Short Communication

DNA barcoding of Chinese native chicken breeds through COI gene

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Abstract

This study was done to investigate the viability and effectiveness of cytochrome c oxidase I (COI) gene in DNA barcoding for identification of Chinese native chickens. Two sequences of the COI gene of 64 chickens of four Chinese native breeds (16 of each breed) were analyzed by amplification and sequencing. Results showed that the bar1 sequence of COI had lower polymorphism compared to bar2. Bar1 had 1 polymorphic site and 2 haplotypes, while bar2 had 4 polymorphic sites and 8 haplotypes. The nucleotide polymorphism (Pi) in bar2 ranged from 0.00102 to 0.00305. The DNA taxonomy of bar2 was consistent with morphological taxonomy in all four native chicken breeds. The findings indicate that DNA barcoding with COI gene is an effective method and the bar2 sequence of COI gene is a better choice for DNA barcoding to identify Chinese native chicken breeds.

Keywords: cytochrome c oxidase I, DNA barcodes, Chinese native chicken, genetic variation

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Introduction

In China, chickens are the main source of poultry meat and eggs, and are also popularly bred for cockfighting. For meat and egg production, Chinese native chickens are usually raised under the free-range system or in semi-confinement, which are cost-effective ways for many smallholder farmers. However, the native chicken populations are in small number and unfortunately the replacement of modern industrial stock intensifies the loss of genetic resource problem. When populations of the same species progress autonomously for an absolute period of time, it increases the chances of intergenomic conflict between the nuclear and mitochondrial genomic genes (Hebert et al., 2003).

Mammals are the most studied animal group in species identification and DNA sequencing is the major source of information to understand evolution and genetic relationship. Genetic identification has been widely used for investigation into different fields including illicit killing of animals (Wu et al., 2005) and illegal trading of protected species (Gratwicke et al., 2008). In case of compromised morphology, genetic identification is an alternative for identifying unknown evidence sample to a known reference sample by comparing sequences of genes (Dawnay et al., 2007). 'DNA bar-coding' is a new scientific technique for species identification. DNA barcoding means the use of objective DNA as a tool to identify species, and in recent years, it has been the new development of biological taxology. DNA barcode is a short sequence of standardized genomic region and every species has a specific barcode. DNA barcoding is based on the principle that a short standardized sequence can distinguish individuals of a species because genetic variation between species exceeds that within species (Bekker et al., 2016). The genes most commonly used for species identification are cytochrome b (cyt b) and the hyper-variable displacement loop (D-Loop) (Hebert et al., 2003). Recently, it has also been reported that the mtDNA gene cytochrome c oxidase I (COI) can be used as a 'barcode' for most animal life (Hebert et al., 2003) and this is a speedily expanding area of research. The anticipated growth in COI data has recently led one leading journal to form a dedicated barcoding section for COI sequence publication, paving the way for the COI gene to become a key taxonomic identification tool. According to recent findings, an approximately 600 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) could be a good choice for a barcoding gene because it might be involved in speciation (Roe and Sperling, 2007).

The purpose of this study was to investigate the reliability of COI barcodes in identifying Chinese native chickens. In this study, variations of selected COI gene and population genetic structure of four native chicken breeds of China were investigated. Also, genetic diversity of these chicken breeds was studied by using COI gene as DNA barcode.

Materials and Methods

Sampling: Blood samples were obtained randomly from 64 chickens of four different Chinese native breeds from different cities, including Lingnan yellow broilers (LN) from Guangzhou city, Huaixiang chicken (HX) from Xinyi city, Princess chicken (GF) from Zhanjiang city and Gallus gallus spadiceus (GS) from Yunnan (Figure 1).

