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Research Article

Light and Scanning Electron Microscopic Structure of the Pecten Oculi in the Common Barn Owl *(Tyto alba)*

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Abstract

This study was carried out to investigate the structural properties of pecten oculi in the common barn owl *(Tyto alba)* by light and electron microscope. Fourteen eyeballs from seven owls were studied. The pecten oculi was located postero-anteriorly in the retina layer where the optic nerve enters the eye. The pecten oculi that was dark brown and pleated type consisted of 7 (n=4) or 8 (n=10) vascularised pectineal pleats. Histologically, there were numerous vessels of different size and melanocytes in the area of the pleats. Melanocytes were more frequently observed in the periphery of the pecten oculi's pleats. Scanning electron microscopy showed hyalocytes on the surface of the pecten oculi. The results of the study indicated that the pecten oculi of the common barn owl was morphologically similar to that of other nocturnal birds.

Keywords: *Common barn owl, Pecten oculi, Tyto alba, SEM*

Peçeli Baykuşlarda *(Tyto alba)* Pecten Oculi'nin Işık ve Elektron Mikroskopik Yapısı

Özet

Bu çalışma peçeli baykuşlarda *(Tyto alba)* pecten oculi'nin yapısal özelliklerini ışık ve elektron mikroskobunda incelemek amacıyla yapıldı. Çalışmada materyal olarak toplam 7 adet peçeli baykuşa ait 14 adet göz küresi kullanıldı. Yapılan incelemede pecten oculi'nin; nervus opticus'un göze girdiği bölgede, retina tabakası üzerinde bulunduğu ve postero-anterior yönlü yerleşim gösterdiği gözlendi. Koyu kahverengi renkte ve kıvrımlı tipte olan pecten oculi, 7 (n=4) veya 8 (n=10) adet damarlı pecten oculi kıvrımlardan oluşmaktaydı. Histolojik olarak; pecten oculi kıvrımları içerisinde çok sayıda orta çaplı damarlar ve melanositler bulunmaktaydı. Melanositlerin pekten oculi kıvrımlarının periferinde daha sık yer aldığı gözlendi. Elektron mikroskobik incelemede pekten yüzeyinde hyalosit varlığı tespit edildi. Peçeli baykuşlarda pecten oculi'nin morfolojik olarak diğer nocturnal türlere benzer olduğu görülmüştür.

Anahtar sözcükler: *Peçeli baykuş, Pecten oculi, Tyto alba, SEM*

INTRODUCTION

The pecten oculi is an organ found only in the eyes of birds that extends from the retinal entrance of the optical nerve up to the vitreous [1,2]. Although birds have higher metabolic rates and thicker retinas than mammals, they do not possess retinal vessels [3,4]. However, the pecten oculi is rich in vessels and pigments and is thought to play crucial roles regulating intraocular pressure and temperature by regulating blood flow, as well as in the nutrition of the retina, which is thick and avascular [5-8]. It may help regulate intraocular pH [9], stabilise the vitreous [10], reduce intraocular flashing [11], aid navigation based on the Earth's magnetic field [12], maintain the balance and integrity of the intraocular environment [13], and regulate metabolic exchange between ocular vessels and the retina [14]. It may also suppress vascular endothelial growth factor (VEGF) and inhibit vascularisation of the retina by supplying

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the eye with sufficient oxygen [15]. Among bird species three different types of pecten oculi are distinguished as conical type, vaned type and pleated type consisting of varying number of folds [16]. Morphologically, it consists of three different parts: the basal and apical parts and the pleats, which are located between the vascularised plates. Gradually decreasing in height, the folds join the basal plate, forming a bridge with the dorso-nasal end of the pecten oculi. In some bird species, there may be long extensions reaching into the vitreous over this bridge [10]. The number of folds depends on the size and shape of the pecten oculi and is associated with the behaviour and visual pattern of the bird species [17]. For example, the pecten oculi is conical in kiwis *(Apteryx)*, vaned in ostriches *(Struthio)*, and pleated in other bird species [16]. Despite such morphological variation, the pecten oculi has the same basic structure in all birds, consisting of numerous vessels surrounded by pericytes and melanocytes, with connective tissue filling the space between them [1].

