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Genetic Diversity of Thai Native Chicken Inferred from Mitochondrial DNA Sequences

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Abstract.– Native chickens play at least four significant roles for Thai people. In this study, the genetic variation of native chickens in northeastern Thailand was quantified based on the mitochondrial DNA HVS-I sequences. The phylogenetic relationship between these Thai native chickens as well as those of other geographic regions was also evaluated. A high level of genetic variation was found in the native chickens from northeastern Thailand, somewhat similar to that reported in Indian and Vietnamese populations but higher than African and Chinese native chickens. This likely implies a greater effective population size and longer demographic history and is consistent with the hypothesis that Thailand is within one of the places where chickens were first domesticated. Phylogenetic analyses revealed that Thai native chickens were divided into three lineages. The first lineage was closely related to *G. gallus gallus*, *G. g. domesticus* and *G. g. murghi* whilst the other two lineages were closely related to *G. g. spadiceus*. Thus, at least three subspecies, *G. g. gallus*, *G. g. murghi* and *G. g. spadiceus,* are likely to have been involved in chicken domestication in Thailand.

KEY WORDS: Domestic chicken; *Gallus gallus*; Genetic diversity; Mitochondrial DNA

INTRODUCTION

Native chickens play four important roles for Thai people; namely they are used as food, in religious activity, as decoration, and for sport (Takashi and Kazunoba, 2010). Archeological evidence has indicated that chickens had already been domesticated in at least two sites, one in northern China by about 6,000 years BC and the other later in the Indus Valley by about 2,500 years BC (Tixier-Boichard et al., 2011). Early molecular studies originally indicated that domestic chickens were likely to have been derived from the continental red jungle fowl, *Gallus gallus gallus*, and that domestication took place in Thailand and/or adjacent areas (Fumihito et al., 1994, 1996). However, recent molecular studies have suggested likely multiple origins of domestic chickens, and that at least three subspecies contributed to chicken domestication (Liu et al., 2006; Kanginakudru et al., 2008).

In addition to searching for the origin of chicken domestication, quantifying the level of genetic variation is also important, since genetic diversity is critically important for species adaptability. For domestic animals, maintaining genetic diversity assures further breed improvement and selection. This is additionally important for globally cultivated/distributed species and those used for different functions (such as meat versus egg production), both of which are true for domestic chickens, where diverse adaptations

are required to local environmental conditions, pathogen resistance and development (e.g. resource allocation to egg or meat production), for example. Previous studies have shown that the genetic diversity of chickens was relatively high in areas where the origin of chicken domestication was speculated to have occurred (Kanginakudru et al., 2008; Berthouly-Salazar et al., 2010).

Although Thailand has been speculated to be within one of the locations where chickens were first domesticated, only a small sampling of Thai chickens was included in the genetic analyses (Fumihito et al., 1996). A recent molecular study also ignored samples from the northeastern region of Thailand (Riztyan et al., 2012). In this study we, therefore, assessed the genetic variation of native chickens from northeastern Thailand and then examined the genetic relationship between Thai chickens and domestic chickens from other geographic regions based on DNA sequence differences in the mitochondrial DNA hypervariable sequence-I (HVS-I).

MATERIALS AND METHODS

Sampling, DNA extraction, Polymerase Chain Reaction (PCR) and Sequencing.– Domestic chickens were sampled from 21 locations in 10 provinces in Northeastern Thailand (Table 1 and Fig. 1). DNA was extracted using a genomic DNA extraction kit (RBC BioScience, Taiwan) as per the manufacturer's protocol and kept at −20 °C until use. The mitochondrial DNA HVS-I sequence was amplified using primers L16750 (5-AGGACTACGGCTTGAAAAGC-3; Fumihito et al., 1994) and H522 (5-ATGTGCCTGACCGAGGAACCAG-3; Fu et al. 2001), using the PCR conditions of Liu et al. (2006). PCR products were checked by resolving over 1 % (w/v) agarose-TBE gel electrophoresis with ethidium bromide staining and uv-light transillumination, and cleaned using a HiYield™ Gel/PCR DNA Extraction Kit (RBC BioScience, Taiwan) as per the manufacturer's protocol. Direct sequencing on both strands was performed commercially at Macrogen sequencing service (Seoul, Korea), using the same primers (L16750 and H522) as in the PCR but in separate reactions.

