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Mitochondrial DNA Haplotype Analysis of the Endemic and Critically Endangered Philippine Cockatoo (*Cacatua haematuropygia* P.L.S. Müller, 1776)

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The Philippine cockatoo or "katala" (*Cacatua haematuropygia*) is a Critically Endangered parrot species endemic to the Philippines with an estimated wild population of up to 1,120 individuals. Threats to its continued existence include habitat destruction and poaching for the pet trade. While protected populations exist in Palawan under the care of the Katala Foundation Inc., no previous genetic studies aimed at quantifying genetic diversity in the protected populations have been done. This study examines two mitochondrial genes, *ND2* and *COX1*, in protected populations of the katala. *ND2* was sequenced for 101 individuals while for *COX1*, 103 individuals were successfully sequenced. Results show that very few haplotypes are present for both *ND2* and *COX1* and there is a possible reduction of genetic diversity over time. In addition, the nuclear gene *TGFB2* was examined for 31 individuals. *TGFB2* sequences were highly similar, further supporting the hypothesis of a possible genetic bottleneck. Future conservation work and breeding efforts for the species should consider the possible consequences of reduced genetic diversity.

Keywords: conservation, *COX1*, genetic bottleneck, haplotypes, *ND2*, Philippine cockatoo

INTRODUCTION

The Philippine cockatoo – locally known as "katala," "kalangay," or "abukay" – is a small, white bird with red underparts (Boussekey 2000; BirdLife International 2017). Its preferred habitats are riverine or coastal forests, often associated with mangroves and breeds between January and July (Widmann *et al.* 2001; BirdLife International 2017). It feeds on fruits, flowers, and seeds of various tree species such as the horseradish tree (*Moringa oleifera*), "narra" (*Pterocarpus indicus*), and "kapok" (*Ceiba pentandra*) (Widmann *et al.* 2001; Nierves *et al.* 2017; Widmann and Widmann 2017). It was once commonly found throughout the Philippine Archipelago (Boussekey 2000) but is now restricted to a few islands, mainly in Palawan and the islands of the Sulu archipelago, with small populations of less than 20 individuals in the islands of Polilio, Homonhon in Samar province, and Bohol (BirdLife International 2018). Sightings of the species since 1980 have also included the islands of Masbate, Samar, Leyte, Bohol, Siargao, Negros, Siquijor, Mindanao, and Sarangani (Figure 1) (BirdLife International 2001). More recent surveys did not yield any records from southern Luzon, Mindoro, Marinduque, Masbate, Leyte, Negros, or Siargao (Widmann *et al.* 2013) and Subic Bay, Luzon Island (Española *et al.* 2013). Records indicate that several individuals were observed

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at Mt. Isarog in Bicol province in 1988, but these may have been escapees from the pet trade since they were spotted at 1,100 meters above sea level – an unusual altitude for this species (BirdLife International 2001). In total, the remaining wild population is estimated at 640 to 1,120 individuals, with 75% of these found in Palawan (Widmann and Widmann 2017). It is listed as Critically Endangered by the International Union for the Conservation of Nature (IUCN) (BirdLife International 2017). Continued threats to the species include persistent habitat destruction, poaching, and the pet trade (Cruz *et al.* 2007; Widmann and Widmann 2016; Nierves *et al.* 2017; BirdLife International 2018).

Conservation efforts to increase the population of the Philippine cockatoo focus mostly on increasing the numbers of individuals and on habitat protection in Palawan (Widmann *et al.* 2006; Widmann and Widmann 2008), although efforts are underway to reintroduce birds into their historical range (Boussekey 2000; Widmann *et al.* 2013, 2018; Nierves *et al.* 2017; BirdLife International 2018). Due to its fragmented populations, the species is vulnerable to inbreeding depression that could impair its ability to adapt to new environmental pressures brought about by climate change (Edmands 2007). Another Philippine endemic, the Philippine eagle (*Pithecophaga jefferyi*), is in a similar plight. It is a critically endangered raptor found only in four islands in the Philippines with an estimated wild breeding population of around 500 individuals (Salvador and Ibañez 2006; Luczon *et al.* 2014). Luczon *et al.* (2014) found reduced haplotype diversity for Luzon and Samar populations compared to Mindanao populations of the Philippine eagle. This is not surprising, considering the rampant deforestation in the Philippines (Lasco *et al.* 2001). A study using minisatellites for the almost extinct Spix's macaw (*Cyanopsitta spixii*) found almost full-sibling relationships between individuals in a captive population (Caparroz *et al.* 2001). The same situation may exist with the Philippine cockatoo. However, a lack of population genetics studies on the species means that there is no objective way of detecting and quantifying genetic diversity in the protected populations of the katala in Palawan province.

