Sexual dimorphism in eye coloration of Philippine Rufous Hornbills (*Buceros hydrocorax* and *Buceros mindanensis*)

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Abstract

Eye color, particularly iris, plays a role in social signaling such as in mate recognition and sexual selection. In the Asiatic lineage of large frugivorous hornbills, genus Buceros are known to exhibit sexual dichromatism in iris coloration that manifests upon reaching definitive adult stage; except for the Philippine endemic Buceros hydrocorax and Buceros mindanensis, which is yet to be confirmed. Institutions (n = 8) known to have Rufous Hornbills (Buceros hydrocorax) in captivity were surveyed for the subspecies, age, sex, iris coloration. Supplementary materials such as digital images of Rufous Hornbills uploaded on the Internet Bird Collection (https://www.hbw.com/ibc) were also sampled. Combined, a total of 23 individuals were sampled and identified as Buceros hydrocorax (n = 13), Buceros mindanensis mindanensis (n = 8), and Buceros mindanensis semigaleatus (n = 2). Of the 23, 10 (n = 5:4:1) were males while 13 (n = 8:3:2) were females. Images of Rufous Hornbills with complete information from IBC (n = 6); Buceros hydrocorax (n = 4), B. m. mindanensis (n = 1), and B. m. semigaleatus (n = 1). 100% of the males expressed brown coloration in iris (n = 12). Likewise, all females had pale blue iris (n = 17) regardless of the subspecies. Fisher's exact test results ($p < 0.0001$) suggest an association between sex and iris color even with confidence level set at 95%, indicative that the iris colors are mutually exclusive. By selecting images per subspecies, RGB values plotted in Euclidean color space indicate subspecies differentiation between male species.

Keywords: *Buceros hydrocorax*, *Buceros mindanensis*, Rufous Hornbill, dichromatism, RGB values

Introduction

In avian ecology, variation in eye coloration has been explained by consensus hypotheses an essential trait in signaling as a social function per se (Bortolotti, Smits, & Bird, 2003; Goodwin, 1984; Snyder & Snyder, 1974; Trauger, 1974), such as in mate recognition and sexual selection (Davidson, Thornton, and Clayton 2017). Avian iris demonstrate great variability in terms of coloration, displaying a rich color palette from inconspicuous, melanistic hues to vivid tones of red and blue (Erichsen 1985, Oliphant, 1988, Oliphant, Hudon, and Bagnara 1992). Oehme (1969) crudely described the physiology

of this as something associated to the biochemical compound Pteridine, which he posited as the most common of the pigments responsible for eye coloration; later on confirmed by Oliphant (1987) (Dias, Goedert, and Macedo 2009). This prominent phenotypic trait, while varying interspecifically, can also express intraspecific variation whereas it could be attributed to maturation with age (Crook 1964, Pearson 1966, Ervin 1975, Picozzi 1981, Stutterheim 1981, Newton and Marquiss 1982, Wilkinson 1982, Craig 1984, Wilkinson 1988, Peterson 1991, Sweijd and Craig 1991, Craig and Hulley 2004), subspecies differentiation (Negro, Blázquez, and Galván 2017), and sexual dimorphism (Hardy 1973, Hudon and Muir 1996, Pyle 1997, Craig and Hulley 2004). As such, there have been previous studies that used iris coloration as a determinant of age class, as well as a rapid and practical indicator of sex in sexually dimorphic species (Wood and Wood 1972, Trauger 1974, Rosenfield and Bielefeldt 1997, Smith *et al.* 2005, Nogueira and Alves 2008).

