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Morphometric and DNA sexing accurately in male Javan hawk-eagle (*Nisaetus bartelsi*) determination at Kamojang Eagle Conservation Center, West Java, Indonesia

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Abstract. *Withaningsih S, Ilham MF, Rosdianto AM. 2024. Morphometric and DNA sexing accurately in male Javan hawk-eagle* (Nisaetus bartelsi) *determination at Kamojang Eagle Conservation Center, West Java, Indonesia. Biodiversitas 25: 1167-1173.* The Javan hawk-eagle (*Nisaetus bartelsi*) is a protected and endemic species of eagle in Indonesia whose existence is symbolized by the Garuda Pancasila. Raptors act at the top of the predatory chain and as indicators of environmental health. IUCN data states that *N. bartelsi* is red-listed as endangered. It is one of Indonesia's most frequently hunted and traded birds, and this trading practice has changed its natural behavior. Therefore, preservation through conservation –one of which is rehabilitation– programs are needed to maintain their sustainability. The ultimate goal of rehabilitation is to release the Javan hawk-eagle back into its natural habitat. In conservation and breeding programs, accurate determination of the sex of raptors is integral to informing conservation decision-making, population management, and research efforts aimed at protecting these iconic birds of prey and their habitats. Therefore, this study aims to determine the sex of male *N. bartelsi* through morphometric and DNA sexing techniques. The research subjects were seven *N. bartelsi* rehabilitated at Kamojang Eagle Conservation Center, Garut, Indonesia. Morphometric sexing measurements obtained the following results: two males were consistent upon comparing DNA sexing and Kamonjang Eagle Conservation Centre records observations. Analyses were obtained using primer 2550F/2718R that recognized CHD1-Z and CHD1-W as cleaved. Furthermore, the amplification was successfully read a sexing of *N. bartelsi* at Kamonjang Eagle Conservation Centre. The analysis primer sets mainly for raptors can assist conservation programs such as breeding programs and data collection for mapping this raptor. In addition, the PCR method can also assist in the accurate management of in-situ and ex-situ conservation.

Keywords: Conservation, DNA sexing, morphometric sexing, *Nisaetus bartelsi*

INTRODUCTION

Indonesia is a country with the highest biodiversity, especially the faunal diversity of wild bird species recorded at 1,711 species or a percentage of 16% of the total bird species worldwide, and the second highest number of endemic species after Brazilwith 397 species (Prawiradilaga 2017). According to Prawiradilaga's (2019) study, the number of endemic bird species has increased from 2017 to 510 species. However, although Indonesia's endemic bird population continues to increase, its status is still deteriorating due to environmental damage and human activities. Furthermore, 10% of the total bird species, including endemic birds, are in threatened condition status (Prawiradilaga 2019).

Raptors have important roles in the ecosystem. Beyond the well-documented ecosystem services provided by scavengers and predators (O'Bryan et al. 2018), raptors serve as cultural symbols and are indicators of biodiversity and environmental health (Donázar et al. 2016; Butet et al. 2022) and can contribute to ecosystem dynamics through their influence on prey population, nutrient cycling, and trophic cascades (Sergio et al. 2008; Wenny et al. 2011; Hudson et al. 2014). Raptors have been good indicators of habitat quality because of their sensitivity to human disturbance and environmental contamination (Bregman et al. 2014). Raptors are categorized as focal species, sensitive to environmental change, such as impacts on their ecosystems, and vulnerable to pollution, so they serve as icons for conservation initiatives (Pruscini et al. 2016; Withaningsih et al. 2019).

In Indonesia, the latest available data reported 81 diurnal raptor species (Bildstein et al. 1998), with 10 to 17 being endemic species, including notable species like the Javan hawk-eagles (*Nisaetus bartelsi*). The Javan hawk-eagle is recognized as a sacred bird by Indonesians and the current national symbol of Indonesia is called Pancasila. However, no current data is available regarding this status (Supriatna 2012) and the conservation status of this raptor is threatened. Major threats to this raptor include the increase in human population, weak law enforcement, and low public awareness about raptor conservation (Birdlife International 2017).

The current population in Indonesia is 650 males and females. The distribution in West Java in 2016 was 234 males and females scattered in all regions (Azmi et al. 2016; Gunawan et al. 2020). Raptors conservation organizations play an important role in conserving this endangered species through preservation efforts, such as rehabilitation, captive breeding, and, most importantly, sex determination.

The rehabilitation process is an effort to restore the raptor's natural character and conform to its natural habits because animals that have been long kept in cages usually have a behavior change, making them different from animals in nature. Therefore, knowing the sex of the raptors to be released is an important stage in the rehabilitation process, as the success of correct gender identification will contribute to the success of these rehabilitations (Cerit et al. 2007). This is related to the importance of the sex ratio in the environment where it was released: a balanced sex ratio in a small population is important for managing and conserving threatened species (Rudnick et al. 2005).