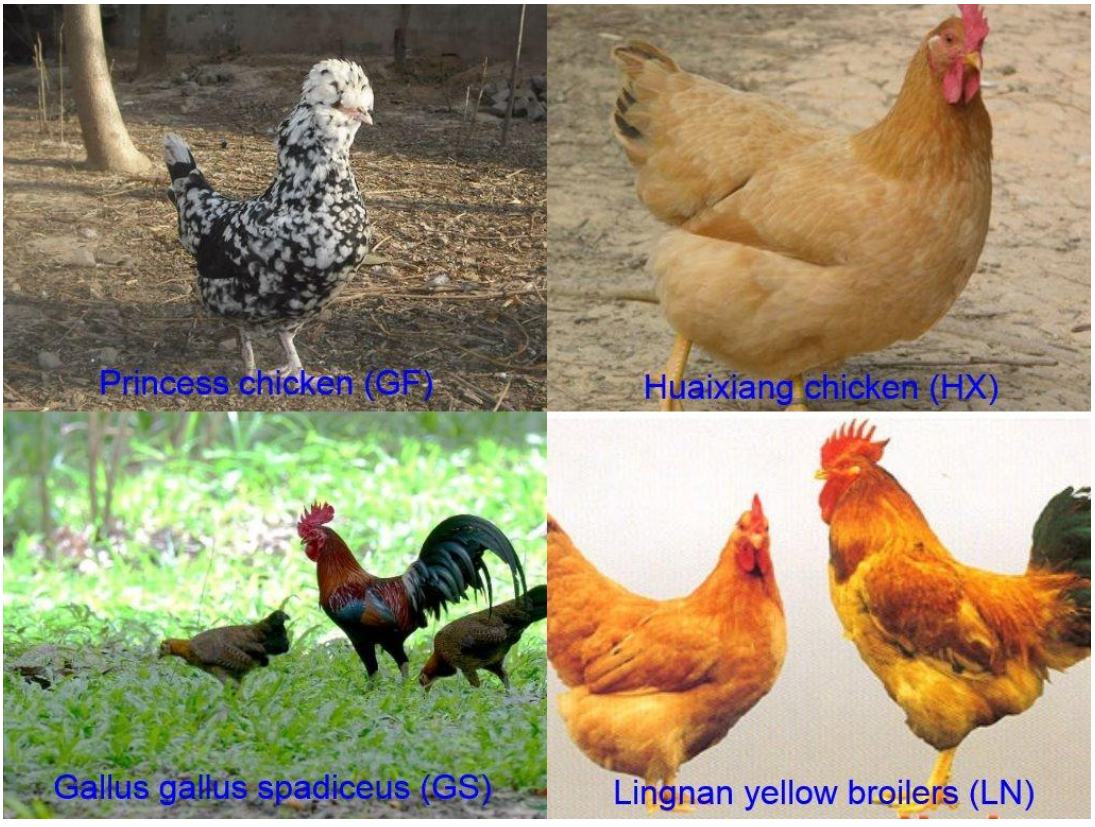

Figure 1 Native chicken breeds in China

Laboratory Analysis: Laboratory protocols for DNA extraction, purification, elution and amplification were developed for poultry specimens at College of Agriculture, Guangdong Ocean University, Zhanjiang, Guangdong, China.

DNA Extraction: Total DNA was extracted from the stored blood samples using standard phenol/chloroform method (Evans, 1990).

DNA Purification and Evolution: Sample discs were washed four to five times with 200 µL of FTA Purification Reagent and rinsed with 200 µL of sterile nanopure water. The sample discs were then dried in a laminar hood overnight. Purity and concentration of the DNA was measured Spectrophotometrically and electrophoretically. The eluted DNA was stored at -20°C for further use.

DNA Amplification and Sequencing: Primers were designed based on the DNA sequence of GS published in GenBank (accession number: AP003322), synthesized by Shanghai Bioengineering Ltd. The designed primers were bar1 (product 590-bp; Loci 371-1021): 5′-GCA CAG GAT GGA CAG TTT AC-3′ (forward); 5′-ATA GCA TAG GGG GGT CTC AT-3′ (antisense); bar2 (product 624 bp; Loci 712-1359): 5′-TCA AGT GAA GCC TGG ACT AC-3′ (forward); 5′-TGC GGA TAC TTG CAT GTA TAT-3′ (antisense). Each PCR amplification volume was 20 µL including 2 µL of 10×buffer, 2 µL of 25 mmol Mg+, 0.8 µL of 10mmol dNTPs, 0.5 µL of 5 U/µL Taq DNA polymerase, 2 µL of 10 pmol/µL each primers, and approximately 2 µL of genomic DNA. PCR thermal cycler condition for the amplifications was one initial denaturation step at 96°C for 3 min; 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; and a final step of primer extension at 72°C for 5 min. Each PCR product was purified using the DNA Fragment Quick Purification/Recover Kit (Ding Guo biotechnology Inc.) according to the manufacturer's instructions and sequenced bi-directionally on an ABI 3730 (Shanghai Bioengineering Ltd.) using the PCR primers.

Statistical Analysis: Sequences were aligned using Clustal X 2.0 (Larkin et al., 2007) and were edited using MEGA 4.0 program (Tamura et al., 2007). Sequence divergences were calculated using the Kimura-2-parameter (K2P) distance model (Kimura, 1980). The neighbor-joining (NJ) method was used for constructing a phylogenetic tree (Zhang and Sun, 2008) and was analyzed by MEGA 4.0. Haplotypes were counted by DNAsp4.50, MEGA 4.0 software statistical analysis of the sequence of the mutation site, the average base composition, percentage and genetic distance. Haplotype diversity (H), nucleotide polymorphism (Pi) and average nucleotide variation (K) of each population were further estimated.