In this study, we have decided to use two mitochondrial genes to explore the genetic diversity of katala populations in Palawan. The first gene is cytochrome c oxidase subunit I (*COX1*) gene, which has previously been used to examine genetic structuring in other bird species, such as Macqueen's bustard (Haghani *et al.* 2018), the Chinese egret (Hwang *et al.* 2018), and the endangered New Zealand Cook's petrel (Rayner *et al.* 2010). The second gene is nicotinamide dehydrogenase subunit 2 (*ND2*). A complete, 1041-bp *ND2* sequence already exists in GenBank for the Philippine cockatoo (Schirtzinger *et al.* 2012). *ND2* has been used to examine genetic structuring and biogeography in other bird species in conjunction with other molecular markers (Billerman *et al.* 2011; Garcia *et al.* 2011; Miller *et al.* 2015), including parrots like the hyacinth macaw (Presti *et al.* 2015). The mitochondrial control region, which may evolve faster, was not used due to duplications present in parrots and cockatoos (Urantówka *et al.* 2018) that made amplifying short, specific segments problematic.

Intron 5 of the transforming growth factor B2 (*TGFB2*) gene was also sequenced using primers designed by Burt and Patton (1991). Together, nuclear and mitochondrial genes should provide a more accurate view of genetic diversity among Philippine Cockatoos than that obtained using only mitochondrial DNA (Ballard and Whitlock 2004).

This study examines the genetic diversity of the katala from four locations in Palawan: Dumaran Island in northern Palawan, Rasa Island, and Iwahig Prison and Penal Farm (IWPP) in central Palawan, and Pandanan Island in southern Palawan (Figure 1). Additionally, feather samples were obtained for a single specimen from Tawi-Tawi province in the Sulu Archipelago (Figure 1).

MATERIALS AND METHODS

Whole blood and feather samples of the Philippine cockatoo were obtained and sent by the Katala Foundation Inc. from four sites in Palawan. These samples were preserved in absolute ethanol inside 1.5 ml tubes. DNA extraction was performed using PureLink™ Genomic DNA Mini Kit, ThermoFisher Scientific (ThermoFisher, USA), or Bioline Genomic DNA Isolate II (Bioline, UK).

ND2 (1,040 bp) was amplified using the primers L5143c (AGGAATCAAAATCCTCCATACTC) and H6313 (ACTCTTRTTTAAGGCTTTGAAGGCS), with a third primer L5758 as an internal primer for Sanger sequencing (GGNGGNTGAATRGGNYTNAAYCARAC). A 920-bp fragment of the *COX1* was amplified using L6615 (CCTCTGTAAAAAGGACTACAGCC) and either H7539c (GATGTAAAGTATGCTCGAGTATCTAC) or H7548 (GTGGCGGACGTGAAGTATGCTCG) as reverse primers. These primers for *ND2* and *COX1* are either taken directly or modified from literature (Sorenson 2003; Sorenson *et al.* 1999). An alternate set of primers for *COX1*, BirdF1 (TTCTCCAACCACAAAGACATTGGCAC) and BirdR1 (ACGTGGGAGATAATTCCAAATCCTG), was used that produced a 750-bp PCR (polymerase chain reaction) product (Hebert *et al*. 2004). A 552-bp fragment of *TGFB2* was also amplified using the primers TGFB2-F (GAAGCGTGCTCTAGATGCTG)

