Short Communication

Genetic variation of captive green peafowl *Pavo muticus* in Thailand based on D-loop sequences

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In the last few decades, it has been reported that the wild populations of *Pavo muticus* in northern and western Thailand have been decreasing dramatically as a result of habitat loss, environmental pollution and human exploitation (Meckvichai et al., 2001). At present *P. muticus* is patchily distributed along the Ping, Yom, Eng and Nan river basins in northern Thailand and in the west is distributed only in the Huai Kha Khang and Mae Klong basins (Meckvichai et al., 2002, 2007) (FIG. 1).

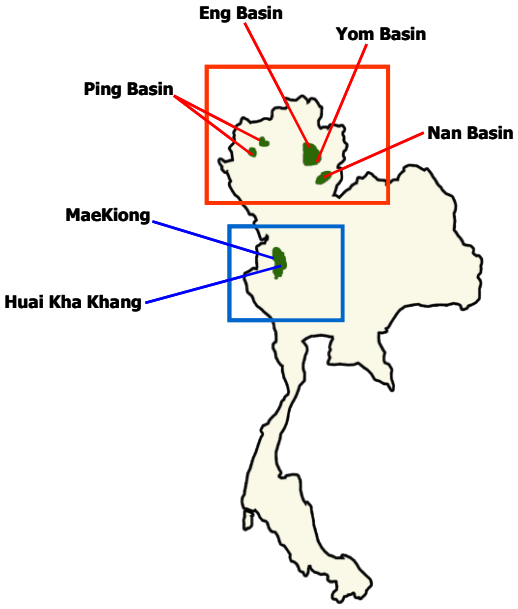

FIG. 1. Current geographical distribution of *P. muticus* in Thailand.

Since it has continued to decline, several captive breeding programmes have been established aiming to ensure its numbers and survival before reintroduction to the wild. In this case, the genetic diversity of captive *P. muticus* would vary among populations depending on the numbers and geographic range of founders. Differences in mitochondrial DNA (mtDNA) haplotypes in a population would indicate multiple origins of maternal lineages of the founders. Thus, study of mtDNA variations would provide insight into the maternal origin of the captive bred individuals and the genetic structure of wild populations. In addition, it may provide some evidence on the historical hybridization via mtDNA introgression between *P. muticus* and its closely related species.

In this study, we therefore aimed to estimate the level of genetic variation of captive *P. muticus* in Thailand using partial sequences of the mtDNA D-loop gene.

Methods

Sample collection and PCR amplification

The feathers of captive *P. muticus* were collected from 5 localities in northern and eastern Thailand (see detail in TABLE 1 and FIG. 3B). The total DNA was extracted from the feather tips by using the QIAamp® Mini kit (Qiagen, USA). A 330 bps fragment of the D-loop gene was amplified using species-specific primers: GPDF (5'-GGGGGGTATACTATGCATAATCGTG-3') and GPDR (5'-AAAGAATGGGCCTGAAGCTAGT-3'). The PCR mixture and amplification were performed following the protocol of Plubcharoensook (2000) using 35 cycles with the following conditions: denaturing at 94°C for 30 sec, annealing at 57°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min.

Data analyses

DNA sequencing was performed by the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA). The obtained sequences were aligned using CLUSTAL W (Thompson et al., 1994) via the program included in version 5.0 of MEGA software (Tamura et al., 2011). After visual inspection,

minor modifications were made for alignment accuracy.

Genetic diversity was estimated using DnaSp version 5.10.00 programme (Librado & Rozas, 2009). The phylogenetic relationship among captive *P. muticus* was reconstructed using the neighbor-joining method via MEGA programme. The confidences in a topology were assessed by 1,000 bootstrap replications. The DNA sequences of *Afropavo congensis* (DQ834507.1) and *P. cristatus* (AF104050) were used as the outgroups. We also performed a haplotype network approach for examining the relationships among mtDNA haplotypes using the NETWORK version 4.5.1.6 programme (Bandelt & Forster, 1999).

TABLE 1 Lists of collection sites, abbreviations, provinces and the total number of individuals collected from each location.

Location Name	Abbreviation	Province	Sample size
Doi Thung Wildlife Breeding Station	DT	Chiang Rai	5
Huai Hong Krai Royal Development Study Center	HK	Chiang Mai	5
Chiang Mai Zoo	CM	Chiang Mai	2
Khao Khew Open Zoo	KK	Chonburi	4
Khao Soi Dow Breeding Station	SD	Chantaburi	33

Results

Genetic diversity

A total of *c.* 309 bps of the D-loop gene were successfully sequenced for 48 individuals of 5 breeding stations. In all samples studies, the haplotype (*hd* = 0.901 ± 0.026) and nucleotide (π = 0.026 ± 0.0029) diversities were high.