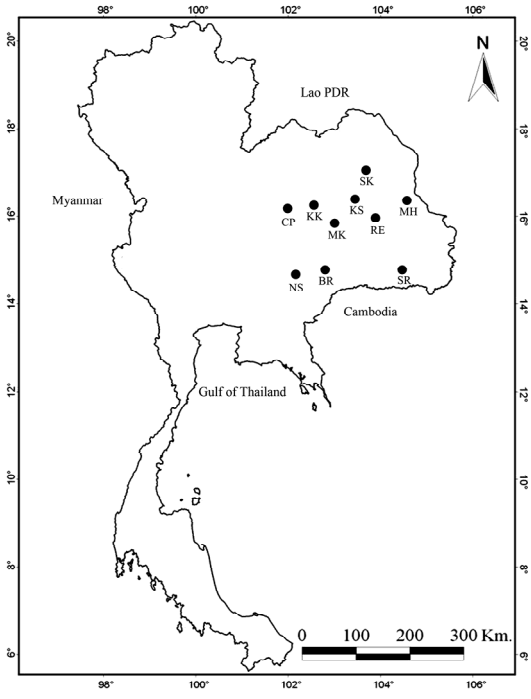

FIGURE 1. Collection sites, within 10 provinces, for Thai native chickens in northeastern Thailand. Details of the sampling locations are given in Table 1.

TABLE 1. Sampling locations and number of samples of native chickens from northeastern Thailand use in the present study.

Sampling location (Code)	Sample size	Number of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)
Si sa ket Province (SR)				
Ban Donlee, Prangku District	5			
Ban Pimai, Prangku District	2			
All	7	7	1.000	0.0147 ± 0.0089
Maha Sarakham Province (MK)				
Ban Nong Wang, Mueang Maha Sarakham District	2			
Ban Nong No, Mueang Maha Sarakham District	2			
Ban Dindam, Mueang Maha Sarakham District	1			
All	5	5	1.000	0.0191 ± 0.0124
Kalasin Province (KS)				
Ban Huai Mek, Huai Mek District	2			
Ban Nong Chum Sang, Nong Kung Si District	1			
Ban Naku, Khao Wong District	3			
All	6	6	1.000	0.0142 ± 0.0089
Chaiyaphum Province (CP)				
Ban Nong Chiangcha, Phu Khiao District	2			
All	2	2	1.000	0.0093 ± 0.0102
Khon Kaen Province (KK)				
Ban Phai District	2			
All	2	2	1.000	0.0093 ± 0.0102
Buriram Province (BR)				
Ban Na Pho, Phutthaisong District	2			
Ban Jaluay, Satuek District	1			
All	3	3	1.000	0.0213 ± 0.0167
Nakhon Ratchasima Province (NR)				
Ban Dang Noi, Bua Lai District	1			
All	1	1	-	-
Roi-Et Province (RE)				
Ban Mek, Changhan District	1			
Ban Pho Thong, Selaphum District	1			
All	2	2	1.000	0.0226 ± 0.0235
Sakon Nakhon Province (SK)				
Ban Tad Phu Wong, Waritchaphum District	1			
Ban Kham Bo, Waritchaphum District	1			
Ban Nong Pan, Waritchaphum District	1			
Ban Non Na Kham, Waritchaphum District	2			
Ban Tao Ngoi, Tao Ngoi District	1			
All	6	4	0.800 ± 0.172	0.0117 ± 0.0074
Mukdahan Province (MH)				
Ban Na Ta Bang, Nong Sung District	11			
All	11	3	0.691 ± 0.086	0.0072 ± 0.0044
Total	**45**	**30**	**0.970 ± 0.013**	**0.0156 ± 0.0082**

Data analysis.– The consensus DNA sequences were aligned using CLUSTAL X (Thompson et al. 1997), with a final visual inspection. Representative haplotypes were deposited in GenBank under accession numbers KC817504 - KC817533. Haplotype diversity (h) and nucleotide diversity (π) were estimated in Arlequin ver. 3.5 (Excoffier and Lischer, 2010).

Phylogenetic relationships were analyzed based on the mtDNA HVS-I sequences of the 30 haplotypes (from 45 samples) of Thai native chickens derived in this study plus those sequences previously published in Genbank (Table 2). Phylogenetic analysis

TABLE 2. List of sequences obtained from Genbank used in the phylogenetic analysis.

