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Diversity of The Ornate Lorikeet (*Trichoglossus ornatus*) Birds Based on Mitochondrial DNA Protein Coding Gene

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Abstract

Ornate lorikeet (*Trichoglossus ornatus*) is an endemic bird in Sulawesi. Endemism is one of the factors in declining bird's population. In the case of the birds conservation programme, information about gene diversity is important for basic strategy. Mitochondrial DNA of animals consists of protein coding genes including ND2 gene. This study informs diversity of the Ornate Lorikeet (*Trichoglossus ornatus*) birds based on DNA sequences of ND2 gene. DNA total was extracted from blood samples of 21 birds. PCR (Polymerase Chain Reaction) was performed and successfully amplified a single DNA fragment of ND2 gene for all birds. DNA fragments were sequenced and totally 997 base pairs were analyzed. NJ tree was constructed using MEGA5. All DNA sequence data showed that between the birds there were 20 polymorphic (segregating) sites with mean genetic distance was 0.004 ± 0.002 (ranged from 0,000 – 0,008), and had 17 sequence haplotypes (HTor1- HTo17). Haplotype diversity (Hd) was 0.967 ± 0.30387 and nucleotide diversity (Pi) was 0.00439 ± 0.0012. Genetic diversity information could be potential relevance to the breeding management for conservation of the birds.

How to Cite

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INTRODUCTION

Ornate lorikeet (*Trichoglossus ornatus*) bird is one of parrot birds and belongs to the family Psittacidae (Forshaw and Cooper, 1989 Juniper and Parr, 1998). The bird is endemic in the Sulawesi island of Indonesia. They spread in Sulawesi and surrounding islands including the islands of Togian, Peleng, Banggai and Tukang Besi (Juniper and Parr, 1998; Forshaw, 2010).

Its endemic presence is in an island and its capture is for trade and as a pet, as well as habitat destruction and fragmented habitats may be the cause of the decline of its population in nature. Currently, this bird population is declining (BirdLife International, 2016) and the conservation status by the IUCN Red Least is Least Concern ver 3.1 (IUCN, 2017) and included in CITES Appendix II.

The population decline of these birds can lead to a decrease in its genetic diversity (Rivers *et al.*, 2014). Genetic diversity is one of the three levels of biodiversity (genetics, species, and ecosystems) required in organism conservation (Sharma and Sharma, 2013) and as a basic or essential for the survival and adaptability of organisms to environmental change (Boettcher *et al.*, 2010; Pauls *et al.*, 2013). The effects of declining of genetic diversity or low genetic variation in wildlife and livestock can affect to a decrease in the ability of birds to adapt to their environment (Barrett and Schluter, 2008) and to the disease attacks (Toro *et al.*, 2011), so that its ability to survive becomes decreasing. The inability to adapt to changing conditions greatly increases the risk of extinction (Riversa *et al.*, 2014). Genetic variation and diversity within a population explain the presence of different alleles in the population for specific genes. The presence of genetic variation indicates that individuals of a population have varied alleles, meaning that individuals can differ in genotypes (Toro and Caballero, 2005), so that in conservation or breeding selection, variations of individuals is more concerned (Toro *et al.*, 2011). Perhaps the most important is how to make local breeds more able to breed through conservation measures and how to prioritize conservation. In both cases, the assessment and management of genetic diversity are key aspects (Hiemstra *et al.*, 2010; Soini *et al.*, 2012; Boettcher *et al.*, 2015).

The use of genetic markers is necessary and important in estimating genetic diversity, either between individuals or within species (Avise, 2004). Mitochondrial DNA is one of the genetic markers (Arif and Khan, 2009; Barker *et al.*, 2012) in which there is a ND2 protein coding gene. The ND2 gene has been widely used as a genetic marker for uncovering genetic diversity of birds and for the study of phylogeny in vertebrates including birds (Townsend *et al.*, 2007; Verónica *et al.* 2009, Mu *et al.*, 2012). This paper takes into account the diversity of Ornate lorikeet (*T. ornatus*) birds which are endemic in Sulawesi Island based on DNA sequences of the mitochondrial protein coding ND2 gene. This genetic diversity information of *T. ornatus* can be used as basic information for its breeding strategies and conservation action plan either in-situ or ex-situ to keep the population in order to be sustainable.