Results and Discussion

Characteristics of Sequences: Two COI barcodes of 64 different chicken breeds of China were analyzed. Results showed that the average length of the analyzed sequences of bar1 and bar2 were 590 bp and 624 bp, respectively. Among the four breeds, only in LN chicken one variable site was found in bar1. In bar1, the COI sequence divergence and average COI sequence difference among the breeds were 0.05% and 0.17%, respectively. In contrast, in bar2, there were four variable sites and the COI sequence divergence among species (0.64%) was only twice as much as the differences within species (0.56%). However, it was reported that the COI sequence difference among closely related species was 19 times higher than the differences within species (KERR et al., 2007)

Bar1 of all four breeds presented two haplotypes, one of which was only present in LN, which could provide a foundation for variety identification, while the other one was present in all breeds. In contrast, in bar2 totally eight haplotypes were present (Table 3). H1 and H2 shared the haplotype of LN and GS, while H3 and H4 shared the haplotype of GF and HX, indicating that the native chicken breeds had a hereditary similarity. The reason may be that when these breeds formed and evolved, cross-breeding and reciprocal influence happened among the different chicken breeds. The variable sites of bar2 haplotype in the 4 chicken breeds are presented in Figure 2.

			4	4
		3	5	5
	1	3	5	6
Hap-1	T	A	C	A
Hap-2	- -	G	T	G
Hap-3	C	G	T	G
Hap-4	C	- -	- -	- -
Hap-5	C	G	- -	- -
Hap-6	C	G	- -	G
Hap-7	- -	G	- -	- -
Hap-8	- -	G	- -	G

Figure 2 Variable sites of bar2 in 4 chicken breeds

Haplotype Diversity (H): Haplotype diversity means the uniqueness of a particular haplotype in a given population. The higher H and nucleotide polymorphism (Pi) mean higher nucleotide diversity and richer genetic diversity in population. In this study, it was found that the H in LN and HX chicken was higher in bar2 (Table 1); similar result was reported in a previous study (Yu-shi et al., 2011). The nucleotide polymorphism (Pi) in bar2 ranged from 0.00102 to 0.00305, similar to that reported in other species of chicken by Yu-Shi et al. (2011), but lower than that reported in other species of chicken by Yun-jie et al. (2011).

Genetic Distances: Genetic distance represents the phylogenetic evolution of the population. The genetic distance was estimated by Kimura-2-parameter model, and the calculated results of bar2 are presented in Table 2. According to results, the intrapopulation genetic distances ranged between 0.005-0.002. The genetic distances of Japanese quail (*Coturnix japonica*) have been published in GenBank (AP003195); the interpopulation genetic distances ranged from 0.996-1.006. The interpopulation genetic distance of LN was 1.006 and was the largest of all. Similar results were also reported for other domestic chickens and quails (Fu et al., 2001). The intrapopulation genetic distances were less than the interpopulation genetic distances. This result indicates that the degree of differentiation is low in the Chinese native chicken breeds.

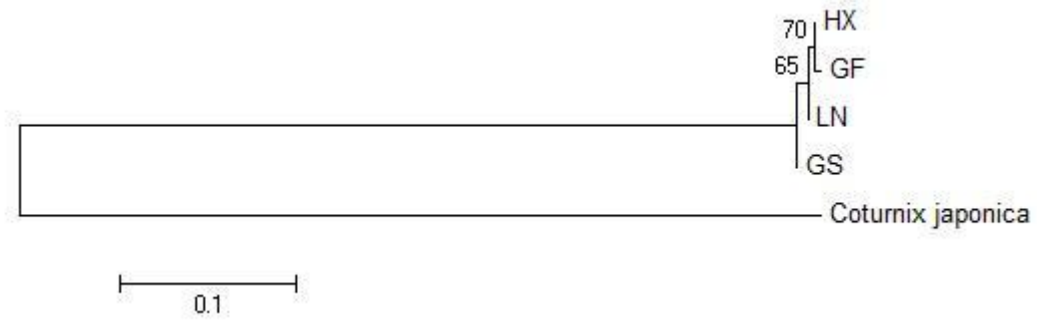

Figure 3 Neighbor-joining (NJ) trees based on Kimura-2-parameter distances for bar2 COI gene of chicken breeds