Species/subspecies	Location	GenBank accession code	Reference
Gallus varius	Bali, Indonesia	D82912	Fumihito et al. (1996)
G. gallus	India	GU902237	Singh and Kumar
	India	GU902221	(unpublished data)
	Egypt	EF586879	
	Vietnam	HM462085	Ramadan et al. (2011)
	Vietnam	HM462217	Berthouly-Salazar et al.
	Sichuan Province, China	AF512337	(2010)
	Sichuan Province, China	AF512336	
	Yunnan province, China	AF512327	Liu, et al. (2006)
	Yunnan Province, China	AF512077	
	Sichuan Province, China	AF512091	
	Yunnan Province, China	AF512108	
	Guizhou Province, China	AF512128	
	Henan Province, China	AF512139	
	Yunnan Province, China	AF512151	
	Hubei Province, China	AF512316	
G. gallus murghi	India	EU847798	Kanginakudru et al. (2008)
	India	EU847791	
	India	EU847784	
G. g. gallus	Thailand	D82900	Fumihito et al. (1996)
	Tokyo, Japan	D82902	
	Palembang, South Sumatra, Indonesia	D82903	
	Palembang, South Sumatra, Indonesia	D82904	
	Palembang, South Sumatra, Indonesia	D82905	Berthouly-Salazar et al.
	Vietnam	HM462088	(2010)
	Vietnam	HM462090	
G. g. domesticus	West Sumatra, Indonesia	D82917	Fumihito et al. (1996)
G. g. bankiva	Singaraja, Bali, Indonesia	D82897	Fumihito et al. (1996)
	West Java, Indonesia	D82898	
	Lanpung, East Sumatra, Indonesia	D82899	
		AB007718	Miyake (unpublished data)
G. g. spadiceus	Thailand	D82907	Fumihito et al. (1996)
	Vientiane, Lao PDR	AB007721	Nishibori et al. (2005)
	China: Yunan Province	AF512154	Liu et al. (2006)

was then performed using neighbor-joining (NJ), maximum parsimony (MP) and Bayesian inference (BI) analyses. The distance based NJ analysis was performed in PAUP* (Swofford, 2002) using the best-fit selected model. Bootstrap support was estimated using 1,000 replicates. MP analyses was also performed in PAUP*, using a heuristic search with 1,000 random addition sequence replicates, TBR branch swapping, and MulTrees effect. Bootstrap support was estimated for 1,000 replicates. BI analysis was performed using MRBAYES 3.04b (Huelsenbeck and Ronquist, 2001). The best-fit model for Bayesian analysis was selected by hierarchical likelihood ratio tests implemented in MrModeltest (Nylander, 2004). The general time-reversible (GTR) model (Rodriguez et al., 1990) with a gamma distribution shape parameter of 0.6624 and a proportion of invariable sites of 0.6729 was selected. The BI analysis was run for 2,000,000 generations, with a sampling frequency of 100 generations. Tracer version 1.5 (Rambaut and Drummond, 2004) was used for visual inspection of the point where

the log likelihood is stationary. Trees sampled before this point were discarded as burn-in. The remaining trees of two simultaneous runs were included in posterior probability calculations.

In all cases the corresponding sequence from *Gallus varius* was used as the outgroup.

RESULTS

Mitochondrial DNA sequence variation.– A 545 bp fragment of the mtDNA HVS-I was obtained from 45 samples of domestic chickens from 21 locations in 10 provinces (populations) in northeastern Thailand. There were 34 base substitutions, comprised of 28 transitions and six transversions. No insertion/deletions (indels) were detected in our sequences. A total of 30 haplotypes were identified. Seven of the nine populations that had more than two samples had a haplotype diversity of 1.00, with one population only having a single sample. Of the two other populations that from Mukdahan Province showed the lowest haplotype diversity (0.691) followed by that from Sakon Nakhon at 0.80. The overall haplotype diversity for the 10 populations (45 samples) was 0.970±0.013 (Table 1).

The nucleotide diversity in the populations differed between all nine populations that had more than two samples bar two (Chaiyaphum and Khon Kean) and ranged from 0.0072 (Mukdahan) to 0.0226 (Roi Et) with an overall nucleotide diversity for the 10 populations (45 samples) of 0.0156±0.0082 (Table 1).