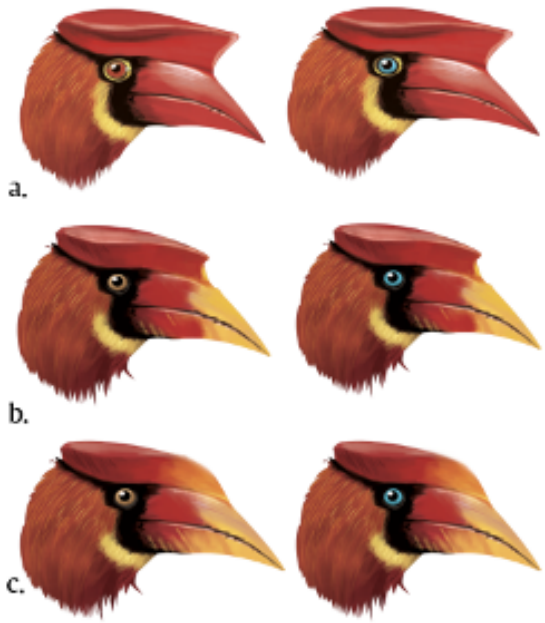

Figure 1: Proposed iris colorations for each taxa of Rufous Hornbills (left, male; right, female): a. *Buceros hydrocorax*, b. *Buceros mindanensis mindanensis*, and c. *Buceros mindanensis semigaleatus*.

The Asiatic lineage of large frugivorous hornbills, genus *Buceros*, are known to exhibit sexual dichromatism in iris coloration that manifests upon reaching definitive adult stage (Trauger 1974, Chamutpong, Ponglikitmongkol, Charoennitikul, Mudsri, and Poonswad 2013); except for the Philippine endemic *Buceros hydrocorax*, which is yet to be confirmed. Both of its congeners *Buceros bicornis* and *Buceros rhinoceros* have red and white iris for males and females, respectively. It remains unreconciled for the Rufous Hornbill as reports have been inconsistent and varying. According to Kemp and Woodcock (1995), *B. hydrocorax*'s iris colors are purportedly exhibiting the same dichromatism as its congeners, although it was noted that further confirmation was necessary. Its conspecifics, the Mindanao and Samar Rufous Hornbills, were described to have different manifestations. Witmer (1988) noted that the male's iris is not red for the *Buceros mindanensis mindanensis,* and were rather pale blue-grey or green in both sexes. On the other hand, Tweeddale (1877) noted that an adult the *Buceros mindanensis semigaleatus* male specimen from Southern Leyte, Philippines had a light-yellow iris, while another of the same sex from the island of Panaon, Southern Leyte had pale blue. To add to the complexity, recent records of digital photographs published on online media databases depict colors differently from these aforementioned studies (Figure 1). Furthermore, if there exists a dimorphism, no observations have been made on the age the trait onsets.

Whether or not the Rufous Hornbill and its conspecifics truly express sexual dichromatism in iris coloration is yet to be clarified, and if there is actually subspecies differentiation in this phenotypic trait as described by previous studies.Therefore, to settle this longstanding issue, the objectives of

this study are to (i) determine and formalize the iris coloration; (ii) clarify whether there really is subspecies differentiation; (iii) and establish the age range the trait manifests in both sexes. By tapping on zoological institutions known to have captive Rufous Hornbill(s), this study proposes that sexual dimorphism exists among the conspecifics, and that there is subspecies differentiation in iris coloration. If proven correct, iris coloration as a criterion will be a rapid and convenient method for sexing, especially for field applications.

Methods

Survey on zoological institutions

Institutions known to have Rufous Hornbills (*Buceros hydrocorax* and *Buceros mindanensis*) (Handbook of the Birds of the World and BirdLife International, 2019) in captivity were surveyed and queried for the subspecies, age, sex, iris coloration. Supplementary materials such as digital images and species reports were also requested upon availability.

Additional images

Digital images of Rufous Hornbills uploaded on the Internet Bird Collection (*https://www.hbw.com/ibc*) were selected according to the completeness of the information supplemented by the author; key details were subspecies, sex, and iris color.

Data analysis

Test for independence

Individuals were classified according to sex, and iris coloration which were defined based on crude observations of the photographs provided. Data were arranged into a contingency table fit for bivariate analyses. Analyses to test for independence was conducted by using a combination of tests; chi-square test and Fisher's exact test. Since there were structural zeroes in the data, the chi-square test was applied with Yates continuity correction for the structural zero values, while Fisher's exact test was recommended for such cases in previous studies (West and Hankin 2008). All analyses were conducted on *R* software ver. 3.5.2 (R Core Team 2013).