Table 1 Statistics information of COI[1] barcode in 4 chicken breeds

Sites	Breeds	No.	Haplotypes	Haplotype diversity (Hd)	Average number of nucleotide differences (K)	Nucleotide diversity (Pi)
bar1	HX	16	1	0	0	0
	GF	16	1	0	0	0
	GS	16	1	0	0	0
	LN	16	2	0.035±0.034	0.035	0.00006
total			2	0.035±0.034	0.035	0.00006
bar2	HX	16	4	0.733±0.600	1.178	0.00199
	GF	16	2	0.200±0.154	0.600	0.00102
	GS	16	2	0.600±0.175	1.800	0.00305
	LN	16	4	0.733±0.101	1.089	0.00184
total			8	0.835±0.036	1.929	0.00328

[1]COI=cytochrome c oxidase I
GF=Princess chicken, GS=*Gallus gallus spadiceus*, HX=Huaixiang chicken, LN=Lingnan yellow broilers

Table 2 Kimura-2-parameter distances among chicken breeds

Breeds	Breeds				
	AC	GS	GF	HX	LN
AC					
GS	1.005				
GF	0.996	0.005			
HX	0.997	0.004	0.003		
LN	1.006	0.002	0.005	0.004	

AC=*Coturnix japonica*, GF=Princess chicken, GS=*Gallus gallus spadiceus*, HX=Huaixiang chicken, LN=Lingnan yellow broilers

Table 3 Number of bar2 haplotypes in chicken breeds

Haplotypes	Breeds			
	LN	GF	HX	GS
Hap-1	4			3
Hap-2	1			2
Hap-3		9	2	
Hap-4		1	2	
Hap-5			5	
Hap-6			1	
Hap-7	4			
Hap-8	1			

GF=Princess chicken, GS=*Gallus gallus spadiceus*, HX=Huaixiang chicken, LN=Lingnan yellow broilers

Phylogenetic Tree of COI Barcode: MEGA 4.0 was used for constructing a phylogenetic tree. All breeds were divided into three groups (Figure 3). GS and LN were placed in the same group, while GF and HX were in separate groups. The native chicken, GS, and Japanese quail were clustered into different groups based on the NJ clustering. It was found that there was a close relationship among the four native chicken breeds, while the chicken breeds had a distant relationship with the quail. This is because although chicken and quail both belong to the pheasant (*Phasiamidae*) family in chicken (*Calliformes*) item of bird (*Aves*), they belong to different genera. The result of the present study confirmed the findings of Fu et al. (2001). The phylogenetic approaches suggest that artificial selection plays an important role in genetic differentiation of chicken breeds.

Conclusion

Based on the present results, COI gene can be used in barcoding. In addition, bar2 sequence of COI gene is a better choice for DNA barcoding and, therefore, could be an important theoretical basis for future investigation into population diversities, genetic structures and confirmation of species classification.

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บทคัดย่อ

ดีเอ็นเอบาร์โค้ด ของยีน COI ในไก่พื้นเมืองจีน

ฮ่องยาง คู ฟาหา อิบติชาม จง ซู หังกวง หวัง ยิง สู*

การศึกษาครั้งนี้ ได้ศึกษาความเป็นไปได้และประสิทธิภาพของยีน cytochrome c oxidase I (COI) เพื่อนำมาใช้เป็นดีเอ็นเอบาร์โค้ด ในการตรวจสายพันธ์ไก่พื้นเมืองจีน โดยศึกษารหัสพันธุกรรมของยีน COI ในไก่พื้นเมืองในประเทศจีน 4 สายพันธุ์ จำนวน 64 ตัว ผลการศึกษาพบว่ารหัสพันธุกรรม Bar1 บนยีน COI มีความหลากหลาย (polymorphism) น้อยกว่า Bar2 โดยรหัสพันธุกรรม Bar1 มี polymorphic site เพียง 1 แห่ง และมี haplotypes เพียง 2 แบบ ในขณะที่ Bar2 มี polymorphic sites 4 แห่ง และมี haplotypes 8 แบบ โดยค่า nucleotide polymorphism (Pi) ของรหัสพันธุกรรม Bar2 มีค่าอยู่ระหว่าง 0.00102 ถึง 0.00305 และผลการจำแนกอนุกรมวิธานโดยรหัสพันธุกรรม Bar2 ให้ผลสอดคล้องกับการจำแนกการจากรูปร่างภายนอกของไก่พื้นเมืองทั้ง 4 สายพันธุ์ ผลการศึกษาครั้งนี้แสดงให้เห็นว่าการทำดีเอ็นเอบาร์โค้ด ของยีน COI ใช้ได้ดีในการตรวจสายพันธุ์ไก่พื้นเมืองในประเทศจีน

คำสำคัญ: cytochrome c oxidase I ดีเอ็นเอบาร์โค้ด ไก่พื้นเมืองจีน ความหลากหลายทางพันธุกรรม

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