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Exploring Shape Variance in Waterbirds' Pad Feet: A Geometric Morphometric Analysis

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Abstract: Waterbirds usually have webbed feet, which help them move easily through water. These pad feet fall into four main categories: palmate, semipalmate, totipalmate, and lobate. In this study, morphological diversity among the pad feet of different waterbird breeds such as the West Indian whistling duck (*Anas bahamensis*), mandarin duck (*Aix galericulata*), red-breasted goose (*Branta ruficollis*), wood duck (*Aix sponsa*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), Pekin duck (*Anas platyrhynchos domesticus*), redhead duck (*Aythya americana*), Egyptian goose (*Alopochen aegyptiaca*), and pelican (*Pelecanus onocrotalus*) was examined by the geometric morphometric method. 2D images of 12 waterbirds' pad feet from different parts of Türkiye were analyzed from a dorsal view. In total thirteen landmarks were used. The analysis focused on principal component 1 and principal component 2 values. Principal component 1 shows slightly greater changes occurring on the lateral toes II and IV, as well as in the interdigital webbing below the average. Principal component 2 also reveals greater shape changes on the toes II and IV, which are more lateral. Geometric morphometric analysis proves valuable in identifying variations in the shape of the pad feet among various breeds of waterbirds, making it an effective tool for taxonomic purposes.

Keywords: Anatomy, Avian, Difference, Evaluation, Foot, Form.

Su Kuşlarının Ayak Şekil Varyasyonlarının İncelenmesi: Geometrik Morfometrik Analiz

Özet: Su kuşları genellikle suyun içinde kolayca hareket etmelerine yardımcı olan perdeli ayaklara sahiptir. Bu perdeli ayaklar dört ana kategoriye ayrılır: palmate, semipalmate, totipalmate ve lobate. Bu çalışmada, Batı Hint düdükçü ördeği (*Anas bahamensis*), mandarin ördeği (*Aix galericulata*), kızıl göğüslü kaz (*Branta ruficollis*), ağaç ördeği (*Aix sponsa*), mute kuğusu (*Cygnus olor*), boz kaz (*Anser anser*), yeşilbaş ördek (*Anas platyrhynchos*), Pekin ördeği (Anas platyrhynchos domesticus), kızılbaş ördek (*Aythya americana*), Mısır kazı (*Alopochen aegyptiaca*) ve pelikan (*Pelecanus onocrotalus*) gibi farklı su kuşu türlerinin perdeli ayaklarındaki morfolojik çeşitlilik geometrik morfometrik yöntem ile incelenmiştir. Türkiye'nin farklı bölgelerinden alınan 12 su kuşunun perdeli ayaklarının dorsal görünümden 2D görüntüleri analiz edilmiştir. Toplamda on üç belirleyici nokta kullanılmıştır. Analiz, temel bileşen 1 ve temel bileşen 2 değerlerine odaklanmıştır. Temel bileşen 1, yan parmaklar II ve IV ile ortalama altındaki parmak arası perdenin olduğu bölgede meydana gelen hafif değişiklikleri gösterirken, temel bileşen 2, daha yanlarda yer alan parmaklar II ve IV'teki şekil değişikliklerini ortaya koymaktadır. Geometrik morfometrik analiz, su kuşlarının perdeli ayaklarının şekil varyasyonlarını belirlemede değerli bir araç olduğunu ve bu analizlerin taksonomik amaçlar için etkili olduğunu kanıtlamaktadır.

Anahtar Kelimeler: Anatomi, Ayak, Değerlendirme, Fark, Kanatlı, Şekil.

Introduction

Waterbirds typically possess webbed feet, enabling them to easily propel themselves through water. These webbed feet are classified into four main types: palmate, semipalmate, totipalmate, and lobate. Among these, palmate feet are the most prevalent among waterbirds, characterized by the complete connection of the three front-facing toes (toes II, III, and IV) through webbing (Lovette et al., 2016; Raikow, 1985; Tokita et al., 2020).

