UNIVERSIDADE DE BRASÍLIA
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA

HISTOMORFOMETRIA DA CÓRNEA DE AVES

RAFAELA ALVES RIBON TOZETTI

TESE DE DOUTORADO EM CIÊNCIAS ANIMAIS

BRASÍLIA/DF
JULHO DE 2023

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ALUNA: RAFAELA ALVES RIBON TOZETTI

ORIENTADORA: PROFESORA DRª PAULA DINIZ GALERA

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PUBLICAÇÃO:

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RAFAELA ALVES RIBON TOZETTI

TESE DE DOUTORADO SUBMETIDA AO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS ANIMAIS, COMO PARTE DOS REQUISITOS NECESSÁRIOS À OBTENÇÃO DO GRAU DE DOUTORA EM CIÊNCIAS ANIMAIS.

APROVADA POR:

_____
Paula Diniz Galera, Dra. (Universidade de Brasília).
(ORIENTADORA)

_____
Márcio Botelho de Castro, Dr. (Universidade de Brasília).
(EXAMINADOR INTERNO)

_____
Giane Regina Paludo, Dra. (Universidade de Brasília).
(EXAMINADOR INTERNO)

_____
Angélica de Mendonça Vaz Safatle, Dra. (Universidade de São Paulo)
(EXAMINADOR EXTERNO)

_____
Fabiano Montiani-Ferreira, Dr. (Universidade Federal do Paraná).
(EXAMINADOR EXTERNO)

BRASÍLIA/DF, 31 de julho de 2023

REFERÊNCIA BIBLIOGRÁFICA E CATALOGAÇÃO

TOZETTI, R. A. R. **Histomorfometria da córnea de aves**. Brasília: Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, 2023, (nº páginas- 95p.). Tese de Doutorado.

FICHA CATALOGRÁFICA

TOZETTI, Rafaela Alves Ribon. **Histomorfometria da córnea de aves**. Brasília: Faculdade de Agronomia e Medicina Veterinária da Universidade de Brasília, 2022. 95p. Tese de Doutorado –Faculdade de Agronomia e Medicina Veterinária da Universidade de Brasília, 2022.

1.Córnea. 2. Aves. 3. Histologia. 4. Microscopia eletrônica de varredura.

EPÍGRAFE

“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota”.

Madre Teresa de Calcutá

DEDICATÓRIA

Dedico ao meu pai, Geraldo Tozetti, e à minha mãe, Sandra Darc Alves, que moldaram minha personalidade e poliram meu caráter, dentro dos ensinamentos de Deus.
Dedico também ao meu filho, Henrique, para que isso o inspire a seguir sempre pelo caminho do conhecimento, da ciência e do respeito à vida plural do nosso planeta.

AGRADECIMENTOS

Agradeço a equipe de animais silvestres do Hospital Veterinário da UnB, por me proporcionar os meios para que eu pudesse realizar essa pesquisa, em especial à Evelyn e à Professora Drª Líria Aquino.

Agradeço a Ana Carolina Rodarte, por seu auxilio com as amostras.

Agradeço a Rose, que esteve sempre comigo, instruindo e auxiliando diretamente nos processos para análise das amostras. Por ter me incentivado e me inspirado.

Agradeço ao Matheus Loes, por ser solícito e por seu trabalho impecável com as amostras histológicas.

Agradeço ao professor Dr. Moore, por se disponibilizar a ler e contribuir na escrita dos artigos científicos.

Agradeço aos Professores da banca, Dr.ª Angélica de Mendonça Vaz Safatle, Dr. Fabiano Montiani-Ferreira, Dr. Márcio Botelho e Dr.ª Giane Regina Paludo, que aceitaram o convite para avaliar e contribuir para o aprimoramento desse estudo, e assim torná-lo possível.

Agradeço a Professora Dr.ª Paula, por sua paciência, insistência e por ter acreditado em mim quando nem eu mais acreditava. Por sua tutoria e orientação.

Agradeço ao meu marido, Lucas, por ter pegado na minha mão e caminhado comigo até aqui. Me dando forças e ajudando em tudo que fosse necessário para que eu conseguisse desenvolver esse trabalho.

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RESUMO

Cada estrutura ocular sofre variações, não só entre as classes de vertebrados, mas também entre as ordens aviárias, correlacionadas com seus diferentes hábitos e habitat, a exemplo da córnea. Este trabalho descreveu as estruturas histomorfométricas da córnea das seguintes aves: *Asio stygius, Crotophaga ani, Pitangus sulphuratus, Turdus rufiventris, Ramphastos toco, Rhea americana, Ara macao, Nyctidromus albicollis e Nyctibius griseus*; habitantes da região Centro-Oeste brasileiro. Adicionalmente, avaliaram-se as características do endotélio corneano do *Nyctidromus albicollis,* por meio da microscopia eletrônica de varredura. Os resultados nos permitiram observar que a composição da córnea é a mesma em todas as aves, mas diferem entre si na espessura das camadas corneanas, na espessura entre as regiões centrais e periféricas, e no número de camadas epiteliais. Estes resultados auxiliam na compreensão da fisiologia da visão e das exigências visuais destas espécies, favorecendo esforços em prol da sua conservação, bem como na interpretação de alterações patológicas da córnea das aves.

Palavras-chave: olho de aves, córnea, endotélio, histologia, morfologia, morfometria.

ABSTRACT

Each ocular structure undergoes variations not only among vertebrate classes, but also among avian orders, correlated with their different habits and habitats, such as the cornea. This work described the histomorphometric structures of the cornea of the following birds: *Asio stygius, Crotophaga ani, Pitangus sulphuratus, Turdus rufiventris, Ramphastos toco, Rhea americana, Ara macao, Nyctidromus albicollis and Nyctibius griseus*; all inhabitants of the Brazilian Midwest region. Additionally, it evaluated the characteristics of the corneal endothelium of *Nyctidromus albicollis*, through scanning electron microscopy. The results allowed us to observe that the composition of the cornea is the same in all birds, but they differ in the thickness of the corneal layers, in the thickness between the central and peripheral regions and in the number of epithelial layers. These results will help understanding the physiology of vision and visual requirements and contribute towards the conservation of different avian species, improving the interpretation of pathological changes in the cornea of birds.

Keywords: avian eye, cornea, endothelium, histology, morphology, morphometry

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INTRODUÇÃO

Contextualização

A classe das aves inclui 23 ordens e mais de 9.000 espécies com múltiplos modos de vida, sendo o clado de vertebrados com maior variedade de espécies. Ao longo dos últimos 20 anos as aves tornaram-se parte importante da clínica de oftalmologia veterinária, não só porque são frequentemente mantidas como animais de estimação, mas também porque há uma crescente consciência de conservação ambiental; e uma boa visão é essencial para aves de vida livre, já que tem influência direta no voo, alimentação e reprodução. Esses animais possuem um complexo sistema visual e cada estrutura ocular tem um papel específico e fundamental possibilitando a recepção de alta qualidade de informação visual (BAYÓN; ALMELA; TALAVERA, 2008; MOORE et al., 2022).

O sistema visual das aves e sua percepção do ambiente variam entre as espécies, de acordo com os diferentes nichos ecológicos habitados. Embora o olho assemelhe-se aos demais vertebrados, notadamente há diferenças estruturais e fisiológicas. Logo, a posição dos olhos no crânio, a amplitude de campo visual, o formato do bulbo ocular, a curvatura da córnea, a distribuição das células da retina e o espectro visual das cores, entre muitos outros exemplos, ajudam a interpretar a interação entre a visão das aves e o meio onde elas vivem, garantindo a sobrevivência das espécies (GARAMSZEGI; MØLLER; ERRITZØE, 2002; HALL; ROSS, 2007; CARVALHO et al., 2018; AUSPREY; NEWELL; ROBINSON, 2020).

Problemática e relevância

Buscando-se aprimorar o conhecimento das estruturas oculares e da capacidade visual das aves, faz-se necessário o registro de informações sobre diferentes espécies, obtidos com auxílio de métodos que se complementam. Dadas as diferenças oculares entre o grupo da classe aves, com grande distinção entre as ordens, famílias e espécies, a compreensão de suas particularidades será material para diagnósticos e terapêuticas mais precisas.

Objetivo

Objetivou-se descrever morfologicamente córneas saudáveis de aves. No primeiro trabalho, realizou-se a análise morfométrica da estrutura histológica da córnea de oito

espécies, por meio da microscopia de luz. No segundo estudo, objetivou-se analisar a córnea do bacurau, ave da espécie *Nyctidromus albicollis*, por microscopia de luz, e avaliar a morfologia e morfometria do endotélio pela da microscopia eletrônica de varredura.

REVISÃO BIBLIOGRÁFICA

A visão das aves

O sistema visual das aves e sua percepção do meio ambiente variam tanto quanto a quantidade de espécies. Compreender a interação das aves com seu meio auxilia na interpretação dos hábitos de caça, fuga e acasalamento desses animais (AUSPREY; NEWELL; ROBINSON, 2020). Apesar de possuírem outros sentidos para realizar suas atividades diárias - como campos magnéticos e sonoros, tato, olfato e audição - a visão parece ser de mais alta relevância na maioria das espécies aviárias (GRIGG et al., 2017; IWANIUK; WYLIE, 2020; POTIER; MITKUS; KELBER, 2020; WAGENER; NIEDER, 2020; PUSCH et al., 2023). Bacuraus e urutaus, da família caprimulgiformes, tem a tática de assentar e esperar que os insetos passem perto de suas cabeças para abocanhá-los, contando com suas fendas palpebrais, por onde essas aves podem enxergar mesmo de olhos fechados, e com a função tátil das vibrissas de seus bicos (DELAUNAY et al., 2020; SALAZAR et al., 2020). Águias, falcões, gaviões e urubus-pretos voam alto, onde suas capacidades olfativas e auditivas não alcançam; logo, acessam suas presas pela da habilidade visual. Já o urubu-rei, se guia principalmente pelo olfato, para encontrar animais em decomposição (GRIGG et al., 2017; POTIER; MITKUS; KELBER, 2020).

Suas vantagens visuais compreendem até a retina, onde a variedade de fotorreceptores traz uma visão tetra cromática a esses animais (MEYER, 1977; GÜNTHER et al., 2021). O quarto tipo de cone, presente na retina das aves, confere a captação da luz no espectro ultravioleta, proporcionando melhor localização dos alimentos e de seus parceiros (KAMMERLING et al., 2018; ESPINHEIRA GOMES et al., 2020). Além de enxergar um maior espectro de cores, ainda há uma variação na predominância de fotorreceptores, sendo que as aves noturnas possuem mais bastonetes e as aves diurnas possuem maior densidade de cones em suas retinas (BORGES et al., 2019; ZUEVA et al., 2019; POTIER; MITKUS; KELBER, 2020). Isso porque as aves diurnas, mais coloridas, tem necessidade visual de identificar diferentes padrões e intensidades de cores, assim como maior detalhamento das imagens, funções que competem aos cones. Já as aves noturnas requerem maior habilidade de captação da pouca luz ambiente, função que compete aos bastonetes. Os quais, juntamente

com o formato tubular do bulbo ocular de corujas, bacuraus e urutaus permitem que a luz permaneça por mais tempo no interior ocular, intensificando sua captação pelos fotorreceptores (ROJAS et al., 2004ª; ROSS, 2007; BORGES et al., 2019; HALL; IWANIUK; WYLIE, 2020;).

Alguns exemplos de rapinantes diurnos ainda podem contar com duas fóveas em cada olho. A fóvea é a região da retina onde se localiza a maior densidade de cones, levando a uma alta resolução espacial que permite avistar as presas mesmo em longas distâncias (TYRRELL; FERNÁNDEZ-JURICIC, 2017a). A região da retina responsável pela resolução espacial difere com os hábitos de caça e o campo visual de cada espécie. Nos caprimulgiformes a fóvea se encontra na região ventrotemporal da retina, tendo seu campo visual binocular direcionado para a porção dorsal do seu bico (MARTIN et al., 2004; SALAZAR et al., 2020).

O campo visual varia conforme a posição das órbitas no crânio, e isso define a amplitude da visão monocular e binocular. A visão binocular é responsável pela visão estereoscópica, ou seja, a capacidade visual tridimensional (TYRRELL; FERNÁNDEZ-JURICIC, 2017b). Sabe-se que aves com os olhos posicionados frontalmente têm maior amplitude horizontal binocular, como as corujas. E quanto mais lateralizada for a posição das órbitas no crânio, mais estreito será o campo visual binocular frontal (MARTIN, 2007). Porém, diferente do que acontece em mamíferos, a visão estereoscópica não é essencial nas aves. A principal necessidade da visão binocular para as aves é observar o próprio bico, podendo controlar seus movimentos para apreensão das presas (MARTIN, 2009).

O comprimento do bico também influencia no campo visual, já que o mesmo causa um ponto cego logo a frente dele. Em aves com os olhos lateralizados, as que têm bicos curtos tem uma área cega rostral maior que as de bicos longos (TYRRELL; FERNÁNDEZ-JURICIC, 2017b), inclusive águias e urubus, tem uma extensa área cega acima de suas cabeças, o que os colocam em risco de colisão em pleno voo (TYRRELL; FERNÁNDEZ-JURICIC, 2017ª; POTIER; MITKUS; KELBER, 2020; MARTIN, 2022). Portanto, devido às limitações de campo visual, as aves desenvolveram mecanismos compensatórios de acordo com a necessidade das espécies. Seus olhos tem movimentos coordenados e em direções contrárias (mesmo tendo pouca movimentação dentro das órbitas); sua cabeça tem alta amplitude de moção e são movidas repetidamente e rapidamente para fixar o objeto de imagem; ainda contam com o movimento de "*head-bobbing*", claramente visível em pombos, que acontece ao caminhar para estabilizar a imagem na retina (VOSS; BISCHOF, 2009;

GUNJI; FUJITA; HIGUCHI, 2013; BUTLER; TEMPLETON; FERNÁNDEZ-JURICIC, 2018; KAMMERLING et al., 2018; YORZINSKI, 2019).

Estruturas Oculares

Órbita

Ambas as cavidades orbitárias delimitam o espaço preenchido pelos bulbos oculares e alguns de seus anexos, tendo como objetivo abrigar e proteger esses órgãos. Os olhos das aves se acomodam estreitamente na órbita, sendo que em muitos casos os bulbos encaixam-se incompletamente na cavidade, pelo grande tamanho do segmento posterior desses olhos (MOORE et al., 2022a). Por esse mesmo motivo, as órbitas ocupam uma grande porção do crânio, levando a um deslocamento caudal do cérebro, e também a um septo ósseo interorbital estreito. Os ossos que formam essa estrutura são: o frontal, pré-frontal, esfenoide, etmoide (septo interorbital), palatino, quadrado e arco jugal (MCLELLAND, 1991; JONES; PIERCE; WARD, 2007).

