	14.11
Dorsoventral length of pupilla	7.65 ± 0.88	7.86 ± 1.01	7.76 ± 0.78	7.03	8.58
Temporonasal length of pupilla	7.69 ± 0.68	7.11 ± 0.73	7.40 ± 0.67	6.59	8.17
Dorsoventral length of lens	9.03 ± 0.37	8.54 ± 0.11	8.79 ± 0.36	8.47	9.30
Temporonasal length of lens	8.81 ± 0.21	8.21 ± 0.01	8.51 ± 0.39	8.20	9.03
Thickness of lens	5.31 ± 0.07	5.49 ± 0.57	5.40 ± 0.35	5.09	5.90
Width of ciliary body	4.11 ± 0.48	3.72 ± 0.35	3.92 ± 0.41	3.48	4.45
Diameter of optic nerve	2.57 ± 0.39	2.30 ± 0.12	2.44 ± 0.28	2.22	2.85

SD: Standard deviation; **g:** Gram; **mm**: Millimeter.

produces an almost completely round bulb shape. Figure 2B shows a caudal view of the ciliary process.

The lens had a biconvex aspect, with rounded surfaces, yellowish color, good transparency, and firm consistency (Figure 2C). The ciliary body was formed by the merging of the ciliary process, which settled caudally on the lens and extended radially.

The tapetum lucidum was yellow-green and triangular; it was located anterior to the optic nerve.

3.2 Histological results

In the histological examination of the tissues, the tunica fibrosa bulbi, tunica vasculosa bulbi, and tunica interna bulbi layer structures were observed.

The exterior eye layer, the tunica fibrosa bulbi, consisted of many collagen fiber bundles (Figure 3A). There were blood vessels, fibroblasts, and a few melanocytes between the collagen fiber bundles. This fibrous layer consisted of 3 sublayers: the episclera, substantia propria, and suprachoroid layer (lamina fusca) (Figure 3B). The outermost episclera had a loose connective tissue structure and consisted of irregular collagen fiber bundles. The substantia propria had a tight connective tissue structure consisting of thick collagen fiber bundles running parallel to each other. The innermost lamina fusca consisted of thinner collagen threads and connected the sclera and choroid.

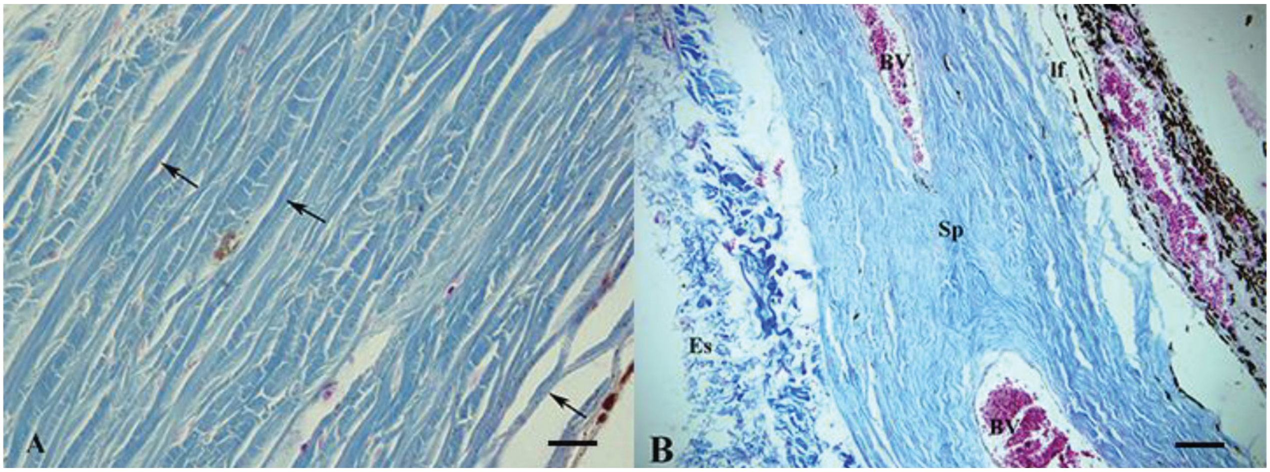

Figure 3. Sclera. A) Arrows: Collagen fibers. Triple Stain, 40× objective magnification, Bar: 50 µm. B) Es: Episclera, Sp: Substantia propria, lf: Lamina fusca, BV: Blood vessel. Triple Stain, 4× objective magnification, Bar: 500 µm.