Special feet known as pad feet are central to their ability to navigate water surfaces and wetland habitats. These remarkable anatomical structures distinguish water birds from their terrestrial counterparts, offering unique advantages for life in and around water (Birkhead et al., 2017). Pad feet, characterized by their flattened shape and webbed toes, serve as multifunctional tools for water birds, facilitating activities such as swimming, walking on mud or vegetation, and perching on floating objects (Koenig et al., 2016). The diversity of pad feet adaptations reflects the varied ecological niches birds water birds occupy, from the elegant swan gliding across serene lakes to the agile heron stalking its prey in marshy wetlands (Birkhead and Van Balen, 2008). These adaptations enable water birds to easily navigate wetland habitats, whether wading through shallow waters, paddling across lakes, or diving beneath the surface in search of prey (Proctor and Lynch, 1993).

Geometric morphometry (GM) is a shape analysis approach that relies on the examination of anatomical curves, points, and contours, utilizing data derived from two- or three-dimensional Cartesian coordinates (Aytek, 2017; Bookstein, 1997; Boz et al., 2023; Demircioğlu et al., 2021; Gündemir et al., 2020; Manuta et al., 2024; Szara et al., 2022). At its core, GM focuses on shape analysis, discerning subtle differences by tracking the displacement of biologically homologous landmarks (Bookstein, 1991; Zelditch et al., 2004), thereby explicitly defining "shape" in terms of proportions and relative arrangements of parts that remain consistent regardless of scaling, thereby providing a quantitative analysis (Rohlf et al., 1993). Principal Component Analysis (PCA) emerges as one of the most commonly employed methods for exploratory multivariate analysis. It serves to visualize the primary features of shape variation within a dataset and functions as an ordination method to unveil patterns in the relationships among observations (Klingenberg et al., 2000).

The geometric morphometric analyses conducted can elucidate how variations in the toe structures of water birds are correlated with food sources, hunting strategies, social structures, and other ecological factors. These findings contribute to a better understanding of the biological diversity and adaptation processes among water birds, providing deeper insights into their evolutionary processes and life strategies. In this way, by better understanding and managing the diversity and ecological adaptations among water birds, we can more effectively contribute to the sustainability of these species and their roles in ecosystems. However, the focus of this study is to examine how changes in finger shapes are associated with the evolutionary processes and life strategies of water birds.

In conclusion, numerous studies have highlighted the distinct genotypes of bird feet pads, showcasing variations in physiological, morphological, and behavioral traits compared to their ancestors (Höfling and Abourachid, 2021; Rico-Guevara et al., 2019; Tokita et al., 2020; Winkler and Leisler, 1985). In this study, we will examine the finger shapes of water birds such as the West Indian whistling duck (*Anas bahamensis*), mandarin duck (*Aix galericulata*), red-breasted goose (*Branta ruficollis*), wood duck (*Aix sponsa*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), Pekin duck (*Anas platyrhynchos domesticus*), redhead duck (*Aythya americana*), Egyptian goose (*Alopochen aegyptiaca*), and pelican (*Pelecanus onocrotalus*) using geometric morphometric analysis (GMA). This article delves into the fascinating realm of pad feet in water birds across different breeds, employing landmark-based GMA to explore their diversity and functionality. Despite limitations in our dataset, the research underscores the efficacy of geometric morphometrics in revealing subtle shape differences in pad feet. With this method, we will investigate how the finger structures of water birds change in relation to their life strategies and ecological impacts. The investigation, focusing on waterbirds, adds to the broader discourse on avian morphology, highlighting the importance of geometric morphometrics in elucidating the complexities of pad feet diversity in Turkish waterbirds.

Material and Methods

Animals: A total of 12 birds' feet were utilized in the study obtained by the Istanbul University-Cerrahpaşa Faculty of Veterinary Medicine, Department of Wildlife Diseases and Ecology. These include West Indian whistling duck (*Anas bahamensis*), mandarin duck (*Aix galericulata*), red-breasted goose (*Branta ruficollis*), wood duck (*Aix sponsa*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), Pekin duck (*Anas platyrhynchos domesticus*), redhead duck (*Aythya americana*), Egyptian goose (*Alopochen aegyptiaca*), and pelican (*Pelecanus onocrotalus*). All animals showed no pathological lesions, and all individuals used for the study were adults. Clinical examinations were provided by specialists before the samples were collected.