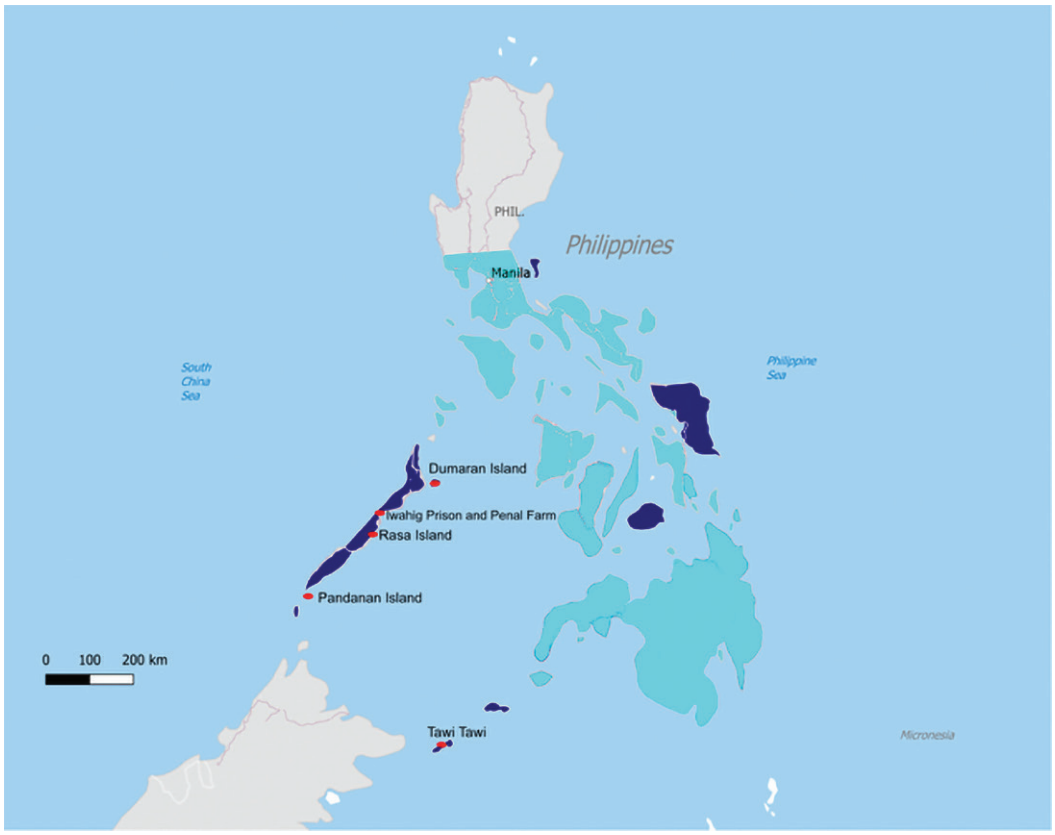

Figure 1. Distribution of the Philippine cockatoo. Areas in dark blue represent areas where there are extant populations of the katala while areas in cyan represent its former range (BirdLife International 2001; Española *et al.* 2013; Widmann *et al.* 2013, 2018). Locations of origin for the katala samples used in this study are marked in red ellipses.

and TGFB2-R (AGGCAGCAATTATCCTGCAC) (Burt and Paton 1991).

For all genes, a 22–25-µL PCR mix was prepared using the following components: 12.875 µL of ultra-pure water (upH_2O), 5 µL of 10X MyTaq™ PCR Buffer (Bioline, UK), 0.75 µL of each of the appropriate forward and reverse primer (10µM), 0.5 µL of 50-mM $MgCl_2$ (Bioline, UK), 0.125 µL of MyTaq™ DNA Polymerase (5U/µL) (Bioline, UK), and 2 µL (for *ND2* and *COX1*) or 5 µL (for *TGFB2*) of DNA template.

The primers for *ND2* bind to tRNAs flanking the gene; these were removed and only the 1,040 bp *ND2* gene was used for analysis. The amplified fragments of *COX1* were trimmed to 564 bp. The region used for *COX1* is the barcoding region for birds developed by Hebert and colleagues (2004).

For *ND2* and *COX1* (using L6615 and H7539c or H7548), initial denaturation was at 94 °C for 2 min, followed by 40–43 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 1 (*COX1*) or 1.5 min (*ND2*), and a final extension step at 72 °C for 2 min.

When BirdF1/BirdR1 was used to amplify *COX1*, a two-stage protocol (Hebert *et al*. 2004) was used: initial denaturation at 94 °C for 1 min, followed by five cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1.5 min, and extension at 72 °C for 1.5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 1.5 min, and extension at 72 °C for 1.5 min, followed by a final extension at 72 °C for 5 min.