Knowledge of sex is important for wildlife conservation, as it affects both the sex ratio of the population and the captive breeding programs. More males will affect social behavior, mainly caused by male aggression, leading to competition with other males. In addition, the sex ratio imbalance can impact population density and growth rates, demographic problems, and, worst-case scenario, extinction (Annisa et al. 2021).

The research on sex determination continues to evolve the difficulty level based on four scientific literature through morphometric sexing representation, and it provides a correct percentage results of 86% (*Buteo jamaicensis, B. lineatus,* and *Accipiter cooperii, Aquila chrysaetos*) to 95% (*A. trivirgatus*) for each species (Pitzer et al. 2008; Muriel et al. 2010; Choi 2013; Harmata and Montopoli 2013).

Although the success percentage of morphometric sexing measurement is high, it requires specific species reference parameters. Another method, Polymerase Chain Reaction (PCR), is consistent and highly accurate, especially for determining sexing (Matagarka 2018); for example, determining sexing for wildlife had an accurate result of 95% from 118 individuals. In addition, PCR examined on the foxes had an accuracy of 96% and even increased to 99.6% (Ramsey et al. 2015). Therefore, this research was carried out for two main reasons: the importance of Javan hawk-eagles and the importance of obtaining information about its sex.

The importance of Javan hawk-eagles is twofold. Firstly, this animal represents the national symbol of Indonesia, Garuda. Secondly, it is one of the endemic bird species in Java, a keystone species, and it currently has endangered status based on the IUCN Redlist. Therefore, it is crucial to continue to maintain its existence in nature as a top predator that maintains the balance of the ecosystem and, at the same time, maintains the existence of symbols of the Indonesian state in nature.

In order to maintain Javan hawk-eagle's existence in nature, it is essential to obtain information regarding its sex. This information will facilitate the selection of individuals to be released, thus affecting the sex ratio in the place where the animals will be released. Understanding the sex ratio of the population undergoing rehabilitation can provide valuable insight into the overall health and dynamics of the species, aiding conservation efforts in the long term.

MATERIALS AND METHODS

Samples

The total subject of this study is seven *Nisaetus bartelsi* in order Accipitriformes; family Accipitridae owned BBKSDA (Balai Besar Konservasi Sumber Daya Alam/Nature Conservation Agency) West Java at Kamonjang Eagle Conservation Centre (*Pusat Konservasi Elang Kamojang* or PKEK). The number of Javan eagle individuals in PKEK was actually eight individuals, but only seven individuals were used as research objects because one individual was included in the inclusion criteria.

The research subject used was *N. bartelsi* in rehabilitation (Table 1). The sex determination with the protocol followed using the PCR blood sample examined with the Qiagen DNeasy® Blood Tissue Kit and Mytaq HS red mix. Blood collection was done by first handling the eagles using a towel. Although this was invasive to the eagles, this method calmed them down to the existing facilities. Blood was drawn from the brachial vein by spreading one of the wings, then the blood vessels would be visible along the humerus to the ulna (crossing the joint of the humerus and ulna). We then sprayed the area with alcohol to sterilize it, and prepared a 3 cc syringe and a 27G needle. The blood collection process was carried out by inserting the needle at an angle of <30 degrees, with the beval position of the needle facing upwards. The blood was then collected using an EDTA (Ethylenediaminetetraacetic Acid) tube which aimed to prevent the blood from coagulating.

Table 1. List of data subject *Nisaetus bartelsi*

Named eagle	Species	Age category	Arrival date	Examination date	Origin
Arimba	*N. bartelsi*	Young	29/05/2021	19/06/2023	BKSDA Jabar
Hayati	*N. bartelsi*	Adult	12/11/2021	19/06/2023	BKSDA Jabar
Tegar	*N. bartelsi*	Adult	15/06/2019	19/06/2023	BKSDA Jatim
Mario	*N. bartelsi*	Adult	15/06/2019	19/06/2023	BKSDA Jatim
Jono	*N. bartelsi*	Young	03/02/2018	20/06/2023	BKSDA Banten
Aki	*N. bartelsi*	Adult	14/08/2020	20/06/2023	BKSDA Jabar
Gagah	*N. bartelsi*	Adult	24/10/2014	20/06/2023	PPS Cikananga

Table 2. Protocol of parameters measurement on morphometric

Measurement parameters	Size (mm)
Body length	610 mm
Wingspan length*	358 mm
Tail centrale length	247 mm
Tarsus length	74-80 mm
Digit III length	55 mm
Digit nails III length	22 mm
Culmen length	27 mm
Body mass	~
Body temperature (°C)	37.5-40.5 (*Rest-phase)*
	38.2-1.8 (*Active phase)*
Basic behaviour	Active/passive

Morphometric method

Morphometric sexing was carried out by measuring all parameters represented in (Table 2). The measurements used a caliper, gauge meter, scales, and thermometer, and then the results were recorded in a protocol. These parameters were adapted from different sources (Kuroda 1933; Ferguson-Lees and Christie 2001; Harmata and Montopoli 2013; McKechnie 2022).