Landmarks: Analyses of foot pad shape were performed on dorsal photographs of each bird. Photographs were taken from a distance of 25 centimeters. Subsequently, the images were digitized using the 'tps' extension with tpsUtil (version 1.74) (Rohlf, 2004). If necessary, the images were rotated to reduce accidental variation in landmark placement. For this study, a specific set of landmarks (LMs) was placed on the foot pad images using tpsDig version 2.29 (Rohlf, 1997). In total, 13 landmarks (LMs) were positioned along the dorsal view of the feet.

Geometric morphometrics: In the study, the results of the dorsal view of woterbird feet pads were subjected to geometric morphometric analyses, which were recorded separately. All analyses were conducted using MorphoJ software version 1.07a (Klingenberg, 2011). MorphoJ is a program package designed for geometric morphometric analysis of two- and three-dimensional landmark data (Klingenberg et al., 2002). After obtaining the Cartesian x, y coordinates for all landmarks, shape data was extracted using a full Procrustes fit (Dryden and Mardia, 1998; Rohlf and Slice, 1990). Subsequently, Generalized Procrustes Analysis (GPA) was applied to the imported landmark (LM) data before analysis to account for the shape variations in the foot pads. Principal Component Analysis (PCA) was then performed to explore the overall morphological variability of foot shape among breeds (Gündemir et al., 2020; Klingenberg and McIntyre, 1998; Klingenberg et al., 2002; Manuta et al., 2023). The data in the study were obtained from the TÜBİTAK 2209A project titled "Investigation of the Foot and Toe Shapes of Waterfowl Using Geometric Morphometric Analysis.

Results

Digitus II, III, and IV: The other three toes on the underside of the foot. These toes point forward and merge with the interdigital membrane. *Torus metatarsalis*: A swollen, cushion-like structure located at the back of the foot. This structure aids birds in maintaining balance on their feet. Interdigital membrane (*Tela interdigitalis*): Flap-like skin structures between the second, third, and fourth toes. These structures assist water birds in swimming and moving on water surfaces. The landmarks used in our study are shown in Figure 1, and the description of each landmark is provided below.
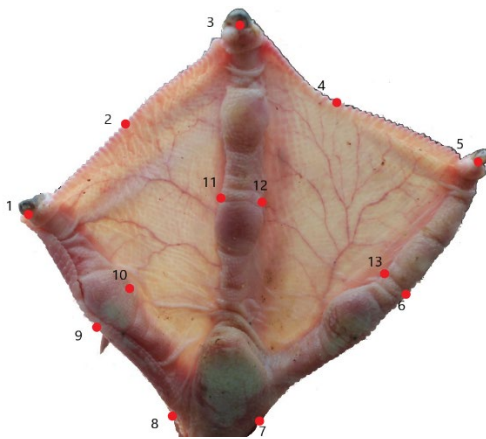

Figure 1. Landmarks used for the study of waterbirds pad feet in dorsal view. In total, 13 landmarks two-dimensional landmarks were used.

1. The point between the claw (*unguis*) and distal phalanx (*phalanx distalis*) of the second digit (*digitus secundus*).
2. The midpoint of the medial interdigital webbing (*tela interdigitalis medialis*).
3. The point between the claw (*unguis*) and distal phalanx (*phalanx distalis*) of the third digit (*digitus tertius*).

