

## Polymorphic sequence in the ND3 region of Java endemic Ploceidae birds mitochondrial DNA

**R. SUSANTI\***

Department of Biology, Faculty of Mathematics and Natural Sciences, Semarang State University. Bld. D6, Fl. 1. Sekaran Campus, Gunungpati, Semarang 50229, Central Java, Indonesia. Tel & Fax. +62-24-8508033. \*email: rsant\_ti@yahoo.com; rsant\_ti@staff.unnes.ac.id

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### ABSTRACT

Susanti R (2011) Polymorphic sequence in the ND3 region of Java endemic Ploceidae birds mitochondrial DNA. *Biodiversitas* 12: 70-75. As part of biodiversity, Ploceidae bird family must be kept away from extinction and degradation of gene-diversity. This research was aimed to analyze ND3 gene from mitochondrial DNA of Java Island endemic of Ploceidae bird. Each species of Ploceidae birds family was identified based on their morphological character, then the blood sample was taken from the birds nail vein. DNA was isolated from blood using Dixit method. Fragment of ND3 gene was amplified using PCR method with specific primer pairs and sequenced using dideoxy termination method with ABI automatic sequencer. Multiple alignment of ND3 nucleotide sequences were analyzed using ClustalW of MEGA-3.1 program. Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2 –parameter. The result of Java Island endemic of Ploceidae bird family exploration showed that *Erythrura hyperythra* and *Lonchura ferruginosa* can not be found anymore in nature, but the *Lonchura malacca* that are not actually Java island endemic was also found. Nucleotide sequence of mitochondrial ND3 gene of Ploceidae bird family showed a quite high polymorphism, with 122 substitutions from 334 nucleotides analyzed. Phylogenetic tree of nucleotide sequence of Ploceidae bird family formed 2 clusters. One cluster consisted of the *Ploceus hypoxanthus*, *Ploceus philippinus*, *Ploceus manyar* and *Passer montanus*, and the others species were included in the second cluster. ND3 gene sequence data from this Ploceidae family need to be analyzed further to see possible relationship with a particular phenotype.

**Key words:** Ploceidae, ND3 gene, mitochondrial DNA.

### INTRODUCTION

Indonesia is rich countries in biodiversity of birds, 1539 species of birds have spread in various regions in Indonesia. Indonesian endemic birds recorded around 494 species spread across the island of Java, consists of 368 species of settlers birds and 126 species visitors (nomads) birds (McKinnon et al. 1993). Latest publication from the International Union for Conservation of Natural Resources (IUCN) in 2000 stated that Indonesia has 324 bird species including the red list of threatened species (Dono 2002). Ploceidae bird family in Indonesia consist of approximately 41 species and 13 species of which was located on the island of Java (Iskandar 1989; McKinnon et al. 1993). Although until now, only Java sparrow (*Padda oryzivora*) that experienced a shift from an agricultural pest predator, but the decline in the number of the population of other bird species of this family should be considered.

As part of biodiversity, Ploceidae birds family needs to be protected from extinction and decline in species diversity. Members of Ploceidae birds family increasingly difficult to find in nature all around us, so it needs to promote conservation. One effort to provide the basis of germplasm conservation strategy is through the study of genetic diversity (Susanto et al. 2004). According Wartono et al. (2000), the concept of conservation today is directed to gene conservation. This is partly because the gene is the

basic unit in natural selection and gene variations are directly related to individual fitness or adaptability to environmental conditions. Animal adaptation to the environment can result in unique combinations of alleles for a particular type, and the circumstances in this difficult re-formed. The types that are different from other types needed to be conserved genes and gene combinations that bring very useful in the future (Christianti et al. 2003). The benefits of genetic diversity in the future, efforts to save biodiversity from extinction should be done immediately. Preservation of biodiversity, including genetic resources will ensure the availability of genetic material for the development of science and technology.