A órbita é aberta ventralmente na maioria das espécies aviárias, sendo essa margem inferior delimitada pelo ligamento subortbital (WILLIAMS, 2012; CARVALHO et al., 2018), embora em alguns psittaciformes a encontramos fechada pelo arco subortbital (MACHADO; DOS SANTOS SCHMIDT; MONTIANI-FERREIRA, 2006). Na junção caudal do septo interorbital (paredes medial e ventral) encontra-se o forame óptico por onde passa o nervo óptico. Ventralmente a esse, três pequenos forames permitem a passagem dos nervos oculomotor, troclear e o ramo oftálmico do nervo trigêmeo. Os ossos envolvidos na composição da órbita são pneumáticos, por isso são mais frágeis e suscetíveis a fraturas (WILLIAMS, 2012).

A porção ventral da órbita faz fronteira com os seios paranasais e com o divertículo do seio infraorbitário (VELADIANO et al., 2016). Em muitas aves (psittaciformes, galloanserae e passeriformes insetívoros), os seios infraorbitais direito e esquerdo se comunicam, mas não em passeriformes não insetívoros (MOORE et al., 2022a). Devido à proximidade com o bulbo, a sinusite do seio paranasal e do divertículo infraorbitário podem levar a alterações oftálmicas atingindo órbita, periórbita e bulbo ocular, causando edema, compressão, exoftalmia, conjuntivite e uveíte (WILLIS; WILKIE, 1999; CARVALHO et al., 2018).

A vascularização da órbita é realizada pela artéria témporo-orbitária, que tem origem da artéria carótida interna. Essa se ramifica em artérias infraorbitária, oftalmotemporal e

supraorbitária, que fazem o suprimento de sangue arterial, enquanto a drenagem é realizada paralelamente por veias com os mesmos nomes, que direcionam o sangue venoso para a veia maxilar e cefálica rostral (PORTER; WITMER, 2016).

Anexos Oculares

Fazem parte dos anexos oculares, pálpebras, músculos, aparato lacrimal e glândulas. As pálpebras são três: superior, inferior e terceira pálpebra. A pálpebra inferior contém uma placa tarsal fibrosa, é sempre maior, mais móvel, mais transparente e delgada que a superior, sendo a principal responsável pela oclusão da comissura palpebral (CARVALHO et al., 2018; MOORE et al., 2022a). Essas características não se aplicam às corujas (RODARTE-ALMEIDA et al., 2013). A porção mais externa das pálpebras superior e inferior pode ser recoberta por filoplumas ou estarem com a pele à mostra, como é o caso das araras. A estrutura da plumagem peripalpebral é especializada e variável entre as espécies aviárias, dependendo das necessidades protetoras e sensoriais de cada uma. Na maioria das aves de rapina, por exemplo, há uma crista superciliar bem desenvolvida onde as penas dessa região se estendem a cima e a frente dos olhos, com o objetivo de diminuir a incidência ou reflexo da luz solar diminuindo o reflexo de ofuscamento (JONES; PIERCE; WARD, 2007). A porção média das pálpebras é composta por músculos, glândulas de Zeiss e de Moll, sebáceas e sudoríparas respectivamente. Aves não possuem glândulas de meibômio. Três músculos estriados permitem a movimentação, o elevador da pálpebra superior, o retrator da pálpebra inferior e o músculo orbicular presente em ambas as pálpebras. Esses são inervados respectivamente pelo oculomotor e pelo nervo mandibular. A camada interna é a conjuntiva palpebral, onde se localizam as células caliciformes e o tecido linfoide associado à conjuntiva (BAYÓN; ALMELA; TALAVERA, 2008; MONÇÃO-SILVA et al., 2016).

As pálpebras também caracterizam dois tipos de aves de acordo com sua eclosão. Quando as pálpebras estão bem desenvolvidas e a fissura palpebral está aberta no momento da eclosão, são chamadas aves precociais; galináceos e anatídeos são exemplos, pois são filhotes relativamente maduros e capazes de seguir a progenitora em busca de alimento. Enquanto que pálpebras seladas e incompletamente desenvolvidas são características de aves altriciais, espécies eclodidas sem plumagem, sem capacidade de andar e dependente dos indivíduos adultos. O tempo de abertura das pálpebras em aves altriciais é variável: no caso das cacatuas ocorre entre 10-17 dias após a eclosão e nas araras 17-26 dias. A separação palpebral inicia-se centralmente, progredindo medial e lateralmente (SCHEIBER et al., 2017; CHEN et al., 2019a, 2019b).

A terceira pálpebra é uma membrana delgada e muitas vezes transparente, altamente móvel, que cobre toda a córnea partindo da porção dorsonasal para a ventrotemporal, também chamada de membrana nictante. O músculo piramidal, que move a terceira pálpebra, origina-se na esclera posterior e circunda o nervo óptico por meio do ligamento do músculo quadrado (WILLIAMS, 2012). A membrana nictante é responsável por espalhar o filme lacrimal, remover debris e corpos estranhos, e principalmente proteger e evitar danos à córnea, permitindo o voo e/ou mergulho de olhos abertos, pois não interfere na refração da córnea quando a recobre (SIVAK; BOBIER; LEVY, 1978).

As glândulas das aves são de dois tipos: a lacrimal, que produz o fluido lacrimal aquoso, e a glândula de Harder, que produz secreção mista. A glândula lacrimal tem menor relevância nas aves que nos mamíferos, e algumas espécies como corujas nem a possuem. Já as aves semiaquáticas possuem uma grande glândula lacrimal, localizada ventrolateralmente ao bulbo, e seus micro ductos se abrem no saco conjuntival, na porção lateral da pálpebra inferior, por onde secretam a porção aquosa do filme lacrimal. A glândula de Harder é maior, mais desenvolvida e a principal fonte lacrimal nas aves (BAYÓN; ALMELA; TALAVERA, 2008; WILLIAMS, 2012). Ela está localizada medialmente ao bulbo, entre os músculos oblíquo ventral e reto medial. Seus ductos liberam substâncias lipídicas e seromucóides que compõe o filme lacrimal, depositado entre a membrana da terceira pálpebra e a córnea, nutrindo diretamente a córnea e desempenhando papel de defesa imunológica (MOORE et al., 2022a). Há ainda, em algumas aves marinhas, a glândula de sal, que faz a eliminação de eletrólitos absorvidos durante a imersão subaquática (MOORE et al., 2022a).

O sistema de drenagem lacrimal inclui dois óstios e canalículos lacrimais, assim como nos mamíferos e répteis, e estão posicionados próximos à comissura lacrimal medial das pálpebras superior e inferior. Os canalículos se unem ao ducto nasolacrimal que desemboca na cavidade oronasal dorsal, rostralmente às coanas (WILLIS; WILKIE, 1999; WILLIAMS, 2012).

Os músculos extraoculares são seis, sendo quatro retos – dorsal, ventral, medial e lateral – e dois oblíquos – ventral e dorsal. Nas aves, o músculo retrator do bulbo está ausente e os músculos extraoculares existentes são pouco desenvolvidos em relação aos mamíferos. Pois além de o bulbo ocupar quase toda órbita, ainda há a porção óssea da esclera que é aderida à margem orbitária, limitando a movimentação do olho. A inervação dos músculos é realizada pelo ramo dorsal do nervo oculomotor (reto dorsal), ramo ventral do oculomotor

(retos medial e ventral, e oblíquo ventral), nervo troclear (oblíquo dorsal) e nervo abducente (reto lateral) (MOORE et al., 2022a).

Bulbo ocular

O olho é divido em três camadas: a externa, a média e a interna, também conhecidas como túnicas fibrosa, vascular e nervosa, respectivamente. O bulbo também é divido em segmentos e câmaras. No segmento anterior está presente a córnea, câmara anterior e câmara posterior preenchidas por humor aquoso, íris, lente e corpo ciliar. O segmento posterior é maior que o anterior, e nas aves chega a ser 2 a 3 vezes maior. Nele estão presentes coroide, retina, vítreo, pécten ocular e nervo óptico (MOORE et al., 2022a).

O bulbo ocular nas aves é tão grande que, em algumas espécies, o equador do bulbo excede as margens da órbita, como nas corujas. O seu formato varia entre as espécies, podendo ser três: achatado, globoso e tubular (BAYÓN; ALMELA; TALAVERA, 2008). O olho achatado tem o eixo anteroposterior curto em relação aos outros formatos, assim como a região da câmara anterior e corpo ciliar, pois tanto a córnea (convexa) como a região média do bulbo (côncava) tem sua curvatura diminuída, próximo ao eixo plano. É o formato mais comum e presente na maioria das aves diurnas, como em psitacídeos, passeriformes e aves aquáticas. O comprimento anteroposterior intermediário é encontrado no formato globoso, onde a córnea é mais convexa e a região do corpo ciliar tem maior concavidade que o formato anterior. Esse formato é encontrado em aves diurnas com a cabeça mais larga, que tem uma necessidade visual mais exigente, como aves insetívoras, de rapina e avestruzes (MARTIN; ASHASH; KATZIR, 2001; BAYÓN; ALMELA; TALAVERA, 2008; CARVALHO et al., 2018; MOORE et al., 2022a). Em aves noturnas, nas quais é necessário maior aproveitamento da luz presente, o comprimento do bulbo se faz maior com o formato tubular. A região média dos bulbos tubulares é longa e côncava, formando um túnel para o segmento posterior. O equador do bulbo se encontra rostralmente à órbita, com córneas grandes e bem convexas, como no caso das corujas (BAYÓN; ALMELA; TALAVERA, 2008; RODARTE-ALMEIDA et al., 2013).

O suprimento vascular do bulbo das aves é dado pela artéria oftálmica externa, ramo da subdivisão interna da artéria carótida comum. Essa dá origem à artéria oftalmotemporal, e seus ramos são a principal irrigação do olho. Esse modelo vascular é o mesmo conhecido em mamíferos (HOSSLER; OLSON, 1984; PORTER; WITMER, 2016; DOWNIE et al., 2021). Para o suprimento do pécten, um ramo específico da artéria oftalmotemporal foi

desenvolvido, a artéria *pectinis oculi* (FERREIRA; GIANNICO; MONTIANI-FERREIRA, 2016).

A inervação do bulbo ocular é realizada pelo nervo oftálmico, ramo do nervo trigêmeo, e pelo nervo óptico. O nervo oftálmico se ramifica em nervos ciliares curtos e longos que adentram o equador da esclera, provendo controle voluntário da íris, e resposta sensorial a córnea e corpo ciliar. O nervo óptico é responsável por transmitir ao cérebro a informação de imagem. Já a inervação autônoma vem do gânglio cervical, projetando mais fibras simpáticas do que parassimpáticas (LACERDA et al., 2014; HE; PHAM; BAZAN, 2022; WU; ZHAO; ZHANG, 2022).

Esclera

A túnica fibrosa ou externa, é composta pela esclera e córnea. Elas formam um envoltório fibroso, como o nome sugere, que recobre todo o bulbo e tem como principal função a proteção das estruturas internas, também é a camada que confere formato e rigidez ao bulbo. A esclera é uma camada densa, branca, vascularizada e formada por fibras colágenas. Diferente de outros vertebrados, a esclera das aves possui um anel escleral formado por pequenos ossos pneumáticos que circundam o equador do bulbo, nomeados de ossículos esclerais. Essa é a região que se adere ao periósteo da órbita, e onde os músculos ciliares se conectam (FISCHER; SCHOENEMANN, 2019; ZEHTABVAR et al., 2022).

A córnea será amplamente discutida na secção *A córnea das aves*.

Lente

A lente é uma estrutura biconvexa, transparente, flexível e macia. Nas aves tem formato quase esférico em olhos tubulares, e alongado em olhos chatos e globosos, tendo a porção anterior mais aplanada e a posterior mais abaulada. Possui uma almofada anular fibrosa em seu equador que permite uma resistente conexão com o corpo ciliar e os músculos ciliares (GLASSER; HOWLAND, 1996; BEEBE; COATS, 2000; MONÇÃO-SILVA et al., 2016).

A principal função da lente é permitir a chegada da luz na retina, por meio da sua propriedade refrativa. Para que isso ocorra, além de transparência, é necessário o ajuste da distância focal, realizado pelo mecanismo de acomodação. Esse mecanismo é a capacidade de mudança da curvatura da lente e da córnea das aves, alterando o poder de refração da luz (MEYER, 1977; GLASSER; HOWLAND, 1996; SIVAK, 2004; POTIER; MITKUS;

KELBER, 2020). As aves aquáticas têm suas córneas quase planas e não flexíveis, então deixam a lente com toda a função de acomodação sub aquática (SIVAK, 2004; COLLIN; COLLIN, 2021). A acomodação é promovida por músculos ciliares estriados, denominados músculo de Crampton, músculo de Brucke e músculo de Müller, e o músculo esfíncter da íris. O primeiro tem ação na córnea, e os demais na lente. Em aves mergulhadoras, esfíncter pupilar potencializa a contração do músculo ciliar para acomodação da lente, encurtando-a até o formato próximo ao esférico, e possibilitando o ajuste do foco subaquático para caça e fuga (HOWLAND, 1983; HOWLAND, 1987; MURPHY; SIVAK, 2004; BAYÓN; ALMELA; TALAVERA, 2008; SCHAEFFEL; WILLIAMS, 2012).

Íris, corpo ciliar, coroide e pécten

A túnica vascular ou média é composta pelas estruturas responsáveis pelo suprimento vascular e função imunológica do olho. A íris e o corpo ciliar suprem a porção anterior do bulbo ocular, enquanto a coroide e o pécten são responsáveis pela retina (KIAMA et al., 2001; REESE; HORST; LIEBICH, 2005; JONES; PIERCE; WARD, 2007; ALBINI; DAVIS, 2015).

A íris é a estrutura responsável por controlar a incidência de luz na retina. Sua face anterior é o estroma, composto por vasos sanguíneos, músculos, pigmentos carotenoides, purinas, pteridinas, e melanócitos, que dão cor a íris. Essa pigmentação nas aves varia entre as espécies, e também entre a idade e sexo da mesma espécie. A face posterior é composta por mioepitélio colunar pigmentado (JONES; PIERCE; WARD, 2007; BAYÓN; ALMELA; TALAVERA, 2008; MONÇÃO-SILVA et al., 2016). Pode-se dividir a íris em duas regiões, a borda ciliar, porção mais externa que está ligada ao corpo ciliar, e a borda pupilar, região que forma a pupila. Na borda pupilar há o músculo esfíncter que contrai fazendo o fechamento da pupila, ou miose. Na borda ciliar está presente o músculo dilatador da pupila, responsável pela midríase. A pupila apresenta uma grande variedade de formatos nos vertebrados, e o que confere essa distinção é a disposição das fibras musculares na borda pupilar. O mecanismo de contração e dilatação da pupila se dá por músculos estriados, ou seja, nas aves o movimento da íris é voluntário. Isso permite alcançar maior profundidade de foco através da miose voluntária (SIVAK, 2004; WILLIAMS, 2012).