4. The midpoint of the lateral interdigital webbing (*tela interdigitalis lateralis*).
5. The point between the claw (*unguis*) and distal phalanx (*phalanx distalis*) of the fourth digit (*digitus quartus*).
6. The midpoint of the medial side of the fourth digit (*digitus quartus*).
7. The midpoint of the metatarsal tubercle (*torus metatarsalis*) is lateral.
8. The midpoint of the metatarsal tubercle (*torus metatarsalis*) medial.
9. The midpoint of the lateral side of the second digit (*digitus secundus*).
10. The midpoint of the medial side of the second digit (*digitus secundus*).
11. The midpoint of the lateral side of the third digit (*digitus tertius*).
12. The midpoint of the medial side of the third digit (*digitus tertius*).
13. The midpoint of the medial side of the fourth digit (*digitus quartus*).

Displays the shape changes associated with the first two principal components of the PCA with wire-frames for the extreme positive and negative values for each PC for the pooled sample (Figure 2). The shape variation between samples was analyzed by principal component analysis (PCA) using 13 landmarks in 2 dimensions in different feet pads (Table 1). The results of PCA using the landmark coordinates,

Table 1. Results of Principal Component Analysis (PCA).

Principal Component	Eigenvalues	Variance %	Cumulative %
PC1	0,00406770	42,59	42,59
PC2	0,00188852	19,77	62,37
PC3	0,00118962	12,45	74,82
PC4	0,00098774	10,34	85,17
PC5	0,00049664	5,20	90,37

determined in the water bird feet, are shown in (Figure 2). Accordingly, the first principal component (PC1) explained 42,59% of the total shape variance, and the first four principal components (PC1 + PC2 + PC3) explained the rest of 74,82 %. The analysis focused on PC1 and PC2 values. Using wire-frame warp plots for visualization, we can observe the intricate structures and variations in thearrangements, webbing, and overall foot shape among waterbird species. PC1 shows slightly greater changes occurring on the lateral toes II and IV, as well as in the interdigital webbing below the average. PC2 reveals greater shape changes on the toes II and IV, which are more lateral. Additionally, a smaller metatarsal tubercle was observed.

Discussion and Conclusion

Pad feet, the specialized structures found in water birds, possess unique physical traits specifically adapted for survival in aquatic habitats. With their graceful movements and effortless navigation of aquatic realms, water birds are marvels of avian adaptation (Birkhead, 2018). The aim of this study was to investigate differences in pad feet across

