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ความหลากหลายทางพันธุกรรมของประชากรในธรรมชาติของนกกระติ๊ดขี้หมู (*Lonchura punctulata*)

Genetic Diversity in Wild Populations of the Scaly-breasted Munia (*Lonchura punctulata*)

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บทคัดย่อ

การวิเคราะห์เพื่อเปรียบเทียบลำดับนิวคลีโอไทด์ของยีน NADH dehydrogenase subunit 3 (ND3) ในไมโทคอนเดรียความยาวขนาด 290 คู่เบส ระหว่างนกกระติ๊ดขี้หมู (*Lonchura punctulata*) จำนวน 29 ตัวอย่าง จาก 3 กลุ่มประชากรธรรมชาติในภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย พบตำแหน่งที่มีความแปรผันของนิวคลีโอไทด์ 11 ตำแหน่ง สามารถจำแนกออกได้เป็น 14 แฮปโพลไทป์ แต่ไม่พบความแตกต่างทางพันธุกรรมอย่างมีนัยสำคัญระหว่างทั้ง 3 กลุ่มประชากร และไม่มีการแบ่งโครงสร้างทางพันธุกรรมที่สัมพันธ์กับพื้นที่อย่างชัดเจน คาดว่าอาจมีสาเหตุมาจากการจับนกชนิดนี้เพื่อนำไปขายในพื้นที่ต่าง ๆ สำหรับการปล่อยสัตว์ทำบุญเป็นจำนวนมาก อย่างไรก็ตามพบว่านกกระติ๊ดขี้หมูจากเกาะชวาที่มีข้อมูลลำดับนิวคลีโอไทด์อยู่ในฐานข้อมูลนั้น มีความแตกต่างทางพันธุกรรมกับนกกระติ๊ดขี้หมูในประเทศไทยค่อนข้างสูง ชี้ให้เห็นว่านกชนิดนี้อาจจะไม่สามารถอพยพหรือย้ายถิ่นฐานระหว่างพื้นที่ที่มีระยะห่างกันมาก ๆ นอกจากนี้ ข้อมูลที่ได้ยังบ่งชี้ว่า นกกระติ๊ดขี้หมูในเอเชียตะวันออกเฉียงใต้รวมทั้งในประเทศไทยน่าจะเป็นสปีชีส์ซับซ้อน เพื่อให้เกิดความเข้าใจเกี่ยวกับความหลากหลายและโครงสร้างทางพันธุกรรมของนกกระติ๊ดขี้หมูมากขึ้น จึงจำเป็นอย่างยิ่งที่จะต้องมีการศึกษาในเชิงลึก ทั้งทางด้านสัณฐานวิทยา ชีววิทยา และพันธุกรรม ให้ครอบคลุมพื้นที่การกระจายพันธุ์ของประชากรนกกระติ๊ดขี้หมูให้มากขึ้น

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ABSTRACT

We analyzed 290 nucleotide sequences of the mitochondrial NADH dehydrogenase subunit 3 (ND3) gene from 29 samples of Scaly-breasted Munia (*Lonchura punctulata*) from three natural populations from the north and northeast of Thailand. Eleven variable nucleotide positions were observed. Based on these variations, 14 haplotypes were generated. Low genetic difference among three populations in Thailand was observed and there was no population sub-structuring related to their geographic distribution. This may be influenced by trading in this species in order to allow its release during Buddhist ceremonies. However, an isolate from Java was highly genetically distinct from those from Thailand, indicating that large geographic distances act as a natural barrier and play an important role in genetic differentiation. Our finding thus provides evidence of a species complex of Scaly-breasted Munia in Southeast Asia. A more comprehensive analysis based on the morphology, biology and genetics of scaly-breasted munia from throughout its distribution will be required in order to determine the number of genetically divergent groups present.