The choroid and ciliary body parts of the tunica vasculosa bulbi layer were observed. The edge of the choroid adjacent to the retina was located between the retinal pigment epithelium and Bruch's membrane. The retinal pigment epithelium consisted of a single-layer cuboidal epithelium; the epithelial cells contained abundant melanin granules (Figure 4A). In the areas close to the optic nerve, the tapetum lucidum layer was determined to be adjacent to the part of the choroid that was rich in blood vessels and melanocytes. This layer had a cellular structure and consisted of several rows of polygonal tapetal cells. Epithelial cells covering the tapetum lucidum were found to contain no pigment (Figure 4B). There were many melanocytes in the choroid, which were located in the connective tissue consisting of collagen and elastic fibers and which contained abundant melanin pigment. Abundant blood vessels were found in the choroid (Figure 4C). The ciliary process and ciliary body were detected in relation to the tunica vasculosa bulbi (Figure 5A). There were blood vessels in the ciliary process, which was surrounded by 2 layers of epithelial cells without interior pigment and with exterior pigment (Figure 5B). The epithelial cells were columnar or cuboidal.

The tunica interna bulbi (retina) layer consisted of several substrata (Figures 4A and 6). The following layers were observed: the retinal pigment epithelium layer, rod and cone cell layer (Figure 4A), membrane limitans externa, outer nuclear layer, outer plexiform layer, inner granular nuclear layer, inner plexiform layer, ganglion cell layer, and nerve fiber layer interna sublayers (Figure 6). The optic nerve was observed on the posterior aspect of the bulbus oculi (Figure 7A). In the cross-section of the optic nerve, many nerve fibers and epineurium and perineurium parts, which are connective tissue sheaths, were observed (Figure 7B). Numerous blood vessels were observed in the epineurium, which consisted of thick collagen fiber bundles that surrounded the nerve from the outside. The perineurium layer was inside the epineurium, surrounding smaller bundles of nerve fibers and containing less collagen fiber.

4. Discussion

The eye is the organ of vision responsible for receiving light stimuli from the environment, converting them into electrical signals, and transmitting them to the brain. These structures consist of the bulbus oculi, optic nerve, and additional structures (e.g., the ocular muscles, eyelids, and lacrimal apparatus). The shape and size and some features of these structures vary among species and individuals [5]. In carnivores, the lateral wall of the orbit does not fully develop [4], as was seen in the brown bear, which has an orbital bone structure typical of carnivores in general. The shape of the eye is almost spherical in canines [9]; that of the brown bears was almost spherical, too. Several studies have been performed on the bulbus oculi to examine differences between the right and left eyes and among sexes and species [16,17,18]. The weight of the bulbus oculi has been reported to differ on the left and right sides [16]. The right eye of the brown bear weighed 11.50 ± 2.12 g; the left was heavier, weighing 12.00 ± 2.83 g. The results of other studies show that the weight of the bulbus oculi can vary between the right and left sides. The axis bulbi externus length was reported to be a minimum 13.84 mm (in the Pomeranian) and a maximum 23.95 mm (in the Rottweiler) in a study conducted in different canine breeds [18]. The axis bulbi externus length of the brown bear was recorded as a minimum of 16.85 mm and a maximum of 20.64 mm. The minimum value was higher in the brown bear, while the maximum value was higher in canines. However, the measurements presented may not be