From the abovementioned first, until the ninth parameters to determine the sexes of the Javan hawk-eagle using the morphometric sexing, three parameters were used to measure the ratio of the male and female individuals. These three parameters – wing length, culmen length and digit III length – were chosen in this study because they had the most objective potential of all the parameters measured.

Molecular method with PCR procedure

Molecular sex determination techniques used primer sets as amplification for DNA templates. The primer set uses 2550F with base [5'GTT ACT GAT TCG TCT ACG AGA -3'] and 2718R [5'ATT GAA ATG ATC CAG TGC TTG -3']. PCR cycle was used during the amplification process: pre-denaturation 94°C for 300 seconds, denaturation 94°C for 45 seconds, annealing 46°C for 45 seconds, extension 72°C for 90 seconds, final extension for 10 minutes, and total the cycle PCR were 35 cycles. On the other hand, product PCR was then run by agarose gel 2% with added safe-green as staining.

Data analysis

We analyzed the result by collecting more scientific theories, especially for the morphometric sexing method. On the other hand, PCR product was analyzed through a UV transilluminator and compared based on the scientific literature on the same testing molecular sexing in raptors.

RESULTS AND DISCUSSION

Morphometric sexing

Based on research results, there was no difference between male and female Javan hawk-eagles because they were a monomorphic type of bird. Therefore, to differentiate the sex of this bird, a morphometric sexing examination was carried out which was confirmed through DNA sexing. Some speculation states that females have larger sizes than smaller males (Mueller and Meyer 1985).

The ratio measurement of the chosen three parameters – wing length, culmen length and digit III length – was used as a reference to see the comparison between the size of males and females. For example, the comparison results between Gagah (male) and Tegar (female) show that ratios of the length of the left wing, the length of the culmen and the length of the digits look were different, with values of 1:1.13; 1:0.93; 1:1.34 respectively. Therefore, the results of this research show that there was no significant difference in size parameters between male and female Javan hawk-eagles in the study area.

The morphometrics and DNA sexing of *N. bartelsi* obtained the best measurement parameters based on the previous discussion. For DNA sexing, the analysis was carried out using blood samples and analyzed through the PCR method. Both parameters were then compared with sex data already known through data from PKEK (Table 3)

Table 3. List of morphometric sexing results in *Nisaetus bartelsi*

Parameters characteristic	Arimba	Hayati	Tegar	Mario	Jono	*Aki	*Gagah
Right wing	530	530	570	530	535	520	520
Left wing	530	535	580	580	535	540	510
Body length	530	600	595	610	515	580	570
Tail length	280	280	290	290	260	270	260
Tarsus length	90	90	90	90	90	90	95
Culmen length	29.59	30	25.45	28,35	27.40	30	27
Digit III length	48.52	68.85	58.70	47.61	41.40	52.80	44.4
Digit nails III Length	23.51	23.45	24.45	24.57	22.5	21.0	22.4
Age	Juvenile	Juvenile	Adult	Adult	Juvenile	Adult	Adult

Males *N. bartelsi* have a wingspan of 358 mm which is shorter than that of females at 371-372 mm (Ferguson-Lees and Christie 2001). In this study, the wing span was measured from the humerus to the longest tip of the primary feathers. The mean value obtained from this study was 535 mm. *Nisaetus bartelsi* that have wingspan, below this value were determined to be male, while those above the value were determined to be female. For culmen length, male *N. bartelsi* were on average longer than females, while for digit III length, male *N. bartelsi* were on average shorter than females (Backus et al. 2015).

Table 3 shows that all parameters were selected based on theoretical analysis from several scientific literature and examined the factors that differentiate them from other parameters. They were said to be the most accurate in measurement. Those parameters were then carefully analyzed in depth to obtain consistent parameters to determine gender regardless of other factors (adaptation from Kuroda 1933; Ferguson-Lees and Christie 2001; Harmata and Montopoli 2013; McKechnie 2022)

Molecular sexing

The PCR product showed in the presence of each *N. bartelsi* sample as a long band (Figure 1). Bands produced through agarose with a UV transilluminator showed the results of 2 band patterns as chromosomes CHD-1W and CHD-Z. The CHD gene in birds is largely a sexing marker (Fridolfsson and Ellegren 1999). Their discovery identified the W-linked gen as encoding a DNA-binding protein. Later, Griffiths and Korn (1997) found the CHD-Z gen was believed to be found mostly in birds. The female gene is heterogamy (CHD1-ZW), and the male is homogamy (CHD1-ZZ) (Griffiths and Korn 1997; Osman et al. 2020).