Color extraction for subspecies differentiation

In order to visualize the subspecies differentiation between iris coloration, available digital images from samples were used to measure RGB (red, green, blue) values of cropped iris region using R package `colordistance' (Weller, 2019). By selecting images per subspecies, randomly selected pixels (n = 10000) from each of the cropped irises were plotted in a Euclidean color space separately to capture all possible colors since the images did not undergo any essential image calibration methods such as white balancing and radiance normalization. Color clusters were extracted from pixels (n = 20000) by k-means clustering to minimize the Euclidean distance in RGB space and to parse common color motifs. The resulting k-means color clusters from each sample were plotted in a Euclidean color space to represent the closest matching colors of iris color for each subspecies. All analyses were conducted on *R* software ver. 3.5.2 (R Core Team 2013).

Results

A total of eight institutions were surveyed (Table 1). Combined, a total of 23 individuals were sampled and identified as *Buceros hydrocorax* (n = 13), *Buceros mindanensis mindanensis* (n = 8),

Table 1. List of surveyed zoological institutions and their captive Rufous Hornbills.

Institution	Location	Taxa	Sex	Age [a]	Iris color
Attica Zoological Park	At Yalou Spata, **Αθήνα** 190 04, Greece	*Buceros hydrocorax*	♂	9.8	Brown
		Buceros hydrocorax	♀	8.7	Pale blue
		Buceros hydrocorax	♀	N/A	Pale blue
Colchester Zoo	Maldon Road, Stanway, Colchester, Essex, United Kingdom	*Buceros mindanensis mindanensis*	♂	10.5	Brown
		Buceros hydrocorax	♀	10.5	Pale blue
Lagos Zoo	Barão de São João, Portugal	*Buceros mindanensis mindanensis*	♂	7.6	Brown
		Buceros mindanensis mindanensis	♀	8.9	Pale blue
Manila Zoo	M. Adriatico Street, Malate, Manila, Philippines	*Buceros hydrocorax*	♂	N/A	Brown
Novosibirsk Zoo	Ulitsa Timiryazeva, 71/1, Novosibirsk, Novosibirsk Oblast, Russia, 630001	*Buceros hydrocorax*	♂	8	Brown
		Buceros hydrocorax	♀	8	Pale blue
		Buceros hydrocorax	♀	7.4	Pale blue
Ouwehands Dierenpark	Rhenen, Utrecht, Netherlands	*Buceros hydrocorax*	♂	12.4	Brown
		Buceros hydrocorax	♀	8.8	Pale blue
Talarak Foundation	Kabangkalan City, Negros Occidental, Philippines	*Buceros mindanensis semigaleatus*	♂	N/A	Brown
		Buceros mindanensis semigaleatus	♀	N/A	Pale blue
		Buceros mindanensis mindanensis	♂	N/A	Brown

Institution	Location	Taxa	Sex	Age [a]	Iris color
Talarak Foundation	Kabangkalan City, Negros Occidental, Philippines	*Buceros mindanensis mindanensis*	♂	N/A	Brown
		Buceros mindanensis mindanensis	♀	N/A	Pale blue
		Buceros mindanensis mindanensis	♀	N/A	Pale blue
		Buceros mindanensis mindanensis	♀	N/A	Pale blue
Vogelpark Avifauna	Alphen aan den Rijn, South Holland, Netherlands	*Buceros hydrocorax*	♀	8.8	Pale blue
		Buceros hydrocorax	♀	14	Pale blue
		Buceros hydrocorax	♀	27	Brown
			Total	23	Brown = 12 Pale blue = 17

[a] Age in years.

and *Buceros mindanensis semigaleatus* (*n* = 2). Of the 23, a total of 10 (*n* = 5:4:1) were males while 13 (*n* = 8:3:2) were females. All sexes were confirmed through molecular sexing based on the species reports provided by the institutions. Mean age was 11.0, calculated from the samples whose age were provided by the institutions (*n* = 14). For males alone, mean age was 12.6 (*n* = 6) while female mean age was 9.8 (*n* = 8). As such, all individuals were classified as adults based on age, in addition to the morphological traits described by Kemp and Woodcock (1995). In addition, images of Rufous Hornbills with complete information from IBC (*n* = 6) were regarded as supplementary samples (Table 2); *Buceros hydrocorax* (*n* = 4) (Gonzalez 2010a, Doron 2011, Wentworth 2013a, 2013b), *Buceros mindanensis mindanensis* (*n* = 1) (Gonzalez 2010b), and *Buceros mindanensis semigaleatus* (*n* = 1) (Gonzalez 2010c) were obtained from the website. Of these were 2 males (*n* = 1:1:0) and 4 females (*n* = 3:0:1). Exact ages, however, were not provided by the authors.