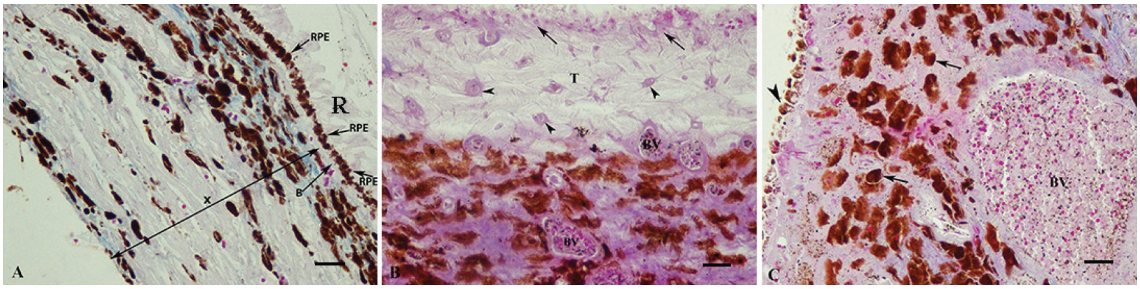

Figure 4. Choroid. A) General view of choroid. X: Choroid, B: Bruch's membrane, RPE: Retinal pigmented epithelium (retina), R: Outer segment of rod and cone cells layer (retina). Triple Stain, 40× objective magnification, Bar: 50 µm. B) BV: Blood vessel, T: Tapetum lucidum, Arrow heads: Tapetal cells, Arrows: Nonpigmented epithelium. Triple Stain, 40× objective magnification, Bar: 50 µm. C) BV: Blood vessel, Arrows: Melanosit, Arrow heads: Retinal pigmented epithelium. Triple Stain, 40× objective magnification, Bar: 50 µm.

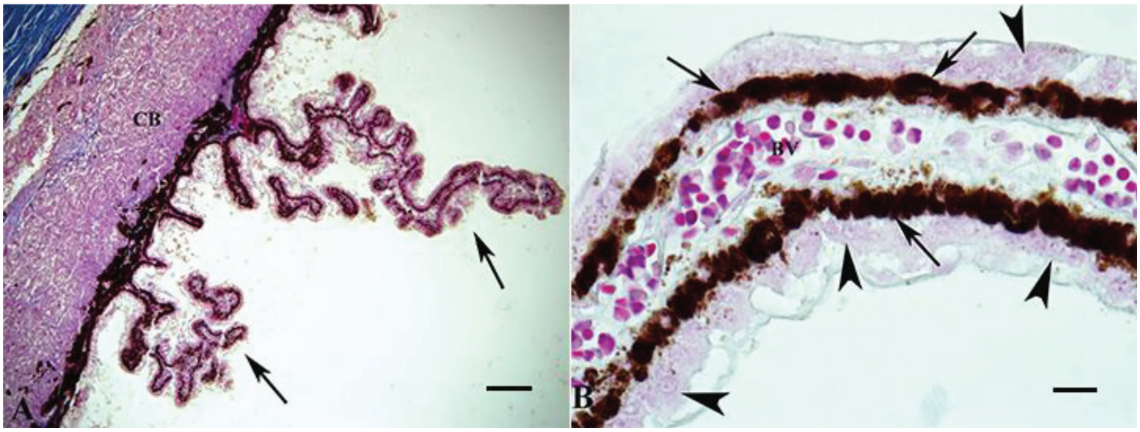

Figure 5. Ciliary body. A) Arrows: Ciliary processes, CB: Ciliary body. Triple Stain, 10× objective magnification, Bar: 200 µm. B) Arrows: Pigmented epithelium, Arrow heads: Nonpigmented epithelium, BV: Blood vessel. Triple Stain, 80× objective magnification, Bar: 25 µm.

considered standard, as only 4 bulbus oculi were evaluated in total. However, it can be said that the size of the bear's eye is small in relation to its body size. In a study on dogs, the length of the equator axis was between 16.00 mm (in Pomeranian) and 27.48 mm (in Kangal), while that in brown bears was between 17.40 and 19.50 mm. The range between the minimum and maximum values was larger in canines. The axis bulbi externus length (dorsoventral axis) was greater than the equator length. A study conducted on different canine breeds revealed that the equator length was higher than the axis bulbi externus length [18]. Our findings did not match those of the canine study. The mean equator length was 21.44 ± 0.75 mm in different canine breeds and 18.28 ± 0.96 mm in brown bears. The axis bulbi externus length in brown bears measured 18.65 ± 1.56 mm, showing very close values between the 2 measurements.