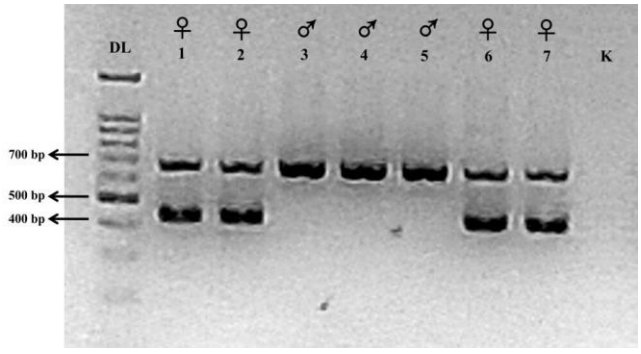

Figure 1. Result of PCR identification molecular sexing of Javan hawk-eagle. (1-7: *Nisaetus bartelsi* samples, DL: DNA ladder)

Based on the sexing markers from PCR results using primers 2550F/2718R, three samples were found to be males (Arimba, Jono, Gagah), and four were females (Hayati, Tegar, Mario, Aki). The electrophoresis represented visual chromosomes. Sample of *N. bartelsi* 1 showed chromosome CHD1-ZW (female) with a long size CHD1-Z range of 600-700 bp and a long CHD1-W range size of 400-500 bp; sample *N. bartelsi* 2 has a long CHD-Z range size of 600-700 bp and have a CHD-Z size range of 600-700 bp and a CHD-W size range of 400-500 bp; *N. bartelsi* 6 sample had a CHD-Z length of 600-800 bp and a CHD-W size range of 400 bp; *N. bartelsi* 7 sample had a CHD-Z size range of 600-700 bp and a CHD-W size range of 400 bp. Sample *N. bartelsi* 3 had a CHD-Z chromosome (male) only with a length range of 600-700 bp; *N. bartelsi* 4 had a CHD-Z length of 600 bp; and *N. bartelsi* 5 had a CHD-Z length of 600 bp.

Comparison morphometric sexing and molecular sexing

Several parameters for morphometric sexing are deemed to have a good level of accuracy: wing length, culmen and digit III (Kuroda 1933; Ferguson-Lees and Cristie 2001; Muriel et al. 2010; Harmata and Montopoli 2013; Ganbold et al. 2019; Outomuro et al. 2021). The results of morphometric sexing measurements using these parameters were then compared with the results of DNA sexing. The determination of the sexes based on the dominant results of these measurements is shown in Table 4.

From Table 4, it is clearly seen that sex determination of *N. bartelsi* using DNA sexing is more accurate than using morphometric paramaters in that DNA sexing provides a definitive answer based on genetic analysis, whereas morphometric sexing relies on physical characteristics that may vary within a species and can be influenced by factors such as age, nutrition, and geographic location. DNA sexing eliminates subjectivity and potential errors associated with visual assessment. Secondly, DNA sexing can determine the sex of raptors from a very early age, often even before physical sexual dimorphism becomes apparent. This is particularly useful in conservation breeding programs where it is important to know the sex of individuals as soon as possible to manage breeding pairs effectively. Lastly, DNA sexing methods are highly accurate and rarely produce ambiguous results. They can detect the presence of sex chromosomes (e.g., Z and W chromosomes in birds) with high precision, providing reliable information about an individual's sex.

Table 4. Comparison morphometric sexing and DNA sexing of *Nisaetus bartelsi*

Name of raptors	Morphometric parameters			Dominant results of morphometric parameters	Dna sexing	Matched
	Wing length*	Culmen length	Digit III length			
Arimba	Male	Female	Male	Male	Male	Matched
Hayati	Male	Female	Female	Female	Female	Matched
Tegar	Female	Male	Female	Female	Female	Matched
Mario	Female	Female	Male	Female	Female	Matched
Jono	Male	Male	Male	Male	Male	Matched
*Aki	Male	Female	Male	Male	Female	Did not match
*Gagah	Male	Male	Male	Male	Male	Match

Discussion

The successful visualization of PCR products using primers 2550F/2718R was reported by Fridolfsson and Ellegren (1999). In this study, the application that was carried out on various bird species throughout the aves phylogeny successfully amplified the CHD1-Z chromosome with sizes ranging from 600 to 650 bp. In a recent study, it was reported that the length of CHD1-Z in DNA sexing of *N. bartelsi* and *N. cirrhatus* ranged from 640 to 758 bp (*N. bartelsi*) and 690 to 758 bp (*N. cirrhatus*). Another study reported that the length of CHD1-Z in *N. cirrhatus* and *B. liventer* ranged from 638-725 bp (*N. cirrhatus*) and 600-700 only (*B. liventer*) (Sitohang 2017; Annisa et al. 2021; Kaewhom and Srikijkasemwat 2021). It can be interpreted that the results of PCR products obtained from this study were not significantly different from previous studies that conducted molecular sexing in raptors.