Genetic markers used in studies of animal genetic diversity analysis are the conventional genetic markers and DNA markers. Conventional genetic marker is a frequently used marker phenotype, for example markers that are determined on the basis of phenotypic characteristics can be observed, such as color, body color patterns, shape of the feet, beak and morphometric analysis (Rahayu 1998). According Christianti et al. (2003), morphometric analysis is strongly influenced by environmental factors. Progress in biotechnology has made it possible to get other markings other than morphological markers, proteins and DNA (McCouch and Tanksley 1991). Studies of DNA variation are more accurate than the study of proteins, because the

variation of DNA does not necessarily indicate protein variations (Christianti et al. 2003). The use of DNA as a genetic marker, providing more accurate data and can immediately detects the variation in the genetic material.

Mitochondrial DNA (mtDNA) are easily extracted, relatively small size (Shadel and Clayton 1997), has a mutation rate ten times faster than nuclear DNA (Christianti et al. 2003), and contain more variation than nuclear DNA (Wood and Phua (1996), so widely used in the analysis of genetic diversity. The variation of human mtDNA variation shows the effect on health-related phenotypes, such as involvement in degenerative diseases, aging and reproduction properties. Pedigree maternal affect growth, reproduction and lactation, even reported that the mtDNA variation associated with milk production in dairy cows. To study the effect of mtDNA on the phenotype required the listing of several generations of phenotypic data (Christianti et al. 2003).

Sequences of mtDNA encodes seven subunits of complex I (NADH dehydrogenase), electron transport chain (oxidative phosphorylation), a subunit of complex III (cytochrome b-c1 complex), three subunits of complex IV (cytochrome oxidase) and two subunits of ATP synthase complex. OXPHOS disease is a clinical illness associated with the components of oxidative phosphorylation. Mutations in the gene of NADH dehydrogenase subunit 4 (ND4) reported Wallace et al. (1995) causes the disease's hereditary optic neuropathy leber (LHON). ND3 gene is a gene that encodes the enzyme NADH dehydrogenase subunit 3, which is one of the seven subunits of complex I subunits of oxidative phosphorylation (Marks et al. 1996).

Previous research indicates that some birds Java Sparrow (*Padda oryzivora*) showed the diversity of ND3 gene sequences (Susanti 2008). Diversity of information based on ND3 gene sequence analysis is the underlying basis for detecting the gene mutations that occur from generation to generation, and also genetic diversity is the basis for studying the relationship between diversity ND3 gene sequences with a particular phenotype such as resistance to disease.

## MATERIALS AND METHODS

### Ploceidae birds

Twelve species birds of the endemic Java island Ploceidae used in this study: *Amandava amandava*, *Erythrura hyperythra*, *Erythrura prasina*, *Lonchura ferruginosa*, *Lonchura leucogastra*, *Lonchura leucogastroides*, *Lonchura maja*, *Lonchura malacca*, *Lonchura punctulata*, *Padda oryzivora*, *Passer montanus*, *Ploceus hypoxanthus*, *Ploceus manyar*, and *Ploceus philippinus*. Each species of Ploceidae bird family was identified based on their morphological character, then the blood sample was taken from the birds nail vein.

### Amplification of ND3 gene

DNA was isolated from blood using Dixit method. The polymerase chain reaction (PCR) amplification was prepared at amount of 30 µl with composition of 15 µL 2x

reaction mix buffer (Fermentas), 10 pmol of primers H11151 dan L10775, 0.6 µL of genomic DNA (12 ng/µL), and ultrapure H<sub>2</sub>O until reaching 30 µL. Primer used was the primer pair that flanking cleavage site region, they are H11151 (5'GATTTGAGCCGAAATCAAC 3') and L10775 (5'GACCAATCTTTAAAATCTGG 3'). PCR program consists of, pre-denaturation 95°C for 5 minutes, 40 cycles consist of denaturation 95°C for 30 seconds, annealing 58°C for 30 seconds, extension 72°C for 1 minutes, and post-extension 72°C for 10 minutes (Sulandari and Zein 2002). The specific DNA band resulted from PCR was identified by electrophoresis on 2% agarose gel.