Based on the digital images obtained from both the institutions and IBC, two iris colorations were observed from the samples; Pale blue and Brown. 100% of the males expressed brown coloration in iris (*n* = 12). Likewise, 100% of females had pale blue iris (*n* = 17) regardless of the subspecies. Pearson's chi-squared test

with Yates' continuity correction resulted to a strong association of the variables ($p < 0.0001$), suggesting the null hypothesis (H_0) be rejected (Table 3). Similarly, Fisher's exact test results ($p < 0.0001$) suggest an association between the variables sex and iris color even with confidence level set at 95%, indicative that the iris colors are mutually exclusive. It is important to emphasize, however, that of the observed brown trait, two sub classifications were recorded from the samples, as two shades of brown were observed; a dark, amber occurring in all *Buceros hydrocorax* species and a lighter beige occurring in all *Buceros mindanensis mindanensis* and *Buceros mindanensis semigaleatus*. However, since sexual dimorphism was being tested, all browns were classified under a single variable as it only occurred in male species. Although no post hoc analysis was carried out, subspecies differentiation between all the males is evident from the observed colors of brown; dark amber ($n = 6$) and beige ($n = 6$).

The sub classifications in brown iris coloration observed in males were analyzed by selecting three images each for *Buceros hydrocorax* and *Buceros mindanensis mindanensis* (See Appendices). Although the trait was observed in *Buceros mindanensis semigaleatus*, it was excluded from the analysis due to insufficient image sample ($n = 1$). Coordinates and quantified RGB values of pixels were plotted in a Euclidean color space to illustrate the closest approximation of the actual colors of iris of the Rufous Hornbill and its subspecies (Figure 2).

Euclidean distances between the pixels were minimized by *k*-means clustering and the closest matching colors were plotted in separate histograms for each image sample

Table 2. List of digital images of Rufous Hornbills sampled from the Internet Bird Collection database.

Institution	Location	Subspecies	Sex	Author *	Iris color
Biodiversity Conservation Centre (NFEFI)	Bacolod City, Negros, Philippines	*Buceros mindanensis mindanensis*	♂	Gonzalez (2010b)	Brown
DENR-PAWB Wildlife Rescue Center	Quezon City, Philippines	*Buceros hydrocorax*	♀	Gonzalez (2010a)	Pale blue
Parc Pairi Daiza (Paradisio)	Cambron-Casteau, Brugelette, Hainaut Province, Belgium	*Buceros hydrocorax*	♂	Wentworth (2013a)	Brown
		Buceros hydrocorax	♀	Wentworth (2013b)	Pale blue
USLS Ecopark	Bacolod City, Negros, Philippines	*Buceros mindanensis semigaleatus*	♀	Gonzalez (2010c)	Pale blue
Vogelpark Walsrode	Walsrode, Lower Saxony, Germany	*Buceros hydrocorax*	♀	Doron (2011)	Pale blue

*Authors from the Internet Bird Collection.

Table 3. Summary of tests of independence.

Test	p-value
Pearson's Chi-squared test (with Yates' continuity correction)	5.662e-07
Fisher's Exact Test	1.927e-08

(Figure 3). Parsed common color values extracted from each samples were combined and visualized in a single Euclidean color space, individually for each sample groups.