DNA strand formation occurs when DNA polymerase extends the base groups of DNA molecules. In the process, DNA polymerase is an enzyme that will synthesize the precursor of DNA molecules, namely deoxynucleotide triphosphate (dNTP), by reading the DNA template strand and creating a new strand. The DNA cluster's length is increased by adding nucleotides at the 3' end of the newly formed strand; this will cause an extension of the base group from the 5' end to the 3' end. However, this nucleotide extension can only add strands without being able to start new strands, so primers are needed to add new strands of DNA (Inshino and Inshino 2014).

However, referring back to the visualized results, the shape of the CHD1 chromosome successfully showed the length of the band and the number of bands. Another contributing factor can be indicated by the amount of DNA polymerase enzyme that affects non-specific results in PCR amplicons. Even so, several factors that may have affected the result have been reported; low or high amounts of DNA polymerase enzymes need to be adjusted, in the case of high polymerase enzymes have the potential to produce more amplified non-specific PCR amplicons (Inshino and Inshino 2014).

From the analysis of morphometric measurements, the best parameters were obtained, where the comparison results for the male sex, based on the explanation in the previous discussion, that most male eagles have a smaller body size and are best for comparing sex. The size of the body frame is based on body size, although body size is not recommended to be included in the examination for determination (Outomuro et al. 2021). In the morphometric measurement of sexing, based on previous studies, it is explained that some variables have a good level of accuracy, such as wing length, culmen, and digit III. Some other variables are not included in the good variables due to several considerations and factors explained in the previous discussion.

The wing span length parameter obtained results that did not follow the protocol that had been made; therefore, the wing length measurement results were longer than the length specified in the protocol. The factor determining the discrepancy in the wing length was that the adapted measurement technique did not provide a specific benchmark. Parameters from some literature show that wing length is measured with specific benchmarks such as flattened wing or wing chord measurement by measuring the carpal joint to the tip of the primary wing and forearm length (Pitzer et al. 2008; Muriel et al. 2010). However, field measurements showed differences in size that point to males. The results of sexes identification were successful in identifying males and females. Male *N. bartelsi* tended to have a wing length in the range of 510-535 mm and females' are longer (535-580 mm) based on the measurements of the *N. bartelsi* subjects.

Based on the results, it was found that morphometric sexing through the variable measurement of the span length of the left-wing, resulted in 3 male *N. bartelsi* (Arimba, Jono, Gagah). Although not much literature can be adapted for morphometric sexing in *N. bartelsi*, it still has the potential to determine the male sex. The accuracy of the wing length variable compared to the DNA sexing results had a highly accurate result, with a correct percentage of all samples of 71.42% (5 out of 7 *N. bartelsi*). The molting period in birds can significantly differ from measurements; young birds will experience molting activity, replacing young to adult feathers (Muriel et al. 2010).

Sex determination on the culmen length variable resulted in 2 males after being confirmed through DNA sexing of all samples with an overall percentage of 57.14%. A total of 2 male *N. bartelsi* matched the measurement values based on the protocol with the values of 27.4 mm and 27 mm. While sex determination through digit length III gets similar results with wing length, namely 5 out of 7 *N. bartelsi* with a percentage of 71.42% after being confirmed through DNA sexing, the overall correct results got 3 males correctly. Morphometric measurements of sexing on the length of male culmen following the protocol resulted in 3 males measuring 48.52 mm, 41.40 mm, and 44.40 mm, respectively.

In the embryonic to hatching phase in aves, male development begins with the Mullerian duct development characterized by thickening of the coelomic epithelium in contact with the Wolffian duct. The formation begins as early as stage 19 HH (Humberger and Hamilton adaptation). The development of the duct leading to sex formation is influenced by the Wolffian (mesonephric) duct; however, in males, it degenerates under the influence of anti-Mullerian hormones formed by Sertoli cells in early development (Jacob et al. 1999).

In male birds with two Z chromosomes, Zhang et al. (2021) reported gene expression levels in males are twice as high as in females. The male gonads are associated with the expression of the Double-sex and Mab-3 Related Transcription Factor 1 (DMRT1) gene; the DMRT1 gene in males has two copies, while one in females (Zhang et al. 2021). To sum up, the DMRT1 gene returns to the influence of anti-Mullerian hormones that affect male growth during the embryonic phase.