Phylogenetic relationship.– All three phylogenetic analyses (NJ, MP and BI) revealed a similar tree topology with *G. gallus* forming two main clades (Fig. 2 – 4). Most of the samples belonged to clade I, with just *G. g. bankiva* from Indonesia forming clade II. Clade I was further divided into four subclades (I-1, I-2, I-3 and I-4) and the Thai *G. gallus* samples were found in subclades I-1, I-2 and I-3 but not in subclade I-4 that contained only *G. g. gallus* from Indonesia. Two subspecies of *G. gallus*, namely, *G. g. domesticus* from Indonesia and *G. g.murghi* from India also clustered with *G. g. gallus* in subclade I-1. *G. g. spadiceus* from China clustered with *G. g. gallus* in subclade I-2 and *G. g. spadiceus* from Thailand and Lao PDR clustered with *G. g. gallus* in subclade I-3. The results strongly suggested that the taxonomic status of these subspecies needs further examination.

DISCUSSION

Genetic variation is important for species adaptability. For domestic animals, it is important for breed improvement and selection. Previous studies have revealed considerable levels of genetic variation in native chickens in regions where the origins of domestication were speculated to have occurred. High levels of genetic diversity (nucleotide diversity of 0.018) were found in the native chicken populations in India based on mitochondrial HVS-I sequences (Kanginakudru et al., 2008), and broadly similar results were reported from Vietnamese chickens with an observed nucleotide diversity of 0.013 (Berthouly-Salazar et al., 2010). This contrasts with the low genetic diversity reported from chickens in Africa (0.00745), which is consistent with the historical evidence that suggested the introduction of chickens into this region was far more recent at approximately 700 BC (Mwacharo et al., 2011).