Numerous studies have examined the morphological and histological structures of the pecten oculi using both light and electron microscopy in the great horned owl *(Bubo virginianus)* [18], red-tailed hawk *(Buteo jamaicensis)* [19], common buzzard *(Buteo buteo)* [20], budgerigar *(Melopsittacus undulates)* [21], black kite *(Milvus migrans)* [22], quail *(Coturnix coturnix japonica)* [23], pigeon *(Columba livia)* [10], stork *(Ciconia ciconia)* [24], and jungle crow *(Corvus macrorhynchos)* [25]. Studies on the morphological and histological structures of the pecten oculi in different owl species including nighthawk *(Chordeiles minor)* [1], great horned owl *(Bubo virginianus)* [18], barred owl *(Strix varia)* [26], spotted eagle owl *(Bubo africanus)* [27] have been published. Strobel [28] examined the pecten oculi of common barn owl *(Tyto alba)* by using ultrasonography. However to our knowledge no study on the light and electron microscopical structure of pecten oculi common barn owl *(Tyto alba)* have been published. We investigated the morphometric and histological structure of the pecten oculi in the common barn owl *(Tyto alba),* a nocturnal bird species.

MATERIAL and METHODS

Sample Collection

The study material consisted of 14 eyeballs from seven adult common barn owls with an average body weight of 509±18 g. The owls had been brought to the Harran University Faculty of Veterinary Medicine clinic in Sanliurfa Province, Turkey, for treatment and were euthanised because of negative prognoses. The experimental procedures were approved by the General Directorate of Nature Conservation and National Parks-Turkey (Approval no. 70525) and Harran University Animal Experimentation Local Ethics Committee (Approval no. 2017/003/01). The eyeballs were harvested immediately after the death of the animals, which had no detectable visual problems clinically or pathologically. To facilitate diffusion of the fixative solution, 10% formaldehyde was injected into each eyeball. Then the specimens were kept in 10% formaldehyde until the morphological and histological examinations were performed.

Morphometric Analysis

The eyeballs were cut equatorially and the pecten oculi, which is located in the posterior eyeball, was examined morphometrically using a stereomicroscope (Olympus-SZX7, Olympus Optical, Japan) with an attached camera (Olympus Cam-SC50). Nomina Anatomica Avium [29] was used for the nomenclature. Descriptions of the morphometric measurements on bulbus oculi including axial globe length, equatorial diameter and corneal diameter as well as on pecten oculi including height of pecten, length of apical border and length of basal border were shown in *Fig. 1*. Weight of the bulbus oculi was measured by using a balance of 0.0001 g sensitivity.

Histological Examination

Four eyeballs from two owls were used for the histological examination. The specimens were washed in flowing water and then fixed in 10% formaldehyde for 24 h. After dehydration through a series of graded alcohols, the tissue samples were cleared in xylene and embedded in paraffin. The paraffin blocks were cut into serial sections of 5 µm thickness. After deparaffinisation and rehydration, the sections were stained using Crossmann's modification of Mallory's trichrome method [30]. Six serial cross-sections in 5 µm intervals for each sample were examined using the Bs200Pro image analysis program (BAB software) for measuring the diameters of the vessels. The average diameters of vessels belonging to each group were determined with standard errors.

