ZOOLOGICAL SCIENCE **25**: 30–35 (2008)

Molecular Evidence for Species Status of the Endangered Hainan Peacock Pheasant

Jiang Chang[1], Biao Wang[1], Yang-Yun Zhang[1], Ying Liu[1], Wei Liang[2], Ji-Chao Wang[2] , Hai-Tao Shi[2], Wen-Ba Su[3] and Zheng-Wang Zhang[1]*

[1]*Ministry of Education Key Laboratory for Biodiversity Sciences and Ecological Engineering; College of Life Sciences, Beijing Normal University, Beijing 100875, China*
[2]*Department of Biology, Hainan Normal University, Haikou 571158, Hainan, China*
[3]*Forestry Department of Hainan Province, Haikou 570203, Hainan, China*

The Hainan peacock pheasant is an endangered taxon found only on Hainan Island of China. Due to lack of detailed taxonomic studies, whether it is a subspecies of the grey peacock pheasant (*Polyplectron bicalcaratum katsumatae*) or a full species (*Polyplectron katsumatae*) remained unclear. We used molecular markers, including the complete mitochondrial cytochrome *b* gene and intron G of the nuclear ovomucoid gene, to reevaluate the taxonomy of the Hainan peacock pheasant. The results showed phylogeographic monophyly and large genetic distance between the Hainan peacock pheasant and the grey peacock pheasant. Sequence differences corroborated the species-level distinction between these two peacock pheasants, which were inferred to have diverged about 1.4±0.3 million years ago, near the time Hainan Island became separated from mainland China. Because the population density of the Hainan peacock pheasant is very low in its tropical forest on the island and the wild population is declining, it is now becoming severely endangered and should be ranked as the rarest species in the Order Galliformes in China. Our results increase the urgency of getting more morphological data to support the classification of the Hainan peacock pheasant as a distinct species and taking more conservation action immediately to protect this endangered island species.

Key words: Hainan peacock, pheasant, *Polyplectron katsumatae*, taxonomy, mitochondrial DNA, intron, conservation

INTRODUCTION

The peacock pheasants (*Polyplectron* spp.), comprising of six or seven species, is a group of small, relatively somber forest pheasants of tropical Asia (Madge and McGowan, 2002). Two taxa of peacock pheasants are distributed in China, the grey peacock pheasant in the west and southwest of Yunnan province (Delacour, 1977; Cheng, 1978; Johnsgard, 1999; Madge and McGowan, 2002; Zheng, 2005) and the Hainan peacock pheasant that is endemic to Hainan Island (Cheng, 1978; Gao and Yu, 1990; Zheng, 2005) (Fig.1). However, the taxonomic status of the Hainan peacock pheasant is controversial. It was first described in 1906 and was treated as a full species, *Polyplectron katsumatae* (Rothschild, 1906). Delacour (1977) later lumped it with the grey peacock pheasant, *Polyplectron bicalcaratum*. Delacour's taxonomic treatment became widely accepted (Cheng 1978, 1987, 1994; Gao and Yu, 1990; Lu, 1991; Johnsgard, 1986, 1999, del Hoyo *et al.*, 1994; Clements, 2000; Dickinson, 2003); however, some ornithologists still considered the Hainan peacock pheasant as a full species (Sibley, 1996; Mackinnon *et al*., 2000; Madge and McGowan, 2002; Zheng, 2002; 2005). Becausethe Hainan peacock pheasant is generally considered a subspecies of the grey peacock pheasant, which is distributed in a relatively large range, it is recognized as non-threatened on the IUCN Red List (IUCN, 2006).