O corpo ciliar tem origem na base da íris e sua extensão vai até onde começa a retina. É formado por duas regiões, a *pars* plicata, onde estão os processos ciliares e ligamentos zonulares que fixam a lente, e a *pars* plana que segue para o segmento posterior e se encontra

com a retina. Assim como nos demais vertebrados, o corpo ciliar das aves tem a função de produzir humor aquoso, sustentar a lente e participar da defesa imune dos olhos (REESE; HORST; LIEBICH, 2005; JONES; PIERCE; WARD, 2007). A drenagem do humor aquoso ocorre pelo ângulo iridocorneano, que é bem desenvolvido nas aves, e seus ligamentos pectinados são claramente definidos, em algumas aves podem ser observados sem lente de gonioscopia (HARRIS et al., 2008; RODARTE-ALMEIDA et al., 2013; SOKOLENKO et al., 2021)

A coroide se inicia com o término do corpo ciliar, se encontra justaposta e externamente à retina, localizada no segmento posterior. É composta por 4 camadas, da mais externa para a mais interna: a lâmina supracoroide, que está em contato com a esclera; a camada vascular, que contém grandes vasos sanguíneos; a camada de coriocapilares, formada por uma rede de capilares oriundos da camada anterior, que tem papel essencial na nutrição da retina; e a lâmina basal sobre a qual repousa a retina (JONES; PIERCE; WARD, 2007; PLATZL et al., 2022). A lâmina supracoroide, ou membrana fusca, das aves é mais desenvolvida que em mamíferos, sendo mais espessa e flexível. Isso auxilia na adesão da coroide com a esclera, e na absorção de impactos no interior dos olhos de aves mergulhadoras e aves que atingem altas distâncias de voos (DE STEFANO; MUGNAINI, 1997). O fundo do olho das aves é normalmente descrito como atapetal, pois não é visualizado um *tapetum* verdadeiro no exame direto da retina ou histologicamente (ROJAS et al., 2004b; MARTIN et al., 2014; CARVALHO et al., 2018; MOORE et al., 2022b).

O pécten, peculiaridade das aves, é uma membrana pregueada, altamente vascularizada. Essa estrutura se origina da coroide e adere à retina. Sua base repousa sobre a papila óptica, recobrindo-a, e seu corpo se projeta para o interior do vítreo direcionada para o centro do bulbo. É composto por capilares e células estromais pigmentadas extra vascularmente. É delimitado por uma fina lâmina basal contínua e a membrana limitante interna da retina (KIAMA et al., 2001; MONÇÃO-SILVA et al., 2016). O pécten é responsável por complementar o suporte nutricional da retina, assim como realizar sua barreira imune. Também produz humor aquoso e reduz o ofuscamento, diminuindo a incidência de luz direta na retina e papila óptica (FERREIRA; GIANNICO; MONTIANI-FERREIRA, 2016; FERREIRA et al., 2019). O pécten possui variações em seu formato e na quantidade de pregas de acordo com a espécie aviária. A quantidade de pregas demonstra estar relacionado às atividades noturnas (com menor número) ou diurnas (com maior número) das aves (ABUMANDOUR; BASSUONI; HANAFY, 2021). São descritos 3 formatos de

pécten: o cônico, presente em aves kiwis; em formato denominado “vanned”, descrito em avestruzes; e o plissado, pertencentes a maioria das aves (DAYAN; OZAYDN, 2013; YILMAZ et al., 2021).

Retina e nervo óptico

Ainda no segmento posterior, encontramos a túnica nervosa ou interna, que compreende a retina e o nervo óptico. Conectado a túnica nervosa está o vítreo, corpo denso de colágeno transparente que ocupa toda a câmara vítrea e aloja o pécten em seu interior (JONES; PIERCE; WARD, 2007).

A retina das aves é anangiótica, e recebe nutrientes e oxigênio através da coroide e do pécten. Assim como as demais estruturas oculares, a retina também tem especificidades nas aves. Porém suas camadas recebem a mesma nomenclatura e tem as mesmas constituições celulares básicas que a retina dos mamíferos (RUGGERI et al., 2010; POTIER; MITKUS; KELBER, 2020). A retina tem a função de captar a luz e converter os estímulos luminosos em sinais elétricos que serão transmitidos ao cérebro. A captação dos espectros luminosos é realizada pelos fotorreceptores, cones e bastonetes (EGBUNIWE; AYO, 2016; ZUEVA et al., 2019). As aves possuem um tipo de bastonete, um cone duplo, e quatro tipos de cones simples. Os cones duplos são predominantes em aves diurnas. Cada tipo de cone possui uma opsonina, que capta uma frequência luminosa diferente, e gotículas de óleo em seu interior. Essas gotículas de óleo filtram a luz e tornam o comprimento de onda mais longo, aumentando a sensibilidade espectral dos cones (MITKUS et al., 2018; GÜNTHER et al., 2021). A distribuição e a densidade dos fotorreceptores na retina variam de acordo com os hábitos de cada espécie aviária. Não só sobre visão escotópia (predominância de bastonetes) ou fotópica (predominância de cones), mas também variam a distribuição de acordo com o campo visual de suas necessidades para caça. A alta densidade de fotorreceptores leva a um espessamento da retina, sendo mais espessa no centro. Aves de rapina diurnas tem suas retinas consideravelmente mais espessa que outras espécies de aves (ROJAS et al., 2004b; EGBUNIWE; AYO, 2016; TYRRELL; FERNÁNDEZ-JURICIC, 2017a; MITKUS et al., 2018; POTIER; MITKUS; KELBER, 2020). Há, ainda na região central da retina, uma alta concentração de cones e células ganglionares. Quando há invaginação da retina essa região é denominada fóvea, sem a invaginação denomina-se área *centralis*. Em águias, gaviões e falcões, encontram-se duas fóveas, a segunda ocupa a região temporal da retina. Já urubus e condores, tem apenas a fóvea central, mas na região temporal eles possuem uma área *temporalis*, local com alta densidade de cones. Algumas aves têm apenas a área *centralis*.

Toda função que a fóvea exerce ainda é objeto de estudo, mas é sabido que essa estrutura é responsável por fixar a imagem no campo binocular e aumentar a resolução da mesma (TYRRELL; FERNÁNDEZ-JURICIC, 2017a; POTIER et al., 2020a, 2020b; POTIER; MITKUS; KELBER, 2020).

O nervo óptico é formado pelos axônios das células ganglionares da retina, que se tornam mielinizadas à medida que penetram a esclera. As fibras seguem caudalmente através do forame óptico e decussam quase completamente no quiasma óptico, para então seguir o caminho até o córtex visual. O diâmetro transversal do nervo óptico das aves é maior que o da medula espinhal cervical (OROSZ; BRADSHAW, 2007). O modelo de decussação do nervo óptico das aves levava a acreditar que esses animais não tinham reflexo pupilar consensual, e sim um reflexo pupilar direto devido a iluminação que atingia o olho contralateral através do septo interorbital, que é delgado a ponto de permitir a passagem da luz de uma órbita a outra. Porém foi demonstrado experimentalmente que o reflexo pupilar consensual realmente está presente em aves (LI; HOWLAND, 1999; MOORE et al., 2022a).

A córnea das aves

A córnea é a estrutura mais externa do bulbo ocular, responsável por prover proteção às estruturas internas e poder de refração da imagem. Para que isso seja possível, a córnea precisa ser transparente, convexa e ter uma relativa rigidez (ABDELFTAH et al., 2021; DOWNIE et al., 2021). Sua convexidade, ou curvatura, varia de acordo com o formato do bulbo ocular, sendo levemente aplanada em bulbos achatados, e próximas a uma semiesfera em bulbos tubulares. A curvatura associada à transparência desempenha a função de refração e convergência da luz, sendo a córnea a primeira superfície óptica que a luz ultrapassa para chegar à retina e possibilitar a formação da imagem (JONES; PIERCE; WARD, 2007; MARTIN, 2022). O poder refrativo da córnea chega a ser igual ao da lente em aves com olhos globosos ou tubulares; enquanto nas aves de bulbo ocular achatado, a lente exerce o maior poder de refração (MARTIN; ASHASH; KATZIR, 2001; LIU et al., 2016). Embora a córnea seja rígida, para proteção contra impactos externos e sustentação das estruturas internas, essa rigidez não é absoluta. A córnea tem propriedades reológicas, para que seja possível sua deformação (atenuação ou acentuação da curvatura) diante do mecanismo de acomodação para focalizar a imagem (LIU et al., 2016; ABDELFTAH et al., 2021; COLLIN; COLLIN, 2021).

A transparência da córnea é possível por ser avascular, por ter seu estroma com fibras de colágeno organizadas paralelamente - camada responsável pela maior parte de sua espessura-, e por fim, pela manutenção do estado de deturgescência, responsável pelo epitélio e endotélio (MATTHYSSEN et al., 2018; COYO et al., 2019; COLLIN; COLLIN, 2021). Como não há presença de vasos sanguíneo ou linfáticos na córnea, o fornecimento de oxigênio e nutrientes é proveniente do filme lacrimal, humor aquoso e vascularização perilímbica. A maior parte do suprimento de oxigênio é oriundo do ar atmosférico, transportado pela lágrima. Enquanto o humor aquoso é a principal fonte de glicose e aminoácidos essenciais (DOWNIE et al., 2021).

Assim como nos mamíferos, a inervação sensorial e autônoma da córnea é feita pelo ramo oftálmico do nervo trigêmeo, que se ramifica em nervos ciliares curtos e longos. Seus feixes nervosos adentram a esclera e a coroide, e alcançam a região periférica da córnea, onde se ramificam densamente por toda circunferência corneana e a penetram radialmente pelo estroma. Os feixes continuam se ramificando em direção ao centro e à superfície (epitélio) da córnea, ocupando-a de forma homogênea (LACERDA et al., 2014; DOWNIE et al., 2021; HE; PHAM; BAZAN, 2022; WU; ZHAO; ZHANG, 2022). Como particularidade das aves, a densidade de fibras nervosas na periferia da córnea é maior que em mamíferos. Essas fibras também não fazem anastomose no centro do estroma, sendo que em mamíferos há formação de uma malha nervosa estromal. Outra diferença é que nas aves os nervos não formam um formato centrípeto em direção ao centro corneano, e sim percorrem radialmente a córnea (HE; PHAM; BAZAN, 2022). Na presença de estímulos térmicos, mecânicos e químicos, há uma resposta motora do nervo facial, gerando o reflexo de piscar, e uma resposta autonômica (principalmente simpática) para secreção lacrimal, garantindo a lubrificação e integridade da córnea (LACERDA et al., 2014; LABETOULLE et al., 2019; WU; ZHAO; ZHANG, 2022).

A estrutura histológica da córnea das aves segue a mesma arquitetura dos demais vertebrados, sendo composta mandatoriamente por 4 camadas: mais externamente o epitélio estratificado não queratinizado; após, o estroma, camada que confere espessura à córnea, formado por lamelas de colágeno; a membrana de Descemet, membrana basal acelular da última camada; por fim e mais internamente o endotélio, camada única de células hexagonais. A maioria das aves também apresenta uma quinta camada pertencente ao estroma, a membrana de Bowman, que está logo abaixo da membrana basal do epitélio. As camadas da córnea ainda sofrem variações espécie-específicas, de acordo com o nicho ecológico ocupado pelas aves (KAFARNIK; FRITSCHE; REESE, 2007; TSUKAHARA et al., 2010;

ABDELFTAH et al., 2021; COLLIN; COLLIN, 2021). Já a espessura da córnea das aves varia não só com a espécie, mas também com o formato do bulbo ocular e de acordo com a região central ou periférica da córnea. Olhos de formato tubular tem córneas marcadamente mais finas no centro do que na periferia (MURPHY; DUBIELZIG, 1993; WERTHER; CANDIOTO; KORBEL, 2017). Diferente de aves com olhos achatados, onde a espessura do centro e da periferia são aproximadas (CHARD; GUNDLACH, 1938; MOORE et al., 2019). A espessura das córneas também não obedece a uma proporção de alometria, já que a mesma não está relacionada ao tamanho e/ou peso da ave (LIU et al., 2016; POPOVA et al., 2022).

Epitélio

O epitélio é a camada superficial da córnea, composta por camadas de células escamosas estratificadas e não queratinizadas. A camada basal consiste em células de formato cuboide ou colunar, sendo coberta por camadas de células que se tornam mais largas e achatadas à medida que se afastam da base. Essas células, que apresentam formas variadas, são chamadas de células poliédricas. As células escamosas mais externas são achatadas, conhecidas como células guarda-chuva ou células alares, devido à sua sobreposição aos ápices de mais de uma célula (COLLIN; COLLIN, 2000; MONÇÃO-SILVA et al., 2016; NAUTSCHER et al., 2016; PINTO et al., 2016; BERGMANSON, 2019; SOKOLENKO et al., 2021). Outra característica das células epiteliais é a presença de microvilos, que são projeções perpendiculares da membrana celular. Os microvilos são responsáveis por aumentar a área de superfície da célula, aumentando o contato com o fluido lacrimal, assim, podendo usufruir melhor de seus benefícios e diminuindo a velocidade de evaporação do mesmo (COLLIN; COLLIN, 2000, 2006). Entre as espécies de aves há variações da espessura do epitélio, da densidade das células epiteliais e o número de camadas epiteliais. Pinguins, aves de rapinas, e aves domésticas entre outros, já foram estudados, e a quantidade de camadas epiteliais varia de 3 a 8 (CHARD; GUNDLACH, 1938; MURPHY; DUBIELZIG, 1993; PINTO et al., 2016; WERTHER; CANDIOTO; KORBEL, 2017; SOKOLENKO et al., 2021; COLLIN; COLLIN, 2021). A espessura do epitélio das aves varia de 5 a 10% da espessura total da córnea (ABDELFTAH et al., 2021; POPOVA et al., 2022). A densidade das células epiteliais demonstra ser maior em aves voadores do que em aves terrestres, já que essas poderiam sofrer com uma maior velocidade de evaporação da lágrima (COLLIN; COLLIN, 2006).

Membrana de Bowman

A membrana de Bowman é uma camada de fibras colágenas condensadas, também chamada de membrana limitante anterior, devido a sua localização no estroma anterior. É a camada que sofre maior variação morfológica entre as espécies de aves e de outros vertebrados, podendo inclusive, não estar presente na córnea (HAYASHI; OSAWA; TOHYAMA, 2002; KAFARNIK; MERINDANO et al., 2002; FRITSCHE; REESE, 2007; POPOVA et al., 2022). A membrana de Bowman é descrita em diversas espécies de aves, como galinhas, codornas, patos, pelicanos, aves de rapina, psitacídeos e passeriformes (MURPHY; DUBIELZIG, 1993; KAFARNIK; FRITSCHE; REESE, 2007; GONÇALVES et al., 2016; PINTO et al., 2016; ABDELFTAH et al., 2021; POPOVA et al., 2022). Essa tem similaridades com a membrana de humanos, por isso, a córnea de aves é modelo de pesquisa para cirurgias refrativas de humanos (REESE, 2007; GONÇALVES et al., 2016; KAFARNIK; FRITSCHE; LIU et al., 2016). Porém, ela é considerada rudimentar por alguns autores, já que comparada aos humanos e outros primatas, a membrana de Bowman das aves não tem a mesma expressão e definição de suas margens na histologia da córnea (MERINDANO et al., 2002; POPOVA et al., 2022).