Within populations, *P. muticus* from HK exhibited the highest *hd* and π whereas those from KK and DT showed the lowest *hd* and π respectively (TABLE 2)**.**

TABLE 2 Genetic diversity in each collected captive *P. muticus* population. The abbreviations used in this table are: n = number of sequences; m = total number of mutations; k = the average number of pairwise nucleotide differences; h = number of haplotype; *hd* = haplotype diversity and its standard deviation (± SD) and π = nucleotide diversity and its standard deviation (± SD). The underlined haplotypes indicate the shared haplotypes.

Location	Haplotypes	***P. muticus***					
		n	**m**	**k**	**h**	***hd* ± S.D.**	**π ± S.D.**
DT	H1, H2, H3	5	4	1.6	3	0.70 ± 0.22	0.01 ± 0.002
CM	H1	2	0	0	1	0	0
KK	H4, H5	3	5	3.3	2	0.67 ± 0.31	0.01 ± 0.01
HK	H1, H6, H7, H8, H9	5	28	12	5	1.00 ± 0.13	0.04 ± 0.02
SD	H10, H11, H12, H13, H14, H15, H16, H17, H18	33	21	8.3	9	0.82 ± 0.05	0.03 ± 0.002

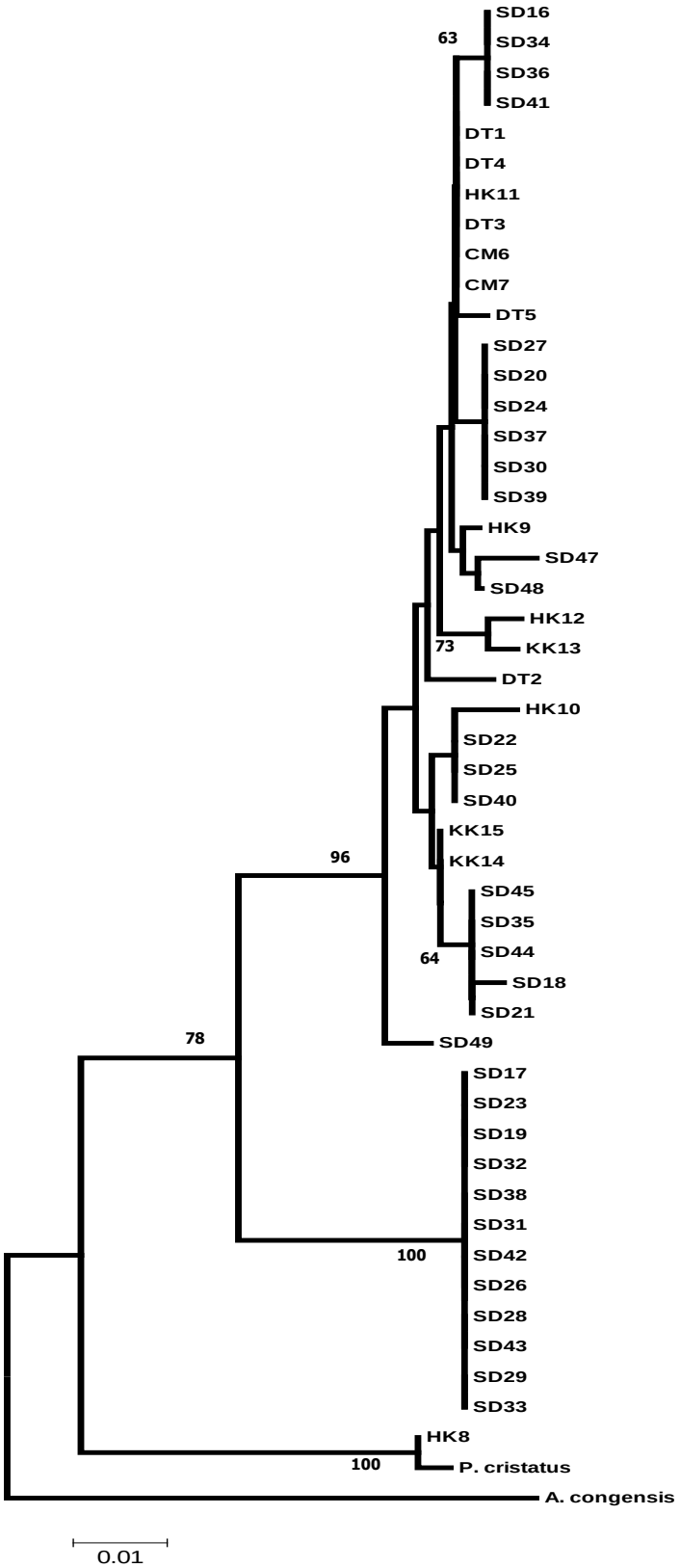

FIG. 2 Neighbour-joining phylogenetic tree of captive *P. muticus* based on the 309 nucleotide sequences of D-loop gene. Bootstrap probabilities in 1000 replicates are shown at nodes. The abbreviations for locations correspond to the name of locations listed in TABLE 1.