As in tropical areas worldwide, the rainforest on Hainan Island that the peacock pheasant inhabits has suffered considerably by human destruction. By the late 1980s, the tropical rainforest on Hainan Island was reduced to its historically lowest area, around 3,000 km^2 (Lin and Zhang, 2001). Due to habitat loss and illegal hunting, the population of Hainan peacock pheasant declined from about 2,700 in 1990 (Gao and Yu, 1990) to only about 300 individuals in 2000 (China State Forestry Administration, unpublished reports of national wildlife surveys from 1995–2000). As species are the common currency for conservation efforts,

* Corresponding author. Phone: +86-10-58809666;
Fax : +86-10-58807721;
E-mail : zzw@bnu.edu.cn
doi:10.2108/zsj.25.30

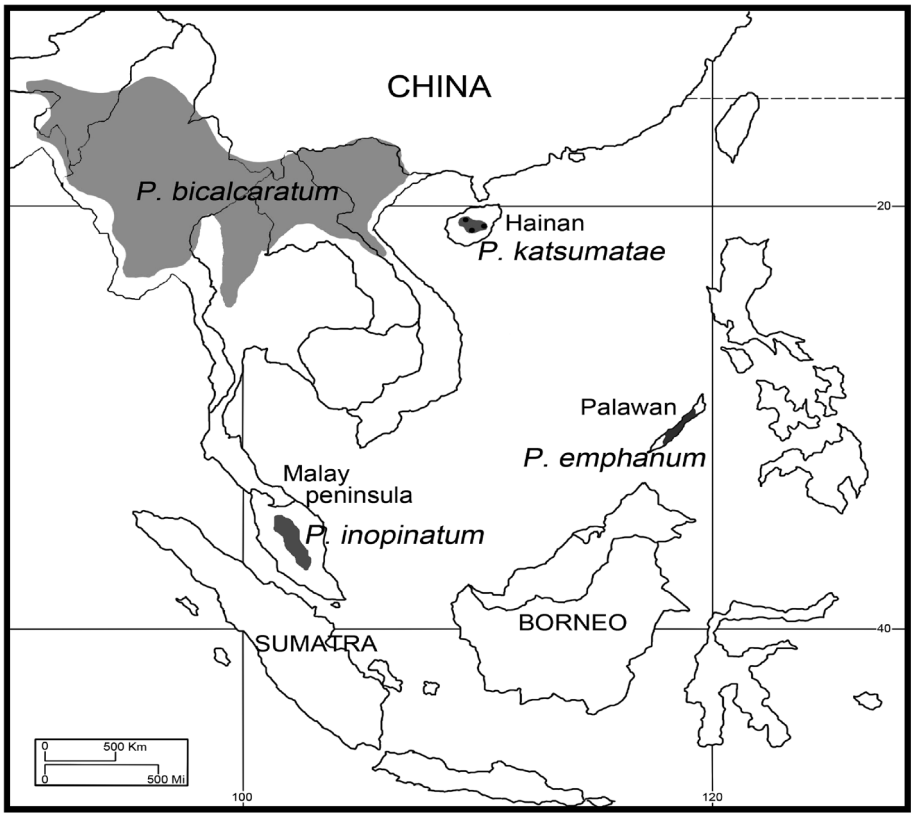

Fig. 1. Geographic distribution of the peacock pheasants used in this study.

their accurate description is essential for effective preservation. The taxonomic uncertainty of the Hainan peacock pheasant is highly relevant to its conservation status. Considering its small population size, if the Hainan peacock pheasant should prove to be a full species, its conservation status should be upgraded immediately to prevent loss of this island endemic.

In the last few decades, genetic information has successfully assisted in resolving taxonomic uncertainties in a number of animals (Mindell, 1997; Blaxter and Floyd, 2003; Mace, 2004; Vogler and Monaghan, 2007), including insects (Monaghan *et al*., 2006; Pons *et al*., 2006), hwamei (Li *et al*., 2006), and clouded leopards (Buckley-Beason *et al*., 2006). In this paper, we analyzed nucleotide sequences from complete mitochondrial cytochrome *b* (cyt *b*) gene and intron G of the nuclear ovomucoid gene (OVOG) of the Hainan peacock pheasant, grey peacock pheasant, and two other well-recognized peacock pheasants to address the following questions: (1) Is the Hainan peacock pheasant a distinct full species or not? (2) When did the Hainan peacock pheasant diverge from the grey peacock pheasant?