Estroma

O estroma, ou substancia própria, é um tecido conectivo denso, formado por lamelas de colágeno sobrepostas e alinhadas paralelamente a superfície, e com queratócitos dispersos entre as lamelas (MEEK; LEONARD, 1993; TSUKAHARA et al., 2010). Nas aves, as lamelas de colágeno são alinhadas entre si formando um arranjo ortogonal preciso e regular, com grande quantidade de ramificações promovendo anastomose dos feixes, desenhando o formato de uma grade. Esse arranjo é mais evidente na porção anterior e média da substância própria, e é associado à maior rigidez mecânica e melhor transmitância da luz no espectro UV – significa que a luz solar que bate na córnea sofre maior dispersão, diminuindo o ofuscamento (TSUKAHARA et al., 2010; BOOTE et al., 2011; KOUDOUNA et al., 2018). O estroma representa a espessura da córnea, já que corresponde a uma média de 90% da espessura total da córnea (NAUTSCHER et al., 2016; KOUDOUNA et al., 2018; ABDELFTAH et al., 2021). Portanto, é o estroma que leva a diferenças de espessuras entre as regiões da córnea (HENRIKSSON; BRON; BERGMANSON, 2012; BERGMANSON; BURNS; WALKER, 2019; BERGMANSON; BURNS; NAROO, 2021).

Membrana de Descemet

A membrana de Descemet é bem desenvolvida nas aves. É a membrana basal do endotélio, e tem a função de auxiliar essa camada. Por ser composta de colágeno concentrado, ela confere resistência mecânica ao endotélio, e também modula a entrada de humor aquoso no estroma anterior. A espessura da membrana de Descemet é variável com a idade da ave, tendo sua espessura aumentada pelo envelhecimento do indivíduo (COLLIN; COLLIN, 2021).

Endotélio

O endotélio é representado como uma monocamada de células principalmente hexagonais de tamanho e forma relativamente uniformes. As células endoteliais têm a capacidade de aumentar de tamanho (polimegatismo) e alterar sua forma (pleomorfismo) para manter o estado de deturgescência da córnea. Esse mecanismo ocorre devido à incapacidade de replicação das células endoteliais e à necessidade de suprir eventuais defeitos. Portanto, é possível visualizar células endoteliais pentagonais, heptagonais, octogonais e até quadradas (COLLIN; COLLIN, 1998; PIGATTO et al., 2004, 2009; DOUGHTY, 2006, 2018; TAMAYO-ARANGO et al., 2009; FRANZEN et al., 2010; ALBUQUERQUE; FREITAS; PIGATTO, 2015; COYO et al., 2019). Em aves jovens, há uma discreta regeneração tecidual, enquanto em adultos e senis, o polimegatismo é evidente. Aves senis podem atingir uma quantidade de 40% ou mais de células endoteliais sem sua forma original (LAING et al., 1976; KAFARNIK; FRITSCHE; REESE, 2007; ALBUQUERQUE; FREITAS; PIGATTO, 2015; COYO et al., 2015; COLLIN; COLLIN, 2021). As células endoteliais das aves também possuem microvilosidades que emergem de suas margens e superfícies (COLLIN; COLLIN, 1998, 2021; PIGATTO et al., 2018). Por ser uma camada unicelular, as variações interespecíficas do endotélio são avaliadas principalmente de acordo com densidade celular e área celular média. Para avaliar a saúde endotelial, a porcentagem de células hexagonais e o coeficiente de variação da área celular se mostram uteis (DOUGHTY, 1989, 2008). O coeficiente de variação da área celular corresponde ao polimegatismo, a taxa mede a variação do tamanho original das células. E a porcentagem de células hexagonais demonstra o número de células endoteliais que estão em sua forma primária. Portanto, quanto mais próximo de zero estiver o coeficiente de variação, e mais próximo de 100% for a porcentagem de células hexagonais, mais saudável e jovem é esse endotélio (PIGATTO et al., 2005, 2009; ALBUQUERQUE; FREITAS; PIGATTO, 2015; COYO et al., 2019).

CAPÍTULO II

Original Article (*Anatomia, Histologia, Embryologia*)

Corneal histomorphometry of birds from the Brazilian Midwest

Rafaela Alves R. Tozetti[1], Matheus Vilardo L. Moreira [2], Rosélia de Lima S. Araújo[1], Liria Queiroz Luz Hirano[3], Bret A. Moore[4] and Paula Diniz Galera[1].

[1]Post-graduate Program in Animal Sciences, Faculty of Agronomy and Veterinary Medicine, University of Brasília, Distrito Federal, Brazil;
[2] MVL Patologia Veterinária, Belo Horizonte, Brazil;
[3]Department of Wild Animals, Faculty of Agronomy and Veterinary Medicine, University of Brasília, Distrito Federal, Brazil
[4] Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.

Correspondence
Paula Diniz Galera, Small Animals Veterinarian Hospital of the University of Brasilia (UnB), L4 Norte, Campus Darcy Ribeiro, UnB – Asa Norte, DF. 70636-200.
Phone number: +55 (61) 99266-0799
Email: dra.paulagalera@gmail.com

Acknowledgment

This study was funded by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil – Funding code 001, and by Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF), Brazil – proc.n.* 00193.00001303/2019-10).

Abstract

The visual structures of birds vary according to their environment, habits, needs of breeding, feeding and predators presence. The cornea is one of the structures in which it is possible to observe clear variations, it is responsible for protecting the inside of the eye, assisting in focusing and refracting the image. Based on the need to know the visual and structural differences between bird species, this work aimed to describe the corneal histomorphometry structures of the following birds: *Asio stygius, Crotophaga ani, Pitangus sulphuratus, Turdus rufiventris, Ramphastos toco, Rhea americana, Ara macao* and *Nyctibius griseus.* These are present in the Brazilian Midwest, an area in which the Cerrado is the predominant vegetation. Through the results it was possible to observe that the gross corneal composition is the same in all birds. Although, they differ from each other in the thickness of the central and peripheral regions, even in the total cornea and in its layers, and in number of epithelial lines.

Keywords: avian; eye; histology; morphometry; ophthalmology; vision.

Introduction

The visual system of birds varies as much as the broad variety of species within these taxa, where the morphology and physiology of vision meets the needs for success in the specific ecological niche they occupy. The cornea is a specialized component of the vertebrate eye with essential functions for avian life, providing protection, refractive power, transparency for image formation, and mechanical support (Abdelftah et al., 2021; Bergmanson, 2019; Jones, Pierce, & Ward, 2007; Meek & Fullwood, 2001). It is one such structure of the eye where interspecific variation can clearly be observed, despite its similarity in histological composition to that of other vertebrates (Collin & Collin, 2006; Meyer, 1977). Even sharing the same habitat, it is possible to observe birds with different visual behaviors, which can be reflected in the morphology of their corneas.

The cornea of birds is composed of five histological layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. However, in some birds, the Bowman's layer has not been identified (Abdelftah et al., 2021; Kafarnik et al., 2007; Popova et al., 2022; Sokolenko et al., 2021). Although the gross structure does not differ among most birds, a distinction can be seen in the diameter, thickness and in number of layers of epithelial cells (Collin & Collin, 2021; Moore, Fernandez-Juricic, Hawkins, Montiani-Ferreira, & Lange, 2022; Tamayo-Arango et al., 2009). Due to the plurality of avian species, seeking knowledge of interspecific differences is important to understanding their visual demands, behaviors, and ecology. This study aimed to describe the histomorphometry of the cornea of eight ecologically and phylogenetically diverse avian species from the Midwest region of Brazil bird: *Asio stygius, Crotophaga ani, Pitangus sulphuratus, Turdus rufiventris, Ramphastos toco, Rhea americana, Ara macao and Nyctibius griseus*.

In the Brazilian cerrado, Scarlet Macaws *Ara macao* and Toco Toucans *Ramphastos toco* mainly inhabit forests and feed on fruits, seeds and insects (BirdLife International, 2016a, 2017; Massari et al., 2020). The Rufous-bellied Thrush *Turdus rufiventris* and the Great Kiskadee *Pitangus sulphuratus* are two examples of passerines; while belonging to different families, both have an excellent adaptation to urban life (BirdLife International, 2016c, 2018; Calegaro-Marques & Amato, 2014; Gasperin & Aurélio Pizo, 2009; Lago-Paiva, 1996). The Greater Rheas *Rhea americana* are omnivorous birds that do not have the ability to fly and differ from most other birds in that they have proportionally smaller eyes when compared to the skull (BirdLife International, 2016b; Pérez Orrico & Sabater González, 2022). The Smooth-billed Ani *Crotophaga ani* is a cuculiform, a diurnal bird that feeds on

insects, arthropods and can catch small fish, usually grabbing prey in mid-flight (Burger & Gochfeld, 2001; Cooke, Haskell, van Rees, & Fessl, 2019; Reavill & Dorrestein, 2018). Stygian Owls *Asio stygius* and Common Potoos *Nyctibius griseus* are nocturnal birds, which have large adapted eyes, providing them high visual sensitivity in dim-light environments (BirdLife International, 2020; Mitkus, Potier, Martin, Duriez, & Kelber, 2018; Potier, Mitkus, & Kelber, 2020).

Materials and methods

Animals

Eight adult birds of different species were included in this study. Only the healthy eyes were evaluated, totaling 11 eyes. Two eyes of Stygian Owl (*Asio stygius*), two eyes of Smooth-Billed Ani (*Crotophaga ani*), one eye of Great Kiskadee (*Pitangus sulphuratus*), one eye of Toco Toucan (*Ramphastos toco*), one eye of a Rufous-bellied Thrush (*Turdus rufiventris*), one eye of a Greater Rhea (*Rhea americana*), one eye of a Scarlet Macaw (*Ara macao*) and finally, two eyes of a Common Potoo (*Nyctibius griseus*) (order, family and popular names described in Table 1). The birds studied were from the Department of Wild Animals (Faculty of Agronomy and Veterinary Medicine of the University of Brasília), where they arrived after being rescued by the Wild Animal Screening Center (Centro de Triagem de Animais Silvestres do Distrito Federal – CETAS), all found in unhealthy conditions. The birds which died naturally from their injuries were evaluated for the integrity of their eyes on presentation. Only eyes that did not show evidence of ophthalmic disease were selected and collected. The collection of biological material from wild native species, for scientific purposes, was authorized by Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade - SISBIO), with protocol number SISBIO n.79141-2.

Sample collection and processing

The eyes were removed within a maximum of 30 minutes after death, with the use of transconjunctival enucleation technique. This technique consisted of a 360° perilimbic incision, dissection of the ocular attachments to isolate the globe, and transection of the optic nerve. The eyes were placed in 10% formaldehyde solution and sent to the Veterinary Pathology Laboratory (MVL Patologia Veterinária, Belo Horizonte, Brazil), where the samples were processed and analyzed by light microscopy.

Light microscopy (LM)

All 11 eyes were processed after fixation. They were embedded in paraffin blocks, then were subjected to serial cuts with a thickness of 4 μm, sections placed on slides, and stained with Hematoxylin and Eosin (HE) (Luna, 1968).

Histological analysis and description

The slides were analyzed using light microscopy for a description of the corneal layers. For birds that had both eyes evaluated, the average value of both corneas was calculated. The structures were measured in the central region and periphery of the cornea, and identified as follows: Central Cornea Full Thickness (CCFT), Peripherical Corneal Full Thickness (PCFT), Central Epithelium (CEp), Peripherical Epithelium (PEp), Central Bowman's Layer (CBL), Peripheral Bowman's Layer (PBL), Central Stroma (CS), Peripheral Stroma (PS), Central Descemet's Layer (CDL) and Peripheral Descemet's Layer (PDL). Values given in micrometers (μm). Endothelial thickness was not measured, as it was lost during histological processing.

Results

The evaluated corneas revealed an avascular tissue composed of four layers as previous described in other species, from anterior to posterior: the epithelium, Bowman's layer, stroma, Descemet's membrane. The internal endothelium was absent due to histological processing. Total corneal thickness varied between bird species, and between the corneal regions. The central region of the cornea was thinner than the peripheral region in A*sio stygius, Crotophaga ani, Turdus rufiventris, Ramphastos toco, Rhea americana* and *Nyctibius griseus*; and thicker in *Pitangus sulphuratus* and *Ara macao*. Table 2 summarizes the values found in the measurements of the corneal layers.

The epithelium is formed by nonkeratinizing stratified squamous cells. The number of epithelial layers is species-specific and corneal region specific, and in this study ranged from three to six cell layers, being composed of one layer of posterior basal cells, 1 to 3 layers of middle polyhedral squamous cells, and 1 to 3 layers of anterior flattened squamous cells. The number of layers and the thickness of the epithelium varied in the central and peripheral regions of the cornea. Table 3 shows the number of epithelial layers in each corneal region of the studied species.

The Bowman's layer was possible to be observed in all 8 birds studied. It also showed variation in its thickness in the center and periphery, and was thinner in the central cornea in *Turdus rufiventris, Ramphastos toco, Rhea americana, Ara macao, Pitangus sulphuratu* and *Nyctibius griseus* (Table 2).

The stroma represents the thickest portion of the cornea in the birds studied. With the exception of *Pitangus sulphuratu* and *Ara macao*, all birds had a central stroma thinner than the peripheral one (Table 2).

Attached to the innermost part of the stroma, the Descemet's membrane is found. The thickness of Descemet's membrane varied slightly between the regions of the cornea, and between the evaluated species (Table 2).

Discussion

Compared to intraspecific studies of retinal morphology, corneal morphometry has been much less studied, with the total thickness of the cornea being the most common measurement among researchers and clinicians. Through different microscopy models, thicknesses can be obtained, and the scientific purposes include veterinary medicine, human medicine, and biological sciences (Chard & Gundlach, 1938; Gonzalez-Alonso-Alegre, Martinez-Nevado, Caro-Vadillo, & Rodriguez-Alvaro, 2015; Liu et al., 2016; Moraes, 2018). Table 4 summarizes the results of studies carried out previously and the findings in this study, and shows that the cornea varies widely among birds. There is likely a correlation between the habitat and such morphological differences in the cornea (Abdelftah et al., 2021; Hall, 2008; Potier et al., 2020). The total thickness of the cornea can also vary significantly between animals of the same species, in relation to age, breed and regions of the cornea (Coyo et al., 2015; Montiani-Ferreira, Cardoso, & Petersen-Jones, 2004). Slight variation can be seen between left and right eyes, and males and females (Werther, Candioto, & Korbel, 2017). The curvature of the cornea also influences the thickness (Figure 1); birds with a flatter cornea have little difference between the center and the periphery (Collin & Collin, 2021). On the other hand, in birds with a large corneal curvature, such as the Golden Eagle *Aquila chrysaetos* (Murphy & Dubielzig, 1993), the Stygian Owl *Asio stygius* and Common Potoo *Nyctibius griseus* in this study, the periphery is substantially thicker than the center (Brooke, Hanley, & Laughlin, 1999; Murphy & Howland, 1983).