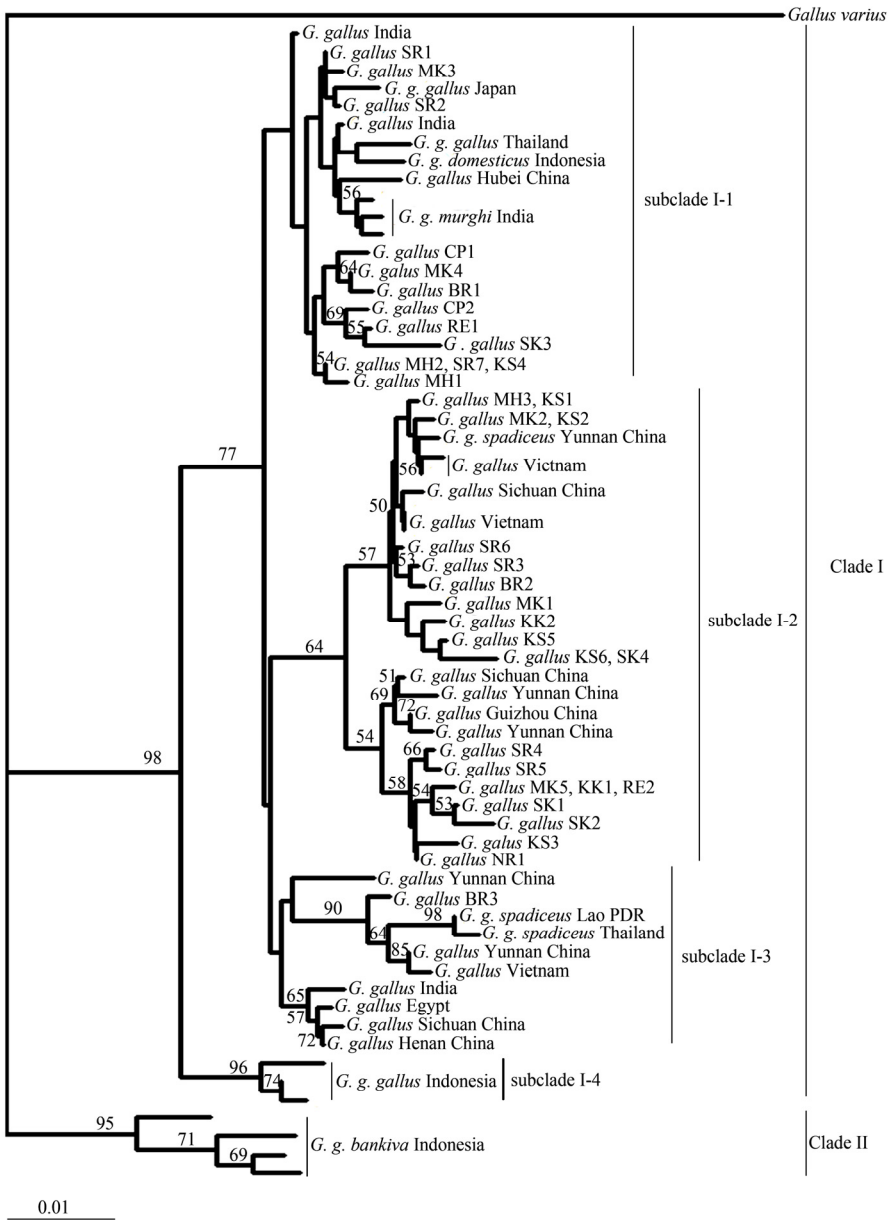

FIGURE 2. Neighbor-joining phylogenetic tree of chickens based on 545 bp of mitochondrial HVS-1 sequences. Bootstrap values are shown above or near the branch. Native chickens from northeastern Thailand were labeled with a code representing the province where the samples were collected as SR, Si sa ket; SK, Sakon Nakhon; MK, Maha Sarakham, CP, Chaiyaphum; BR, Buriram; RE, Roi-Et, KK, Khon Kaen; KS, Kalasin; NR, Nakhon Ratchasima; MH, Mukdahan. The scale bar represents 0.01 substitution per nucleotide position.