METHODS

This study was conducted on 2015 and used blood samples from 21 Ornate lorikeets (*T. ornatus*) an endemic bird of Sulawesi. The samples were genetic material collection of Genetic Laboratory of Zoology Division, Research Center for Biology - LIPI. Each blood sample was preserved in absolute ethanol (96%) and kept in refrigerator at 4 ° C. The DNA total was extracted from about 10 mg of blood using a QIAGEN Mini Kit and follows the procedures provided by manufacture.

The quality and quantity of DNA was checked in agarose gel through the electrophoresis process then visualized using photos under UV light. The extracted DNA solution was then diluted to a certain concentration for further use in PCR analysis (Polymerase Chain Reaction). DNA fragments of the ND2 gene from each bird were amplified through the PCR process using a pair of DNA nucleotide primer L5216 and H6313 under PCR conditions following Sorenson *et al.* (1999). Each PCR product then sequenced. DNA sequencing process is done by using the services of First Base company.

All DNA sequence data from all birds studied, then aligned together in a conventional alignment process using eyes on MEGA5 (software). Nucleotide composition, nucleotide variation including number and their position, nucleotide sites, and parsimony information sites were calculated using MEGA5. The number of sequence haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (Pi) were calculated using the DnSP software. A neighbor-joining (NJ) analysis was then performed to create a NJ tree and to know the groupings of the individual birds. The grouping confidence value (bootstrap value) is obtained by 1000 repetitions on MEGA 5. *Trichoglossus haematodus , Psittaculirostris edwardsii,* and *P. desmarestii* were *ougroup species.*

RESULTS AND DISSCUSION

The PCR process successfully amplified the single DNA fragment of the ND2 gene which the length was 1040 base pairs of all 21 samples of *Trichoglossus ornatus*. DNA sequence data obtained from each sample varied in length, therefore only 997 base pairs were used for genetic diversity analysis. There were no insertion, deletion, and stop codon in all sequence data analyzed. The absence of a stop codon indicates that the DNA sequences are from the protein coding gene (Li and Gaur, 1991). DNA is extracted from blood samples, the consequence is that DNA sequence data can be contaminated by nuclear pseudogene (Sorenson and Fleicher, 1996). The absence of insertion and deletion indicated that there was no pseudogene contamination in all *T. ornatus* sequence data analyzed in this study. This means that the amplified DNA fragment is the true target fragment of the ND2 gene.

DNA sequence contained AT-rich; where the composition of A + T (53.90%) was higher than C + G (46.10%) (Figure 1) as found in birds and other vertebrates (Udin *et al.*, 2015). The composition of the nucleotide base (A, C, G, T) in the total codon positions of the DNA sequence of the ND2 in *T. ornatus* studied were higest in cytosine (T), followed by adenine (A), thymine (T), and guanine (G) respectively (Figure 1). The DNA sequences produced the lowest of guanine (G) as found in other vertebrates including bird, fish, and mammal. Cytosine (C) was highest as it occurs in others aves and fish (Udin *et al.*, 2015). These results are also consistent with those of the Javan parakeets (*Psittacula alexandri alexandri*) (Astuti, 2017) and Litlle spiderhunter (*Arachnothera longirostra*) of passerine birds (Moyle *et al.*, 2011; Prijono *et al.*, 2017).