Not only does the total thickness of the cornea have variation, but the individual corneal layers vary in thickness as well. The epithelium is the corneal anterior surface is

178 composed by layers of stratified squamous and non-keratinized cells. The basal cell layer has
179 a cuboidal or columnar shape, covered by multiple layers of cells that become wider and
180 flatter as they move away from the base, which are polyhedral. The most superficial
181 squamous cells are almost completely flattened. They are called umbrella or wing cells, as
182 their extent overlaps the apices of more than one cell. This epithelial pattern is found in
183 previously studied mammals and birds, including the birds in this study (Figure 2)
184 (Bergmanson, 2019; Collin & Collin, 2000, 2006, 2021; Mayakkannan, Ramesh, Kumaravel,
185 Venkatesan, & Kannan, 2018; Monção-Silva et al., 2016; Nautscher et al., 2016; Pinto, Cruz,
186 Teixeira, Couto, & Carvalho, 2016; Sokolenko et al., 2021). The number of epithelial cell
187 layers varies between species, and among the birds studied here, a variation from 3 to 6 layers
188 was observed (Table 3). In the Little Penguin *Eudyptula minor*, 5 to 6 layers were found in
189 the epithelium (Collin & Collin, 2021). The African Penguin *Spheniscus demersus* was found
190 to have 4 layers of epithelial cells and an epithelial thickness 15 µm (Sokolenko et al., 2021).
191 Raptor epithelium ranges from 2 to 5 layers thick (Pinto et al., 2016), except in the Golden
192 Eagle where an epithelium of 8 layers and 50 µm of thickness was found (Murphy &
193 Dubielzig, 1993). In this study, the thickest epithelium was observed in the Greater Rhea
194 *Rhea americana* (strictly terrestrial and diurnal bird), with up to 6 epithelial layers, and the
195 thinnest being the Stygian Owl and Common Potoo (nocturnal birds), having a maximum of 4
196 lines. The Greater Rhea´s cornea also had a high epithelial proportion with respect to the total
197 thickness of the cornea, being 10.3% (Figure 3). In a study by Popova *et al.* (2022), the ratio
198 of the epithelium thickness to the total corneal thickness was similarly defined, where the
199 Hyacinth Macaw *Anodorhynchus hyacinthinus* demonstrated the highest epithelial proportion
200 in the group of birds at 9.9% of the corneal thickness (Popova et al., 2022). In the present
201 study, the Scarlet Macaw *Ara macao* demonstrated that 8.09% of the total thickness of the
202 cornea corresponds to the epithelium (Figure 3). The Smooth-billed Ani *Crotophaga ani* was
203 the bird that showed the highest epithelium *vs.* total cornea ratio at 16% (Figure 3). The
204 Passeriformes Rufous-bellied Thrush *Turdus rufiventris* and Great Kiskadee *Pitangus*
205 *sulphuratus*, studied here, presented 10.78% and 8.88% of epithelium *vs.* total cornea ratio, as
206 observed in the Java Sparrow *Lonchura oryzivora*, also passerine, described by Popova *et al.*
207 (2022), with 8.9% of epithelium. Past hypotheses for differential epithelial thicknesses relate
208 to the habitat of different species, where those with greater risk of trauma (diving birds, birds
209 in arid environments, or those living in dense fauna) may have greater epithelial thickness,
210 particularly in proportion to total epithelial thickness (Almubrad & Akhtar, 2012; Collin &
211 Collin, 2006; Nautscher et al., 2016; Popova et al., 2022; Swamynathan, Crawford, Robison,

Kanungo, & Piatigorsky, 2003). The total size of the bird also likely plays a role in total epithelial thickness.

The Bowman's layer (BL), also referred to as the Anterior Limiting Lamina, is a continuous meshwork of condensed collagen fibers located in the anterior stroma, beneath the epithelium (Bergmanson, 2019; Hayashi, Osawa, & Tohyama, 2002; Meek & Fullwood, 2001). This layer is the corneal structure that has the greatest morphological variation between species, with a chance of not even being present (Abdelftah et al., 2021; Hayashi et al., 2002; Kafarnik et al., 2007; Merindano, Costa, Canals, Potau, & Ruano, 2002; Moore et al., 2022; Popova et al., 2022). More developed mammals have a well-defined BL, as observed in deers, giraffes, humans and other primates (Hayashi et al., 2002; Merindano et al., 2002; Popova et al., 2022; Wilson, 2020). This layer is also described in several species of birds, such as chickens, quails, ducks, pelicans, birds of prey, penguins, parrots and Passeriformes (Abdelftah et al., 2021; Carvalho, Rodarte-Almeida, Santana, & Galera, 2018; Collin & Collin, 2021; Gonçalves, Pérez-Merino, Martínez-García, Barcía, & Merayo-Loves, 2016; Kafarnik et al., 2007; Mayakkannan et al., 2018; Moore et al., 2022; Murphy & Dubielzig, 1993; Pinto et al., 2016; Popova et al., 2022; Willis & Wilkie, 1999). However, there is no consensus on the definition of BL in animals. Popova *et al.* (2022) and Merindano *et al.* (2002), consider that BL in birds is rudimentary, since when compared to humans and other primates, it is not clearly defined. Alternatively, Kafarnik *et al.* (2007), described the BL of birds as similar to that of primates, being acellular and with homogeneous reflectivity, observed through *in vivo* confocal microscopy. This is in corroboration with Gonçalves *et al.* (2016), who suggested that the chicken cornea is an excellent research model for refractive surgeries in humans, due to the similarity of corneal structures, with an emphasis on the Bowman's layer.

In this study, it was possible to observe that the outermost margin of the BL is distinct, as it borders the basement membrane of the epithelium. However, its innermost limit is not as distinct, as the margin is progressively incorporated into the stroma, making it challenging to measure the thickness of this layer. Collin & Collin (2021) describe not having clear enough definition to measure the extent of the BL in the Little Penguin, but stated that it is located 5µm deep in the stroma. In another study, by Sokolenko et al. (2021), the African Penguin´s BL was not described. Among the birds studied here, we found that the Great Kiskadee, the Stygian Owl, the Greater Rhea and the Rufous-bellied Thrush, have a BL with visible anterior and posterior delimitation, but with low contrast in relation to the stroma (Figure 4). It was observed in many histological samples of this study that the stroma suffers from the presence

of artifacts in its interior caused by the penetration of the processing substances (Figure 5). These artifacts are randomly present in the substantia propria, but do not extend into the BL. The same pattern was observed in histological images from other studies (Bastola, Song, Gilger, & Hirsch, 2020; Feizi, 2018; Merindano et al., 2002; Pinto et al., 2016; Popova et al., 2022). It is possible to suggest that this happens due to the high condensation of collagen fibers in the BL, making it more difficult for them to break.

The stroma, or substantia propria, is a dense connective tissue meshwork formed by overlapping collagen fibril lamellae aligned parallel to the corneal surface, with scattered keratocytes between them. The density, orientation of the lamellae, and the concentration of keratocytes vary between the regions and the stroma depth, as well as between the species (Abdelftah et al., 2021; Bergmanson, 2019; Crespo-Moral, García-Posadas, López-García, & Diebold, 2020; Kafarnik et al., 2007; Meek & Fullwood, 2001; Meek & Knupp, 2015; Nautscher et al., 2016; Tsukahara et al., 2010). In birds, the collagen lamellae are aligned with each other, forming a precise and regular orthogonal arrangement, with a large number of branches promoting anastomosis of the bundles. This arrangement is most evident in the anterior and middle portion of the substantia propria, and is associated with greater mechanical rigidity and better light transmittance in the UV spectrum – meaning that the sunlight in contact with the cornea is more scattered, decreasing the amount of light entering the eyes (Boote et al., 2011; Collin & Collin, 2021; Gonçalves et al., 2016; Koudouna et al., 2018; Tsukahara et al., 2010). Tsukahara *et al.* (2010) compared the corneas of birds with mammals, demonstrating that birds have a lower density of keratocytes distributed in the stroma, which are more concentrated in the anterior portion. In histological images from this study, it is also possible to identify that Smooth-billed Ani and Rufous-bellied Thrush have more keratocytes in the anterior stroma (Figure 5). Birds also have thicker collagen lamellae than mammals, with greater lamella thickness indicating better light refraction power (Boote et al., 2011; Tsukahara et al., 2010). Another characteristic of the stroma is that it represents the thickest portion of the cornea, measuring greater than 90% of the total corneal thickness (Abdelftah et al., 2021; Boote et al., 2011; Crespo-Moral et al., 2020; Gonçalves et al., 2016; Koudouna et al., 2018; Nautscher et al., 2016). According to the present study, five birds demonstrated a stromal thickness between 91 and 95% of the total thickness of the cornea, while three, Rufous-bellied Thrush, Scarlet Macaw and Smooth-billed Ani, demonstrated 76, 78 and 79%, respectively (Figure 3).

In most animals, the stroma is thinner at the center than at the periphery of the cornea, thus making the central cornea thinner than the peripheral cornea (Bergmanson, Burns, &

Walker, 2019; Meek & Leonard, 1993; Werther et al., 2017). However, some species demonstrate a thinner peripheral cornea than the center, while others do not demonstrate significant differences between the thicknesses of the regions (Collin & Collin, 1998, 2021; Coyo et al., 2015; Henriksson, Bron, & Bergmanson, 2012; Moore et al., 2019). In the present study, the Scarlet Macaw and the Great Kiskadee showed a slightly thinner periphery than the center, with a mean variation of 14 µm. The Rufous-bellied Thrush's cornea also varied slightly, but in an opposite manner, being 13 µm thicker at the periphery. The Stygian Owl, the Toco Toucan, the Greater Rhea and the Common Potoo, have a more pronounced variation between the regions, being between 128 and 387 µm thicker in the periphery, respectively. Reasons why the stroma undergoes size changes between corneal regions have not yet been well explained, but it is known that collagen lamellae undergo variations in size, thickness and quantity according to the stromal region and depth (Bergmanson, Burns, & Naroo, 2021; Boote et al., 2011; Henriksson et al., 2012; Meek & Fullwood, 2001).

Conclusion

The histomorphometric description of the cornea, through light microscopy, proved to be efficient for the study of different birds' species from Brazil, being an accessible method. Similarities were found between the cornea of birds and other vertebrates, but with specific differences in the metrics of each species. A better understanding of the structures can help to explain the form of visual interaction of birds with their environment, as well as improve knowledge on how to interpret pathological changes in the birds' cornea.

Acknowledgment

This study was funded by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil – Funding code 001, and by Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF), Brazil – proc.n.* 00193.00001303/2019-10).

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Figure 1. Macroscopy of the ocular bulbs demonstrating the variation of the corneal curvature, of Crotophaga ani (A) with a low degree of curvature, and of *Nyctibius griseus* (B), with high convexity.

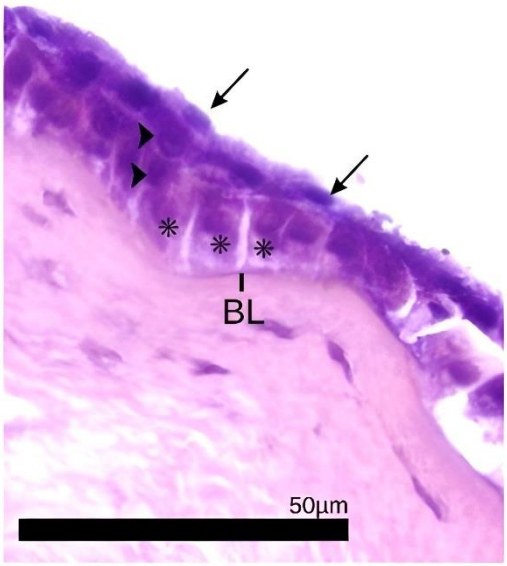

Figure 2. Cornea of the *Turdus rufiventris*. Columnar-shaped basal epithelial squamous cells (asterisk), polyhedral cells (arrowhead), and wing cells (arrows). The Bowman's membrane (BL) can be visualized in the image below. Measurements were carried out using the Opticam O500R microscope, with OPTHD software, 400x magnification.

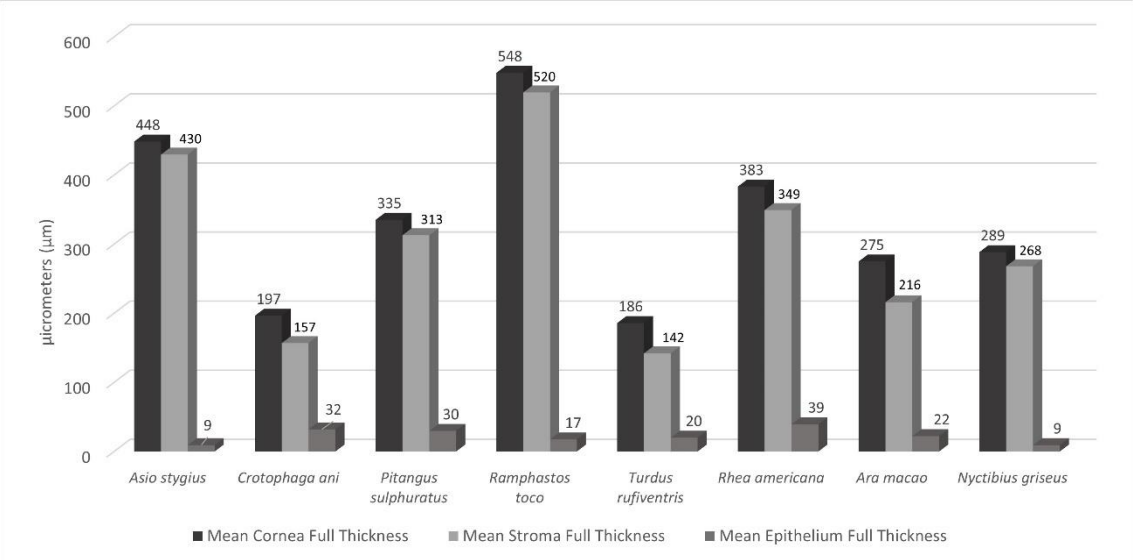

Figure 3. Representation of the percentage of epithelium and stroma in relation to the total corneal thickness of the birds.

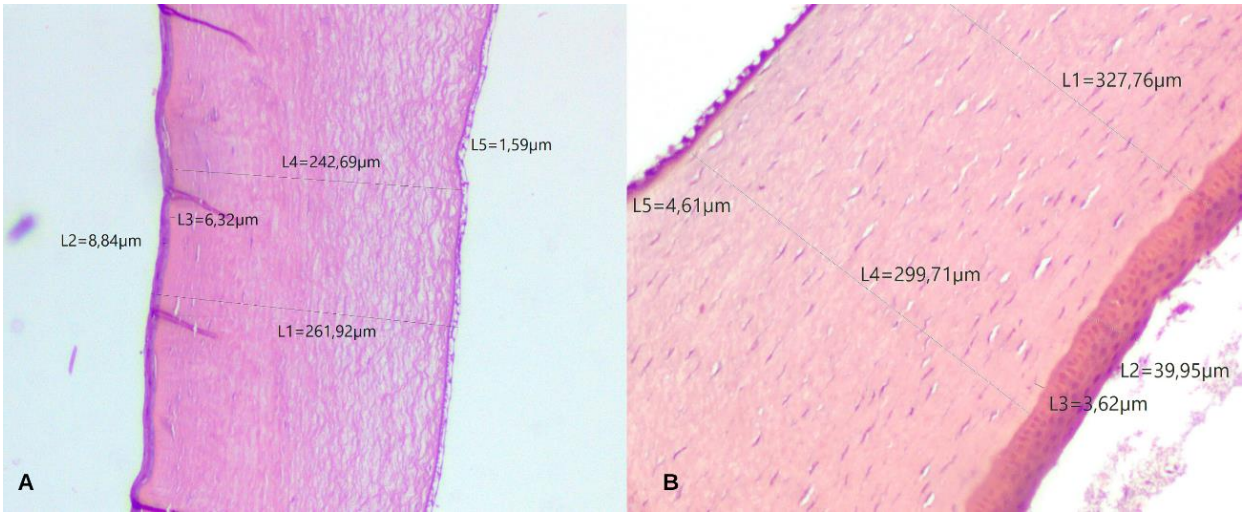

Figure 4. Image of the Image J software interface demonstrating the measurement of total corneal thickness (L1) and its individual layers; Epithelium (L2), Bowman's membrane (L3), stroma (L4), Descemet's membrane (L5). A) Central region of the *Asio stygius* cornea; B) Central corneal region of *Rhea americana*. Measurements were carried out using the Opticam O500R microscope, with OPTHD software, 100x magnification.