Meanwhile, when comparing all data, the results for the male sex are consistent (found in 2 *N. bartelsi*, namely Arimba, and Jono). Both were confirmed to be correct based on morphometric sexing tests and even through

accurate DNA sexing tests. A highly probable reason for the consistent results in the 2 *N. bartelsi* was that the confirmation and DNA sexing on both methods were very good/appropriate. It is reported that the sexual traits of a living creature depend on age and conditions from the past to the times it is sampled (Soulsbury 2018). For example, physiologically, the hormonal status of birds will be influenced by the environment, and it is reported that males are photoperiodic and develop their reproductive systems in response to aging continuously (Davies et al. 2015). Thus, it can be said that the consistency of 2 confirmed male *N. bartelsi* was because they were in a phase where male sexual expression was observed.

Limitations of study

Firstly, the sex determination of female *N. bartelsi* in this study was obtained from the result of the sex determination of the male *N. bartelsi*. The researchers understand that understanding the sex ratio of the population undergoing rehabilitation can provide valuable insight into overall health and dynamic of the species, aiding conservation effort in the long term. While it is equally important to determine female *N. bartelsi* sex in rehabilitation programs, the focus of this study was on the male due to specific conservation priorities, research interests, and budget limitations. Thus, future studies focusing on female *N. bartelsi* are needed to gain a fuller comprehension of the condition at Kamojang Eagle Conservation Centre.

Secondly, although the researchers chose morphometric and DNA sexing method techniques as they were the most commonly used and generally reliable, they may not always provide definite results, especially in certain cases or with specific species.

Thirdly, being a semi-quantitative study, this study did not calculate the significant differences in the parameters for the morphometric sexing using statistics. Rather, it was differentiated through size ratios and also grouped based on age and gender and was then compared to literature references. Future quantitative studies using statistics to calculate the differences of parameters for morphometric sexing are needed to get more specific results in this area.

Lastly, it is important to adopt a multifaceted approach to conservation. While rehabilitation programs play a crucial role in addressing immediate threats to individual raptors and can contribute to population recovery, they are just components of a broader conservation strategy. It is essential to recognize that sustainable population management requires addressing the underlying factors driving population decline and habitat degradation. To address this concern, the researchers would advocate for the implementation of comprehensive conservation initiatives that focus on habitat protection, restoration and management. This may include measures such as habitat conservation agreements and reforestation programs.

In conclusion, the Javan Hawk-eagle (*N. bartelsi*) is an endemic species of eagle in Indonesia that is red-listed as endangered by IUCN. It is vital that this species is protected through conservation to maintain its sustainability, one of which is rehabilitation. The ultimate goal of rehabilitation is to release the Javan hawk-eagle back into its natural habitat. In conservation and breeding programs, accurate determination of the sex of raptors is integral to making decisions, managing the population, and researching. Two of the ways to determine the sex *N. bartelsi* are morphometric and DNA sexing techniques. The research subjects were seven *N. bartelsi* rehabilitated at Kamojang Eagle Conservation Center, Garut, Indonesia. Morphometric sexing measurements obtained the following results: two males were consistent upon comparing DNA sexing and Kamonjang Eagle Conservation Centre records observations. Based on the findings, it can be concluded that DNA sexing examination produces band patterns (CHD-ZZ and CHD-ZW gene expression) through the visualization of PCR products. In comparison, the morphometric sexing accurately identifies both sexes using parameter protocols such as wing length, culmen length, and length of digit III. Each parameter produces correct results for wing length and length of digit III (71.42%), with culmen length obtaining an accuracy rate of 57.14%. These consistent results are revealed across all three parameters, with two males identified. Overall, DNA sexing offers a reliable, accurate, and non-invasive method for determining the sex of raptors, making it an invaluable tool in research, conservation, and management efforts aimed at protecting these magnificent birds of prey.

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REFERENCES

Annisa, Fikriyanti M, Withaningsih S. 2021. DNA sexing for gender determination of changeable hawk-eagle (*Nisaetus cirrhatus*, Gmelin, 1788). E3S Web Conf 248: 2-5. DOI: 10.1051/e3sconf/202124903012.

Azmi N, Syartinilia, Mulyani YA. 2016. The spatial distribution model of Javan Hawk-eagle's (*Nisaetus bartelsi*) remnants habitat in West Java. Media Konservasi 2 (1): 9-18. [Indonesian]

Backus SB, Sutaita D, Odhner LU, Dollar AM. 2015. Mechanical analysis of avian feet: Multiarticular muscles in grasping and perching. R Soc Open Sci 2: 140350. DOI: 10.1098/rsos.140350.

Bildstein KL, Schelsky W, Zalles J, Ellis S. 1998. Conservation status of tropical raptors. J Raptor Res 32 (1): 3-18. DOI: 10.3356/JRR-10-110.1.

Birdlife International. 2017. *Nisaetus bartelsi*, Javan Hawk-Eagle. The IUCN Red List of Threatened.