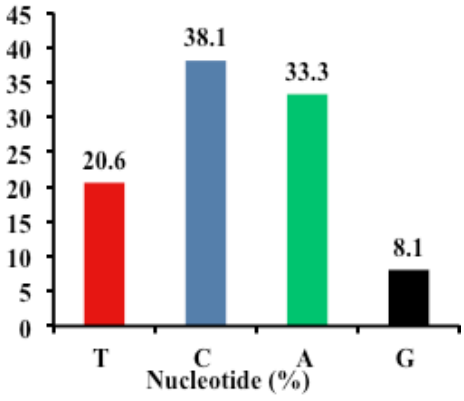

Figure 1 . Graph of nucleotide base composition of 977 bp (total codon position) of the ND2 gene in Ornate lorikeet (*Trichoglossus ornatus*).

Each of the ND2 codon position in the *T. ornatus* birds differed in the highest base composition. The first codon position was dominated by thymine (T), the second codon by adenine (C), the third codon by adenine (A), and the lowest guanine (G) (Figure 2) as it occurs in other birds, fish, and mammals (Udin *et al.*, 2015).

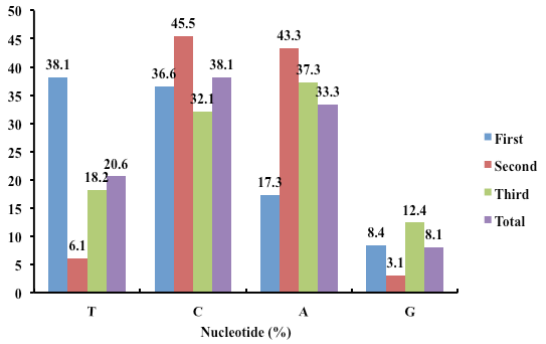

Figure 2. Graph of nucleotide base composition (%) at each codon position in the 997 bp of ND2 gene sequence of the Ornate lorikeet (*Trichoglossus ornatus*)

The number of nucleotide variable and parsimony sites were most widely at the third codon position, followed by the first codon position and second codon position, respectively (Table 1). Most variable in the third codon position because the substitution of the base nucleotide is synonymous and has the same amino acids (Li and Gaur, 1991).

The number of synonymous polymorphic sites is 15 sites, more abundant than non synonymous polymorphic (5 sites) (Table 5), since substitution of nucleotide bases is easier to occur than non-synonymous one. In synonymous polymorphic, although nucleotide base substitution and nucleotide base component of amino acids are different but the type or name of amino acids is the same. The substitution of nucleotide base is more common in the position of the third codon than in the first codon position or in the second codon (Li and Gaur, 1991).

Table 1. The number of variable sites and the parsimony informative sites (PI) in each codon position of the ND2 gene of Ornate lorikeet (*Trichoglossus ornatus*)

Codon position	Variable site	Invariable site	Total base nucleotide
1st position	6 (PI =4, singleton =2)	327	333
2nd position	5 (PI = 3, singleton = 2)	327	332
3rd position	9 (PI = 5, singleton =4)	323	332

Note: PI = Parsimony Informative

The total number of substitutions (varia-

Table 2. Number of transition and transversion substitutions and its ratio at each codon position

Codon position	Identic	Transition Substitution	Transversion Substitution	si/sv Ratio	Number of
	(ii)	(si)	(sv)	(R)	nucleotide
1st codon	331.00	1.00	1.00	0.63	333.00
2nd codon	331.00	1.00	0.00	3.05	332.00
3rd codon	330.00	2.00	0.00	4.56	332.00
Total codon	993.00	4.00	1.00	1.80	997.00

tions) of nucleotide occurred at 20 sites; consisting of 15 transitional sites and 5 transversional sites (Table 2). Transitional substitution was more abundant than transversional substitution. This fenomena is quite possible occured because transitional substitution is nucleotide substitutions of purine bases to other purine bases or from pyrimidine bases to other pyrimidine bases. Transversional substitution occurs from the purine bases to the pyrimidine or from pyrimidine to purin bases. Therefore, transitional substitutions are more frequent or easier to occur than trasversional substitutions (Li and Gaur, 1991), and the nucleotide variations were mainly related to transitions (Mu *et al.*, 2012). Transitional substution occurring in T. ornatus birds in recent study of 4, ie 2 substitutions from C to T and the otherwise C to T. While, the transversional substitution was only 1 that is from G to C.