Discussion

Of the total 29 individuals, 41% were males while 59% were females. Nearly half of the sample group was constrained in definitive adult stage as evident from the mean age (11.0). The other half whose ages were not specified can be classified as adults as well without having to determine the actual ages from a morphological perspective based on Kemp and Woodcock (1995)'s description. Both tests of independence yielded extreme p-values that can be expected since the observed character states of iris coloration, pale blue and brown, were mutually exclusive to the corresponding sex, females and males respectively, across all samples. Even at 95% confidence level, the significance of correlation was still extremely high with a p-value of 1.927e-08, providing a strong support to claim that sexual dimorphism in iris coloration does indeed manifest in Rufous hornbills. However, since the only age group of the samples are adults, the age at which the trait manifests has not been delineated, and whether it is similar among all the conspecifics. In a report about the development of soft parts, beak, and feather by C. Barwick (pers. comm., May 27, 2019) from Colchester Zoo, he

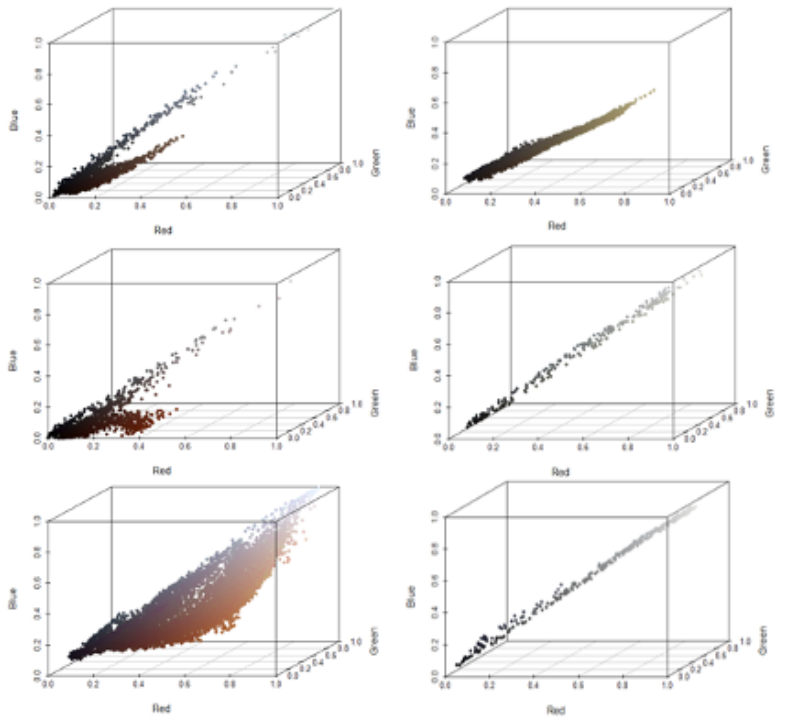

Figure 2: RGB values of iris from pixels of cropped images in Euclidean space of Rufous Hornbills; (a-c) *Buceros hydrocorax* and (d-f) *Buceros mindanensis mindanensis*.