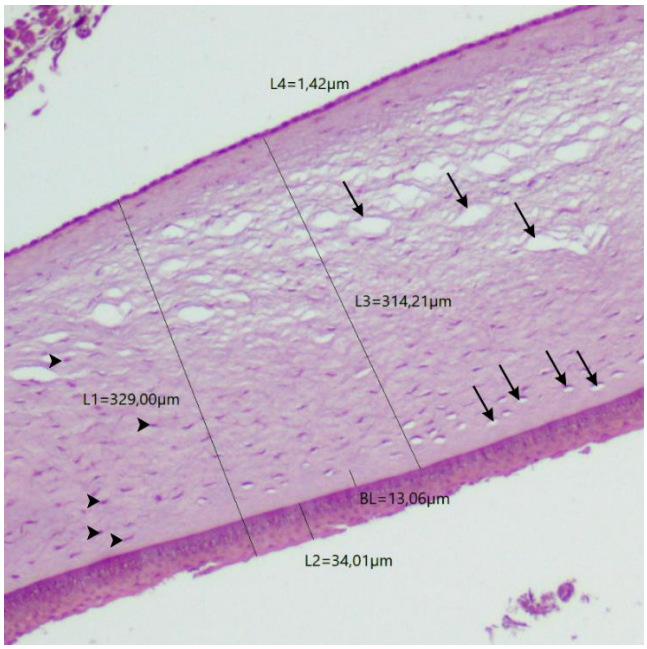

Figure 5. Peripheral region of the *Pitangus sulphuratus* cornea. Eosinophilic keratocyte nuclei (arrowhead) and artifacts (arrows) scattered throughout the stroma. Measurements referring to the thickness of the total cornea (L1), epithelium (L2), Bowman's membrane (BL), stroma (L3) and Descemet's membrane (L4). Measurements were carried out using the Opticam O500R microscope, with OPTHD software, 100x magnification.

Table 1. Order, family, and popular names of bird species.

Species	Order	Family	Brazilian popular names	Popular names in IUCN Red List
Asio stygius (Wagler, 1832)	Strigiformes	Strigidae	Mocho-preto	Stygian Owl
Crotophaga ani (Linnaeus 1758)	Cuculiformes	Cuculidae	Anu-preto	Smooth-billed Ani
Pitangus sulphuratus (Linnaeus, 1766)	Passeriformes	Tyrannidae	Bem-te-vi	Great Kiskadee
Ramphastos toco (Statius Muller, 1776)	Piciformes	Ramphastidae	Tucanuçu	Toco Toucan
Turdus rufiventris (Vieillot, 1818)	Passeriformes	Turdidae	Sabiá-laranjeira	Rufous-bellied Thrush
Rhea americana (Linnaeus, 1758)	Rheiformes	Rheidae	Ema	Greater Rhea
Ara macao (Linnaeus, 1758)	Psittaciformes	Psitacidae	Araracanga	Scarlet Macaw
Nyctibius griseus (Gmelin, 1789)	Nyctibiiformes	Nyctibiidae	Urutau	Common Potoo

Table 2. Measurement of the total cornea and its layers from avian species, in micrometers (µm).

	Cornea Full Thickness		**Epithelium**		**Bowman's Layer**		**Estroma**		**Descemet's Layer**	
Species	Central	Peripherical	Central	Peripherical	Central	Peripherical	Central	Peripherical	Central	Peripherical
Asio stygius	254.76	642.2	9.53	8.91	4.3	3.02	236.08	623.19	1.92	2.33
Crotophaga ani	172.27	220.75	37.71	26.32	4.24	4.04	131.39	182.40	1.59	1.27
Pitangus sulphuratus	341.45	329	25.58	34	10.16	13.06	311.64	314.21	2.01	1.42
Ramphastos toco	374.36	721.38	18.65	16.31	6.27	9.3	344.2	694.87	3.06	3.27
Turdus rufiventris	179.33	192.25	19	21.09	3	3.34	140.45	143.45	3.14	2.11
Rhea americana	327.76	439	39.95	39.03	3.62	4.32	299.71	399.14	4.61	3.17
Ara macao	282.88	266.97	21.08	23.43	3.52	7.84	232.06	200.07	19.72	13.94
Nyctibius griseus	224.5	352.5	8.5	10	2.81	4.48	206.6	329.44	3.37	1.55

Table 3. Number of layers and characteristics of the corneal epithelium from avian species.

	Epithelial layers	
Species	Central	Peripherical
Asio stygius	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)
Crotophaga ani	5 to 6 layers (1 basal, 2 to 3 polyhedral squamous and 2 flat squamous)	4 to 5 layers (1 basal, 1 to 2 polyhedral squamous and 2 flat squamous)
Pitangus sulphuratus	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)	4 to 5 layers (1 basal, 1 to 2 polyhedral squamous and 2 flat squamous)
Ramphastos toco	3 to 5 layers (1 basal, 1 to 3 polyhedral squamous and 1 flat squamous)	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)
Turdus rufiventris	3 to 5 layers (1 basal, 1 to 3 polyhedral squamous and 1 flat squamous)	3 to 5 layers (1 basal, 1 to 3 polyhedral squamous and 1 flat squamous)
Rhea americana	3 to 6 layers (1 basal, 1 to 3 polyhedral squamous and 1 to 2 flat squamous)	3 to 6 layers (1 basal, 1 to 3 polyhedral squamous and 1 to 2 flat squamous)
Ara macao	3 to 5 layers (1 basal, 1 to 3 polyhedral squamous and 1 flat squamous)	3 to 5 layers (1 basal, 1 to 3 polyhedral squamous and 1 flat squamous)
Nyctibius griseus	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)	3 to 4 layers (1 basal, 1 to 2 polyhedral squamous and 1 flat squamous)

Table 4. Total corneal thickness of previously studied bird species and of the birds studied here, including their sizes/weights, habits and feeding.

Species (Popular Name)	Size & Weight [1]	Habits [1,2]	Feeding [1,2]	Total Corneal Thickness	Source
***Eudyptula minor* (Little Penguin)**	30 cm 1.1 - 1.2 kg	Diurnal, amphibious, flightless	Piscivore	380 ± 54 µm (central region)	(Collin & Collin, 2021)
***Spheniscus demersus* (African Penguin)**	45 cm 3.1 kg	Diurnal, amphibious, flightless	Piscivore	450 µm (region not specified)	(Sokolenko *et al.*, 2021)
***Spheniscus demersus* (African Penguin)**	45 cm 3.1 kg	Diurnal, amphibious, flightless	Piscivore	384 ± 30 µm (central region)	(Gonzalez-Alonso-Alegre *et al.*, 2015)
***Spheniscus humboldti* (Humboldt Penguin)**	66 - 70 cm 4 - 5 kg	Diurnal, amphibious, flightless	Piscivore	636 µm† (region not specified)	(Popova *et al.*, 2022)
***Gallus gallus domesticus* (Domestic chickens)**	40 - 60 cm 2580.2 g	Diurnal, terrestrial, domestic	Granivore and insectivore	242 µm (central region)	(Montiani-Ferreira *et al.*, 2004)
***Gallus gallus domesticus* (Domestic chickens)**	40 - 60 cm 2.6 - 4.5 kg	Diurnal, terrestrial, domestic	Granivore and insectivore	225.3 ± 30 µm (region not specified)	(Gonçalves *et al.*, 2016)
***Coturnix coturnix* (Common Quail)**	17.5 cm 70 - 155 g	Diurnal, terrestrial, grassland	Granivore	154 ± 17.7 µm (region not specified)	(Gonçalves *et al.*, 2016)

Coturnix japonica **(Japanese Quail)**	16-18 cm 90 - 115 g	Diurnal, terrestrial, grassland	Granivore	138.64 µm (region not specified)	(Mayakkannan *et al.*, 2018)
Ostrich (species not described by the author)	180 - 270 cm 90 - 130 kg	Diurnal, terrestrial, flightless	Omnivore	550 ± 35 µm (central region)	(Liu *et al.*, 2016)
Rhea americana **(Greater Rhea)**	1.34 - 1.70m 26 - 36 kg	Diurnal, terrestrial, flightless	Omnivore	327.76 µm (central region) 439 µm (peripherical region)	This study
Harpia harpyja **(Harpy Eagle)**	89 - 102 cm 6 - 9 kg	Diurnal, raptor, rainforests	Carnivore	563 µm (central region)	(Moraes, 2018)
Aquila chrysaetos **(Golden Eagle)**	70 - 84 cm 3 - 6.125 kg	Diurnal, raptor, open or semi-open areas	Carnivore	640 µm (central region) 1200 µm (peripherical region)	(Murphy & Dubielzig, 1993)
Falcon tinnunculus **(Common Kestrel)**	36 - 58 cm 907 g	Diurnal, raptor, open or semi-open areas	Carnivore	129 µm (central region) Varies from 197 to 210.8 µm (peripherical region)	(Werther *et al.*, 2017)
Asio stygius **(Stygian Owl)**	38 - 46 cm 400 - 675 g	Nocturnal, raptor, open or semi-open areas	Carnivore	254.76 µm (central region) 642.2 µm (peripherical region)	This study
Nyctibius griseus **(Common Potoo)**	34 - 38 cm 160 - 190 g	Nocturnal, open or semi-open areas	Insectivore	224.5 µm (central region) 352.5 µm (peripherical region)	This study
Columba livia **(Domestic pigeon)**	29 - 35 cm 315 - 410 g	Diurnal, domestic, urban areas	Granivore	157 µm (central region) 188 µm and 169 µm (peripherical nasal and temporal regions)	(Chard & Gundlach, 1938)
Calypte anna **(Anna's Hummingbird)**	10 cm 4 – 4.5 g	Diurnal, scrub forest	Nectarivore	59 µm (central region) 48 µm (peripherical region)	(Moore *et al.*, 2019)

Pitangus sulphuratus **(Great Kiskadee)**	21 - 26 cm 52 - 68 g	Diurnal, rainforests, urban areas	Omnivore	341.45 µm (central region) 329 µm (peripherical region)	This study
Turdus rufiventris **(Rufous-bellied Thrush)**	25 cm 68 g	Diurnal, rainforests, urban areas	Omnivore	179.33 µm (central region) 192.25 µm (peripherical region)	This study
Lonchura oryzivora **(Java Sparrow)**	15 - 17 cm 24.5 g	Diurnal, open grassland	Granivore	166 ± 5 µm (region not specified)	(Popova *et al.*, 2022)
Crotophaga ani **(Smooth-billed Ani)**	35 cm 115 g	Diurnal, rainforests, urban areas	Omnivore	172.27 µm (central region) 220.75 µm (peripherical region)	This study
Ramphastos toco **(Toco Toucan)**	61 cm 592 - 760 g	Diurnal, scrub forests	Omnivore	374.36 µm (central region) 721.38 µm (peripherical region)	This study
Ara macao **(Scarlet Macaw)**	89 cm 1.2 kg	Diurnal, rainforests	Frugivore	282.88 µm (central region) 266.97 µm (peripherical region)	This study
Anodorhynchus hyacinthinus **(Hyacinth Macaw)**	1 m 1.2 - 1.7 kg	Diurnal, rainforests	Frugivore	472 µm† (region not specified)	(Popova *et al.*, 2022)
Platalea leucorodia **(Eurasian Spoonbill)**	80 - 90 cm 1.7 - 2 kg	Diurnal, wetlands	Piscivore	436 µm† (region not specified)	(Popova *et al.*, 2022)

†Approximate mean total corneal thickness.

[1]https://animaldiversity.org/

[2]https://www.iucnredlist.org/

Gmail **Rafaela Tozetti <rafaelartozetti@gmail.com>**

Anatomia, Histologia, Embryologia - Manuscript ID AHE-06-23-OA-154 [email ref: SE-6-a]

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Cc: dra.paulagalera@gmail.com, rafaelartozetti@gmail.com, mvlpatologiaveterinaria@gmail.com, roselialaraujo@hotmail.com, liriahirano@unb.br, bretskimoore@gmail.com

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Dear Prof. Galera:

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CAPÍTULO III

Original Article (*Anatomia, Histologia, Embryologia*)

Evaluation of the Common Pauraque (*Nyctidromus albicollis*) cornea using light and scanning electron microscopy

Rafaela Alves Ribon Tozetti[1], Rosélia de Lima Sousa Araújo[1], Matheus Vilardo Loes Moreira[2], Larissa Cristina de Souza Akiyama[1], José Raimundo Corrêa[3], Bret A. Moore[4] and Paula Diniz Galera[1].

[1]Comparative Ophthalmology Laboratory, School of Agricultural Sciences and Veterinary Medicine, University of Brasília, Brasília, DF, Brazil.
[2]Laboratory of Veterinary Pathology, MVL Patologia Veterinária, Belo Horizonte, MG, Brazil
[3]Laboratory of Microscopy and Microanalyses, Institute of Biology, University of Brasilia, Distrito Federal, Brazil
[4]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Correspondence
Paula Diniz Galera, University of Brasilia (UnB), Campus Darcy Ribeiro, UnB – Asa Norte, DF, Brazil. 70910-900.
Phone number: +55 (61) 99266-0799
Email: dra.paulagalera@gmail.com

Acknowledgments

This study was funded by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil – Funding code 001, and by Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF), Brazil – proc.n.* 00193.00001303/2019-10). The authors thank the team of the Laboratory of Microscopy and Microanalysis IB/UnB and the Department of Wild Animals FAV/UnB in name of DVM, PhD Liria Queiroz Luz Hirano, for providing their facilities to perform this research.

Abstract

The Common Pauraque *Nyctidromus albicollis* (Gmelin, 1789) is a widespread avian species, however due to its nocturnal habits and reclusive behavior, little is known about their vision and ecology. Most avian species are visually dependent with advanced visual systems providing high spatial resolution, temporal resolution, or sensitivity depending on the species needs. Each ocular structure has a specific role in contributing toward high visual function, and the cornea is the first refractive structure in the visual process. No morphological or morphometric evaluation has been described on the Common Pauraque cornea. This study aims to describe the morphology and morphometry of the Common Pauraque cornea by means of light and scanning electron microscopy to evaluate the cross-sectional anatomy as well as the ultrastructure of the endothelial cells. A better understanding of the Common Pauraque cornea can help us better explain the physiology of vision and the visual requirements of this species. In turn, this will help us better understand how this species successfully interacts with its environment, and will improve our knowledge on how to interpret pathological changes in their cornea in a clinical setting.