Table 3. Variations of nucleotide bases and haplotypes in 997 bp of ND2 gene at Ornate lorikeets (*T. ornatus*)

	Base Nucleotide Sites																				
				1	1	2	3	3	4	5	6	7	8	8	8	8	8	8	9	9	
Individual bird	1	4	4	1	7	0	5	8	6	6	2	4	2	2	4	4	6	9	0	7	Haplotype
	9	2	7	4	1	7	3	1	4	5	8	3	0	9	7	9	8	5	8	9	
T.ornatus-1	C	G	C	C	C	T	G	T	C	C	C	C	C	A	G	T	C	T	A	C	HTo1ND2
T.ornatus-2	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	HTo2ND2
T.ornatus-3	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	A		HTo3ND2
T.ornatus-4	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.		HTo4ND2
T.ornatus-5	T	.	.	T	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.		HTo5ND2
Tornatus-6	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	G	.	.	.		HTo6ND2
T.ornatus-7	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo7ND2
T.ornatus-8	T	.	.	.	T	C	.	.	.	.	.	G	.	.	.	G	C	.	.		HTo8ND2
T.ornatus-9	.	.	.	T	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo9ND2
T.ornatus-10	.	.	T	T	T	C	.	.	.	.	T	G	.	.	.	G	.	.	.		HTo10ND2
T.ornatus-11	.	.	.	.	T	C	.	C	T	.	.	.	.	.	.	.	.	C	.		HTo11ND2
T.ornatus-12	T	C	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo12ND2
T.ornatus-13	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo7ND2
T.ornatus-14	T	C	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo12ND2
T.ornatus-15	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo7ND2
T.ornatus-16	T	C	T	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo13ND2
T.ornatus-17	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.		HTo7ND2
T.ornatus-18	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.			HTo14ND2
T.ornatus-19	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	G	.	.	A		HTo15ND2
T.ornatus-20	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.		Hto16ND2
T.ornatus-21	.	.	.	T	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.		Hto17ND2

The nucleotide base variation of 21 individual birds generated seventeen (17) DNA sequence haplotypes. The haplotype HTo7ND2 was in four individual birds of *T.ornatus*, they were number 7, 13, 15, and 17. The other haplotype (HTo12ND2) was in two individual birds of *T.ornatus*, they were number 12 and 14 (Table 3 and 4). The similarity of haplotypes to some individual birds, in one possibility, is because they are from the same elders.

Table 4. Number of individual *T.ornatus* birds in each haplotype

Haplotype	Haplotype code	Total bird's sample	No. of sample
Haplotype_1	HTo1ND2	1	1
Haplotype_2	HTo2ND2	1	2
Haplotype_3	HTo3ND2	1	3
Haplotype_4	HTo4ND2	1	4
Haplotype_5	HTo5ND2	1	5
Haplotype_6	HTo6ND2	1	6
Haplotype_7	HTo7ND2	4	7. 13. 15, 17
Haplotype_8	HTo8ND2	1	8
Haplotype_9	HTo9ND2	1	9
Haplotype_10	HTo10ND2	1	10
Haplotype_11	HTo11ND2	1	11
Haplotype_12	HTo12ND2	2	12, 14
Haplotype_13	HTo13ND2	1	16
Haplotype_14	HTo14ND2	1	18
Haplotype_15	HTo15ND2	1	19
Haplotype_16	HTo16ND2	1	20
Haplotype_17	HTo17ND2	1	21

The average number of nucleotide differences within 21 ND2 gene sequence data was 4 and the value of Tajima test (D) was 0.79609 with P> 0.01 not significant (Table 5).