### Sequencing and phylogenetic analysis

The PCR products were then sequenced using dideoxy termination method with ABI automatic sequencer (Applied Biosystems). Nucleotide sequence of mitochondrial ND3 gene of Ploceidae birds submitted to GeneBank. Multiple alignment of nucleotide sequences were analyzed using ClustalW of MEGA 3.1 program. Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2-parameter.

## RESULTS AND DISCUSSION

The result of Java Island endemic of Ploceidae birds family exploration showed that *Erythrura hyperythra* and *Lonchura ferruginosa* were not found anymore in nature, but the *Lonchura malacca* that was not Java island endemic (McKinnon et al. 1993) was observed. The loss of two birds species are likely due to population changes in bird habitat ecosystem. As one component of the environment, birds can be used directly or indirectly as an environmental bioindicator to detect environmental changes and can reflect the stability of the habitat. Bird life depends on vegetation, soil and water. The diversity of vegetation and its density determines the number and level of diversity of bird species (Hardy et al. 1987; Peakall and Boyd 1987; Rutschke, 1987). Plants to be much visited by birds and a comfortable place for birds. In addition to the nest and food sources for birds, plants give birds protection from sunlight intensity, stress, excessive heat, low humidity and predators attack (Soendjoto and Gunawan 2003). The loss of bird populations may also be caused by the arrest by humans, because the Ploceidae bird families are eating seeds that are commonly founded in agricultural land to a height of 1500m so easily captured. This is reinforced by the fact that these birds was traded in bird markets in some regions and exported to Japan, Europe and America (Iskandar 2005). Population might also be due to the limited use of pesticides on agricultural land resulting in lower levels of pesticide contamination due to bird health.

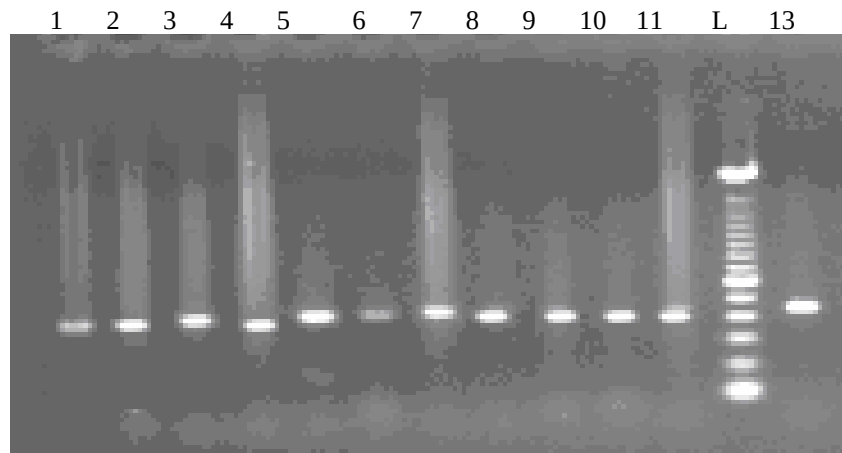
ND3 gene that was amplified by PCR using H11151 and L10755 primers have a high specification, because producing a single band (400bp fragment length) (Figure 1). ND3 nucleotide sequences of 12 species of Java endemic Ploceidae bird families can be accessed at

GenBank with accession numbers EF102496-EF1022485. Nucleotide alignment with ClustalW (MEGA 3.1) indicated that 122 substitution from 334 nucleotides analyzed (Figure 2).