Keywords: avian eye, bird, cornea, endothelium, histology, morphological.

Introduction

The Common Pauraque *Nyctidromus albicollis* (Gmelin, 1789) is a small bird of the caprimulgiform order, weighting 50 to 70g and measuring 20 to 30 cm in length. It can be found throughout Central and South America, largely in tropical regions and barring mountainous regions, and to a lesser degree in the Southern tip of Texas, United States (BirdLife International, 2020; Guilherme & Lima, 2020; Thurber, 2003; Trupkiewicz, Garner, & Juan-Sallés, 2018). This species is insectivorous, hunting along brushwood and forest edges, and has a crepuscular to nocturnal activity pattern (Cadbury, 1981; Pérez-Granados & Schuchmann, 2020). Insectivorous birds generally need high spatial and temporal resolution to successfully capture prey. Caprimulgiformes, however, have the added need for visual specializations that enable successful foraging in scotopic conditions, such as proportionally large eyes positioned to optimize the dorsal visual field, large palpebral openings, and high rod-concentration in the retina (Delaunay, Larsen, Lloyd, Sullivan, & Grant, 2020; Martin, Rojas, Figueroa, & McNeilo, 2004; Moore, Montiani-Ferreira, & Gardner, 2022; Salazar *et al*., 2020). Additionally, high visual resolution may benefit detection of predators in addition to their camouflage brown and gray plumage to help remain conspicuous while nesting on the ground among leaves (Guilherme & Lima, 2020; Pérez-Granados & Schuchmann, 2020; Sandoval & Escalante, 2011; Thurber, 2003).

Generally, birds have complex visual systems that provide them with excellent visual acuity, and each ocular structure has its specific role in enabling high acuity (Butler, Templeton, & Fernández-Juricic, 2018; Fernández-Juricic, 2012; Martin, 2022; Salazar *et al*., 2020). However, despite being a common and widespread avian species, there is still much to learn about pauraque vision and ecology (Guilherme & Lima, 2020; Pérez-Granados & Schuchmann, 2020; Thurber, 2003). No morphological and morphometric evaluations have been performed describing the pauraque cornea. The goal of the present study is to describe the normal structure of the Common Pauraque cornea, through light microscopy and scanning electron microscopy, to contribute toward our understanding of their visual biology. Primarily, evaluation of the corneal layers and thickness, the average cell area and cell densities, coefficient of variation of the average cell area, and the morphology of the corneal endothelium are described.

Materials and methods

Animals

Six Common Pauraques *Nyctidromus albicollis* (Caprimulgidae) were included, from which 12 eyes were evaluated. The birds were from the Department of Wild Animals (Faculty of Agronomy and Veterinary Medicine of the University of Brasília). They arrived in unhealthy conditions after being rescued by the Wild Animal Screening Center (Centro de Triagem de Animais Silvestres do Distrito Federal – CETAS). The exact age was not able to be determined since they were free-living animals, although all were adults. The birds died naturally after arrival from their poor condition. The collection of biological material for scientific purposes was authorized by Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade - SISBIO), with protocol number SISBIO n.79141-2. At the time of death, the eyes were evaluated and did not show evidence of ophthalmic disease.

Sample collection and processing

The eyes were removed via transconjunctival enucleation within a maximum of 30 minutes after death. The technique consisted of a 360° perilimbal incision, dissection of the ocular attachments to isolate the globe, and transection of the optic nerve. Ten eyes were evaluated via light microscopy, and two eyes by scanning electron microscopy.

Light microscopy (LM)

Ten eyes were placed in 10% formaldehyde and were sent to the Veterinary Pathology Laboratory (MVL Patologia Veterinária, Belo Horizonte, Brazil) for light microscopic analysis. After fixation, they were embedded in paraffin. The blocks containing the sample were subjected to serial cuts with a thickness of 4 μm, which were placed on slides and stained with hematoxylin and eosin (HE) (Luna, 1968).

Scanning electron microscopy (SEM)

Two eyes were sent to the Microscopy and Microanalyses Laboratory (Institute of Biology at the University of Brasilia) for preparation of scanning electron microscopy (SEM). The cornea was separated from the globe, followed by fixation in 2% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), for 24 h at room temperature.

After washing in 0.1 M sodium cacodylate buffer (pH 7.2), corneal samples were fixed in 2% osmium tetroxide, 1.6% potassium ferricyanide (1:1 v/v) and 5 mM CaCl2 in sodium cacodylate buffer for 1 hour at room temperature, followed by washing in 0.1 M sodium cacodylate buffer. The samples were kept for 24 hours in 0.5% aqueous uranyl acetate solution at 4°C, washed in distilled water and dehydrated in an ascending series of acetone (30, 50, 70, 90 and 100%). Subsequently, the corneas were dried at a critical point (Critical Point Drying 0 CPD 0 Balzers) in liquid CO2 and fixed in metallic *stubs*, with the endothelium facing up, using double-sided carbon tape. Subsequently, they were metallized with a layer of gold at 20 nm, using a high vacuum metallizer (Leica EM SCD500). Sample analysis was performed using a Jeol JSM-7000F Field Emission Scanning Electron Microscope (Jeol Ltd.).

Only images of the corneal endothelial cells were segmented and analyzed by SEM. The evaluated parameters were the mean cell area (MCA - μm^2), endothelial cell density (CD - cell/mm^2), polymegathism (measured by the coefficient of cell variation - CV) (Doughty, 2008) and pleomorphism (measured by the percentage of hexagonal cells) (Coyo et al., 2019, 2015; Doughty & Oblak, 2008; Franzen, Pigatto, Abib, Albuquerque, & Laus, 2010). In total, 100 endothelial cells were analyzed. The cellular area was determined using Image J Software. Endothelial CD was calculated by dividing the number of cells per 0,033 mm^2. The results were described by Mean ± SD (standard deviation). The coefficient of variation (CV) was determined by dividing the standard deviation of the areas by the average of cellular area. Finally, the percentage of pleomorphism was manually counted in the same group of cells.

Results

Histological analysis with light microscopy

The mean thickness of the pauraque central cornea (146.2 ± 34.5 µm) was slightly thinner than the peripheral area (149.2 ± 35.8 µm) between the central cornea and the limbus. The pauraque cornea was composed of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium, from the outermost to innermost. The epithelium was nonkeratinizing stratified squamous cells and formed by 3 to 4 layers measuring 10.2 ± 3.4 µm in the center, and 2 to 3 layers in peripheral area measuring 8 ± 3.9 µm. Just below it, there was a continuous acellular basement membrane, known as Bowman's layer (center area 3 ± 1.1 µm and peripheral area 3.5 ± 1.2 µm). The stroma represents the thickest portion of the cornea and was composed of collagen fibrils arranged approximately parallel to the

corneal surface (center thickness 126.7 ± 30 µm and peripheral thickness 135 ± 37.5 µm). Attached to the innermost part of the stroma, Descemet's membrane had a central thickness of 2.2 ± 1.2 µm and peripheral thickness of 1.9 ± 0.7 µm). Posterior to Descemet's membrane was a single layer of endothelial cells. Figure 1 exemplifies the measurements performed on the Common Pauraques corneas.

Analysis in Scanning Electron Microscopy

The endothelial cell density, mean cell area, degree of polymegathism and pleomorphism were obtained by SEM. The Common Pauraque corneal endothelium revealed a monolayer of polygonal cells with uniform size and shape (Fig. 2). Interdigitations and microvilli were visualized at the cell borders and in the center (Fig. 3). The mean cell area of the corneal endothelium was 311.659 ± 95.86 μm^2 and the endothelial cell density was 3,032.70 cell/mm^2. The coefficient of variation was 0.30. Most endothelial cells were hexagonal (85%), followed by pentagonal cells (10%), octagonal (2%), heptagonal (2%) and quadrilateral (1%).

Discussion

The present study is the first to describe the characteristics of the Common Pauraque (*Nyctidromus albicollis*) cornea. Light microscopic evaluation showed similarities to most avian species previously studied: it is avascular, formed predominantly by collagen tissue, and composed of five layers from outside to inside: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium (Bayón, Almela, & Talavera, 2008; Carvalho, Rodarte-Almeida, Santana, & Galera, 2018; Collin & Collin, 2021; Kafarnik, Fritsche, & Reese, 2007; Pinto, Cruz, Teixeira, Couto, & Carvalho, 2016; Sokolenko *et al*., 2021; Werther, Candioto, & Korbel, 2017).

The Common Pauraque corneal epithelium consisted of stratified, squamous and non-keratinized cells, similar to that found in other bird species (Collin & Collin, 2021; Monção-Silva *et al*., 2016; Pinto *et al*., 2016; Sokolenko *et al*., 2021). Centrally the epithelium consisted of 3 to 4 layers and measured 10.2 ± 3.4 µm thick, whereas peripherally only 2 to 3 layers were detected measuring 8 ± 3.9 µm thick. Such dimensions are noticeably thinner in relation to other avian species (Collin & Collin, 2021; Murphy & Dubielzig, 1993; Sokolenko *et al*., 2021). For instance, the corneal epithelium of Little Penguins *Eudyptula minor* has a thickness of 26 ± 7 µm and is composed of 5-6 rows of stratified squamous cells (Collin &

Collin, 2021). Yet, the African Penguin *Spheniscus demersus* presented values closer to the Common Pauraque, demonstrating 4 lines of cells and 15 µm of thickness in the epithelium (Sokolenko *et al*., 2021). In the Golden Eagle *Aquila chrysaetos*, Murphy & Dubielzig (1993), found an epithelium with 8 layers and 50 µm thick. The author suggested that the distinct number of cell layers is a result of both size differences between species and their eyes, but also due to the cornea's exposure to adverse environmental conditions depending on a species ecology (e.g. a diving bird vs. a strictly terrestrial species, as in this study) (Almubrad & Akhtar, 2012; Collin & Collin, 2006; Nautscher, Bauer, Steffl, & Amselgruber, 2016).

The avian cornea has been reported to be thinner than the corneas of similarly sized mammals (Moore, Fernandez-Juricic, Hawkins, Montiani-Ferreira, & Lange, 2022; Willis & Wilkie, 1999). Additionally, studies in most species support that the corneal periphery is thicker than the central cornea (Bergmanson, 2019; Bergmanson, Burns, & Naroo, 2021; Collin & Collin, 2021; Coyo *et al*., 2015; Downie *et al*., 2021; Jones, Pierce, & Ward, 2007; Pinto *et al*., 2016). The total corneal thickness of Common Pauraque is 146.2 ± 34.5 µm centrally, and 149.2 ± 35.8 µm in the periphery. In most other avian species, considerably higher values can be seen (Chard & Gundlach, 1938; Collin & Collin, 2021; Gonçalves, Pérez-Merino, Martínez-García, Barcía, & Merayo-Loves, 2016; Gonzalez-Alonso-Alegre, Martinez-Nevado, Caro-Vadillo, & Rodriguez-Alvaro, 2015; Liu *et al*., 2016; Montiani-Ferreira, Cardoso, & Petersen-Jones, 2004; Moore & Montiani-Ferreira, 2022; Moraes, 2018; Murphy & Dubielzig, 1993; Sokolenko *et al*., 2021; Werther *et al*., 2017) (Tab. 1). Species smaller than the Common Pauraque have been shown to have thinner corneas, such as in the Anna's and Black-Chinned Hummingbirds (Moore *et al*., 2019), and in the Japanese Quail (Mayakkannan, Ramesh, Kumaravel, Venkatesan, & Kannan, 2018) (Tab. 1). Similar to corneal epithelial thickness, the total thickness of the cornea likely is dependent on multiple factors including body mass and eye size, and the visual ecology of a given species (Hall, 2008; Hall & Ross, 2007; Tyrrell & Fernández-Juricic, 2017).

In contrast to the thickness of the epithelium, the central corneal stroma (126.7 ± 30 µm) was thinner than in the periphery (135 ± 37.5 µm) in the Common Pauraque. A same pattern was observed in Little Penguins *Eudyptula minor* (center 312 µm and periphery 350 µm) (Collin & Collin, 2021), Common Kestrel *Falco tinnunculus* (center ranged from 96 to 112 µm, and periphery 165 to 198 µm) (Werther *et al*., 2017), and in pigeons *Columba livia domesticus* (center 105 µm, temporal periphery 119 µm and nasal periphery 136 µm) (Chard & Gundlach, 1938). This differs from that of mice, where an experiment showed a thicker

central cornea in both the epithelial and stromal layers (Henriksson, Bron, & Bergmanson, 2012). However, Descemet's membrane and the single layer of endothelial cells showed no significant difference in thickness between the cornea regions. Bergmanson *et al. (*2021) suggested that the difference in thickness of the corneal regions still remains unknown, although refraction demands, stem cell development and local environments, cellular aging and nutrition, to name a few, may be contributing reason for differential thickness across the cornea.

The cornea plays an important role in the refraction of light onto the retina for focused image formation, and for this to occur, the cornea needs to be completely transparent (Crespo-Moral, García-Posadas, López-García, & Diebold, 2020; Jones *et al*., 2007; Martin, 2022; Nautscher *et al*., 2016). Transparency is afforded by the integrity of the epithelial barrier, the parallel arrangement of stromal collagen cells and relative dehydration, the relative acellularity in the stroma, and the efficient active cell membrane fluid pump of the endothelium (Bergmanson, 2019; Collin & Collin, 2021; Jones *et al*., 2007; Liu *et al.*, 2016; Meek & Fullwood, 2001). A healthy endothelium is represented as a monolayer of polygonal cells of relatively uniform size and shape, in which the cells are mostly hexagonal. Endothelial cells can increase in size (polymegathism) and change their shape (pleomorphism) to maintain the cornea in a state of deturgescence. This mechanism occurs due to the limited ability of endothelial cells to replicate and the need to span deficits in this unicellular layer. Therefore, it is possible to visualize changes in cell shape (from hexagonal to pentagonal, octagonal, quadrilateral and heptagonal) and size (Albuquerque, Freitas, & Pigatto, 2015; Collin & Collin, 1998; Coyo et al., 2019; Doughty, 2006, 2018; Franzen et al., 2010; Pigatto et al., 2004, 2009; Tamayo-Arango et al., 2009). In young animals, there is a greater capacity for tissue regeneration, thus with aging, polymegathism and pleomorphism is generally more evident. In fact, senility alone can result in rates of pleomorphism reaching 40% or more (Albuquerque *et al*., 2015; Collin & Collin, 2021; Coyo *et al*., 2015; Kafarnik *et al*., 2007; Laing, Sandstrom, Berrospi, & Leibowitz, 1976).

The viability and health of the endothelium is assessed by visualizing the cellular mosaic of this layer in scanning electron microscopy. Endothelial efficiency can be evaluated from values such as mean cell area, cell density, coefficient of variation and percentage of hexagonal cells. In such manner, it is possible to evaluate endothelial pleomorphism and polymegathism as a marker of past or present endothelial damage or disease (Doughty, 2006, 2018; Doughty & Oblak, 2008). Different methods are used, such as specular microscopy, confocal microscopy and scanning electron microscopy. The latter not only provides

information on cell morphology, but also is high enough to evaluate the three-dimensional cell organization and the ultrastructure of endothelial cells (Coyo *et al*., 2019; Doughty, 1989, 2018; Meek & Fullwood, 2001; Tamayo-Arango *et al*., 2009). The downside is that significant cellular contraction occurs with processing by dehydration with glutaraldehyde. The cellular area obtained using other methods in which dehydration is not required can be from 11 to 39% larger than samples prepared for SEM (Doughty, 1989, 2006; Pigatto *et al*., 2004; Tamayo-Arango *et al*., 2009). Although SEM provides excellent analysis of cellular morphology, interpretation of the results must consider the methods used, including other processing methods during SEM (Doughty, 1989).