Haplotype diversity of *T. ornatus* was high (0.967 ± 0.30387) and nucleotide diversity was 0.00439 ± 0.00125 (Table 5). The percentage of haplotype diversity was relatively high and nucleotide diversity was relatively low. This condition was also shown in the endemic populations of European starling (*Sturnus vulgaris granti*) birds which had high haplotype diversities ranged from 0.767 to 0.900 (Veronica *et al.*, 2009), and was also in the mitochondrial genes in other bird species such as *Cacatua alba* and *C. moluccensis* (Astuti, 2011), *Pterodroma magentae* (Taiko) (Lowrence *et al.*, 2008), and other vertebrate species such as *Lontra felina* (Valqui *et al.*, 2010). High haploty-

pe diversity values indicate high genetic diversity (Wu and Fang, 2005) and apparently low of nucleotide diversity values in T. ornatus studied may be due to the birds having low population histories, such as those occurring in *Muntiacus crinifrons* (Black muntjac) (Ran *et al.*, 2008).

Table 5. Parameters of gene diversity in the Ornate Lorikeets (*T.ornatus*) based on 997 base pairs of ND2 gene sequence.

Parameters of gene diversity	Value
Number of sites	997
Identic (monomorphic) sites	977
Variation (Polymorphic) sites	20
Singleton variations (SI)	7
Parsimony informative sites	13
Synonimous polymotphic	15
Non Synonimous polymorphic	5
Number of haplotype (H)	17
Haplotype diversity (Hd)	0.967 ± 0.30387
Nucleotyde diversity (Pi)	0.00439 ± 0.00125
Mean nucleotides differences	4
Tajima test (D)	-0.79609
	(P> 0.01) Not significant

The genetic distance (genetic divergence) between individuals (intraspecific distance) based on ND2 on *T. ornatus* birds studied ranged from 0.001% to 0.008% (Table 5). This value indicates that the individual birds are still in the same bird species. In other bird species of passerine birds the divergence of ND2 sequence between populations in the different islands was 0.7% (Verónica *et al.*, 2009) lower than divergence in one island (1.2%) % (Townsend *et al.*, 2007). The genetic distance of ND2 between individuals (intraspecific) in *Arachnothera longirostra* birds population were ranged from 0.25% to 0.76 (Java) and 0.10% (Sumatra) (Priyono *et al.*, 2017), in *Hirundo rustica* (European swallow) was 0.25 % - 1.6% (Dor *et al.*, 2010), and 0.24% - 0.58 % were in *Psittacula alexandri alexandri* (Javan parakeets) (Astuti, 2017).

Tabel 6. Genetic distance between individual (intraspesific) of Ornate Lorikeet (*T.ornatus*) birds based on 997 bp of ND2 gene sequences.