MATERIALS AND METHODS

Taxon sampling

Non-destructive samples (blood and feathers) were collected from nine individuals of the Hainan peacock pheasant from Bawangling National Natural Reserve, Hainan Island (18°57'–19°11'N, 109°03'–109°17'E) and from six individuals of the grey peacock pheasant from Yunnan province, with permission from local wildlife management authorities. The complete sequences of cyt *b* and OVOG of the peacock pheasants *P. bicalcaratum, P. emphanum*, and *P. inopinatum*, the green peafowl (*Pavo muticus*), and the great argus (*Argusianus argus*) were obtained from GenBank [AF013761 (Kimball *et al*., 1997); AF028799 (Kimball *et al*., 1999); AF331954, AF331955, AF331958, AF331959, AF330062, AF330064 (Kimball *et al*., 2001); AF170989 (Armstrong *et al*., 2001); DQ010650 (Zhu *et al*., 2004)]. *Pavo muticus* and *Argusianus argus* served as outgroups in the phylogenetic reconstructions.

DNA Extraction, Amplification and Sequencing

Total DNA was extracted from blood samples based on the protocol of Han *et al*. (1999). Feather DNA was extracted according to the protocol of Taberlet and Bouvet (1991) with some modifications (Zhan and Zhang, 2005). Amplification and sequencing of the cyt *b* and OVOG genes were conducted using the primers of Kimball *et al*. (1999). To avoid amplifying mitochondrial DNA homologs from the nuclear genome (numts), we verified the sequences using the methods described by Zhan and Zhang (2005).

The complete cyt *b* and OVOG sequences were obtained by overlapping partial sequences with the software SeqEdit (Applied Biosystems, USA). At least two single amplifications were done and sequenced for each individual. As the cyt *b* sequences were uniform in length, the alignment was straightforward. OVOG sequences were aligned with Clustal X (Jeanmougin *et al*., 1998). Nucleotide composition, variation at various positions, and genetic distances were estimated using MEGA3.1 (Kumar *et al*., 2004).

Phylogenetic analysis

Phylogenetic trees were constructed using the maximum parsimony (MP) and maximum likelihood (ML) methods implemented in PAUP* 4.0b10 (Swofford, 1998). A phylogenetic tree was constructed by the neighbor-joining (NJ) method implemented in MEGA3.1. A Bayesian phylogenetic tree was constructed by

MrBayes3.1.2 (Huelsenbeck and Ronquist, 2001). A maximum parsimony majority-rule (50%) consensus tree was determined (Hillis and Bull, 1993). To test reliability, bootstrap proportions (BP, Felsenstein, 1985) were derived from 1,000 pseudoreplicates by heuristic searches, with 10 random addition sequence replicates for each bootstrap replicate, for both the ML and MP analysis, and clades were considered to be well supported when the BP value was >70% (Hills and Bull, 1993; Kimball *et al.,* 1999). The best-fit model

