The Sex Identification of the Sun Conure (*Aratinga solstitialis)* Using Calamus Based on Polymerase Chain Reaction

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ABSTRACT

Sex identification of Sun Conures (*Aratinga solstitialis*) is crucial for breeding and preservation, as well as increasing sun conure populations. These birds are sexually monomorphic. Therefore, Determination between male and female carried out by their morphology examination. The Polymerase Chain Reaction (PCR) method, utilizing molecular-based technology, was employed to determine the sex of *Aratinga solstitialis* in this study. The P2 and P8 primers were utilized in this method, which has been deemed suitable and accurate for sex identification through calamus samples. The research focused on two 28-month-old *Aratinga solstitialis* birds. Calamus samples were collected and subjected to PCR amplification using the extracted calamus. The resulting PCR products were then visualized using electrophoresis with a 1% agarose gel. In the electrophoresis photo, the presence of two bands indicated a female specimen, whereas a single band indicated a male specimen. The result of the gel electrophoresis research showed that both of the *Aratinga solstitialis* were male with one band of each bird on ranged from 300-400 base pairs. The result show that the Polymerase Chain Reaction method in terms for sex identification on monomorphic birds, especially *Aratinga solstitialis* birds is very effective to differentiate the sex of young birds and the adults.

Keyword: *Aratinga solstitialis*, PCR, sex determination

INTRODUCTION

The Sun Conure bird (*Aratinga solstitialis*) is a type of parrot. It is monomorphic, so the female and male birds are very similar and difficult to distinguish if only based on appearance (Höfling, 2005). The sex of birds can be identified by: (1) observation of behavior, (2) presence or absence of

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brooding patches, (3) differences in morphometric characteristics, (4) examination of the gonads by laparotomy or laparoscopy, and (5) examination of the sex chromosomes (Cerit and Avanus, 2007).

Aves and mammals have different sex chromosomes. Mammals have heterozygous XY sex chromosomes in males and homozygous XX in females. The sex chromosomes in aves are the opposite, namely ZW heterozygous sex chromosomes in females and ZZ homozygous in males (Grant, 2001). Molecular sex determination is a highly accurate method that can be utilized for young birds and monomorphic birds. This approach directly targets the sex chromosomes, resulting in a precise determination of the bird's gender (Holsinger *et al*., 2002).

Molecular techniques for sex identification in birds require DNA samples. DNA is obtained from extracting and purifying a cell, tissue, or organ. Bird DNA can be obtained through calamus (Dubiec and Neubauer, 2006). Calamus in bird can be a source of DNA because the calamus base contains many epithelial cells. Genetically, calamus originate from epidermal origin, while embryologically, it originates from the dermal papillae (Questiau *et al*., 2000). The number of calamus that can be used in determining the sex of a bird is three secondary wing calamus. Inside the calamus are deposits of blood and living cells in the *calamus*. Blood deposits and cells in the *calamus* are used as a source of DNA. DNA isolation in bird can be done with a small amount of blood because bird have red cells with nuclei. (Nugroho and Zein, 2015).

The Chromodomain Helicase DNA binding (CHD) gene serves as a genetic marker for determining the sex of birds. The CHD gene is located on both the W and Z chromosomes, specifically CHD-W on the W chromosome and CHD-Z on the Z chromosome (Dubiec and Zagalska-Neubauer, 2006). The sexing primers often used are P2 and P8, which have been proven that these primers P2 and P8 can determine the sex of 27 birds species from 23 families (Dawson *et al*., 2002). PCR is often used in molecular sexing because it is a practical, precise, and fast method of sexing. PCR also has high sensitivity (Reddy *et al*., 2007).

The identification of sex bird plays a significant role in both poultry farming and the keeping of birds in captivity. Based on the background described above this study aimed to prove that a sample of the Sun Conure's calamus (*Aratinga solstitialis*) can be used to identify the sex of the Sun Conure bird by using PCR.

MATERIALS AND METHOD

The materials for DNA isolation in birds using the DNeasy® Tissue Kit C (No. 69504, Qiagen Germany) include the following components: 200 µl ethanol, 180 µl ATL lysis buffer, 200 µl AL buffer, 20 µl of 10 mg/ml proteinase

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K, 500 µl AW1 buffer, 500 µl AW2 buffer, and 200 µl AE buffer. Additionally, other materials required are a 1% agarose gel, ethidium bromide, loading dye, TBE (Tris-Borate-EDTA) buffer, 12.5 µl of super master mix containing DNTPs, Taq polymerase, PCR reaction buffer, and gel loading buffer. Furthermore, the materials include a 100 bp DNA (lot no. 00066905), 3 µl of distilled water (DW), and the primers P2 and P8.

The tools needed in this study include: PCR machine, tips 100 µl, tips 1000 µl, microcentrifuge tube 10 µl, microcentrifuge 100 µl, eppendorf micropipette 10 µl, eppendorf micropipette 100 µl, eppendorf micropipette 1000 µl, collection tube, electrophoresis apparatus, geldocumentation, stavolt, , microcentrifuge, spin down, UV transilluminator, dry bath.