No	Individu	Genetic distances (below diagonal) and Standard deviation (above diagonal)																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	16	17	18	19	20	21
1	*T.ornatus-1*		0.001	0.001	0.001	0.002	0.001	0.001	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.001	0.002	0.001	0.00
2	*T.ornatus-2*	0.001		0.002	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.001	0.002	0.001	0.00
3	*T.ornatus-3*	0.003	0.004		0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.001	0.001	0.002	0.00
4	*T.ornatus-4*	0.003	0.004	0.004		0.001	0.002	0.001	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.001	0.002	0.002	0.00
5	*T.ornatus-5*	0.004	0.005	0.005	0.003		0.002	0.001	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.002	0.002	0.002	0.00
6	*Tornatus-6*	0.003	0.004	0.004	0.006	0.007		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.00
7	*T.ornatus-7*	0.001	0.002	0.002	0.002	0.003	0.004		0.002	0.001	0.002	0.002	0.002	0,000	0.002	0,000	0.002	0,000	0.001	0.001	0.001	0.00
8	*T.ornatus-8*	0.006	0.007	0.005	0.005	0.006	0.007	0.005		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.00
9	*T.ornatus-9*	0.003	0.004	0.004	0.002	0.003	0.006	0.002	0.005		0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.00
10	*T.ornatus-10*	0.007	0.008	0.006	0.006	0.007	0.008	0.006	0.005	0.004		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.00
11	*T.ornatus-11*	0.005	0.006	0.006	0.006	0.007	0.008	0.004	0.007	0.004	0.008		0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.00
12	*T.ornatus-12*	0.004	0.005	0.007	0.005	0.004	0.007	0.005	0.006	0.003	0.007	0.007		0.002	0.000	0.002	0.001	0.002	0.002	0.002	0.002	0.00
13	*T.ornatus-13*	0.001	0.002	0.002	0.002	0.003	0.004	0.000	0.005	0.002	0.006	0.004	0.005		0.002	0.000	0.002	0.000	0.001	0.001	0.001	0.00
14	*T.ornatus-14*	0.004	0.005	0.007	0.005	0.004	0.007	0.005	0.006	0.003	0.007	0.007	0,000	0.005		0.002	0.001	0.002	0.002	0.002	0.002	0.00
15	*T.ornatus-15*	0.001	0.002	0.002	0.002	0.003	0.004	0.000	0.005	0.002	0.006	0.004	0.005	0,000	0.005		0.002	0,000	0.001	0.001	0.001	0.00
16	*T.ornatus-16*	0.005	0.006	0.006	0.006	0.005	0.008	0.004	0.005	0.004	0.006	0.006	0.003	0.004	0.003	0.004		0.002	0.002	0.002	0.002	0.00
17	*T.ornatus-17*	0.001	0.002	0.002	0.002	0.003	0.004	0.000	0.005	0.002	0.006	0.004	0.005	0,000	0.005	0,000	0.004		0.001	0.001	0.001	0.00
18	*T.ornatus-18*	0.002	0.003	0.003	0.003	0.004	0.005	0.001	0.006	0.003	0.007	0.003	0.006	0.001	0.006	0.001	0.005	0.001		0.002	0.002	0.00
19	*T.ornatus-19*	0.004	0.005	0.001	0.005	0.006	0.005	0.003	0.006	0.005	0.007	0.007	0.008	0.003	0.008	0.003	0.007	0.003	0.004		0.002	0.00
20	*T.ornatus-20*	0.002	0.003	0.005	0.005	0.006	0.005	0.003	0.008	0.005	0.007	0.005	0.006	0.003	0.006	0.003	0.007	0.003	0.004	0.006		0.00
21	*T.ornatus-21*	0.003	0.004	0.006	0.004	0.005	0.006	0.004	0.007	0.002	0.006	0.006	0.003	0.004	0.003	0.004	0.006	0.004	0.005	0.007	0.005	

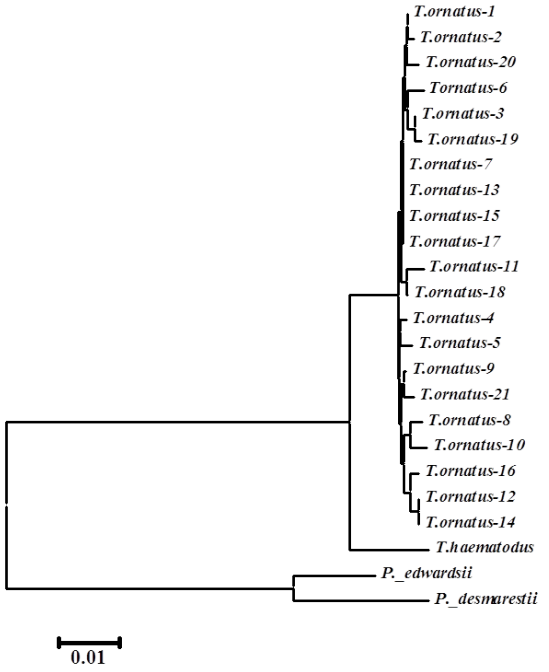

Figure 3. NJ tree of 21 Ornate lorikeet (*T. ornatus*) birds based on 997 base pairs of ND2 gene.