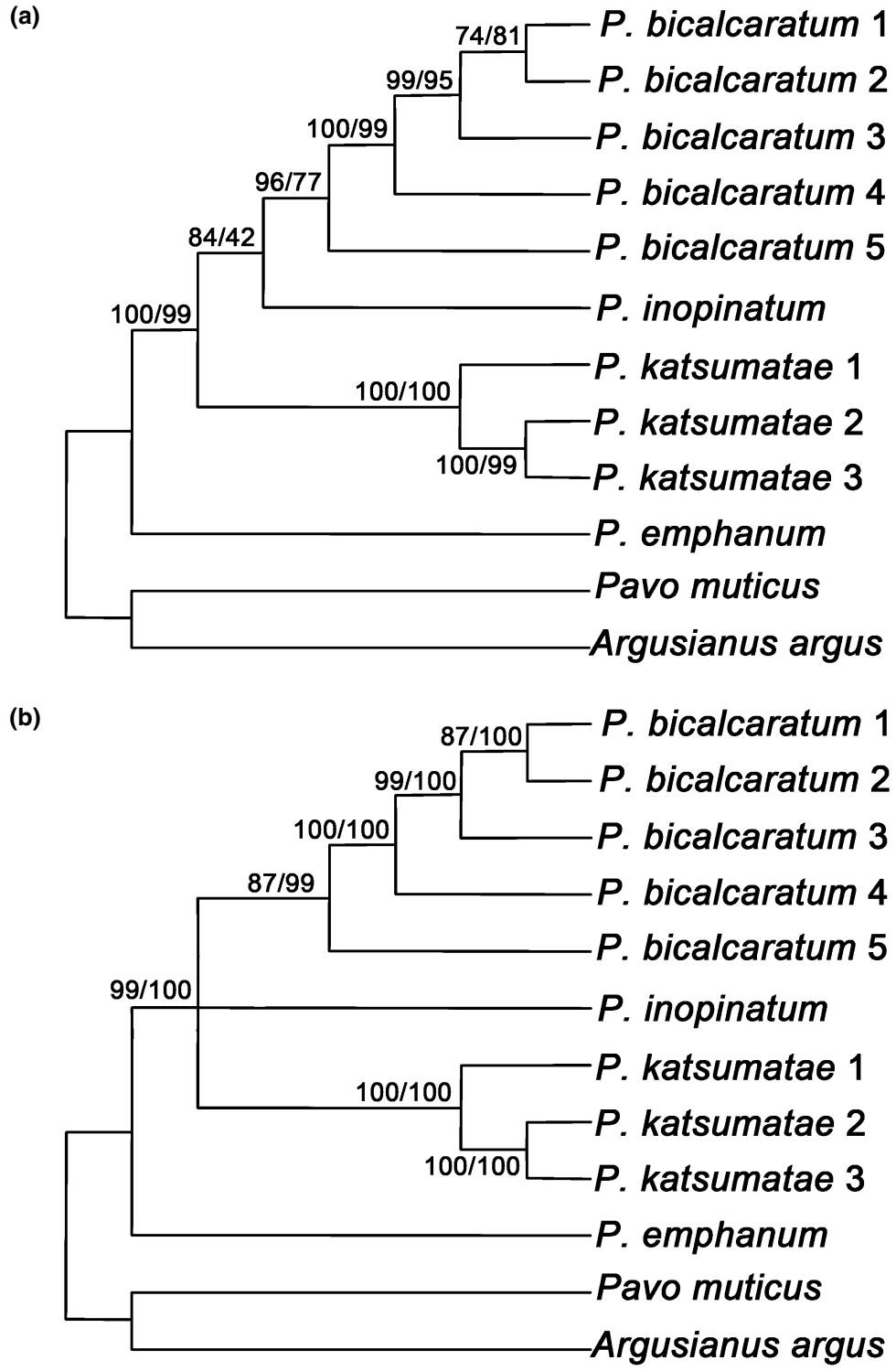

Fig. 2. Phylogenetic relationships among *P. bicalcaratum*, *P. katsumatae*, and two recognized *Polyplectron* species, based on the combined data set (sequences of the complete cyt *b* gene and intron G of the nuclear ovomucoid gene). The numbers represent bootstrap values for major clades. **(a)** The neighbour-joining and maximum-parsimony tree (numbers are percentage bootstrap support for NJ and unweighted parsimony). **(b)** The maximum-likelihood and Bayesian tree (numbers are percentage bootstrap support for ML and posterior possibility (PP) for Bayesian analysis).

Table 1. Morphological differences between *P. bicalcaratum* and *P. katsumatae*.

	Sample Size	Body Length (mm)	Wing Length(mm)	Tail Length (mm)	Bill Length (mm)	Tarsus Length (mm)
			Male ♂			
P. katsumatae	*5*	511.7±24.7	194.3±17.5	259.0±24.3	20.8±2.7	65.8±2.3
P. bicalcaratum	*2*	665.5± 3.5	219.0± 8.5	371.5±12.0	25.0±1.4	76.5±0.7
			Female ♀			
P. katsumatae	*3*	376.3±45.2	164.0± 1.4	163.0±46.0	18.7±1.5	54.7±3.2
P. bicalcaratum	*3*	515.7±51.0	199.7±12.7	250.3±27.5	22.0±1.0	62.7±4.0