Bregman TP, Sekercioglu CH, Tobias JA. 2014. Global patterns and predictors of bird species responses to forest fragmentation:

implications for ecosystem function and conservation. Biol Conserv 169: 372-383. DOI: 10.1016/j.biocon.2013.11.024.

Butet A, Rantier Y, Bergerot B. 2022. Land use changes and raptor population trends: a twelve-year monitoring of two common species in agricultural landscapes of Western France. Glob Ecol Conserv 34: e02027. DOI: 10.1016/j.gecco.2022.e02027.

Cerit H, Avanus K. 2007. Sex identification in avian species using DNA typing methods. World Poult Sci J 63: 91-99. DOI: 10.1017/S0043933907001316.

Choi CY. 2013. Morphometric and sexual dimorphism of chinese goshawk (*Accipiter soloensis*). J Raptor Res 4: 385-391. DOI: 10.3356/JRR-13-00007.1.

Davies S, Behbahaninia H, Giraudeau M, Meddle SL, Waites K. 2015. Advanced seasonal reproductive development in a male urban bird is reflected in earlier plasma luteinizing hormone rise bur not energetic statu. Gen Comp Endocrinol 224: 1-10. DOI: 10.1016/j/ygcen.2015.05.005.

Donázar JA, Cortés-Avizanda A, Fargallo JA, Margalida A, Moleon M, MoralesReyes Z, Moreno-Opo R, Perez-Garcia JM, Sanchez-Zapata JA, Zuberogoitia I, Serrano D. 2016. Roles of raptors in a changing world: from flagships to providers of key ecosystem services, Ardeola 63 (1): 181-234. DOI: 10.13157/arla.63.1.2016.rp8.

Ferguson-Lees J, Christie DA. 2001. Raptors of the World. Christopher Helm Publisher, London.

Fridolfsson, AK, Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30 (1): 116-121. DOI: 10.2307/3677252.

Ganbold O, Reading RP, Wingard GJ, Paek WK, Tsolmonjav P, Jargalsaikhan A, Khuderchuluun O, Azua J. 2019. Reversed sexual size dimorphism: body size patterns in sexes of lesser kestrels (*Falco naumanni*) in the Ikh Nart Nature Reserve, Mongolia. J Asia Pac Biodivers 12 (3): 363-368. DOI: 10.1016/j.japb.2019.04.003.

Griffiths R, Korn RM. 1997. A CHD1 Gene is Z Chromosome linked in the chicken *Gallus domesticus*. Gene 197: 225-229. DOI: 10.1016/s0378-1119(97)00266-7.

Gunawan, Widodo TW, Fajar DNA, Jamil I, Permana D. 2020. Population dynamics of Javan Hawk-Eagle (*Nisaetus bartelsi*) in Gunung Picis and Gunung Sigogor nature reserve areas. Siombiosis 8 (1): 9-16. DOI: 10.24843/JSIMBIOSIS.2020.v08.i01.p02. [Indonesian]

Harmata A, Montopoli G. 2013. Morphometric sex determination of north American golden eagles. J Raptor Res 2: 108-116. DOI: 10/3356/JRR-12-28.1.

Hudson LN, Newbold T, Contu S et al. 2014. The PREDICTS database: A global database of how local terrestrial biodiversity responds to human impacts. Ecol Evol 4 (24): 4701-4735. DOI: 10.1002/ece3.1303.

Inshino S, Inshino Y. 2014. DNA polymerase as useful reagents for biotechnology-the history of developmental research in the field. Front Microbiol 5: 45-65. DOI: 10/3389/fmicb.2014.00465.

Jacob M, Konrad K, Jacob HJ. 1999. Early development of the müllerian duct in avian embryos with reference to the human. An ultrastructural and immunohistochemical study. Cells Tissues Organs 162 (2): 63-81. DOI: 10.1159/000016644.

Kaewhom P, Srikijkasemwat K. 2021. Efficacy of two primer sets used in the sex identification of rufous-winged buzzard (*B. liventer*). Open Vet J 11 (4): 581-586. DOI: 10.5455/OVJ.2021.v11.i4.7.

Kuroda N. 1933. Birds of the island of Java: Volume 1. The authors, Tokyo-Japan.

Matagarka A, Balaskas C, Sotirakoglou K, Ikonomopoulus J. 2018. Comparative evaluation of the performance of the PCR assays commonly used for the determination of sex in avian species. J King Saudi Univ-Sci 32 (1): 228-234. DOI: 10.1016/j.jksus.2018.04.020.

McKechnie AE. 2022. Regulation of body temperature. In: Scanes CG, Dridi S (eds). Sturkie's Avian Physiology: Seven Edition. Academic Press, London, UK. DOI: 10.1016/B978-0-12-819770-7.00025-6.