In the present study, endothelial cellular microvilli were visualized as described in other animals (Collin & Collin, 1998, 2021; Pigatto *et al*., 2018; Tamayo-Arango *et al*., 2009). The endothelium of Common Pauraque had microvilli located mostly at the cell borders, with less emerging to the cell surface (Fig. 3). Quantitative analyses included: 1) the mean cellular area; 2) the cellular density; 3) the coefficient of variation in size (polymegathism); and 4) the percentage of hexagonal cells (Doughty, 1989).

The corneal endothelial mean cell area (311.659 ± 95.86 μm^2) and density (3,032.70 cell/mm^2) varied from those reported in other birds, also analyzed by SEM, whereas the percentage of hexagonal cells (85%) and the coefficient of variation of the area (0.30) was more similar to previous studies (Collin & Collin, 1998, 2021; Doughty, 2006; Pigatto *et al*., 2004, 2009, 2005; Tamayo-Arango *et al*., 2009). A study with four different bird species all showed greater endothelial density than the Common Pauraque: Barred owl *Bubo strix* with 4,713 ± 766 cells/mm^2, South African ostrich *Struthio camelus* CD 9,250 ± 1,080 cells/mm^2, Emu *Dromaius novaehollandiae* CD 11,734 ± 1,687 cells/mm^2, and Australian Galah *Eolophus roseicapillus* CD 9,905 ± 873 cells/mm^2 (Collin & Collin, 1998). The Magellanic Penguin also had a larged CD than the Common Pauraque (3717 cells/mm^2 in the central cornea, and 3731 cells/mm^2 in the peripheral cornea), however the MCA was lower (269 ± 24 μm^2) (Pigatto *et al*., 2005). Additionally, the CV of the Magellanic Penguin (0.08) was less similar to that of the Common Pauraque than that of a non-avian species, the Yacare Caiman (0.22), although the percentage of hexagonal cells was similar across all three species (~80%) (Pigatto *et al*., 2004, 2005). Considering that the present evaluated healthy corneas and adult animals, similar to other studies cited above, the low rate of pleomorphism is expected. However, why some corneal endothelial values differ between species (e.g. commonly CD and MCA) while others are most often similar (CV and the degree of pleomorphism) is poorly understood.

Conclusion

The use of both light microscopy and scanning electron microscopy allowed for a broad study of the Common Pauraque's cornea. Generally, similarities in gross structure of the corneal organization and cellular morphology with other birds was found. However, specific differences in MCA, CD, and CV leave open the need for greater understanding of why such variation exists even among closely related species. Increased understanding may help explain the physiology of vision and the visual requirements for a given species to successfully interact with their environment, as well as improved knowledge about how to interpret pathologic changes in the avian cornea.

Acknowledgments

This study was funded by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil – Funding code 001, and by Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF), Brazil – proc.n.* 00193.00001303/2019-10). The authors thank the team of the Laboratory of Microscopy and Microanalysis IB/UnB and the Department of Wild Animals FAV/UnB in name of DVM, PhD Liria Queiroz Luz Hirano, for providing their facilities to perform this research.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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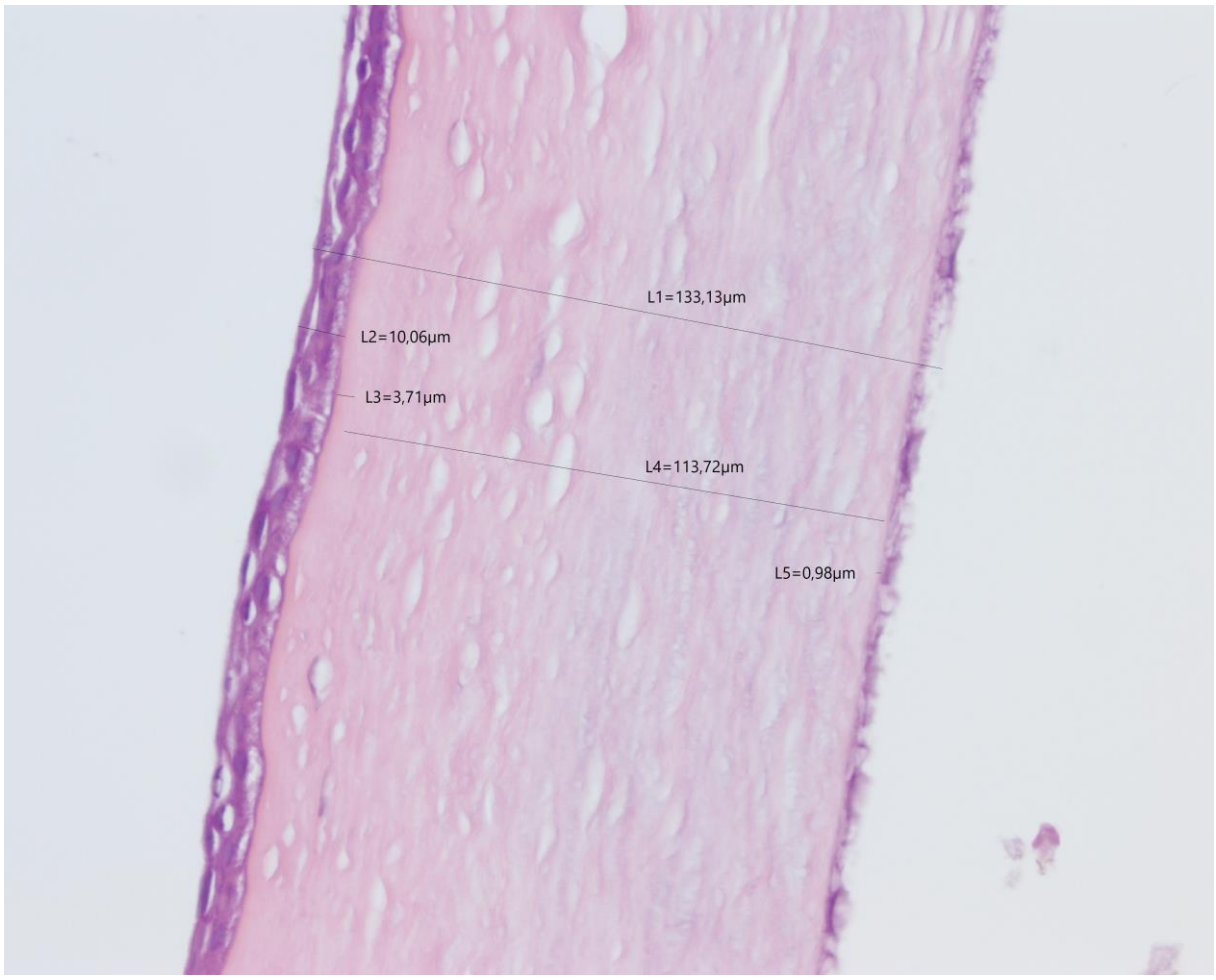

Figure 1. Histology of Common Pauraque cornea. L1 - total corneal thickness; L2 - epithelium thickness; L3 - Bowman's layer thickness; L4- stroma thickness; L5 – Descemet's layer thickness. HE. Measurements were carried out using the Opticam O500R microscope, with OPTHD software, 400x magnification.

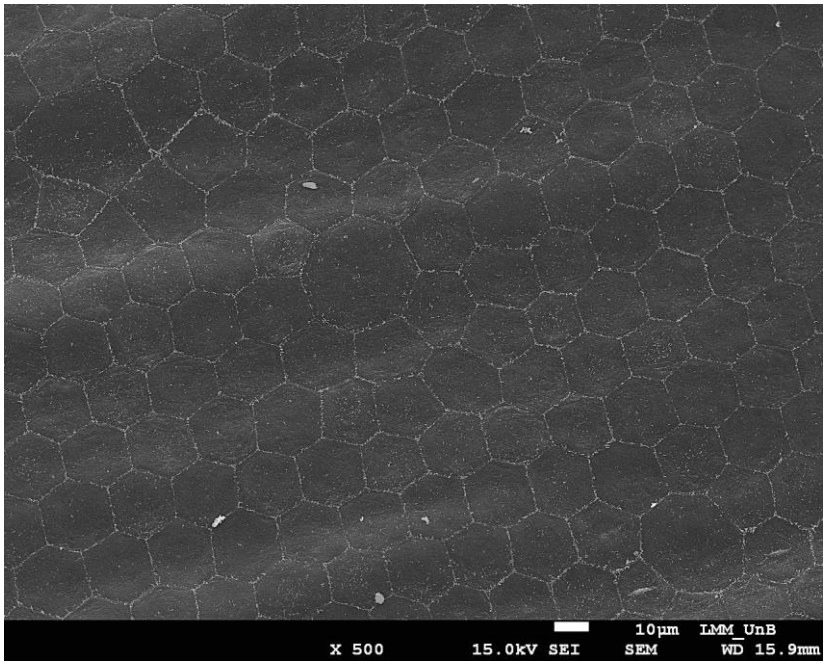

Figure 2. Scanning electron micrograph showing the corneal endothelium of the Common Pauraque (*Nyctidromus albicollis*). Note majority of cells are healthy, small, and hexagonal, compared to scant larger cells with variable shapes.

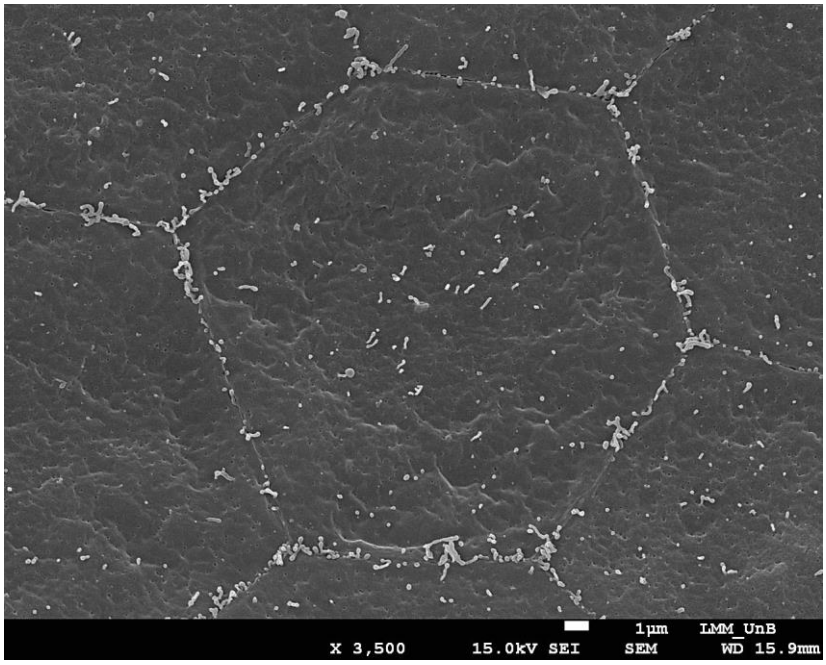

Figure 3. Scanning electron micrograph of normal corneal endothelial cell of the Common Pauraque (*Nyctidromus albicollis*) showing microvilli (white structures). Note the highest density localized at the intercellular junctions.

Table 1. Total corneal thickness of birds described in previous studies.

Species (Popular Name)	Total corneal thickness	Source
Eudyptula minor (Little Penguin)	380 ± 54 µm (central region)	(Collin & Collin, 2021)
Spheniscus demersus (African Penguin)	450 µm (region not specified)	(Sokolenko et al., 2021)
Spheniscus demersus (African Penguin)	384 ± 30 µm (central region)	(Gonzalez-Alonso-Alegre et al., 2015)
Gallus gallus domesticus (Chicken)	242 µm (central region)	(Montiani-Ferreira et al., 2004)
Gallus gallus domesticus (Chicken)	225.3 ± 30 µm (region not specified)	(Gonçalves et al., 2016)
Coturnix coturnix (Common Quail)	154 ± 17.7 µm (region not specified)	(Gonçalves et al., 2016)
Coturnix japonica (Japanese Quail)	138.64 µm (region not specified)	(Mayakkannan et al., 2018)
Ostrich (species not specified)	550 ± 35 µm (central region)	(Liu et al., 2016)
Harpia harpyja (Harpy Eagle)	563 µm (central region)	(Moraes, 2018)

Aquila chrysaetos (Golden Eagle)	640 µm (central region) 1200 µm (peripherical region)	(Murphy & Dubielzig, 1993)
Falcon tinnunculus (Common Kestrel)	129 µm (central region) Varies from 197 to 210.8 µm (peripherical regions)	(Werther et al., 2017)
Columba livia (Domestic pigeon)	157 µm (central region) Varies from 188 to 169 µm (peripherical regions)	(Chard & Gundlach, 1938)
Calypte anna (Anna's Hummingbird)	59 µm (central region) 48 µm (peripherical region)	(Moore et al., 2019)

Gmail **Rafaela Tozetti <rafaelartozetti@gmail.com>**

Anatomia, Histologia, Embryologia - Manuscript ID AHE-12-22-OA-315 [email ref: SE-6-a]

Vigneshwari Loganathan <onbehalfof@manuscriptcentral.com> 30 de maio de 2023 às 08:25
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Cc: dra.paulagalera@gmail.com, rafaelartozetti@gmail.com, roselialaraujo@hotmail.com, mvlpatologiaveterinaria@gmail.com, akiyamapls@gmail.com, joseraimundocorrea@gmail.com, bretskimoore@gmail.com

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Dear Prof. Galera:

Your manuscript entitled "Evaluation of the Common Pauraque (Nyctidromus albicollis) cornea using light and scanning electron microscopy" by Galera, Paula; Tozetti, Rafaela Alves Ribon; Araújo, Rosélia de Lima Sousa; Moreira, Matheus Vilardo Loes; Akiyama, Larissa Cristina de Souza; Corrêa, José Raimundo; Moore, Bret A., has been successfully submitted online and is presently being given full consideration for publication in Anatomia, Histologia, Embryologia.

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CAPÍTULO IV

CONSIDERAÇÕES FINAIS

Com base nas análises e resultados apresentados, além da literatura citada, ao longo do presente manuscrito, pode-se afirmar que os dados morfométricos e morfológicos permitem a descrição e comparação das córneas das aves estudadas. Portanto, embora os dados sejam considerados representativos das espécies aviárias, esses não são suficientes para compreender a variabilidade intra ou interespecífica.

As metodologias utilizadas para a coleta das informações, a microscopia de luz e de varredura, foram eficientes para concluir o objetivo do estudo e podem ser reproduzidas na prática.

Finalmente, gostaríamos de referir que a análise das métricas e da histomorfologia corneana foi feita com vista a oferecer uma descrição elementar dos dados, sem pretender uma inferência estatística, já que apenas um indivíduo de cada espécie foi estudado.

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