for ML analysis was selected by Modeltest 3.06 (Posada and Crandall, 1998) using the Akaike information criterion (AIC). The Bayesian tree was constructed by executing the MCMC (Markov chain Monte Carlo) procedure in MrBayes with four Markov chains for 500,000 generations and sampling every 100 generations. The number of cycles before the chain reached stationarity (burn-in point in MrBayes) was 230. To generate the consensus tree, 230 sampled trees from the burn-in phase were first discarded. The posterior probability (PP) was then considered as an estimate of reliability. The model for Bayesian analysis was selected by MrModeltest version 2.2 (Nylander, 2004) using the AIC standard. To test for congruence among the Cyt *b* and OVOG partitions, the partition homogeneity test (incongruence length difference test, ILD; Farris *et al*., 1995) was conducted in PAUP* 4.0b10, with 1,000 replicates and branch-and-bound searches using all sites.

To examine when the grey peacock pheasant diverged from the Hainan peacock pheasant, we used the NJ branch lengths from the mitochondrial cytochorme *b* data to assess the genetic distance. The molecular clock rate is 2% (Brown *et al*, 1979, Pereira and Baker, 2006), which is the conventional molecular clock used in avian phylogenetic studies.

Genetic distance among studied subspecies and species

To describe the genetic differentiation between the peacock pheasants studied, net genetic distance was used to remove the effect of within-subspecies polymorphism (Nei and Li, 1979). The standard error (se) of net distances was calculated by the bootstrap method with 1,000 replicates. Both the net genetic distance and its standard error were estimated using the program MEGA3.1.

Morphological measurements

Morphological characters (such as body length) of eight Hainan peacock pheasant (five males and three females) and five grey peacock pheasant (two males and three females) were measured to examine the differences between *P. katsumatae* and *P. bicalcaratum*.

RESULTS

Molecular characters of the cyt *b*

Of the complete 1,143 bp of the cyt *b* sequences, we recognized 18 variable sites in *P. b. bicalcaratum* and 13 in *P. katsumatae*. OVOG was 446 bp long, with three variable sites in *P. katsumatae* and 45 in *P. b. bicalcaratum* . There was no apparent trend towards saturation of transitions in the grey and Hainan peacock pheasants (data not shown). The grey and Hainan peacock pheasants each contained distinct haplotypes: for cyt *b*, there were three haplotypes in *P. katsumatae* and four in *P. bicalcaratum*; for OVOG there were three OVOG haplotypes in *P. katsumatae* and five in *P. bicalcaratum*.

Molecular phylogeny

Cyt *b* and OVOG sequences were combined in the phylogenetic analysis because the *p*-value of the partition homogeneity (ILD) tests was 1.00, which indicated no discordance in phylogenetic signal between the data partitions. In addition, comparison of the results between the mtDNA and nuDNA suggested that our final combined data set (Fig. 2) provided higher resolution than separate analyses (data not shown). The base composition were A=0.2577, C=0.3194, G=0.1648, T=0.2581. The best-fit model selected for maximum likelihood (ML) was TVM+I. The best-fit model for the Bayesian analysis was GTR+I. The mean Jukes-Cantor distance in the data was 0.091, which was larger than 0.05. The *p*-distance model was thus not taken into account. All available models for generating a NJ tree, even the complex general time reversible model, were tried. They all got an identical topology, and the bootstrap values were also consistently the same among the results. Since the simpler model will give less statistical error, the Jukes-Cantor model was utilized to generate the NJ tree (Nei and Kumar, 2000).

The neighbor-joining and maximum-parsimony trees showed identical topology (Fig. 2a); the ML and Bayesian methods also yielded trees with identical topology (Fig. 2b).

In the tree in Fig. 2a, samples from *P. inopinatum* and *P. bicalcaratum* formed a monophyletic group, with the Hainan peacock pheasant as the sister taxon. These groups were all monophyletic with high BP and/or PP support. Fig. 2b shows an unresolved polytomy among *P. bicalcaratum*, *P. katsumatae*, and *P. inopinatum.* We estimated that the divergence between *P. katsumatae* and *P. bicalcaratum* occurred at 1.4±0.3 Ma.