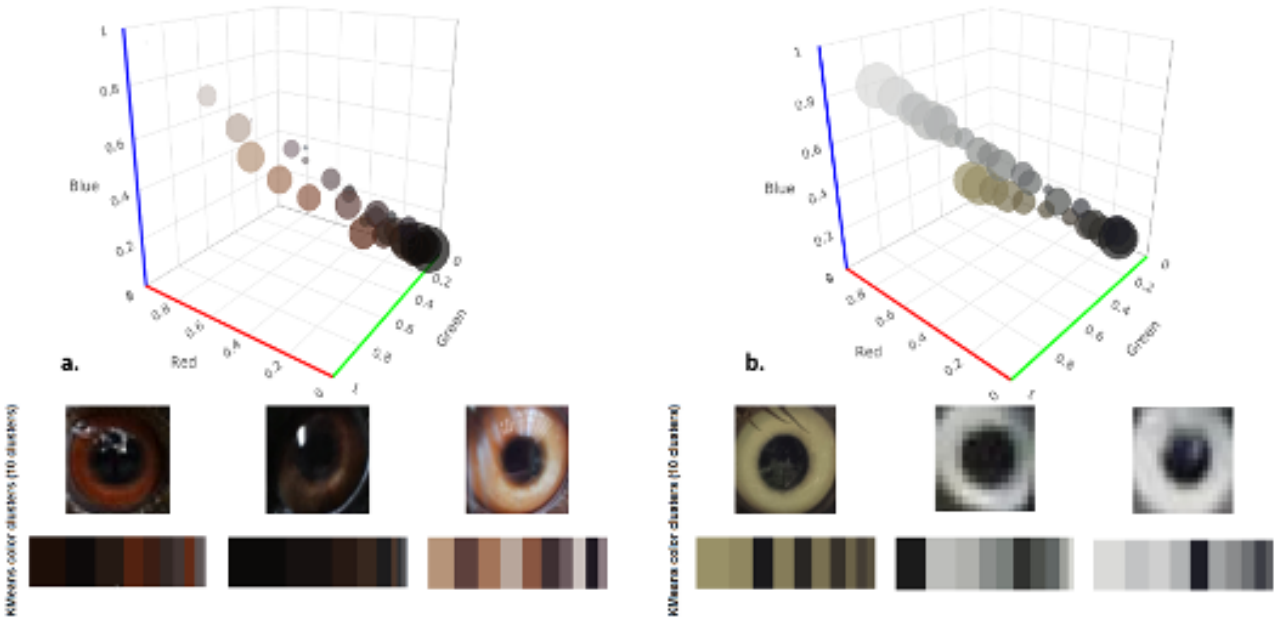

Figure 3: RGB *k*-means clustering in histograms along with cropped images of iris from adult ;/.'Rufous hornbills. Extracted color clusters from k-means clustering were combined and plotted in Euclidean color space: a. *Buceros hydrocorax* (respective ages in years: 8, 12.4, 9.8), b. *Buceros mindanensis mindanenis* (NA, 10.5, 7.6).

observed that the female *Buceros mindanensis mindanensis* had red iris at age 1.5 years while its pair, a male *Buceros mindanensis semigaleatus* of the same age, had a pale brown iris. He noted the subsequent change in iris color of the female to 'milky white' at approximately 5 years of age until it finally turned pale blue at 6 years. On the contrary, the male had a consistent color. The data from this study was unable to confirm this because the lowest age sampled for a female was 7.4, and had already manifested such color. However, no interspecific variation has observed across all female samples as they all possessed pale blue eyes. Males, on the other hand, seem to have subspecies differentiation as it has been observed that the brown iris color has two states, a darker hue found in *Buceros hydrocorax* and lighter for its conspecifics. Plotted RGB values illustrated in Figure 2 display the color composition of pixels from cropped iris images to approximate the actual colors and the difference thereof. From a visual standpoint, the distinction is obvious between the two sets of plots where *Buceros hydrocorax* demonstrated clusters of brown while its conspecific had light to pale color clusters. However, it is important to emphasize that the images were of different sources, so lighting conditions during image capture are unknown. Furthermore, the region of interest, iris, was not refined from the cropped image of the eye and included the pupil on RGB extraction. This might explain the significant proportion of black color clusters in either sets of Euclidean color space. To account for this noise, *k*-means clustering minimized the Euclidean distance by clustering analogous color pairs, illustrated as histograms in Figure 3. These represent the major color clusters because *k*-means capture even the redundant colors as it excludes the breaks in each channel. Therefore, the colors in the histograms illustrate the major actual color composition of the iris images. Shades of brown are noticeably more common in *Buceros hydrocorax* iris across all three samples as compared to its conspecific, *Buceros mindanensis mindanensis*, whose iris colors are apparently lighter at ranges of shades of beige to greyish white. The Euclidean color

space included in Figure 3 illustrates the summary of RGB values with minimized distances from *k*-means clustering.

In the quantitative criteria for species delimitation by Tobias *et al*. (2010), a difference involving contrasting hues (e.g. white/yellow; red/brown; green/blue) in plumage and bare parts has a magnitude of major (score = 3). However, it is not mentioned whether iris coloration is included under the definition of these specified criteria, or if it applies on every covariance in color-related traits. Witmer (1988) and Tweeddale (1877) were not mistaken from their observation that there is indeed a subspecies differentiation among the male species of Rufous hornbills. Although the descriptions were not accurate, the iris color of the Luzon Rufous hornbill is indeed distinct from its conspecifics based on the results.

Conclusion

The results provide a strong support on sexual dimorphism in iris color, and is true for all the conspecifics. In addition, color extraction provided meaningful insights about the subspecies differentiation in male species, and warrant further study. In any case, recognition of this intraspecific dimorphism in the eye color and relative intra-subspecies difference in eye color could prove useful especially in field applications where rapid identification is of great necessity. Furthermore, the results suggest for updates on the description of iris color in Rufous hornbills.

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