For *TGFB2*, a two-stage protocol was followed, consisting of an initial denaturation step at 96 °C for 1 min, followed by 21 cycles of denaturation at 96 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 40 s, followed by 21 cycles of denaturation at 96 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s, and a final extension step at 72 °C for 40 s.

Agarose gel electrophoresis (1% agarose, 1% ethidium bromide) was done to isolate the DNA products, with HyperLadder™ 100 bp (Bioline, UK) or HyperLadder™ 1kb (Bioline, UK) molecular ladder loaded alongside DNA samples in order to measure band molecular weight. Isolated DNA was then purified using Thermo Scientific™ GeneJET Gel Extraction Kit (USA) or Zymoclean™

Gel DNA Recovery Kit (Zymogen, USA) following the manufacturer's protocols and sent to Macrogen in South Korea for Sanger Sequencing.

Sequences were checked for quality and assembled using Pregap4 and Gap 4 (Bonfield *et al.* 1995) of the Staden package (v. 2.0.0b10) (Staden *et al.* 2000), aligned using Clustal Omega (v. 1.2.2) (Sievers *et al.* 2011) and visualized and trimmed in BioEdit (v. 7.0.9) (Hall 1999). Sequences for *COX1* and *ND2* were concatenated using MEGA (v.7.0.26) (Kumar *et al.* 2016). Haplotypes were quantified using the python script hapFrequency, which is part of the haploTools package written by Chafin (2019). Haplotype diversity was calculated using PopSc (Chen *et al.* 2016). Haplotype and nucleotide diversity were computed using Arlequin v.3.5.2.2 (Excoffier and Lischer 2010). Representative sequences for each *ND2* and *TGFB2* haplotype or genotype, respectively, were submitted directly to GenBank, while *COX1* haplotypes were uploaded to the Barcode of Life Database and submitted secondarily to GenBank (Appendix Table I). A haplotype network (Figure 2) was constructed in R using the pegas package v.0.14 (Paradis 2010) while analysis of molecular variance (AMOVA) (Appendix Figure I) was carried out using GenAlex v.6.503 (Peakall and Smouse 2006, 2012).

RESULTS AND DISCUSSION

For *ND2*, 101 individuals were sequenced: 37 from Rasa Island, 36 from Pandanan Island, 16 from IWPP, and 12 from Dumaran Island. An additional sequence for *ND2* (Schirtzinger *et al.* 2012) was downloaded from GenBank and included in the analysis.

A total of six variable sites and eight haplotypes were found for 101 individuals (Appendix Table I). Three of the variable sites are heteroplasmic, with two haplotypes being present in one individual; each heteroplasmic site was treated as unique in its own right, instead of calling it as either or both of the bases it contains to avoid double counting or inflating the counts of other haplotypes. One additional haplotype is represented by the sequence uploaded by Schirtzinger *et al.* (2012) in GenBank; this comes from a preserved specimen collected in 1992 from an unknown locality in the Philippines and currently in the collection of the Smithsonian National Museum of Natural History. It is possible that this unique haplotype arose due to the degradation of DNA and sequencing error given its provenance as a museum specimen. More data from museum specimens will help verify the previous existence of more mtDNA haplotypes.