คำสำคัญ: นกกระติ๊ดขี้หมู *Lonchura punctulata* ความหลากหลายทางพันธุกรรม ประชากรในธรรมชาติ
Keywords: Scaly-breasted Munia, *Lonchura punctulata*, Genetic diversity, Natural populations

Introduction

The Scaly-breasted or Spotted Munia (*Lonchura punctulata*) is a small sparrow-sized estrildid finch native to tropical Asia. It occurs from India and Sri Lanka to Indonesia and the Philippines (Arnaiz-Villena et al., 2009). The species has several generally accepted subspecies across its range that differ slightly in size and color (Collar et al., 2010). The subspecies name *lineoventer* was formerly used for the Indian population, *subundulata* from the eastern Himalayas, *yunnanensis* of southern China, *topela* of Thailand, *cabanisi* of the Philippines and *fretensis* of Singapore and Sumatra. Island populations include *nisoria* (Java, Bali, Lombok, Sumbawa), *particeps* (Sulawesi), *baweana* (Bawean Island), *sumbae* (Sumba), *blasii* (Flores, Timor and Tanimbar) and *holmesi* (Southeast Borneo) (Collar et al., 2010; Clements et al., 2013). This species has also been introduced to other parts of the world due to its popularity as a cage bird and populations have established in the wild (Burton and Burton, 2002).

Scaly-breasted Munias are found in a range of habitats but usually occur close to water and grassland. They are especially common in paddy fields and in many areas it is regarded as an agricultural pest, feeding in

large flocks on cultivated cereals such as rice (Bomford and Sinclair, 2002). They are found mainly on the plains, but occur up to 1,675 m (Robson, 2008). *L. punctulata* is an abundant species which is classified as of "Least Concern" by the International Union for Conservation of Nature (IUCN) (BirdLife International, 2012). The scaly-breasted munia is not globally threatened and is common to very common throughout most of its range. In Southeast Asia, the scaly-breasted munia is trapped in large numbers for Buddhist ceremonies and although most birds are later released in order to gain merit (Gilbirt et al., 2012; Kaewsaensuk, 2013).

Genetic diversity, measured as the level of intraspecific genetic variation, has provided valuable information on the levels of genetic variation, gene flow, population subdivision, historical patterns of population fragmentation, and the evolutionary history of populations (Bates et al., 2003). Mitochondrial DNA has high mutation rate that is ten times faster than that of nuclear DNA (Brown et al., 1979). Thus, mDNA genes are suitable for use as genetic markers to explore the intraspecific genetic variation of many groups of organisms including birds. Brid mitochondrial genome contain sets of functional genes to produce energy through a process called oxidative phosphorylation. A set of enzyme complexes, designated as complexes I-V, carry out oxidative phosphorylation NADH dehydrogenase subunit 3 (ND3) is one of the seven subunits of complex I subunit. In addition, ND3 gene has been proven to be potentially genetic marker for genetic variation investigation in Passerine birds include Scaly-breasted Munia (Susanti, 2011; Kaewsaensuk, 2013). The current study aims to investigate genetic diversity of natural populations of scaly-breasted munia from north and northeast Thailand by using the ND3 gene as a molecular marker. We present the first data on the genetic diversity of Scaly-breasted Munia in Thailand.

Materials and methods

Collection sites and blood sampling

A total of 29 blood samples from scaly-breasted munia were collected from three different geographical populations, namely 11 samples from Nong La Lerng Keng, Nong Song Hong district, Khon Kaen Province (NLK, 15° 39.222' N, 102° 46.979' E), 10 samples from Nam Gliang Village, Wapipathum district, Maha Sarakham Province (BNG, 15° 49.969' N, 103° 10.379' E) and eight samples from Nam Kham Nature Reserve, Chiang Saen district, Chiang Rai Province (NKR, 20° 17.611' N, 100° 02.919' E). Birds were trapped by authorized bird ringers using mist-nets set in rides cut across areas frequented by munias. The birds were ringed with one individually numbered metal ring, supplied by the Department of National Parks, Wildlife and Plant

Conservation, to allow individual identification and to avoid duplicate sampling. Blood was collected from the tarsus vein or brachial vein following the standard protocol (Fair et al., 2010) and stored on filter paper in a dry paper envelope. All birds were released back where they were caught within 30 minutes of catching.

Molecular analysis

To extract DNA, a single blood sample absorbed onto filter paper was cut into small pieces and placed in a 1.5 ml vial. Next a GeneJET Genomic DNA Purification Kit (Fermentas, EU) was used following the manufacturer's instruction. To amplify the mitochondrial ND3 genes, PCR was performed using a primer pairs of H11151 (5'-GATTTGAGCCGAAATCAAC-3') and L10775 (5'-GACCAATCTTTAAAATCTGG-3') with PCR conditions reported by Susanti (2011). All PCR products were gel-purified using Gene Clean II Kit (Q-BIO Gene, Carlsbad, CA, USA). The purified PCR products were cycle-sequenced using ABI BigDye v3.1 (Warrington, UK) and run on an ABI Prism 377 automated sequencer (Perkin-Elmer Corp., Foster City, CA, USA).