Between March and April 2021, this research was conducted on two Sun Conures (*Aratinga solstitialis*) aged 28 months old. Three calamus were obtained from each bird, representing potential male and female specimens, based on their body posture. Polymerase Chain Reaction (PCR) was then employed to analyze the DNA samples. DNA results are used for testing using PCR. The primers were P8 (5'-CTCCCAAGGATGAGRAAYTG-3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3'). The PCR amplification consisted of a pre-denaturation step at 94°C for 5 minutes, 30 cycles consisting of denaturation at 94°C for 1 minute 30 seconds, annealing at 50°C for 1 minute, and extension at 72°C for 45 seconds, and the final stage of extension at 72°C for 5 minutes (Malago *et al*., 2002; Sefc *et al*., 2003). The PCR results are then visualized using 1% agarose gel. The sex of the Sun Conure bird was identified from the number of bands produced at 300bp – 400bp. Male birds will produce one band, while the female sex will produce two bands (Cerit and Avanus, 2007).

RESULT AND DISCUSSION

The Sun Conure (*Aratinga solstitialis*) feather sample used to carry out the PCR test is presented in Figure 1.

Figure 1. The sample of Sun Conure bird calamus. The arrows point to the calamus.

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Calamus in birds have prospects as a source of DNA because the base of the calamus contains epithelial cells, and using calamus samples can reduce stress in birds by avoiding excessive blood loss (Cerit and Avanus, 2007).

The PCR results are then read using electrophoresis, with the reading results in Figure 2.

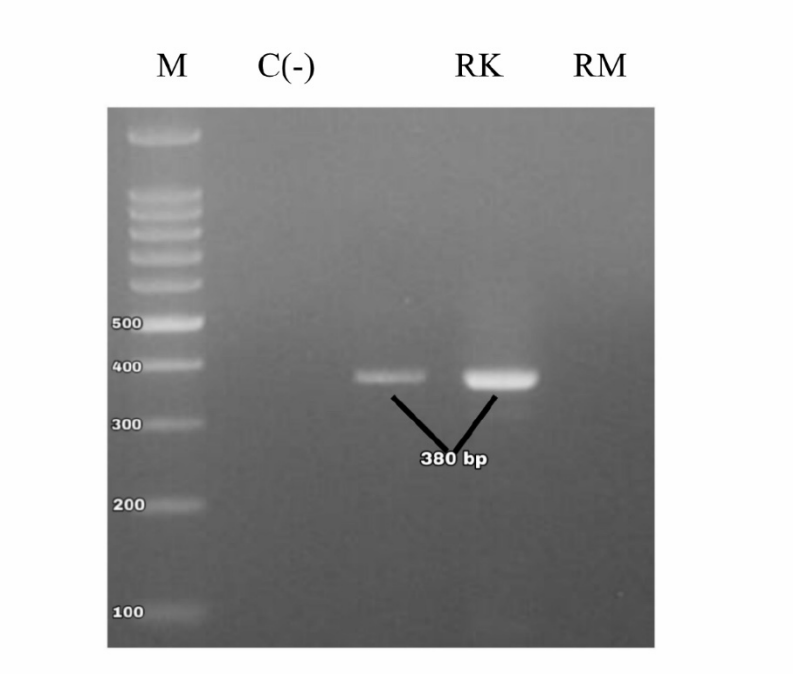

Figure 2. The test results were read using 1% agarose gel electrophoresis.

The results of research on calamus samples obtained by plucking three calamus from each Sun Conure bird (*Aratinga solstitialis*) which owners believe to be male and female after going through the PCR process show that the two calamus samples are male. After the electrophoresis process, the band is in the middle, between 300 bp and 400 bp (380 bp), with a single DNA. PCR results from male and female bird feather samples were expected to show very different results, with a single DNA band in males and two in females (Purwaningrum *et al*., 2019).

Cultivating monomorphic birds, such as the Sun Conure, can be accelerated by carrying out sex identification with the PCR technique because the time to do the PCR technique is very short and does not require a lot of DNA sources, namely only using a few strands of calamus. Hence, the risk of the bird being stressed is minimal (Cerit and Avanus, 2007).

PCR technique using specific primers for sex determination has been known to be used to determine the sex of monomorphic birds. The sexing primers often used to identify the sex of

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birds are primers P2 and P8, designed by Griffiths. The P2 primer is a reverse primer, while the P8 is a forward primer used to sex identification. The base sequence consists of P8 (5'-CTCCCAAGGATGAGRAAYTG-3') while P2, namely (5'-TCTGCATCGCTAAATCCTTT-3'). The base size of the DNA bands produced from PCR ranges from 300 bp to 400 bp, and each species has variations in size (Griffiths *et al.,* 1998).