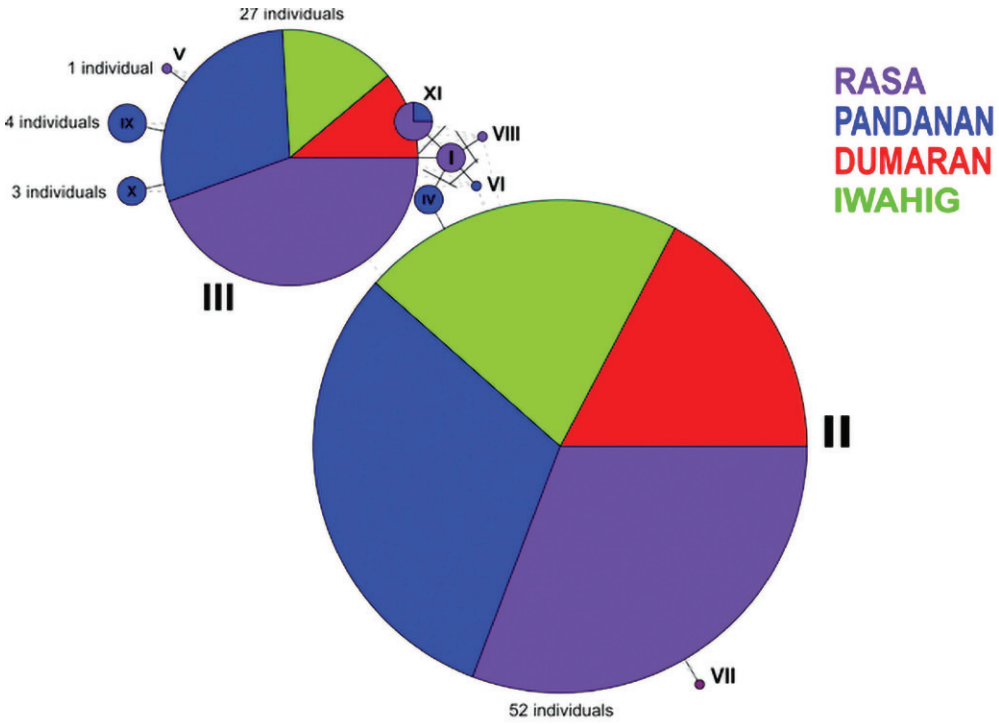

Figure 2. Haplotype network of the concatenated *nd2* and *cox1* dataset. Only Rasa and Pandanan islands contain individuals with unique haplotypes.

For *COX1*, 103 individuals were sequenced: 38 from Rasa, 37 from Pandanan, 16 from IWPP, and 12 from Dumaran. Only three haplotypes were found (Appendix Table II). The single variable site contains a transition from A–G. A total of five individuals are heteroplasmic at this site, forming the third haplotype for this gene. The GenBank sequence for *COX1* for this species corresponds to a different region of *COX1*, so it was not included in this analysis.

Only 31 individuals were sequenced for intron 5 of *TGFB2* due to difficulty in amplifying nuclear DNA: eight from Rasa, 10 from Pandanan, seven from IWPP, and six from Dumaran. A sequence for this gene (Schirtzinger *et al.* 2012) is available in GenBank and was included in the analysis. The final length of the alignment is 552 bp. In all, three genotypes were found for *TGFB2* (Appendix Table II). The differences between genotypes are due to a transition from C–T. Nine individuals were heterozygotes, which constitute the third genotype for this gene. The sequence published by Schirtzinger *et al.* (2012) for this gene is also apparently a heterozygote.

Concatenating *ND2* and *COX1* for 100 individuals results in 11 haplotypes, dominated by haplotypes 2 and 3, containing 52 and 27 individuals, respectively (Appendix Table II). The haplotype diversity is 0.657 (Table 1).

Our results show little diversity in the Palawan populations of the katala. For *ND2*, the population in Pandanan Island has three unique haplotypes in addition to the three haplotypes it shares with other populations, while Rasa Island has two unique haplotypes. These, however, were detected in four or fewer individuals for each island, suggesting that they may be new mutations. A similar pattern is seen with the concatenated alignment, where only Rasa and Pandanan have unique haplotypes that are present in very few individuals (Appendix Table II). For *TGFB2*, only 31 individuals were sequenced, but all belong to three genotypes. Overall, based on the concatenated dataset, the Palawan population has a relatively high haplotype diversity but low nucleotide diversity (Table 1).

Feather samples from a single individual from the province of Tawi-Tawi were also obtained; unfortunately, these were not preserved in ideal conditions and took some time to be transported to the laboratory. Only partial sequences for *ND2* (537 bp) and *COX1* (470 bp) were obtained. The *ND2* gene for this individual differs from the specimen of Schirtzinger *et al.* (2012) in the same base as the Palawan populations (A–T transversion), but it is not long enough to classify into any of the haplotypes found for Palawan. The fragment for *COX1* only has a 338-bp overlap with the barcoding region of the gene used in this study, but it is identical throughout its overlap with the Palawan samples.