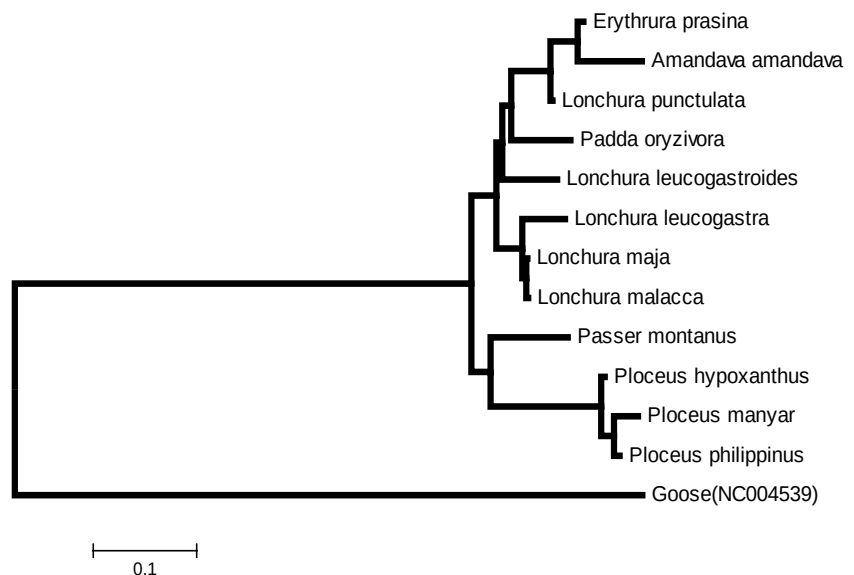
ND3 gene nucleotide analysis using ClustalW program (MEGA 3.1) shows that the 334 nucleotide, there were 142 polymorphic sites (Figure 2). Genetic distance and nucleotide sequence phylogeny was analyzed using neighbor-Joining method and calculation of distance matrix with Kimura 2-parameter model (Kimura 1980), successively seen in Table 1 and Figure 3. Result of phylogenetic analysis indicated that all Ploceidae bird family form two distinct sublineages. One cluster consisted of the *Ploceus hypoxanthus*, *Ploceus philippinus*, *Ploceus manyar* and *Passer montanus*, and the others species birds of this study include in the second cluster (Figure 3).

Mitochondrial DNA has a mutation rate ten times faster than nuclear DNA (Christianti et al. 2003). Most of the mitochondrial gene coding for a mitochondrial protein. Subunit of cytochrome-c oxidase, cytochrome b, and ribosomal genes are widely used in studies of population genetics and phylogeny (Shearer et al. 2002). Genetic diversity based on mtDNA sequence has been successfully carried out on fruit-eating bats (*Chinorax Melanocephalus*) (Zein and Maharadatunkamsi 2003), bats (Wilkinson et al. 1997), woodpeckers (Prychitko and Moore 2000), mammals (Gemmell et al. 1996) and birds (Tuinen et al. 2000). Polymorphism of mtDNA also occur in horses (Ishida et al. 1994), sheep (Heindleder et al. 1991), goats (Upholt and Dawid 1977), buffalo (Bhat et al. 1990) and cows (Christianti et al. 2003). The variation of fragment displacement-loop (D-loop) have been reported between species, even within a species (Gemmell et al. 1996; Wood and Phua 1996; Wilkinson et al. 1997). D-loop fragment is the initiator of transcription and replication (Linberg 1989). Christianti et al. (2003) reported that the D-loop fragment influence the fertility of livestock, milk production, milk fat percentage and health of livestock.

Reported that the ND3 gene sequences in 46 species of birds have one extra nucleotide at position 174. Excess



**Figure 1.** Electrophoregram of ND3 gene PCR of *Ploceidae* birds using L10775 and H11151 primer (product 400bp). Well M: DNA ladder 100bp. Well 1-11, 13 : sample of *Ploceidae* birds



**Figure 3.** A phylogenetic tree of the *Ploceidae* mitochondrial ND3 nucleotide sequence by using the neighbor-joining method.

base is one causes the shift (frameshift) nucleotide bases behind him so that formed two stop codons in the downstream part ND3 gene, resulting in premature translational stops and produces only 68 amino acids (should be 117 amino acids) (Mindell et al. 1998 ). Frameshift due to the substitution of one nucleotide bases mengasilkan different proteins, which may also influence its activity as an enzyme in oxidative phosphorylation. ND3 gene sequence data from Ploceidae families need to be analyzed in further research on possible relationship with a particular phenotype.