Mueller HC, Mayer K. 1985. The evolution of reversed sexual dimorphism in size. In: Johnston RF (ed). Current Ornithology. Current Ornithology, vol 2. Springer, Boston, MA. DOI: 10.1007/978-1-4613-2385-3_2.

Muriel R, Casado E, Schmidt D, Calabuid AP, Ferrer M. 2010. Morphometric sex determination of young Ospreys *Pandion haliaetus* using discriminant analysis. Bird Stud 57: 336-343. DOI: 10.1080/00063651003674953.

O'Bryan CJ, Braczkowski AR, Beyer HL, Carter NH., Watson JEM, McDonald EM. 2018. The contribution of predators and scavengers to human well-being, Nat Ecol Evol 2 (2): 229-236. DOI: 10.1038/s41559-017-0421-2.

Osman MA, Sugnaseelan S, Panandam JM, Ab Ghani NI. 2020. Molecular sex identification of Malaysian white-nest swiftlet (*Aerodramus fuciphagus* Thunberg, 1812). Ecol Evol 10: 10440-10448. DOI: 10.1002/ece3.6699.

Outomuro D, Golab MJ, Johansson F, Sniegula S. 2021. Body and wing size, but nor wing shape, vary along a large-scale latitudinal gradient in a damselfy. Sci Rep 11: 18642. DOI: 10/1038/s41598-021-97829-9.

Pitzer S, Hull J, Ernest HB, Hull AC. 2008. Sex determination of three raptor species using morphology and molecular techniques. J Field Ornothol 79 (1): 71-79. DOI: 10.1111/j.1557-9263.2008.00147.x.

Prawiradilaga DM. 2017. Endemism. Reference Module in Life Sciences. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12- 809633-8.02164-6.

Prawiradilaga DM. 2019. Keanekaragaman dan Strategi Konservasi Burung Endemik Indonesia. LIPI Press, Jakarta. [Indonesian]

Pruscini F, Morelli F, Perna P, Mazzeo R, Cavitolo P, Bertellotti M, Catorci A, Santolini R. 2016. Roadside raptor surveys in valdes Peninsula (Patagonia, Argentina). Hornero 31 (1): 7-11. DOI: 10.56178/eh.v31i1.570.

Ramsey DSL, MacDonald AJ, Quasim S, Barclay C, Sarre SD. 2015. An examination of accuracy of a sequential PCR and sequencing test used to detect the incursion of an invasive species: The case of the red fox in Tasmania. J Appl Ecol 53 (3): 562-570. DOI: 10.1111/1365-2664.12407.

Rudnick JA, Kaatzner TE, Bragin EA, Rhodes OE, Dewoody JA. 2005. Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern Imperial Eagle (*Aquila heliaca*) population from Kazakhstan. Mol Ecol 14: 2959-2967. DOI: 10.1111/j.1365-294x.2005.02641.x.

Sergio F, Caro T, Brown D, Clucas B, Hunter J, Ketchum J, McHugh K, Hiraldo F. 2008. Top predators as conservation tools: ecological rationale, assumptions, and efficacy. Ann Rev Ecol Evol Syst 39: 1-19. DOI: 10.1146/annurev.ecolsys.39.110707.173545.

Sitohang LR, Yuda P, Mursyanti E. 2017. Molecular sexing pada Elang Jawa (*Nisaetus bartelsi* Streseman, 1924) dan Elang Brontok (*Nisaetus cirrhatus* Gmelin,, 1788) hasil sitaan BKSDA di Yogyakarta. E-Journal Universitas Atma Jaya Yogyakarta. Universitas Atma Jaya, Yogyakarta. [Indonesian]

Soulsbury CD, Lipponen A, Wood K, Mein CA, Hoffman JI, Lebigre C. 2018. Age- and quality-dependent DNA methylation correlate with melanin-based coloration in a wild bird. Ecol Evol 8 (13): 654-6557. DOI: 10.1002/ece3.4132.

Supriatna AA. 2012. Current status of diurnal raptors in Indonesia and its conservation challenges. Ornis Mongolica 1: 68-73.

Wenny DG, DeVault TL, Johnson MD, Kelly D, Sekercioglu ÇH, Tomback DF, Whelan CJ. 2011. The need to quantify ecosystem services provided by birds. Auk 128 (1): 1-14. DOI: 10.1525/auk.2011.10248.

Withaningsih S, Iskandar J, Prawiradilaga DM. 2019. Conservation and management strategies for the sustainability of raptors in a human-modified landscape. Intl J Conserv Sci 10 (4): 749-762.

Zhang X, Li J, Chen S, Yang N, Zheng J. 2021. Overview of avian sex reversal. Intl J Mol Sci 24 (9): 8284. DOI: 10.3390/ijms24098284.