These findings are also consistent with previous studies in which rearchers used feather as a potential source for DNA extraction (Bayard de Volo *et al*., 2008; Andleeb *et al*., 2012; Avanus and Koenhemsi, 2018; Purwaningrum *et al*., 2019). Especially the study of DNA extraction used only some parts of the feather such as calamus (Presti *et al*., 2013; Avanus and Koenhemsi, 2018; Changtor and Yimtragool, 2020). The findings suggest that a part of the feather could be used as a source of DNA for biomolecular research.

CONCLUSION

This research concludes that examination of the CHD-Z and CHD-W genes using calamus with the Polymerase Chain Reaction method can be used to identify sex in Sun Conure birds (*Aratinga solstitialis*) with accurate results. The results of the identification of the sex of the Sun Conure bird were based on the test results which showed one band so that it could be concluded that the two Sun Conure birds were male.

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REFERENCES

Andleeb, D.S., S. Shamim, M.N. Awan and R.A. Minhas. 2012. Modified protocol for genomic DNA extraction from newly plucked feathers of (*Lophura leucomelana hamiltoni*) (Galliformes) for genetic studies and its endo-restriction analysis. Pak.J. Sci.Res; 55(2):108-113.

Avanus, K and L. Koenhemsi. 2018. Investigating the usage of molted feather samples as a DNA source with two methods in gender identification of African Grey Parrot (*Psittacus erithacus*) by molecular analyses of CHDW and CHDZ genes. Koc.Vet.J; 11:40-44.

Bayard de Volo, S., R. Reynolds, M. Douglas and M. Antolin. 2008. An improved extraction method to increase DNA yield from molted feathers. Condor; 110:762-766.

Cerit, H and Avanus. 2007. Sex Identification in Avian Species Using

Received: 27-06-2023, Accepted: 23-10-2023, Published online: 08-01-2024
Available at https://e-journal.unair.ac.id/MKH/index

DNA Typing Methods. Wor.Poult.Sci.J; 63.

Changtor, P and N. Yimtragool. 2020. Comparison of DNA extraction methods and selection of primer sets for sex identification of the red-whiskered Bulbul (*Pycnonotus jocosus*). Int.J.Poult.Sci; 19:244-251.

Dawson, A.D., D. Steven., F.M. Hunter., A.P. Krupta., I.L. Jones and B. Terry. 2002. A critique of avian CHD-based molecular sexing protocols illustrated by a Z-chromosome polymorphism detected in auklets. University of Stirling. UK.

Dubiec, A. and M. Zagalska-Neubauer. 2006. Molecular techniques for sex identification in birds. Bio.Lett; 43(1):3-12.

Grant, A. 2001. DNA sexing of brown kiwi (*Apetryx mantelii*) from feather samples. DOC Science Internal Series. Wellington: Department of Conservation.

Griffiths, R., M.C. Double., K. Orr and R.J. Dawson. 1998. A DNA test to sex most birds. Mol.Eco.J; 7:1071-1075.

Höfling, E. 2005. A New Species of Aratinga Parakeet (Psittaciformes: Psittacidae) from Brazil, with Taxonomic Remarks on the *Aratinga solstitialis* Complex. The Auk; 122(1):292-305.

Holsinger, E.K., O.P. Lewis and K.D. Dey. 2002. A Bayesian approach to inferring population structure from dominant markers. Mol.Eco.J.

Malago, Jr.W., M.F. Heitor., Jr.E. Matheucci., A. Medaglia and F. Hendrique-Silva. 2002. Large Scale Sex Typing of Ostriches Using DNA Extracted from Feathers. BMC.Biotec; 2: 19.

Nugroho, H.A dan M.S.A. Zein. 2015. Evaluasi metode penentuan jenis kelamin pada nuri kepala hitam. J.Faun.Trop; 24:83.

Presti, F., J. Meyer, P. Antas, N. Guedes and C. Miyaki. 2013. Non-invasive genetic sampling for molecular sexing and microsatellite genotyping of hyacinth macaw (*Anodorhynchus hyacinthinus*). Genet.Mol.Biol; 36:129-133.

Purwaningrum, M., H.A. Nugroho, M. Asvan, K. Karyanti, B. Alviyanto, R. Kusuma and A. Haryanto. 2019. Molecular techniques for sex identification of captive birds. Vet.World; 12(9):1506-1513.

Questiau., N., M. Escaravage,. C. Eybert and P. Taberlet. 2000. Nestling S ex Ratios in a Population o f B luethroats Luscinia S vecica Inferned from AFLP™ Analysis. J.Avi.Bio; 31:814.

Reddy, A., V. Prakash and S. Shiveji. 2007. A Rapid, Non-Invasive, PCR-Based Method for Identification of Sex of The Endangered Old World Vultures Implications for Captive Breeding Programmes. Current Science.

Sefc K.M., R.B. Payne and M.D. Sorenson. 2003. Microsatellite Amplification from Museum Feather Samples : Effects of Fragment Size and Template Concentration On

Received: 27-06-2023, Accepted: 23-10-2023, Published online: 08-01-2024
Available at https://e-journal.unair.ac.id/MKH/index

Genotyping Errors. The Auk; 120: 982-989.

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