|                           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |       |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| #Lonchura malacca         | ... | .G. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | [195] |
| #Lonchura leucogastra     | ... | CG. | ... | ... | .C. | ... | ... | ... | ... | .T  | ... | ... | ... | .A  | [195] |
| #Amandava amandava        | .A. | .G. | ... | ... | ... | .TC | ... | ... | ... | ... | ... | ... | ... | ... | [195] |
| #Ploceus hypoxanthus      | ... | ... | ... | ... | ... | ... | .T. | .G. | ... | ... | .A. | .C. | ... | ... | [195] |
| #Ploceus manyar           | ... | ... | .G  | ... | ... | ... | .T. | .G. | ... | ... | .G. | .C. | ... | ... | [195] |
| #Ploceus philippinus      | ... | ... | ... | ... | ... | ... | .T. | .G. | ... | ... | .G. | .C. | ... | ... | [195] |
| #Padda oryzivora          | .A. | .G. | ... | ... | ... | ... | .T. | ... | ... | ... | ... | ... | ... | ... | [195] |
| #Lonchura leucogastroides | GAG | CAG | ATC | CCA | GGG | GGT | CAA | ATC | CAC | ATT | CGT | ATG | GGG |     | [234] |
| #Lonchura punctulata      | ... | .G. | ... | .T. | ... | ... | .G. | ... | ... | ... | ... | ... | ... | ... | [234] |
| #Passer montanus          | ... | .T. | .G. | .T. | ... | ... | ... | ... | .G. | ... | ... | ... | ... | ... | [234] |
| #Lonchura maja            | .G. | .G. | .C. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | [234] |
| #Erythrura prasina        | ... | GG. | ... | .T. | ... | ... | .G. | ... | ... | .C  | ... | ... | ... | ... | [234] |
| #Lonchura malacca         | .G. | .G. | .C. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | [234] |
| #Lonchura leucogastra     | .G. | .G. | .CT | ... | .C  | ... | ... | ... | ... | ... | ... | ... | C.  | ... | [234] |
| #Amandava amandava        | ... | A.  | ... | .T. | ... | ... | .G. | ... | ... | .C  | ... | ... | ... | ... | [234] |
| #Ploceus hypoxanthus      | .G. | ... | ... | .T. | ... | ... | .G. | ... | ... | ... | ... | ... | ... | ... | [234] |
| #Ploceus manyar           | .G. | ... | ... | .T. | ... | ... | AG. | ... | ... | ... | ... | A.  | ... | ... | [234] |
| #Ploceus philippinus      | .G. | ... | ... | .T. | ... | ... | AG. | ... | ... | ... | ... | A.  | ... | ... | [234] |
| #Padda oryzivora          | .G. | .G. | ... | .T. | ... | ... | .G. | ... | ... | ... | ... | A.  | ... | ... | [234] |
| #Lonchura leucogastroides | ATA | GTT | TTC | TGC | GTC | TGG | GTT | TGT | TTG | GGC | AAG | TCA | GAG |     | [273] |
| #Lonchura punctulata      | ... | ... | ... | .A  | ... | ... | ... | GA. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Passer montanus          | ... | ... | ... | .G  | ... | ... | ... | .A. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Lonchura maja            | ... | ... | ... | .A  | ... | ... | ... | AA. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Erythrura prasina        | ... | ... | ... | .AA | ... | ... | ... | GA. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Lonchura malacca         | .C. | ... | ... | .A  | ... | ... | ... | AA. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Lonchura leucogastra     | ... | ... | ... | .A  | T.  | ... | ... | AA. | ... | ... | TC. | ... | .A  | ... | [273] |
| #Amandava amandava        | ... | .GG | CC. | .AA | ... | ... | ... | GA. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Ploceus hypoxanthus      | .C. | ... | ... | C.G | ... | C.  | ... | .A. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Ploceus manyar           | .C. | ... | ... | C.G | ... | CT. | ... | .A. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Ploceus philippinus      | .C. | ... | ... | C.G | ... | C.  | ... | .A. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Padda oryzivora          | ... | ... | ... | .G  | A.  | ... | ... | .A. | ... | ... | T.  | ... | .A  | ... | [273] |