Data analysis

The ND3 sequences were multiple aligned using the ClustalW program (Larkin et al., 2007). Haplotype data and a neutrality test were calculated using the DnaSp v5 program (Librado and Rozas, 2009) and Arlequin ver 3.5.1.3 (Excoffier and Lischer, 2010). A maximum parsimony haplotype network was generated by the Network 4.6.1.2 program based on median-joining network (Bandelt et al., 1999). The phylogenetic trees were constructed by using MEGA 5.1 program (Tamura et al., 2011) based on the neighbor-joining method.

Results

Variation within the 290 nucleotide positions of the mitochondrial ND3 sequences comparing the 29 Scaly-breasted Munia samples from Thailand was observed at 11 positions (3.79%). Of these, pyrimidine transitions were observed at seven positions, purine transition at two positions and nucleotide transversion at two positions (Table 1). Based on the variable positions of the ND3 sequence observed in this study, the 29 samples of scaly-breasted munia could be classified into 14 haplotypes (H1–H14) as shown in Table 1. A haplotype network shows no population sub-structuring related to geographical locality. Ten unique haplotypes were observed, i.e. H1, H2, H5, H6 and H7 for the NLK population, H9, H10, H11, H12 and H13 were unique for the BNG population and H14 was unique for the NKR population. The most common haplotype is H3, which was contained in samples from all three populations (Fig. 1).

Table 1. Variable positions in the nucleotide sequence alignments of the partial ND3 sequences of Scaly-breasted Munia from Thailand examined in this study. A dot represents an identical nucleotide of haplotype 1 (H1).

Haplotypes	Nucleotide positions										
	7	95	118	133	142	148	169	207	219	256	262
H1	T	T	C	T	G	T	A	T	C	G	T
H2	.	.	.	.	.	.	T	C	.	.	.
H3	.	.	.	C	.	C	T	C	T	A	A
H4	.	C	.	C	.	C	T	C	T	A	A
H5	C	C	T	.	.	.	.	.	.	.	.
H6	C	C	.	.	.	C	.	.	.	.	A
H7	.	C	T	.	.	.	.	.	T	.	.
H8	.	C	T	.	.	.	.	.	.	.	.
H9	.	.	.	C	.	C	T	C	.	A	A
H10	.	C	.	.	.	C	.	.	.	A	A
H11	.	C	T	.	A	.	.	.	.	.	.
H12	C	C	T	.	A	.	.	.	.	.	.
H13	.	C	.	.	.	.	.	.	.	.	.
H14	.	C	.	.	.	C	.	.	.	.	.