NJ phylogenetic tree presents the groupings of *T.ornatus* birds. Individual bird's number 7, 13, 15, and 17 that shared the HTo7ND2 haplotype (haplotype 7) was clustered together with 0% genetic distance. Individual 12 and 14 who have HTo12ND2 haplotype (haplotype 12) clustered together. All twenty one (21) *T. ornatus* birds grouped together and separated from *T. haematodus* by a genetic distance (divergence) of 3.1 ± 0.05 % and supported by 100% bootstrap value. The value of interspecies divergence in birds varies depending on the bird species and its speciation process. For example, Hirundo birds interspecies divergence ranged from 0.7% to 11.6% (Dor *et al.* 2010), and 8.3% ~ 26.7% between different fish species (Mu et al, 2012). *T.ornatus* and *T. haematodus* are separated from outgroup species (*Psitacula edwarsii* and *P. desmarestii*) with 100% bootstrap value.

The results of this study reveal the genetic diversity of birds *T. ornatus*. This is expected to be useful as basic information for breeding programs and strategies and action plans in its conservation programs both of in-situ and ex-situ. The genetic diversity of *T. ornatus* birds in this study is relatively high. It provides promising hopes and prospects in its conservation so that it can be used sustainably.

CONCLUSION

The haplotype diversity among the 21 birds studied were relatively high indicating that genetic diversity among individuals of the *Trichoglossus ornatus* bird was high as well, even though this bird is an endemic bird. High genetic diversity gives a good prospect for conservation of this bird.

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REFERENCES

Arif, I. A. & Khan, H. A. (2009). Molecular Markers for Biodiversity Analysis of Wildlife Animals: A Brief Review. *Animal Biodiversity and Conservation*, 32(1), 9–17.

Astuti, D. (2011). Variasi Gen Mitokondria Cytochrome b pada Dua Jenis Burung kakatua putih (*Cacatua alba* dan *C. moluccensis*). *Jurnal Biologi Indonesia.* 7(2), 1-5

Astuti, D. (2017). Struktur genetik population burung betet jawa (*Psittacula alexandri alexandri*) berdasarkan sekuen DNA mitokondria gen ND2. *Jurnal Biologi Indonesia*. 13(1), 117-124.

Avise, J. C. (2004). *Molecular* Markers, Natural History, and Evolution (Second Edition). Sinauer, Sunderland, MA.

Barker, F. K., Benesh, M. K., Vandergon, A. J., & Lanyon, S. M. (2012). Contrasting evolutionary dynamics and information content of the avian mitochondrial control region and ND2 gene. *PLoS One*, 7(10), e46403.

Barrett, R. D., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in ecology & evolution*, 23(1), 38-44.

Boettcher, P. J., Tixier-Boichard, M., Toro, M. A., Simianer, H., Eding, H., Gandini, G., ... & Globaldiv Consortium. (2010). Objectives, criteria and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. *Animal Genetics*, 41, 64-77.

Boettcher, P. J., Hoffmann, I., Baumung, R., Drucker, A. G., Mc Manus, C., Berg, P., Stella

Boettcher, P. J., Hoffmann, I., Baumung, R., Drucker, A. G., McManus, C., Berg, P., ... & Thompson, M. C. (2015). Genetic resources and genomics for adaptation of livestock to climate change. *Frontiers in genetics*, 5, 461.

CITES (2017). Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendices I, II and III . valid from 2 January 2017. 69 p.

Dor, R., Safran, R. J., Sheldon, F. H., Winkler, D. W., & Lovette, I. J. (2010). Phylogeny of the genus Hirundo and the Barn Swallow subspecies complex. *Molecular Phylogenetics and Evolution*, 56(1), 409-418.

Forshaw, J. M. & Cooper, W. T. (1989). *Parrots of TheWorld. Third Edition*. Lansdowne Editions, Sydney, Australia.

Forshaw, J. M. (2010). *Parrotsof the World*. Princeton University Press.