The dorsoventral length of the cornea in the male bears was 12.87 ± 0.45 mm; the temporonasal length was

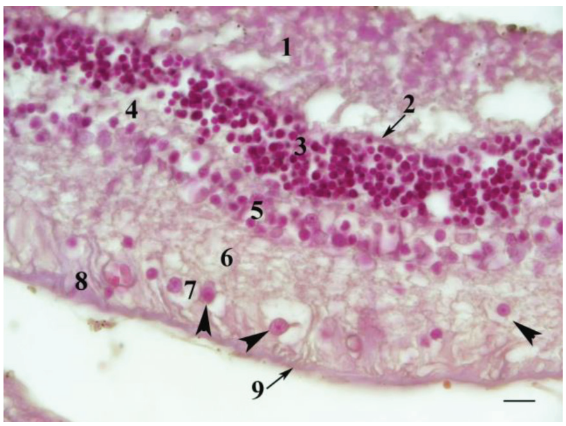

Figure 6. Retina. 1: Inner segments of rod and cone cells layer, 2: External limiting membran, 3: Outer nuclear layer, 4: Outer plexiform layer, 5: Inner nuclear layer, 6: Inner plexiform layer, 7: Ganglion cell layer, 8: Nerve fiber layer, 9: Internal limiting membran. Arrow heads: Ganglion cells. Triple Stain, 60× objective magnification, Bar: 33 µm.

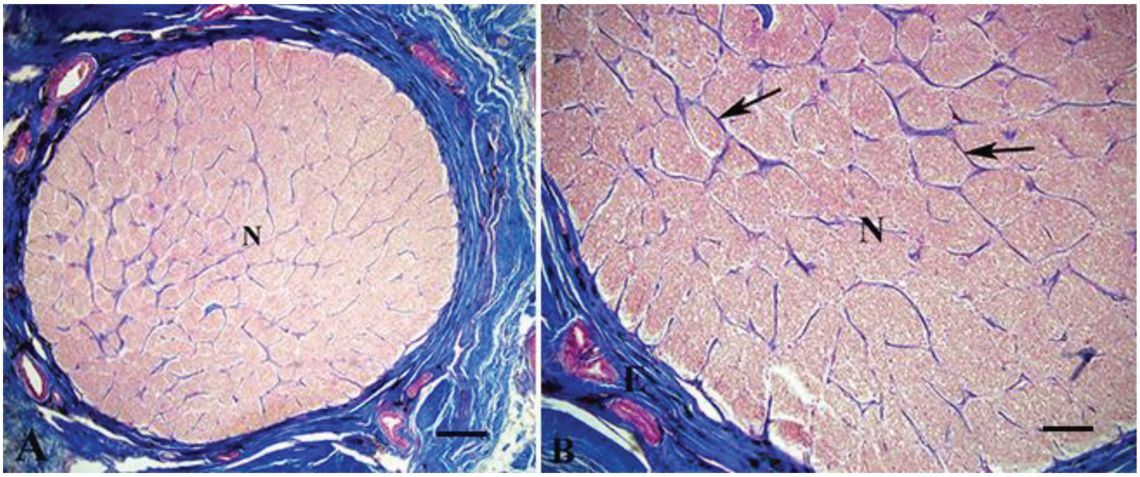

Figure 7. Optic nerve. A) N: Optic nerve. Triple Stain, 4× objective magnification, Bar: 500 µm. B) N: Optic nerve, E: Epineurium, Arrows: Perineurium. Triple Stain, 10× objective magnification, Bar: 200 µm.

16–18 mm in canines [19], 20 mm in large-breed canines, 14 mm in small canine breeds [18], and 13.64 ± 0.51 mm in the bears in our study. The parameters for the brown bear were similar to those of small canine breeds. In a canine study [19], the temporonasal length of the cornea was greater than the dorsoventral length. The results were similar to our findings. The values among domestic mammals decrease from cats to dogs, horses, cattle, and pigs, respectively [5].

In our study, the sclera layer of the brown bear eye was composed of 3 layers, as in other mammals [5,20–24]; furthermore, the sclera layer consisted mainly of parallel collagen fibers and contained melanocytes, as in other mammals [5,8]. In contrast to cattle, buffalo, goats, and donkeys, which have smooth muscle cell bundles in the sclera layer in their eyes [25], no muscle cells were found in the bears' sclerae in our study.