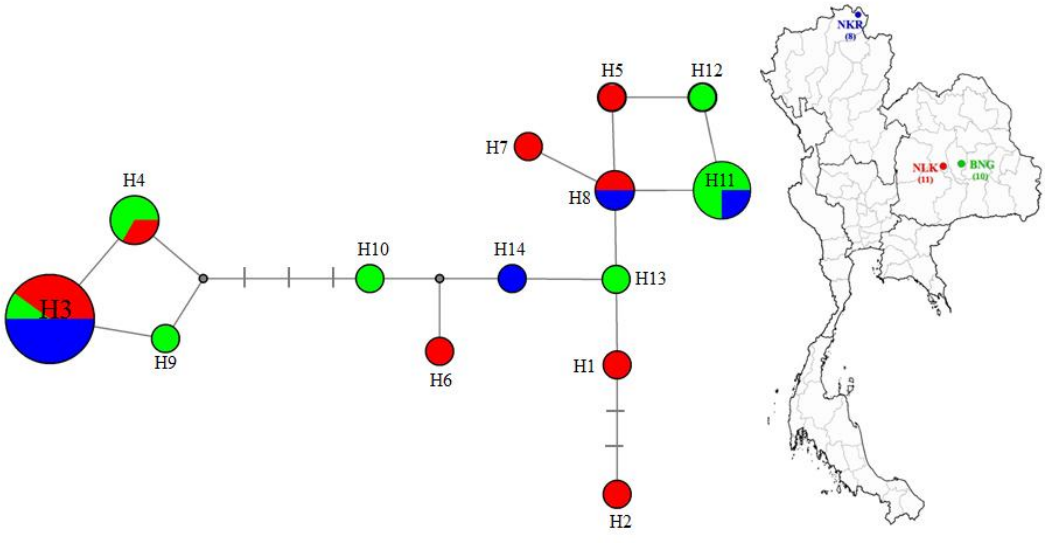

Figure 1. Minimum spanning haplotype network for Scaly-breasted Munia generated based on ND3 sequence. A network of Scaly-breasted Munia corresponds to their geographical localities separate into three populations in Thailand. Area of circles representing the proportion of sample numbers found in each haplotype.

The haplotype and nucleotide diversity were 0.643–0.911 and 0.017–0.019, respectively (Table 2). Tajima's D and Fu's Fs were 1.248–2.135 and -0.573–2.125, respectively. The mismatch distribution analysis was multimodal (Fig. 2), suggesting that *L. p. topela* populations are in demographic equilibrium. A phylogenetic tree (Fig. 3) showed that an available ND3 sequence of scaly-breasted munia subspecies *L. p. nisoria* originating from Java, Indonesia (accession no. EF102487) was highly genetically divergent from all samples in Thailand with F_{ST}s ranging between 0.500–0.607 (Table 3), while the three Thai populations revealed low genetic diversity with F_{ST} ranging between 0.012–0.077 (Table 3).

Table 2. Summary statistics of the genetic diversity indices and neutrality test of ND3 sequence of three Thai populations of the Scaly-breasted Munia.

Populations	n	S	Ts	Tv	H	Uh	Hd ± SD	**ΤΤ**±SD	Tajima's D	Fu's Fs
NLK	11	10	8	2	8	5	0.891±0.008	0.018±0.002	2.135 (0.995)	-1.394 (0.178)
BNG	10	11	9	2	7	4	0.911±0.006	0.019±0.002	1.689 (0.972)	-0.573 (0.342)
NKR	8	10	8	2	4	1	0.643±0.034	0.017±0.004	1.284 (0.916)	2.125 (0.858)

Remark: n = number of samples, S = number of segregating sites, Ts = number of nucleotide transition, Tv = number of nucleotide transversion, H = number of haplotypes, Uh = unique haplotype, Hd = haplotype diversity, **ΤΤ** = nucleotide diversity, SD = standard deviation**,** N/A = Not analyzed and *P*-value is indicated in the bracket. NLK = Nong La Lerng Keng, Nong Song Hong district, Khon Kaen Province, BNG = Nam Gliang Village, Wapipathum district, Maha Sarakham Province, NKR = Nam Kham Nature Reserve, Chiang Saen district, Chiang Rai Province.

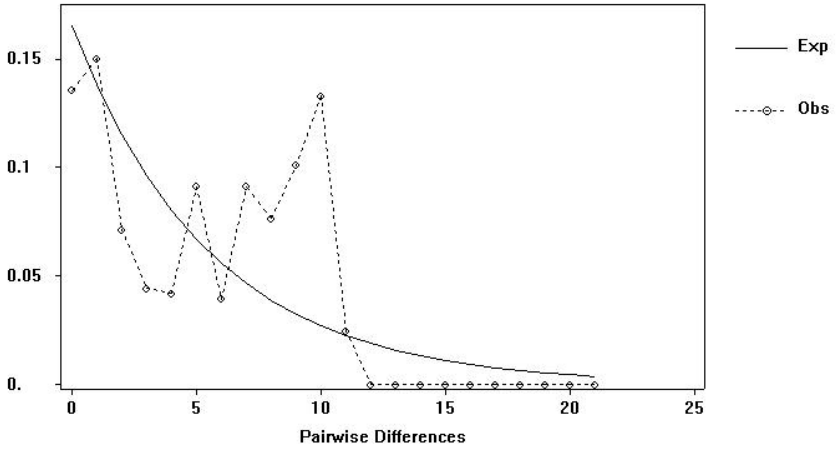

Figure 2. Mismatch distribution of the partial ND3 sequences of Scaly-breasted Munia. The mismatch distribution is the distribution of number of pairwise differences among sequences. The expected distribution under a model of expansion is given as a continuous line, and the observed distribution is given as a dashed line. Exp = expected; Obs = obsereved.