Juniper T. M. & Parr, M. (1998). *Parrots. A Guide to The Parrots of The World*. Yale University Press, New Haven and London.

Hiemstra, S. J. (2011). Cryopreservation strategies for farm animal genetic resources in Europe. Downloaded 4 jan 2018

Lawrence, H. A., Taylor, G. A., Millar, C. D., & Lambert, D. M. (2008). High mitochondrial and nuclear genetic diversity in one of the world's most endangered seabirds, the Chatham Island Taiko (Pterodroma magentae). *Conservation Genetics*, 9(5), 1293-1301.

Li, W. H. & Gaur, D. (1991). *Fundamental of Molecular Evolution*. Sinauer Association Inc., Sunderland, Mass, USA.

Mu, X. D., Wang, X. J., Song, H. M., Yang, Y. X., Luo, D., Gu, D. E., ... & Hu, Y. C. (2012). Mitochondrial DNA as effective molecular markers for the genetic variation and phylogeny of the family Osteoglossidae. *Gene*, 511(2), 320-325.

Pauls, S. U., Nowak, C., Bálint, M., & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and species. *Molecular ecology*, 22(4), 925-946.

Prijono, S. N., Irham, M., & Astuti, D. (2017). Divergence of Mitochondrial DNA in The Little Spiderhunter Birds (*Arachnothera longirostra*) from Indonesia. *Jurnal Biologi Indonesia*, 13(2), 203-212.

Rivers, M. C., Brummitt, N. A., Lughadha, E. N., & Meagher, T. R. (2014). Do species conservation assessments capture genetic diversity?. *Global ecology and conservation*, 2(1), 81-87.

Li, D., Li, D., Fan, L., Li, D., Fan, L., Ran, J., ... & Yue, B. (2008). Genetic diversity analysis of Macaca thibetana based on mitochondrial DNA control region sequences: Full-Length Research article. *DNA Sequence*, 19(5), 446-452.

Sharma, D. K., & Sharma, T. (2013). Biotechnological approaches for biodiversity conservation. *Indian Journal of Scientific Research*, 4(1), 183.

Soini, K., Diaz, C., Gandini, G., De Haas, Y., Lilja, T., Martin-Collado, D., ... & Hiemstra, S. J. (2012). Developing a typology for local cattle breed farmers in Europe. *Journal of Animal Breeding and Genetics*, 129(6), 436-447.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular phylogenetics and evolution*, 12(2), 105-114.

Sorenson, M. D., & Fleischer, R. C. (1996). Multiple independent transpositions of mitochondrial

DNA control region sequences to the nucleus. *Proceedings of the National Academy of Sciences*, 93(26), 15239-15243.

Toro, M. A., & Caballero, A. (2005). Characterization and conservation of genetic diversity in subdivided populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1459), 1367-1378.

Toro, M. A., Fernández, J., Shaat, I., & Mäki-Tanila, A. (2011). Assessing the genetic diversity in small farm animal populations. *Animal*, 5(11), 1669-1683.

Townsend, A. K., Rimmer, C. C., Latta, S. C., & LOVETTE, I. J. (2007). Ancient differentiation in the single-island avian radiation of endemic Hispaniolan chat-tanagers (Aves: Calyptophilus). *Molecular Ecology*, 16(17), 3634-3642.

Valqui, J., Hartl, G. B., & Zachos, F. E. (2010). Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (Lontra felina). *Conservation Genetics*, 11(5), 2067-2072.

Verónica, C., Griffiths, K., Savory, F. R., Furness, R. W., & Mable, B. K. (2009). Are European starlings breeding in the Azores archipelago genetically distinct from birds breeding in mainland Europe?. *European journal of wildlife research*, 56(1), 95-100.

Wu, H. L., & Fang, S. G. (2005). Mitochondrial DNA genetic diversity of black muntjac (Muntiacus crinifrons), an endangered species endemic to China. *Biochemical Genetics*, 43(7-8), 407-416.