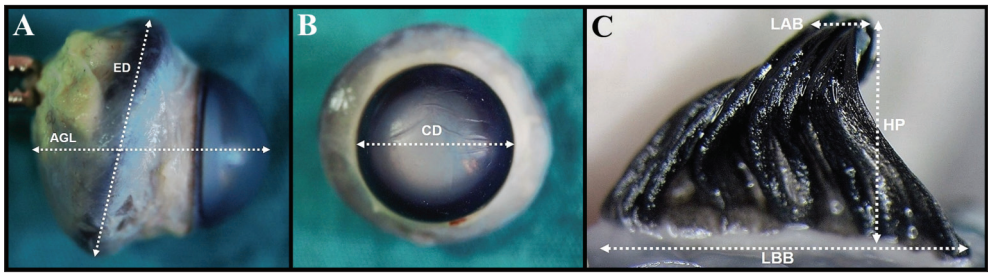

Fig 1. Measurements taken for morphometric analysis of the bulbus oculi (A, B) and pecten oculi (C). AGL: axial globe length, ED: equatorial diameter, CD: corneal diameter, LBB: length of basal border, LAB: length of apical border, HP: height of pecten

Scanning Electron Microscopical (SEM) Examination

For scanning electron microscopy (SEM) analyses, four eyeballs from two owls were used. After dissecting out the eyeballs, they were washed twice with phosphate-buffered saline (0.1 M, pH 7.4) and fixed in 2.5% glutaraldehyde for 48 h. Then the samples were treated with 1% osmium tetroxide for 1 h and dehydrated through a series of increasing concentrations of acetone (25%, 50%, 75%, and 100%, three repetitions each) and dried in a critical point drier. The samples were coated with gold-palladium using a Polaron SC7620 sputter coater and examined with SEM (Leica, LEO 440, UK) at different magnifications.

RESULTS

The dark brown, pleated type pecten oculi was located in the retina layer postero-anteriorly where the optic nerve enters the eye. The pecten oculi consisted of three distinguishable parts: the base, apical (or bridge), and pleats. The basal part was near the optic nerve and was wider than the apical part. Four samples carried pecten oculi of 7 and 10 samples had pecten oculi of 8 vascularised pleats *(Fig. 2-A,B)*.

Minimum-maximum and mean and standard error values of the macroscopic measurements were given in *Table 1*. In stereomicroscopic examinations of the eyeballs, the axial length and equatorial and corneal diameters were 17.56±0.19, 18.01±0.27, and 11.95±0.17 mm, respectively *(Table 1)*. The mean width of the basal part of the pecten oculi was 4.431±0.09 mm, while that of the apical part was 1.447±0.06 mm. The height of the pecten oculi, defined as the distance between the base and the highest point of the apical, was 2.741±0.08 mm. The ratios of the mean height of the pecten oculi to the axial, equatorial and corneal diameters of the eyeball were 0.16, 0.15, and 0.23, respectively.

Histologically, there were numerous vessels of moderate size and melanocytes in areas where pleats extended from the base to the apical *(Fig. 3* and *Fig. 4-A)*. Three different types of vessel were distinguished based on