Genetic distance among studied subspecies and species

The net genetic distance of the combined sequences between *P. bicalcaratum* and *P. katsumatae* was 0.028 (±0.005 se), even higher than that between *P. bicalcaratum* and *P. inopinatum* 0.023(±0.004 se). The net genetic distance between *P. katsumatae* and *P. inopinatum* was 0.037(±0.006 se).

Morphological measurements

The morphological data are listed in Table 1.

DISCUSSION

As a critical step in conserving biodiversity, it is important to gain a better understanding of the systematic status of threatened taxa. An accurately established systematic status will ensure they receive public concern and conservation efforts, and therefore remove them from the brink of extinction (Daugherty *et al*., 1990; Buckley-Beason *et al*., 2006). In birds, the majority of extinct taxa have been island endemics (Myers, 1979). Consequently,

the systematic status of threatened taxa on islands deserves more attention from conservation biologists.

Hainan Island was first separated from the mainland of China in the Early Pleistocene (about 2 million years ago) and experienced at least two continental connections thereafter due to changes in sea level (Zhang, 1999). A variety of species occurring on the adjacent continent and other islands in the Pacific Ocean evolved on Hainan into endemic species, including a newt (*Tylotorition hainanensis*) and the Hainan torrent frog (*Amolops hainanensis*), Hainan sunbeam snake (*Xenopeltis hainanensis*), Hainan hill partridge (*Arborophila ardens*), and Hainan hare (*Lepus hainanus*), which indicated that the isolation of Hainan Island could play an important role in speciation.

We provide the first molecular evidence that the Hainan peacock pheasant is a full species, as follows. (1) Samples from *P. katsumatae* formed a monophyletic group and comprise a distinct taxon clearly phylogenetically discontinuous from *P. bicalcaratum*. (2) Though the ML and Bayesian trees showed an unresolved polytomy between *P. bicalcaratum*, *P. katsumatae*, and *P. inopinatum*, the NJ and MP trees were identical in topology, with high levels of bootstrap support for the monophyly of each; *P. katsumatae* was located outside the group of *P. inopinatum* and *P. bicalcaratum*, which suggests that *P. katsumatae* is evolutionarily older even than *P. bicalcaratum*. (3) The net genetic distance between *P. katsumatae* and *P. bicalcaratum* was 0.028(±0.005 se), even greater than that between *P. inopinatum* and *P. bicalcaratum* [0.023(±0.004 se)].

The Hainan peacock pheasant and the grey peacock pheasant diverged around 1.4±0.3 Ma, within the Pleistocene epoch, which began about 1.8 Ma. Hainan Island first became separated from mainland China in the late Pliocene (about 2 Ma), and we suggest that both geographical isolation and suitable habitat discontinuity during glacial periods were important factors in shaping genetic distinctness and creating opportunities for deeper population isolation between these two peacock pheasants. Similar factors probably influenced the evolutionary history of other species of peacock pheasants and many endemic taxa on islands (Kimball *et al*., 2001; Li *et al*., 2006).

The morphological data (Table 1) indicate that the Hainan peacock pheasant is distinct from the grey peacock pheasant (Delacour, 1977; Yang *et al*., 1995; Madge and McGowan, 2002). The Hainan peacock pheasant is the smallest among allied species of peacock pheasants. In addition, the crest of the Hainan peacock pheasant is obviously shorter than that of grey peacock pheasant. Differences also exist in the color of ocelli, with those of the mantle and wings blue and green, and the tail ocelli have a complete grayish-buff border and a diameter of no more than 15 mm (Cheng, 2002).

The Hainan peacock pheasant was once widely distributed in tropical rainforest over most of Hainan Island (Gao, 1998). As a result of habitat loss and illegal hunting, both the range and population have decreased drastically since the 1950s (Zheng and Wang, 1998), and the extant population of the Hainan peacock pheasant has become fragmented into small, partially isolated populations. The future outlook of this species will be dismal if no urgent conservation policy is taken immediately. Therefore we urge the authorities establish more nature reserves to protect the remaining habitat of the Hainan peacock pheasant, strengthen the management of the extant protection areas, and stop poaching of this endangered species in most of its range.