The present results could be an artefact of uneven sampling. Dumaran Island and IWPP are relatively underrepresented compared to Rasa and Pandanan Island, but these reflect the natural abundance of the Katala for each study site. It is also telling that our results are consistent for all three genes – only a few haplotypes and alleles were found; for the concatenated mtDNA dataset, most individuals belong to two dominant haplotypes. The haplotype network (Figure 2) and AMOVA (Appendix Figure I) show that little population differentiation exists and that while Rasa and Pandanan Island contain unique haplotypes, these are found in small numbers and are derived from the two most numerous haplotypes (Figure 2). While more individuals from Palawan are needed to be certain, we believe that the low genetic diversity and lack of genetic differentiation is not an artefact of sampling for the Palawan populations. While it is possible that other populations of the Katala that are not included in this study are genetically differentiated, the lone Tawi-Tawi sample indicates otherwise.

Over the past century, the range of the katala has shrunk dramatically, from being present throughout most of the archipelago to being relegated to a few island strongholds, notably in Palawan and the islands of Tawi-Tawi and Sulu in the Sulu archipelago (Figure 1) (Boussekey 2000; BirdLife International 2001, 2018; Widmann *et al.* 2013). Outside of Palawan and the Sulu Archipelago, remnant populations are only known from Polillo, Bohol, Samar, and Homonhon (Figure 1); all of these populations are overaged and have to

Table 1. Haplotype and nucleotide diversities of the concatenated *ND2* and *COX1* alignment.

Location	N[a]	Haplotype diversity	Nucleotide diversity	Unique haplotypes
Rasa	37	0.7117	0.000647	4
Pandanan	36	0.7460	0.000762	4
IWPP	15	0.4190	0.000522	0
Dumaran	12	0.4091	0.000510	0
Overall	100	0.6570	0.000675	-

[a]N – number of samples or individuals for each location

be considered as functionally extinct (Widmann *et al.* 2013). This distribution is greatly diminished when compared to its previous distribution all over the Philippines, except for northern Luzon and the islands of Batanes and Babuyan (BirdLife International 2001).

A species with a similar demographic history of population decline is the Philippine eagle (*Pithecophaga jefferyi*) (Salvador and Ibañez 2006). A loss of haplotypes is a hallmark of genetic drift due to small populations (Allendorf 1986). Genetic studies indicate that the Philippine eagle has high haplotype diversity and low nucleotide diversity (H = 0.8960, π = 0.0062, n = 22) (Luczon *et al.* 2014), which is similar to the Philippine cockatoo and consistent with a possible pattern of demographic decline from a previously genetically diverse population (Spielman *et al.* 2004). A decline in genetic diversity is also seen for historical and contemporary populations of the "kākāpō" (*Strigops habroptilus*) and the white-headed duck (*Oxyura leucocephala*) using the mitochondrial control region (Muñoz-Fuentes *et al.* 2005; Bergner *et al.* 2016). In the kākāpō, historical populations had 17 haplotypes (H = 0.82, π = 0.00104, n = 51) while contemporary ones have only three haplotypes, two of which are apparently novel (H = 0.56, π = 0.00114, n = 45) (Bergner *et al.* 2016). In the white-headed duck, historical populations had 10 haplotypes (H = 0.708, π = 0.00539, n = 67) while contemporary populations in Spain (H = 0.456, π = 0.00238, n = 39) and Greece (H = 0.286, π = 0.00149, n = 7) each had only two haplotypes (Muñoz-Fuentes *et al.* 2005). The presence of a unique haplotype for *ND2* in GenBank, taken from a museum specimen by Schirtzinger *et al*. (2012) indicates that greater genetic diversity may also have existed historically for the Philippine Cockatoo.

Other Philippine avian taxa such as the yellow-vented bulbul (*Pycnonotus goiavier*), elegant tit (*Parus elegans*), and white-eared brown dove (*Phapitreron leucotis*) show much higher numbers of *ND2* haplotypes (Appendix Table III). Only eight haplotypes were found for 101 individuals of the Katala, while 12 haplotypes for 29 individuals were seen in the yellow-vented bulbul, 18 haplotypes for 36 individuals of the elegant tit, and 12 haplotypes for 28 individuals of the white-eared Brown dove (Campbell *et al.* 2016). Phylogenetic studies indicate that Philippine birds show higher levels of endemism than is currently acknowledged (Lohman *et al.* 2010; Campbell *et al.* 2016; Hosner *et al.* 2018). On the other hand, our study shows low genetic diversity for the katala.