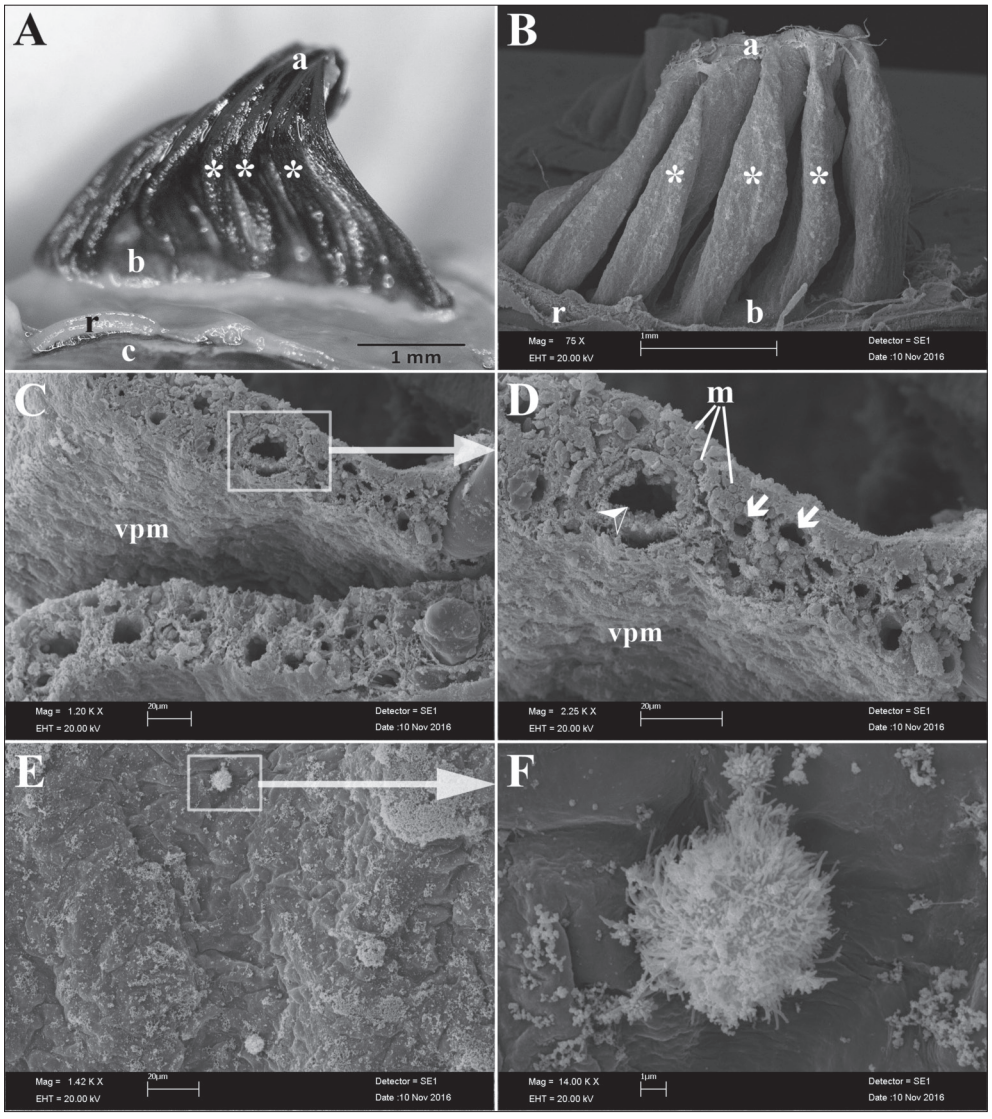

Fig 2. Stereomicroscopic (A) and scanning electron microscopic (B) view of the pecten oculi *in situ*. Scanning electron micrography of the cross-sectional (C, D) and outer (E, F) surface of the pecten oculi. * pectineal folds, a: apical border, b: basal border, r: retina, c: choroidea, m: melanocytes, *arrow:* afferent blood vessels, *arrow head:* efferent blood vessels, vpm: vitreo-pectineal limiting membrane

Table 1. *Body, ocular and pecten oculi measurements of the common barn owls*		
Parameters	**Min - Max**	**Mean ± SE**
Body and ocular measurements		
Body weight (g)	466 - 550	509±18
Bulbus oculi weight (g)	1.96 - 2.28	2.13±0.05
Axial globe length (mm)	16.93 - 18.79	17.56±0.19
Equatorial diameter (mm)	17.04 - 19.70	18.01±0.27
Corneal diameter (mm)	11.09 - 12.58	11.95±0.17
Pecten oculi measurements		
Height of pecten (mm)	2.481 - 3.036	2.741±0.08
Length of apical border (mm)	1.276 - 1.791	1.447±0.06
Length of basal border (mm)	4.158 - 4.811	4.431±0.09