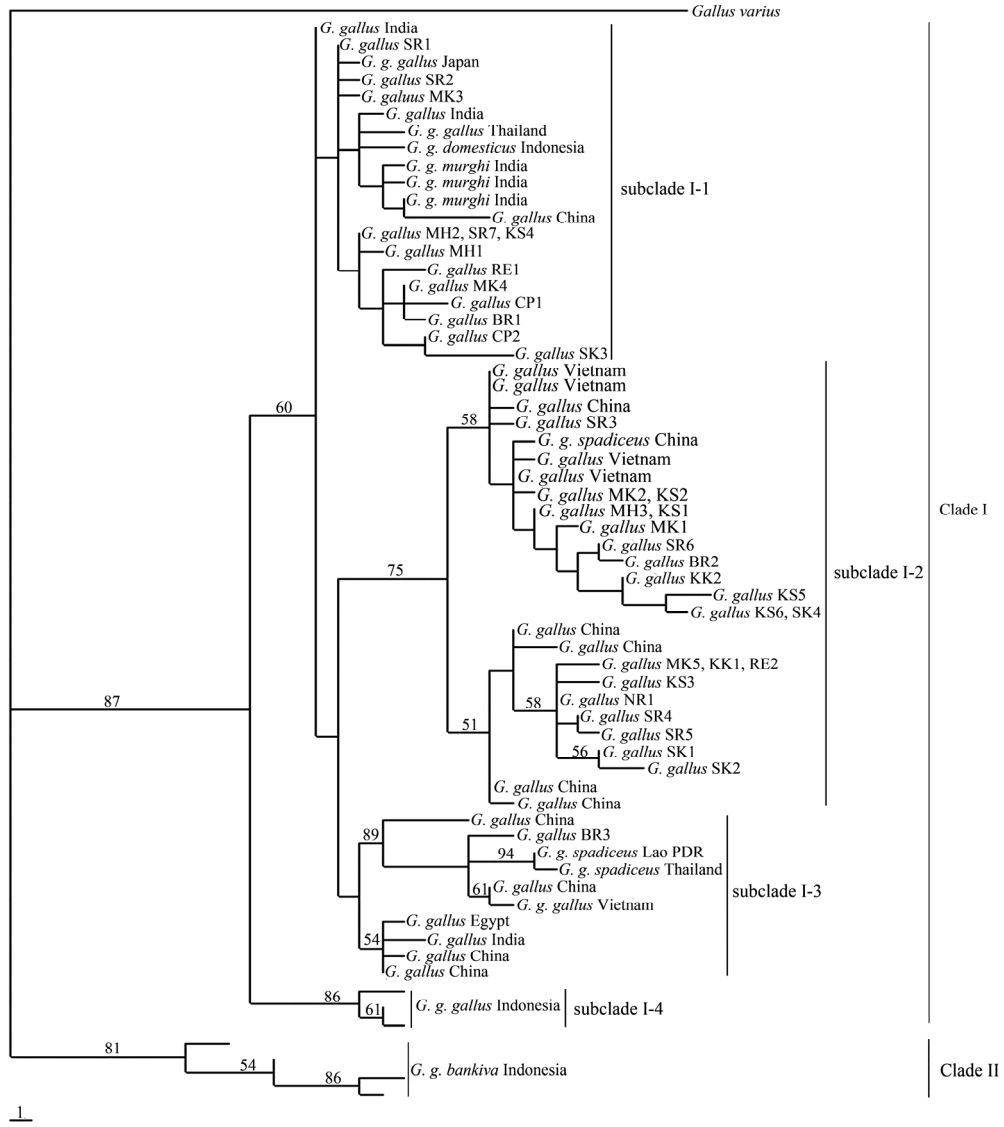

FIGURE 3. Maximum parsimony phylogenetic tree of chickens based on 545 bp of mitochondrial HVS-1 sequences. Bootstrap values are shown above or near the branch. Native chickens from northeastern Thailand were labeled with a code representing the province where the samples were collected as SR, Si sa ket; SK, Sakon Nakhon; MK, Maha Sarakham, CP, Chaiyaphum; BR, Buriram; RE, Roi-Et, KK, Khon Kaen; KS, Kalasin; NR, Nakhon Ratchasima; MH, Mukdahan. The scale bar represents one nucleotide change.