| #Lonchura leucogastroides | GTT | TAA | CGC | GAT | TAG | GAG | GGT | GCT | TAG | GGC | TGT | GGA | TAG |     | [312] |
| #Lonchura punctulata      | .C. | ... | G.T | .G. | ... | .T  | .A. | ... | ... | ... | ... | T.  | ... | ... | [312] |
| #Passer montanus          | ... | .G  | GCT | .G. | ... | T.  | TA. | ... | ... | .A. | GAA | T.  | A.  | ... | [312] |
| #Lonchura maja            | ... | .G  | G.T | .G. | C.  | .C  | AA. | ... | ... | ... | ... | ... | .A  | ... | [312] |
| #Erythrura prasina        | .C. | ... | G.T | .G. | .T  | C.T | .A. | ... | ... | ... | ... | T.  | .T  | ... | [312] |
| #Lonchura malacca         | ... | .G  | G.T | .G. | C.  | .C  | AA. | ... | ... | ... | ... | ... | .A  | ... | [312] |
| #Lonchura leucogastra     | ... | .G  | G.T | .G. | C.  | .C  | AA. | .T. | ... | ... | ... | C.  | .T  | ... | [312] |
| #Amandava amandava        | .C. | ... | GTT | .G. | .T  | C.T | .A. | ... | ... | ... | ... | T.  | .T  | ... | [312] |
| #Ploceus hypoxanthus      | ... | .G  | G.T | .G. | ... | .G. | CA. | ... | ... | .AA | AAA | ... | .C  | ... | [312] |
| #Ploceus manyar           | ... | .G  | G.T | .G. | ... | .G. | CA. | ... | ... | .AA | AAA | ... | .C  | ... | [312] |
| #Ploceus philippinus      | ... | .G  | G.T | .G. | ... | .G. | CA. | ... | ... | .AA | AAA | ... | .C  | ... | [312] |
| #Padda oryzivora          | ... | .G  | G.T | .G. | ... | .GC | AA. | ... | ... | .T  | .C  | ... | ... | ... | [312] |
| #Lonchura leucogastroides | GGT | TAG | TAT | GAA | TAT | AAT | TAT | G   |     |     |     |     |     |     | [334] |
| #Lonchura punctulata      | ... | G.A | ... | ... | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Passer montanus          | TT. | ... | ... | ... | .G  | ... | ... |     |     |     |     |     |     |     | [334] |
| #Lonchura maja            | ... | G.A | C.  | ... | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Erythrura prasina        | ... | G.A | ... | .C. | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Lonchura malacca         | ... | G.A | C.  | ... | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Lonchura leucogastra     | ... | G.A | C.  | ... | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Amandava amandava        | ... | G.A | ... | .C. | ... | ... | ... |     |     |     |     |     |     |     | [334] |
| #Ploceus hypoxanthus      | .C. | CCA | G.G | CT. | .G  | ... | AGG |     |     |     |     |     |     |     | [334] |
| #Ploceus manyar           | TC. | GCA | G.G | CT. | ... | ... | AGG |     |     |     |     |     |     |     | [334] |
| #Ploceus philippinus      | TC. | CCA | G.G | CT. | .G  | ... | AGG |     |     |     |     |     |     |     | [334] |
| #Padda oryzivora          | ... | G.A | ... | ... | ... | G.  | ... |     |     |     |     |     |     |     | [334] |

Figure 2. Polymorphism of the ND3 fragment gene sequences of Ploceidae birds (GenBank ID: EF1022485-EF102496)

## CONCLUSION

Nucleotide alignment of Ploceidae ND3 gene fragment have high polymorphism, with 122 substitutions from 334 nucleotides analyzed. Phylogenetic tree of nucleotide sequence of Ploceidae bird family form 2 clusters. One cluster consisted of the *Ploceus hypoxanthus*, *Ploceus philippinus*, *Ploceus manyar* and *Passer montanus*, and the others were included in the second cluster. Nucleotide sequence of ND3 gene of this Ploceidae bird family needs

to be analysed further to elucidate the possibility of its relationship with certain phenotype

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