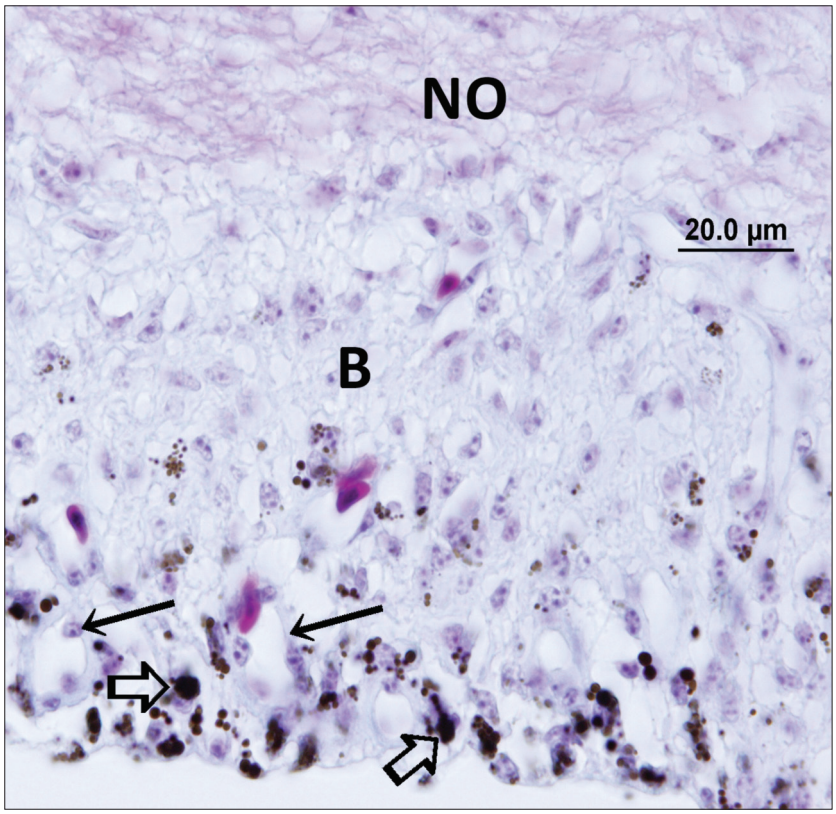

Fig 3. Light microscopic appearance of the basal border of the pecten oculi. B: basal border, NO: optic nerve, *thin arrow:* vessels, *white arrow:* melanocytes

diameter, and defined as primary (largest diameter), secondary (moderate size), and tertiary (small) vessels *(Fig. 4-B,C)*. The primary vessels were central with respect to the pleats, while the secondary vessels were peripheral. Tertiary vessels (capillaries) were observed among the primary and secondary vessels. There were more primary vessels at the basal part, while the number of capillaries increased towards the apical. The average diameters of the primary, secondary, and tertiary vessels were 83.59±19.44, 47.20±12.02, and 23.57±6.59 µm, respectively. Melanocytes were first observed in the area at which the pleats arose from the base and more were found among vessels at the periphery of the pleats *(Fig. 4-C,D)*. No hyalocytes were observed in light microscopy examinations.

SEM showed that the pleats started from the basal part, formed rib-like segments, and merged at the apical part extending into the vitreous *(Fig. 2B)*. The distance between the pleats was greater basally than apically. A vitreo pecteneal limiting membrane separated the pecten oculi from the corpus vitreous. In transverse sections, the pleats contained numerous afferent and efferent vessels along with capillaries *(Fig. 2-C,D)*. Melanin was observed among the capillary net and at the periphery of the pleats *(Fig. 2-D)*. The surface of the pleats appeared rough due to the presence of capillaries. Several star-like hyalocytes with numerous thin, irregular extensions were observed on the outer surface of the pleats *(Fig. 2-E,F)*.