Some caveats temper our conclusions for species-level designation of the Hainan peacock pheasant. First, more molecular markers could be used to provide more evidence. Second, we urgently recommended a comprehensive morphological assessment of the Hainan peacock pheasant and other peacock pheasants to testing the hypothesis that the Hainan peacock pheasant is a full species rather than a subspecies of the grey peacock pheasant. Even well-studied groups may be in need of taxonomic revision before accurate tests of incongruence can be conducted. This study is a useful starting point for conserving the endangered Hainan peacock pheasant, and future studies should afford additional resolution by sequencing more taxa to better understand the phylogenetic status of the Hainan peacock pheasant within *Polyplectron*, and its population-genetic diversity.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 30330050, 30360015, and 39830030). We thank the Bawangling National Nature Reserve for their kind support and Shou-hsien Li, Ming Li, Xiang-jiang Zhan, Simba Chan, and Nigel Collar for useful comments on the manuscript. Dr. Nigel Collar helped correct the English Grammar.

REFERENCES

Armstrong MH, Braun EL, Kimball RT (2001) Phylogenetic utility of avian ovomucoid intron G: a comparison of nuclear and mitochondrial phylogenies in Galliformes. Auk 118: 799–804

Blaxter M, Floyd R (2003) Molecular taxonomics for biodiversity surveys: already a reality. Trends Ecol Evol 18: 268

Brown W, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967–1971

Buckley-Beason V, Johnson W, Nash W, Stanyon R, Menninger J, Driscoll C, Howard J, Bush M, Page J, Roelke M (2006) Molecular evidence for species-level distinctions in clouded leopards. Curr Biol 16: 2371–2376

Cheng TH (1987) A Synopsis of the Avifauna of China. Sciences Press, Beijing

Cheng TH (1994) A Complete Checklist of Species and Subspecies of the Chinese Birds. Science Press, Beijing

Cheng TH (2002) Systematic Index of Birds in China. Science Press, Beijing

Cheng TH, Tan YK, Lu TC, Tang CZ, Bao GJ, Li FL (1978) Fauna Sinica, Aves, Vol 4: Galliformes. Science Press, Beijing

Clements JF (2000) Birds of the World: A Checklist. 5th ed, Pica Press, Sussex

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). Nature 347, 177–179

del Hoyo J, Elliott A, Sargatal J (1994) New World Vultures to Guineafowl. In "Handbook of the Birds of the World Vol 2" Ed by J del Hoyo, A Elliott, J Sargatal, Lynx Editions, Barcelona

Delacour J (1977) The Pheasants of the World. 2nd ed, World Pheasant Association and Spur Publications, London

Dickinson EC (2003) The Howard and Moore Complete Checklist of the Birds of the World. 3rd ed, Christopher Helm, London