The tunica vasculosa bulbi is a thick vascular layer located between the retina and sclera [20,26–28]. It is a continuous structure consisting of the choroid, ciliary body, and iris from the posterior to the anterior [28–33]. In our study, the choroid, ciliary body, and ciliary process belonging to the tunica vasculosa bulbi, which is the middle layer of the brown bear's eye, were observed. Many melanocytes and abundant blood vessels in the collagen and elastic fiber structures of the choroid were observed. In addition, Bruch's membrane, which is located between the choroid and the retinal pigment epithelium, was determined. Another structure belonging to the choroid is the tapetum lucidum. This structure has a reflector function and provides vision in the dark [6]. In previous studies, while the tapetum lucium was found in cows, buffalo, sheep, goats, horses, cats, dogs, and rats [10,34–37], no tapetum lucidum layer was found in primates, birds, squirrels, camels, or pigs [10,38–40]. Kleckowska-Nawrot et al. [8] did not encounter the structure of the tapetum lucidum in their histological study in the Sulawesi bear cuscus. The pigmentation of the choroid is closely related to the development of the tapetum lucidum [41]. While the color of the tapetal area was reported not to be age-related [41], Evans and De Lahunta [9] noted that it changed from a gunpowder color to red-orange in adult canines. Carnivores have been reported to have red-orange [9], yellow [7], or greenish-yellow [41] tapeta lucida; the brown bear had a yellow-greenish tapetum lucidum. Our result was similar to that of the study by Graner et al. [41], in which the authors found a yellow-green color of the tapetum lucidum in most dog breeds, although some breeds showed different colors, such as the Miniature Schnauzer which has green and green-blue colors, and the English Springer Spaniel, which has an orange color. In our study, the cellular tapetum lucidum layer was determined to be adjacent to the part of the choroid rich in blood vessels and melanocytes.

The dorsoventral length of the pupil ranged from a minimum of 5.11 mm (in Miniature Pinscher) to a maximum of 9.56 mm (in Kangal) in different canine breeds, while it was between 7.03 mm and 8.58 mm in the bears of this study. While the temporonasal length of the pupil was between 5.61 mm (in Miniature Pinscher) and 9.87 mm (Kangal) in different canine breeds [18], it measured between 6.59 mm and 8.17 mm in the bears in this study. For the shape of the pupil, felines have a greater dorsoventral length (the flattened side) compared to the temporonasal length [5]; the brown bear had a wider temporonasal length than horse, cattle, or pig (dorsoventral flattened).

As reported in the literature [15], the lens was determined to be round and biconvex. The mean length of the lens (dorsoventral) in canines was 7.83 ± 0.45 mm

[9], and that in the Weddell seal was 16.7 mm [42], while that in brown bears was measured as 8.79 ± 0.36 mm. This value is closer to the value found in dogs than that of seals, perhaps due to the different habitat that seals live in. While the central thickness of the lens in the sloth bear was 5.08 ± 0.15 mm on the right side and 5.16 ± 0.16 mm on the left [15], in brown bears, it was 5.31 ± 0.07 mm on the right and 5.49 ± 0.57 mm on the left. Our values were close with small differences between the right and left sides, but with the low sample number, it is not possible to say whether this is typical for the species. In the Sulawesi bear cuscus, the mediolateral length of the cornea was reported to have a maximum of 10.39 ± 0.2 mm and a minimum of 10.13 ± 0.08 mm [8], while that of brown bears was a minimum of 13 mm and a maximum of 14.11 mm. The mediolateral length of the cornea was greater in the brown bears.

In our study, the retinal layer consisted of 10 layers in brown bears, as in other mammals [5,43–48]. The optic nerve is formed by the axons of multipolar cells in the ganglionic layer of the retina [5]. The diameter of the optic nerve is 1 mm in cats, 2 mm in dogs, and 5 mm in horses [5]. The mean was 2.44 ± 0.28 mm in the brown bear, closest to the value found in dogs.

In conclusion, the anatomical and histological features of the brown bear's eye were identified and it was concluded that the eye resembled that of domestic carnivores, especially dogs. It is thought that this study will contribute to anatomical, histological, clinical, and surgical knowledge in the literature.

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Conflict of interest
The authors have declared no conflicts of interest.

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