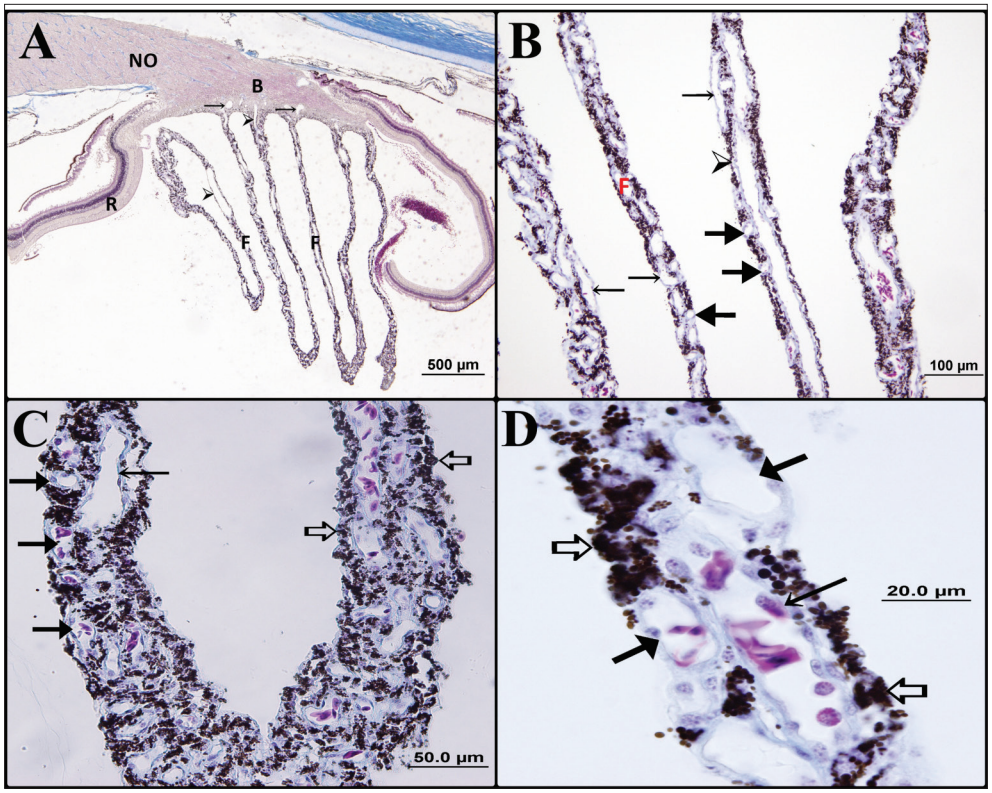

Fig 4. Light microscopic appearance of the pecten oculi (A) and pectineal folds (B-D). R: retina, B: basal border, NO: optic nerve, F: Pectineal folds, *arrow head:* primary vessels, *thin arrow:* secondary vessels, *thick arrow:* tertiary vessels, *white arrow:* melanocytes

DISCUSSION

Similar to other bird species pecten oculi of common barn owl was a pigmented, vascular structure extending into the vitreous located below the postero-temporal region over the optic disc [18,19,22,31]. The pecten oculi of the common barn owl had three different parts, in accordance with the literature: a basal part located near to where the optic nerve enters the eye, pleats arising from the basal part, and an apical part where the pleats merge [21].

Although numerous variants have been described about the morphological structure of the pecten oculi [32], there are generally three different types of the pecten oculi, being conical in kiwis, vaned in ostriches, and pleated in other bird species [16,18,33]. We found that the pecten oculi in common barn owls was of the pleated type, similar to that reported for the great horned and barred owls [18,26]. The physiological significance of the pleats has been explained by different authors so that the presence of the pleats increases the surface area of the pecten oculi without increasing its volume and provides mechanical stability to pecten oculi [27,34].

The size and number of pleats varies among bird species and numerous studies have associated the number of pleats with the function of the pecten oculi. Several studies have indicated that visually more active species have larger pecten oculi consisting of more pleats compared to bird species with less visual activity [21,26,27]. Accordingly, diurnal species, which require visual acuity have larger pecten oculi with more pleats than nocturnal bird species requiring visual sensitivity [17,27]. Among owl species, long-eared, tawny, and eagle owls have short and compact pecten oculi while barn and little owls have longer and narrower pecten oculi [28]. The barred owl [26], great horned owl [18], and emu [35] have a larger pecten oculi relative to the size of their eyeballs. We found that the number of pleats in common barn owl (7-8 pleats) lower than that in barred owl (8-10 pleats) [26] while it was higher than that reported in spotted eagle owl (5-6 pleats) [27] and nighthawk (4-5 pleats) [1]. Pecten oculi of common barn owl was found to be similar to that of great horned owl [18] with respect to number of the pleats. However length of pecten at basal border in barn owl (4.431±0.09) was lower than that in great horned owl (5-6 mm) [18] and higher than that in spotted eagle owl (2.77±0.09) [27] while the pecten hight in barn owl (2.741±0.08) was lower than that great horned owl (5-6 mm) [18] and spotted eagle owl (6.02±0.16) [27]. These results were in accordance with those of Kiama et al.[27] who reported that the number of pleats may not reveal the true size of pecten oculi.