Felsenstein J (1985) Confidence limits on phylogenies: an approach

using the bootstrap. Evolution 39: 783–791
Gao YR (1998) Conservation status of endemic Galliformes on Hainan Island, China. Bird Conserv Intl 9: 411–416
Gao YR, Yu DQ (1990) Hainan peacock pheasant. Chinese J Zool 25: 42–44
Han Z, Li F, Liu B (1999) Detection of intraspecific genetics relationships in crested ibises *Nipponia nippon* through randomly amplified polymorphic DNA analysis. J Yamashina Inst Ornithol 3l: 39–44
Hills DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42: 182–192
Huelsenbeck JP, Ronquist FR (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755
IUCN (2006) The IUCN Red List of Threatened Species. Gland, Switzerland
Johnsgard PA (1986) The Pheasants of the World. Oxford University Press, Oxford
Johnsgard PA (1999) The Pheasants of the World: Biology and Natural History. 2nd ed, Smithsonian Institution Press, Washington, DC
Kimball RT, Braun EL, Ligon JD (1997) Resolution of the phylogenetic position of the Congo peafowl, *Afropavo congensis*: a biogeographic and evolutionary enigma. Proc R Soc Ser B Bio 264: l5l7–1523
Kimball RT, Braun EL, Zwartjes PW, Crowe TM, Ligon JD (1999) A molecular phylogeny of the pheasants and partridges suggests that these lineages are not monophyletic. Mol Phylogenet Evol 11: 38–54
Kimball RT, Braun EL, Ligon JD, Lucchini V, Randi E (2001) A molecular phylogeny of the peacock pheasants (Galliformes: *Polyplectron* spp.) indicates loss and reduction of ornamental traits and display behaviours. Biol J Linn Soc 73: 187–198
Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefs Bioinform 5:150–163
Li SH, Li JW, Han LX, Yao CT, Shi HT, Lei FM, Yen CW (2006) Species delimitation in *Hwamei garrulax canorus*. Ibis 148: 698–706
Lin MZ, Zhang YL (2001) Dynamic changes of tropical forest in Hainan Island. Geogr Res 20: 703–712
Lu TC (1991) The Rare and endangered gamebirds in China. Fujian Science and Technology Press, Fujian
Mace GM (2004) The role of taxonomy in species conservation. Phil T Roy Soc B 359: 711–719
Mackinnon J, Phillipps K (2000) A Field Guide to the Birds of China. Oxford University Press, Oxford
Madge S, McGowan P, Kirwan GM (2002) Pheasants, Partridges and Grouse. Christopher Helm, London
Mindell DP (1997) Avian Molecular Evolution and Systematics. Academic Press, San Diego
Monaghan MT, Balke M, Gregory TR, Vogler AP (2005) DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. Phil T Roy Soc B 360:1925–1933
Moritz C, Dowing TE, Brown WM (1987) Evolution of animal mitochondrlal DNA: relevance for population biology and systematics. Annu Rev Ecol Evol Syst 18:269–292
Myers (1979) The Sinking Ark: A New Look at the Problem of Disappearing Species. Pergamon Press, New York
Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, Oxford
Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76: 269–5273
Nylander JAA (2004) MrModeltest 2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
Pereira SL, Baker AJ (2006) A molecular timescale for galliform birds accounting for uncertainty in time estimates and heterogeneity of rates of DNA substitutions across lineages and sites. Mol Phylogenet Evol 38: 499–509
Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol 55: 595–609
Posada D, Crandall KA (1998) MODLETEST: testing the model of DNA substitution. Bioinformatics 14: 817–818
Sibley CG (1996) Birds of the World, on diskette, Windows version 2.0. Santa Rosa, CA
Swofford DL (1998) PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods) Version 4, Sinauer Associates, Sunderland, Massachusetts
Taberlet P, Bouvet J (1991) A single plucked feather as a source of DNA for bird genetic studies. Auk 108: 959–960
Vogler AP, Monaghan MT (2007) Recent advances in DNA taxonomy. J Zool Syst Evol Res 45: 1–10
Yang L, Wen XJ, Han LX, Yang XJ (1995) The Avifauna of Yunnan China. Yunnan Science and Technology Press, Kunming
Zhan XJ, Zhang ZW (2005) Molecular phylogeny of avian genus *Syrmaticus* based on the mitochondrial cytochrome *b* gene and control region. Zool Sci 22: 427–435
Zhang RZ (1999) Zoogeography of China. Science Press, Beijing (In Chinese)
Zheng GM (2002). A Checklist on the Classification and Distribution of the Birds of the World. Science Press, Beijing
Zheng GM (2005) A Checklist on the Classification and Distribution of the Birds of China. Science Press, Beijing
Zheng GM, Wang QS (1998) China Red Data Book of Endangered Animals (Aves). Science Press, Beijing
Zhu SF, Chang H, Zhang CP, Wang W, Xu QF (2004) Phylogenetic relationships among *Pavo* based on complete mitochondrial cytochrome *b* gene sequence variations. Acta Sci Nat Univ Sunyatseni 43: 45–47

(Received April 16, 2007 / Accepted September 11, 2007)