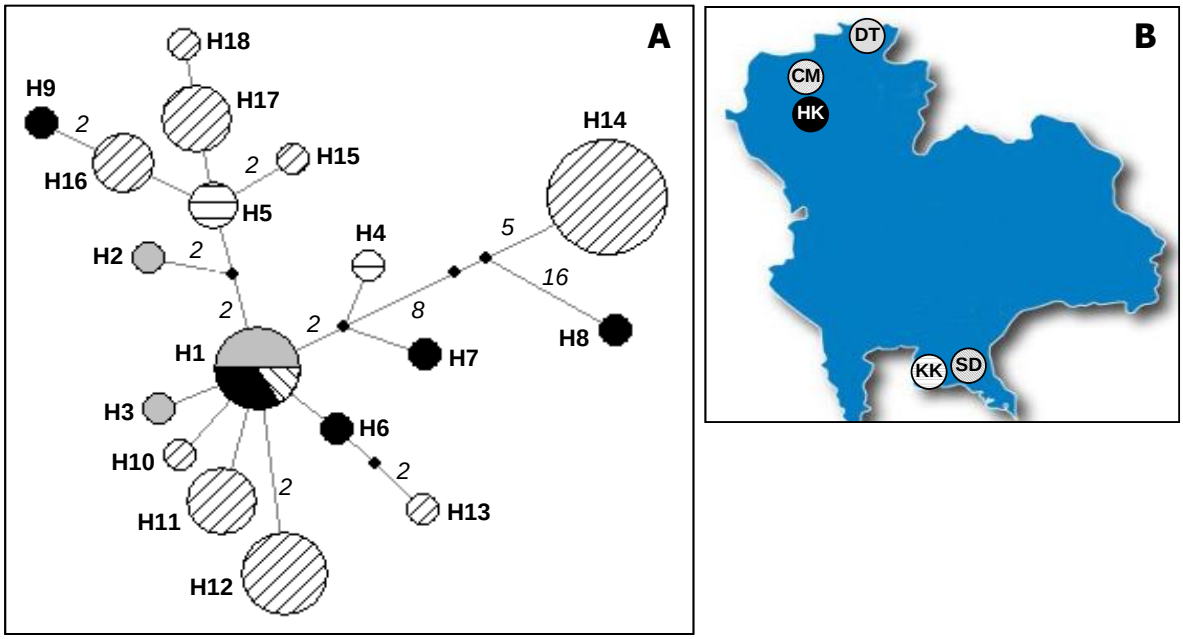

FIG. 3 The median-joining network for mtDNA haplotypes of *P. muticus*. A circle represents the unique haplotypes that are connected by maximum parsimony. Italic numerals beside the links indicate numbers of mutational changes only when the observed mutation is more than one. The size of circles corresponds to the frequency of haplotypes (shared haplotypes).

Phylogenetic and haplotype network analyses

The phylogeny revealed that the Thai population of *P. muticus* can be divided into 2 major clades with moderately high bootstrap values (FIG. 2). The first clade consists of 12 individuals collected from SD only whereas the second one is composed of the rest of the individuals regardless of their locations. Interestingly, we found that the DNA sequence of HK8 was very similar to that of *P. cristatus* than *P. muticus*. As a result, it was nested to neither clade of *P. muticus* (FIG. 2).

In the median-joining network, 18 unique haplotypes were detected among the collected samples based on 36 (11.7%) variable sites (FIG. 3A). There were seven common haplotypes that were shared by more than one individual, in which the H14 haplotype was the most common haplotype. Additionally, we also found that the H1 haplotype was shared by DT, CM and HK.

Discussion

Of 18 haplotypes detected in this study, the H1 haplotype was shared by individuals collected from 3 distinct locations in the north. This suggests that the founders of these individuals in DT, CM and HK possibly come from the same source. Unfortunately, the lack of genetic data of some samples from wild populations makes it impossible to trace the evolutionary history and the place of origin of those individuals. To solve this problem, further study is required.

The finding that a HK8 individual is grouped with *P. cristatus* but not *P. muticus* in the phylogeny indicates that this individual is probably a hybrid between *P. cristatus* and *P. muticus*. This result is made even more likely by the fact that this captive facility maintained both *P. cristatus* and *P. muticus* in the same large cage for visitors in the past. Since mtDNA is generally inherited maternally, the detection of *P. cristatus* mtDNA in the phenotypical *P. muticus* indicates directional introgression of mtDNA from *P. cristatus* (the mother species) to *P. muticus* (the paternal species). The presence of mtDNA introgression is a common consequence of hybridization and is now becoming increasingly recognized in a variety of closely related taxa (Arnold, 2006).

Overall, our results revealed the presence of mtDNA genetic variation both within and between populations of captive *P. muticus* in Thailand. The absence of detection of only a single haplotype in any populations (except in CM), combined with the fact that the *hd* and π are high among the captive populations,

suggests multiple founders from geographically widespread regions, as is often suggested for captive breeding programmes. Moreover, we also suggest that such high genetic diversities could be suitable for a long- term breeding programme but for the reintroduction programme more additional data on DNA sequences of mtDNA of wild populations and that of nuclear genome are needed.

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Biographical sketches

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