It is possible that the unique haplotypes found in Rasa and Pandanan Islands are products of new mutations due to the increasing population of the Philippine cockatoo in both locations (Widmann *et al.* 2018). Contemporary populations of the kākāpō (*Strigops habroptilus*) contain haplotypes not found in museum specimens, although a haplotype network indicates that extant haplotypes are descended from those found in historical populations (Bergner *et al.* 2016). This might be due to new mutations that have occurred when captive breeding and conservation efforts were made to preserve and increase the remaining populations of the species (Bergner *et al.* 2016). Genetic studies using museum specimens of the katala obtained from various provinces of the Philippines similar to that done by Bergner *et al*. (2016) for the kākāpō in New Zealand should be done in order to confirm the occurrence of a genetic bottleneck and possibly detect genetic signatures for population expansion or recovery.

Range and demographic decline have probably led to the reduction of genetic diversity (Frankham 1996), which may impair the ability of the species to adapt to future threats – whether due to disease, climate change, or others (Spielman *et al.* 2004; Edmands 2007). This underscores the need to incorporate genetic tools in efforts to breed and reintroduce the katala to its former range (Frankham 2015; Pierson *et al.* 2016) while maintaining current efforts to protect and restore habitats and extant populations. The populations of the katala in Palawan are not genetically distinct, as indicated by the predominance of two mitochondrial haplotypes (Figure 2) and the shared alleles of *TGFB2* in all locations sampled. Nonetheless, the presence of unique haplotypes in Pandanan and Rasa Islands indicates that some genetic diversity still exists for the species, although their relative rarity leaves them vulnerable to the effects of genetic drift (Frankham 1996).

The increase in katala populations in Rasa and Pandanan Islands, as well as the presence of unique mtDNA haplotypes in both locations, make them an ideal source of founders for any reintroduction of the katala to its former range. Future reintroduction of the species to parts of its former range should include as many haplotypes as possible, while captive breeding should prioritize breeding of individuals with low-frequency mtDNA haplotypes. In addition, individuals from Rasa and Pandanan Island may be allowed to disperse elsewhere in Palawan naturally by maintaining forest corridors throughout the province that enables population dispersion. The forest cover on Dumaran Island and other parts of Palawan are heavily depleted (Widmann *et al.* 2018), making natural dispersion difficult and necessitating artificial translocation. Reforestation of Palawan should be a priority for the conservation of the katala and other elements of Palawan biodiversity. Genetic testing of captive individuals and populations of the katala, including those confiscated by the Biodiversity Management Bureau of the Department of Environment and Natural Resources (DENR), for unique haplotypes

that may be used for reintroduction and captive breeding should also be considered. Such individuals may possess unique haplotypes or alleles that may be used to supplement wild populations. In this context, while we acknowledge and reiterate the importance of genetic diversity to conservation (Frankham 1996, 2015; Spielman *et al.* 2004; Pierson *et al.* 2016), we would not recommend the creation of hybrids between the katala and other species of cockatoos, even for purposes of "conservation" (Chan *et al.* 2019) as this may lead to loss of the parental phenotype and unique identity as a species (Allendorf *et al.* 2001; Roberts *et al.* 2010). The population in Palawan may still serve as a viable source of founders, not to mention other populations in the Sulu Archipelago; there is no need to resort to the creation of hybrids. Moreover, as a threatened taxon, breeding of the Philippine cockatoo may only be lawfully done for scientific and conservation purposes under Republic Act 9147 of the Republic of the Philippines; priority should always be given to preserving and increasing naturally occurring populations of the Philippine cockatoo.

CONCLUSION

Only eight haplotypes were found for *ND2* while three haplotypes were found for *COX1* and three alleles were found for *TGFB2*. One unique haplotype for *ND2* exists in GenBank, taken from a museum specimen currently stored in the Smithsonian Museum of Natural History. Taken together, the results indicate a possible genetic bottleneck has occurred for the Philippine cockatoo, which is supported by historical data indicating the range and demographic decline over the past century. Current breeding efforts for the species need to account for possible inbreeding in protected populations while striving to preserve the remaining natural populations of the Philippine cockatoo.

Future genetics studies should incorporate microsatellite data and other mtDNA genes to gain a fuller picture of the genetic diversity in wild populations.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no competing interests exist.