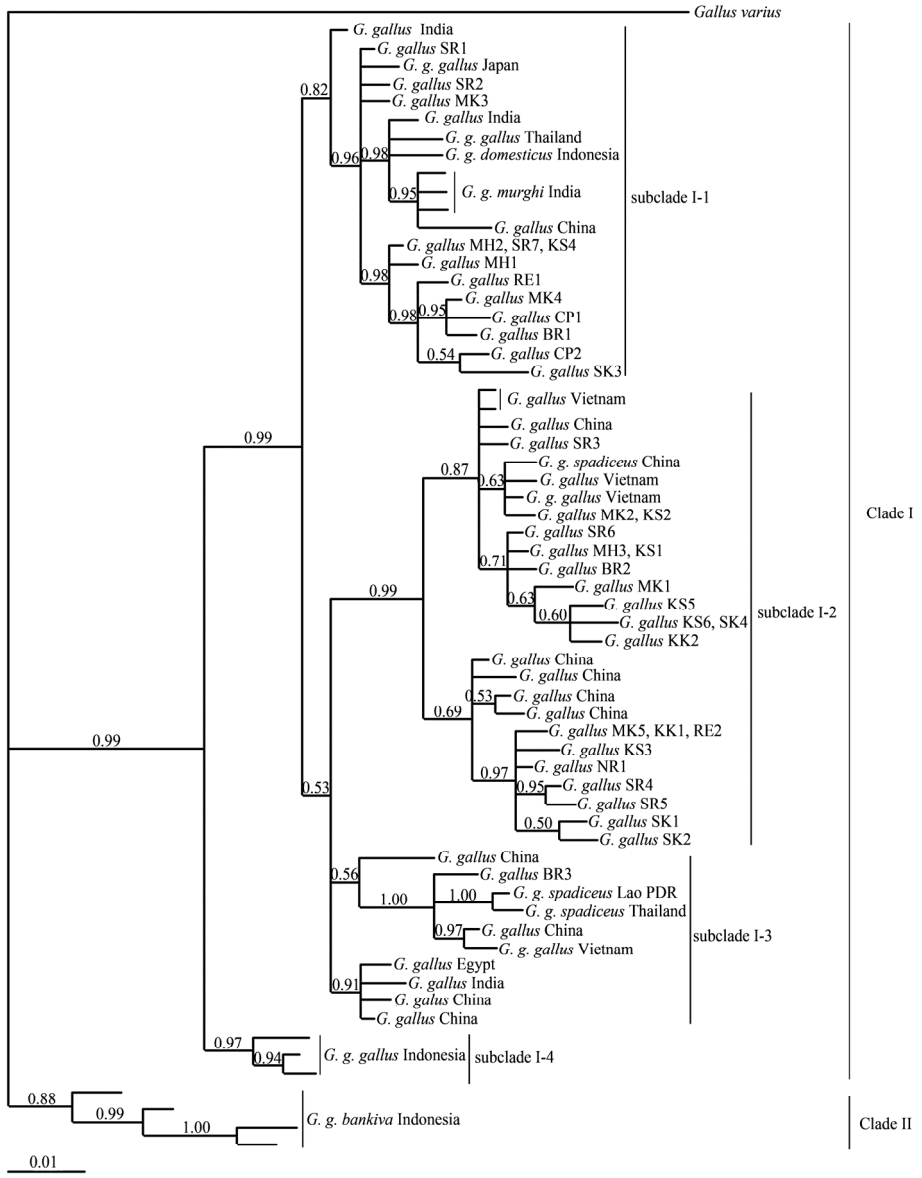

FIGURE 4. Bayesian inference phylogenetic tree of chickens based on 545 bp of mitochondrial HVS-1 sequences. Bootstrap values are shown above or near the branch. Native chickens from northeastern Thailand were labeled with a code representing the province where the samples were collected as SR, Si sa ket; SK, Sakon Nakhon; MK, Maha Sarakham, CP, Chaiyaphum; BR, Buriram; RE, Roi-Et, KK, Khon Kaen; KS, Kalasin; NR, Nakhon Ratchasima; MH, Mukdahan. The scale bar represents 0.01 substitution per nucleotide position.

The genetic diversity of Thai native chicken has been reported previously, based on 98 autosomal SNP markers, to show a similar level of genetic variation to those from neighboring Myanmar (Riztyan et al., 2012). From that reported SNP based analysis, both the Thai and Myanmar native chickens possessed a higher genetic variation than the commercial domestic chicken lines (Riztyan et al., 2012), as expected. Our results for native chickens from the northeast of Thailand, based on mitochondrial HVS-I sequences indicated comparable levels of genetic variation (average nucleotide diversity of 0.0156) to those reported from India and Vietnam. The results support the hypothesis that the area around and including Thailand was one potential location where chicken domestication occurred (Fumihito et al., 1996).

Consistent with the high levels of observed mitochondrial DNA genetic variation, phylogenetic analyses revealed that the mtDNA haplotypes of village chickens in Northeastern Thailand were dispersed in several clades. Each population possessed genetically divergent mtDNA haplotypes. Similar results have been reported for Vietnamese chickens, where a high diversity within populations with diverse mtDNA haplotypes was found (Berthouly-Salazar et al., 2010). On the one hand, the results indicated a high rate of individual movement within the sampling areas. On the other hand, high levels of genetic variation in a single village (i.e. sampling location) is important for the population to maintain genetic variation to counter the effect of genetic drift (Berthouly-Salazar et al., 2010).

Fumihito et al. (1996) speculated that Thailand and / or nearby areas were the location of the origin of chicken domestication. According to this study, the red jungle fowl, *G. gallus gallus* was thought to be the sole ancestor of domestic chickens. In contrast, Liu et al. (2006) demonstrated that domestic chicken could have originated from at least the three independent locations of (i) Yunnan, in South/Southwest China, (ii) the adjacent areas (Vietnam, Myanmar and Thailand), and (iii) India. This study also revealed that the same three subspecies of *G. gallus* (*G. g. spadiceus*, *G. g. gallusi* and *G. g. murghi*) were likely to have been included in the origin of modern domestic chicken, although when they were introgressed is not clear. Similar results obtained from Indian subcontinent chickens (Kanginakudru et al., 2008).

The results of the present study also support multiple origins of the current domestic Thai chickens. Phylogenetic analysis revealed that *G. g. spadiceus* and *G. g. murghi* were genetically closely related to Thai village chicken. On the other hand, *G. g. bankiva* from Indonesia was genetically distinct, suggesting that this subspecies may not have been involved in modern (maternal) chicken domestication. This has also been noticed in other studies (Fumihito et al., 1996; Liu et al., 2006; Kanginakudru et al., 2008).

In conclusion, we found high levels of genetic variation in native chickens from northeastern Thailand, which could be the result of the high rate of chicken movement between villages. Phylogenetic analysis revealed that Thai native chickens were divided into three genetically divergence lineages that were associated with three different subspecies of *G. gallus* (*G. g. spadiceus*, *G. g. gallus* and *G. g. murghi*) and so suggests that at least these three subspecies were included in the maternal origins of the domestication of Thai native chickens, which has been observed previously in native chicken from India.

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