Table 3. Genetic differentiation (F_{ST}) among three populations from Thailand (*L. p. topela*) and one sequence from Java, Indonesia (*L. p. nisoria*) examined by ND3 sequence.

Populations	NLK	BNG	NKR	Java
NLK	_			
BNG	0.017	_		
NKR	0.077	0.012	_	
Java	0.563	0.500	0.607	_

Remark: All F_{ST} value showed $P > 0.05$

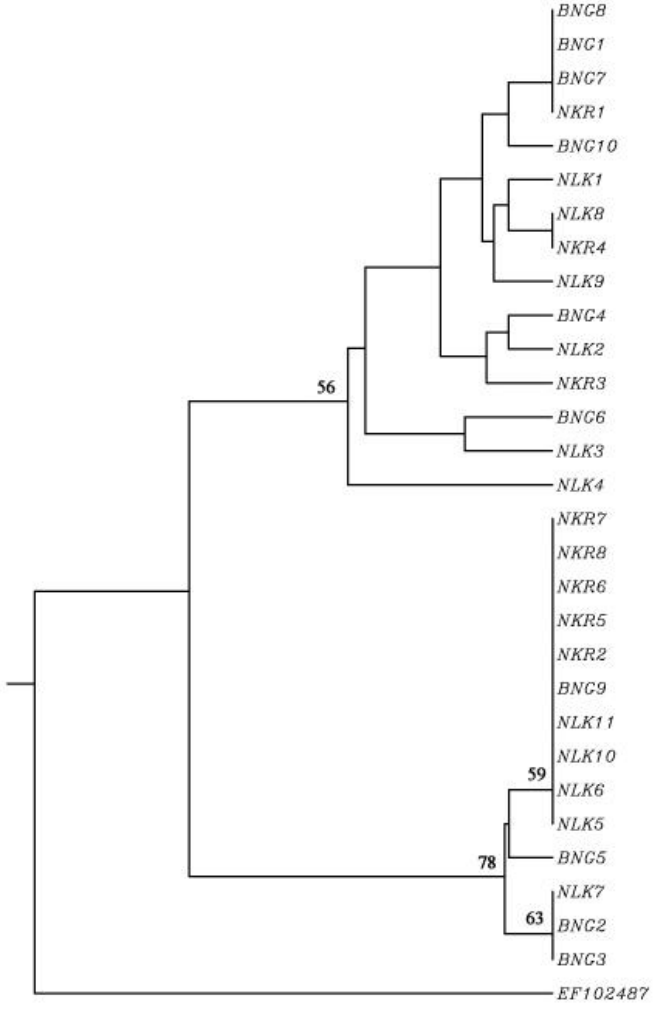

Figure 3. A neighbor-joining tree constructed based on 290 bp of ND3 sequence of Scaly-breasted Munia from Thailand. A sequence of Scaly-breasted Munia originated from Java, Indonesia (EF102487) was compared. Bootstrap values (>50%) are indicated above branches.

Discussion

The Scaly-breasted Munia is a principal species used in trading for Buddhist ceremonies in Southeast Asia, including Thailand (Kaewsaensuk, 2013). This may influence the relatively high genetic diversity and lack of population sub-structuring related to geographical distribution within the Thai populations. This species is usually trapped in large numbers in certain areas and then transferred to many places, such as the famous temples, historical parks and even to large markets in cities for ceremonies on important personal days or for Buddhist celebrations during which most of them are released (Kaewsaengsuk, 2013). In contrast, the European sparrow which is somewhat larger than *L. punctulata* shows significant population sub-structuring related to their geographic location, even at levels of only 10 km (Kekkonen et al., 2011; Wilson et al., 2011). In this case gene flow is low without mixing due to human interference.

In contrast to the low genetic diversity found in the Thai populations, *L. p. nisoria* from Java were highly genetically distinct from Thai *L. p. topela* populations. The large distance between sample sources (around 3,000 km), as well as the natural barriers of the Gulf of Thailand and the Java Sea are most likely to account for these differences. The level of genetic divergence strongly suggests that the subspecific status is erroneous and that the Thai and Javanese populations are actually good species. Nevertheless, a more comprehensive analysis based on the morphology, biology and genetics of scaly-breasted munia subspecies through Southeast Asian countries need to be further investigated in order to determine whether a species complex exists.

Acknowledgements

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