different waterbird breeds, such as the West Indian whistling duck (*Anas bahamensis*), mandarin duck (*Aix galericulata*),
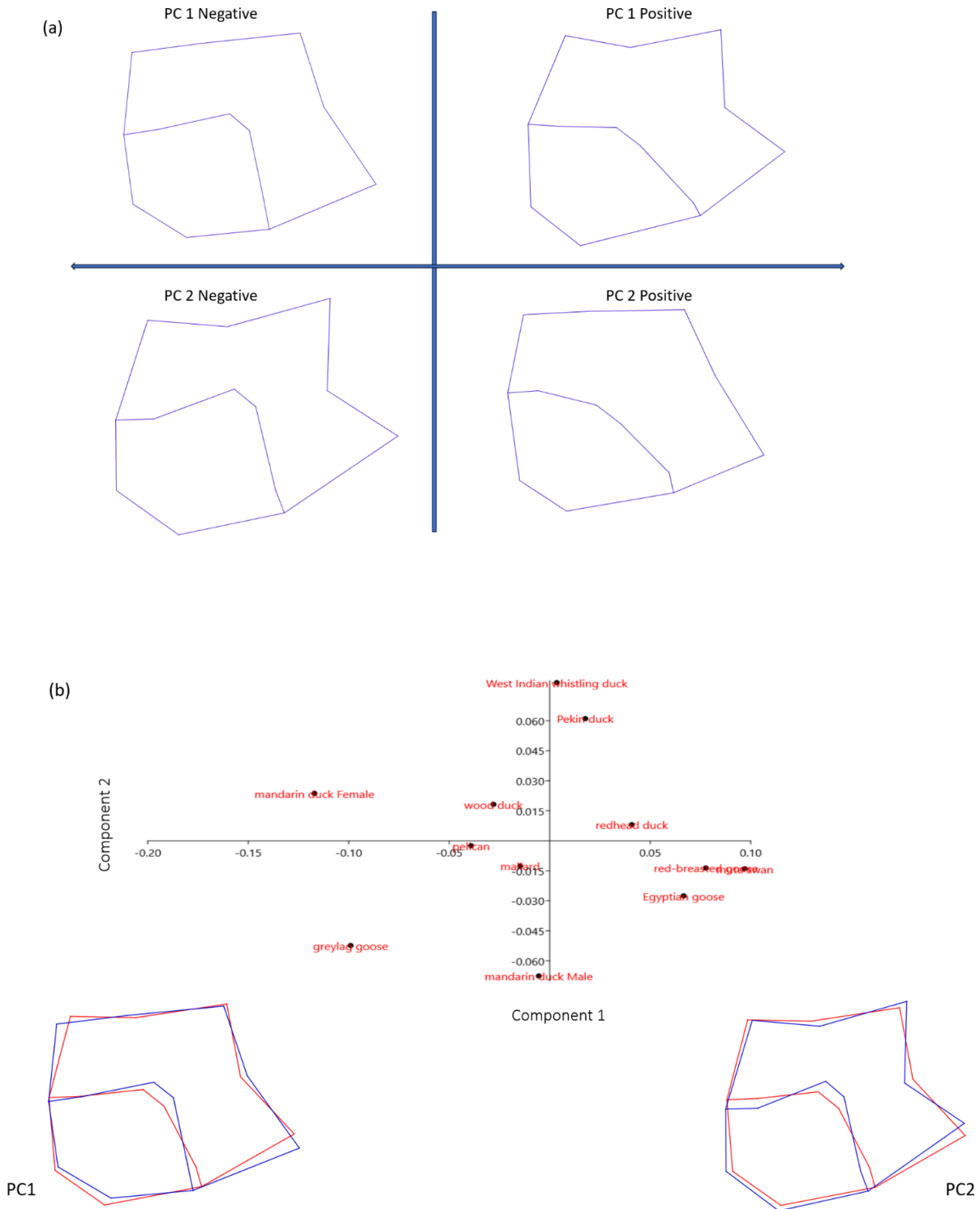

Figure 2. Scatter plot of PC1 and PC2 of the pad feet shape in dorsal view for interspecies. Wire-frames warp plots of shape changes depicting the positive and negative changes associated with PC1 (42,59%), PC2 (19,77%) of changes (top), and PCA showing variation among different breeds of water birds feet pads, as mapped by 13 landmarks (bottom). Blue outlines

represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes.

red-breasted goose (*Branta ruficollis*), wood duck (*Aix sponsa*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), Pekin duck (*Anas platyrhynchos domesticus*), redhead duck (*Aythya americana*), Egyptian goose (*Alopochen aegyptiaca*), and pelican (*Pelecanus onocrotalus*), using Principal Component Analysis (PCA) as a tool of geometric morphometric methods. Specifically, we examined scatter plots and wire-frame representations to elucidate potential variations. Our findings reveal notable distinctions among waterbird species, suggesting diverse morphology pad foot morphology adaptations.

Following geometric morphometry analysis based on landmarks, results showed a wide range of phenotypes in the shape of the pad feet of the specimens used in this study. Scatter plots generated from PCA provide visual representations of morphology pad foot morphology variation across different waterbird species. PCA allowed the extraction of principal components, with the first principal component (PC1) explaining 42.59%, while PC2 explained 19.77% of the total variation. Wire-frame representations further enhance our understanding by illustrating shape differences in pad feet morphology.

The observed variations in pad feet morphology among waterbird species are not superficial; they represent distinct adaptations tailored to each species' specific ecological niches and behaviors. These adaptations play a crucial role in waterbirds' survival and reproductive success by enabling them to effectively exploit their habitats and resources (Lin and Xu, 2017). For instance, species with elongated, webbed toes may exhibit enhanced swimming abilities, allowing them to efficiently navigate through water bodies in search of prey (Segesdi and Pecsics, 2022; Tokita et al., 2020). Conversely, species with shorter, more robust toes may excel in terrestrial locomotion, enabling them to forage on land or traverse different substrates with ease (Brown et al., 2002; Sargata-Vicens et al., 1992). Understanding these morphological adaptations provides valuable insights into waterbirds' evolutionary history and ecological diversification (Lin and Xu, 2017). By elucidating the functional significance of pad feet morphology, we gain a deeper appreciation for the remarkable diversity of avian adaptations and the complex interplay between form and function in the natural world.