By using eye ultrasonography, Strobel [28] has reported that the length of the pecten oculi is 4.03 mm in the common barn owl, 4.04 mm in the long-eared owl, 5.10 mm in the tawny owl, 4.29 mm in the little owl, and 7.52 mm in the eagle owl. Ravelhofer [36] has reported that the length and height of the pecten oculi in vultures are 11.25 and 6.17 mm, respectively. Braekevelt [18] has reported that the basal length, bridge length, and distance between the basal and apical parts are 5-6, 3, and 5-6 mm, respectively,

in the great horned owl *(Bubo virginianus)*. These values were more higher in the common barn owl. The differences might be attributed to the body sizes of the species studied.

Onuk *et al*.[24] determined that the ratio between the height of the pecten oculi and the diameter of the eyeball in storks was 0.4, while we found that the ratio between the height of the pecten oculi and the equatorial diameter was 0.15. This might be attributable to the differences in the habits of the species under study as storks are diurnal species in contrast to common barn owls.

Numerous capillaries along with afferent and efferent vessels are found in pecten pleats [1,7]. We also observed capillaries and vessels of different sizes in the common barn owl. However, in contrast to other bird species, vessels and melanocytes were observed beginning from the basal part of the pecten oculi. Capillaries were more abundant and were located among the primary and secondary vessels. We did not find any lymph vessels in the pecten oculi, unlike in the mallard *(Anas platyrhynchos)* [13]; this is in accordance with studies on other species [21,24].

Melanocytes are frequently observed in the pecten oculi [1,19,24,37]. We also observed melanocytes at the periphery of the pleats beginning from the basal part and becoming more intensive at apical part. Melanocytes have been reported to be more abundant in the apical and peripheral regions of the pleats than at the basal region [1,18,24]. Because no other cell types function as supporting components in the pecten oculi, melanocytes are thought to play a structural role [17,19]. However it has been also suggested that melanocytes regulate the temperature of pecten oculi by absorbing light and contribute to the metabolic function [9,17] or protect the eye from harmful effects of sunlight [33].

Although the presence of a vitreo-pecteneal limiting membrane covering the pecten oculi has been reported in all bird species [9,26,38], macrophage-like hyalocytes around this connective tissue have been observed only in some species including the chicken [38,39], budgerigar [21], quail [23,40], mallard [41] and emu [35] and not in the pigeon [42], red-tailed hawk [19], or nighthawk [1]. We also found a vitreo-pecteneal limiting membrane in the common barn owl. While macrophage-like hyalocytes were not observed by light microscopy, they were detected on the surface of the pleats via SEM. We suggest that there might be two reasons for this observation. Firstly, hyalocytes might be freely circulating within vitreous in birds as in mammals [43]. These hyalocytes can be lost during the tissue following procedures for light microscopic examinations while they can be seen in 3D SEM using different tissue preparation methods. Secondly, the number of hyalocytes in common barn owl might be low so as to be seen by using SEM which is capable of providing a more detailed information compared to light microscope.

In summary, we investigated the anatomical, morphometric, and histological structure of the pecten oculi of the common barn owl in detail and compared it to that of other bird species. The results suggest that the morphological, morphometric and histological properties of pecten oculi in common barn owl was similar to other nocturnal bird species.

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