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APPENDICES

Table I. GenBank accession numbers for representative haplotypes or genotypes of *ND2*, *COX1*, and *TGFB2*.

Gene	Haplotype	Individual (field code)	GenBank accession number
ND2[a]	Haplotype 1	DENR 002-16	MT854313
	Haplotype 2	DENR 004-16	MT854314
	Haplotype 3	DENR 009-16	MT854315
	Haplotype 4	DENR 011-18	MT854316
	Haplotype 5	DENR 014-16	MT854317
	Haplotype 6	DENR 033-18	MT854318
	Haplotype 7	DENR 069-17	MT854319
	Haplotype 8	DENR 072-17	MT854320
COX1	Haplotype 1	DENR 141-17	MT887876
	Haplotype 2	DENR 184-18	MT887875
	Haplotype 3	DENR 188-18	MT887874
TGFB2[b]	Haplotype 1	DENR 051-16	MT854325
	Haplotype 2	DENR 052-16	MT854326
	Haplotype 3	DENR 053-16	MT854327

Table II. Number of haplotypes found for each gene (*ND2*: 1041 bp; *COX1*: 564 bp; *TGFB2*: 552 bp) and the concatenated alignment (1605 bp).

Gene	Haplotype	Rasa	Pandanan	IWPP	Dumaran	Total
ND2[a]	Haplotype 1	3	0	0	0	3
	Haplotype 2	16	16	12	9	53
	Haplotype 3	14	8	4	3	29
	Haplotype 4	3	4	0	0	7
	Haplotype 5	1	0	0	0	1
	Haplotype 6	0	1	0	0	1
	Haplotype 7	0	4	0	0	4
	Haplotype 8	0	3	0	0	3
COX1	Haplotype 1	16	20	5	3	44
	Haplotype 2	18	16	11	9	54
	Haplotype 3	4	1	0	0	5
TGFB2[b]	Haplotype 1	8	7	6	1	22
	Haplotype 2	0	0	0	1	1
	Haplotype 3	0	3	1	4	8

Gene	Haplotype	Rasa	Pandanan	IWPP	Dumaran	Total
Concatenated *ND2* and *COX1*	Haplotype 1	3	0	0	0	3
	Haplotype 2	16	16	11	9	52
	Haplotype 3	12	8	4	3	27
	Haplotype 4	0	3	0	0	3
	Haplotype 5	1	0	0	0	1
	Haplotype 6	0	1	0	0	1
	Haplotype 7	1	0	0	0	1
	Haplotype 8	1	0	0	0	1
	Haplotype 9	0	4	0	0	4
	Haplotype 10	0	3	0	0	3
	Haplotype 11	3	1	0	0	4

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[a]Haplotype 9 is represented by the GenBank sequence HQ629722.
[b]The GenBank sequence for *TGFB2* (HQ629636) for this species belongs to haplotype 3. Both this and the *ND2* sequence (HQ629722) were taken from the same individual by Schirtzinger *et al.* (2012; Appendix Table III).

Table III. Comparison of the number of *ND2* haplotypes between the Katala and several Philippine avian taxa (Campbell *et al.* 2016).

Species	Number of individuals sequenced	Number of haplotypes
Cacatua haematuropygia	101	8
Parus elegans	36	18
Pycnonotus goiavier	29	12
Phapitreron leucotis	28	12

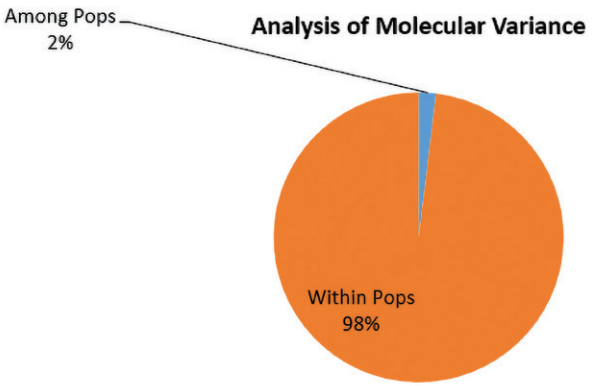

Figure I. Results for analysis of molecular variance for the concatenated *nd2* and *cox1* dataset. Most of the variation in the dataset is explained by variation between individuals rather than among populations.