The West Indian whistling duck is renowned for its strong, agile feet, which enable it to navigate through dense vegetation and shallow water bodies while foraging for aquatic vegetation, insects, and small invertebrates. Additionally, this species typically possesses moderately webbed feet with long, slender toes (Madge and Burn, 1988). Based on the results of our study, we observed the highest positive PC1 value compared to other specimens, indicating significant differences. Conversely, the mandarin duck male exhibited the lowest negative PC1 value. Mandarin ducks have well-developed webbed feet with pronounced webbing between the toes, extending almost to

the tips. Unlike the West Indian whistling duck, mandarin ducks are less reliant on terrestrial locomotion and are primarily adapted for a semi-aquatic lifestyle, spending much of their time on or near water bodies (Kear, 2005). Consistent with research by Johnsgard (2010), our study's PCA results highlight distinct differences between our specimens.

Furthermore, pelican feet are characterized by long, webbed toes with reduced webbing between the front toes, allowing for greater maneuverability in water (Ogden et al., 1983; Tokita et al., 2020). Goose feet may vary in size and shape depending on the species, with moderate webbing between the toes (Kear, 2005; Livezey, 1986). Ducks exhibit varied foot morphology depending on their habitat and feeding behaviors; for instance, dabbling ducks have relatively small, webbed feet adapted for shallow water foraging and dabbling (Cherry and Morris, 2008; Sargata-Vicens et al., 1992). Swans possess large, powerful feet adapted for swimming and walking on land, characterized by long, slender toes with prominent webbing between them, providing strong propulsion in water (Gill, 2007; Johnsgard, 2010).

The ecological implications of pad feet morphology extend beyond individual species to influence community dynamics, ecosystem structure, and conservation strategies (Tokeshi, 2009). Waterbirds, acting as keystone species in aquatic ecosystems, play pivotal roles in nutrient cycling, habitat structure, and prey populations (Tokita et al., 2020). Pad foot morphology can significantly impact a species' foraging behavior, habitat preferences, and competitive interactions with other organisms. For instance, waterbirds with specialized adaptations for diving may exploit deeper water habitats, while those with more agile feet may dominate shallow wetlands. Moreover, changes in pad feet morphology in response to environmental pressures, such as habitat loss, pollution, and climate change, can serve as crucial indicators of ecosystem health and resilience. of monitoring of monitoring these morphological traits over time provides valuable insights into the impacts of anthropogenic disturbances on waterbird populations and their associated habitats (Serrano and Tella, 2018; Smith and Buhl, 2015).

Overall, of comparing pad feet morphology among different waterbird species reveals fascinating adaptations shaped by evolutionary processes and ecological pressures. Through detailed studies and analyses, we can unravel the complexities of avian morphology and gain a deeper understanding of the intricate relationships between form and function in the natural world.

In conclusion, based on these hypotheses, environmental factors may be the reason for the differences in pad feet among our breeds — West Indian whistling duck (*Anas bahamensis*), mandarin duck (*Aix galericulata*), red-breasted goose (*Branta ruficollis*), wood duck (*Aix sponsa*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), Pekin duck (*Anas*

platyrhynchos domesticus), redhead duck (*Aythya americana*), Egyptian goose (*Alopochen aegyptiaca*), and pelican (*Pelecanus onocrotalus*). We believe our study will guide future researchers in employing methods such as biomechanics, comparative genomics, developmental genetics, and functional experiments to fully explain the observed evolutionary transitions in feet morphology and pad feet shape of waterbirds.

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Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on the Working Principles and Procedures of Animal Experimentation Ethics Committees".

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