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Molecular Phylogeny of Avian Genus Syrmaticus Based on the Mitochondrial Cytochrome *b* Gene and Control Region

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ABSTRACT—Mitochondrial DNA cytochrome *b* (cyt *b*) and control region (CR) nucleotide sequences were used to study the molecular phylogeny of the genus *Syrmaticus*. We found that the substitution rates among the three codon positions of cyt *b* were heterogeneous and the transition-transversion ratio was highly biased. As to CR sequences of the genus, most variable sites were in the peripheral domains. All molecular phylogenetic trees based on the two genes showed that: 1) the *Syrmaticus* was monophyletic and included five species with the following cladistic relationship: (*S. reevesii*, (*S. soemmerringii*, (*S. mikado*, (*S. humiae* and *S. ellioti*)))). Using the TN genetic distance of cyt *b*, we inferred the divergence time of the five species according to putative molecular clock and found that values were largely in agreement with the geological scenarios. The origin and speciation processes of the studied group were inferred by combining molecular and biogeographical evidences.

Key words: *Syrmaticus*, molecular phylogeny, mitochondrial DNA, cytochrome *b*, control region, biogeography

INTRODUCION

The *Syrmaticus* (Galliformes: Phasianidae) pheasants refer to a group of small to medium-sized montane pheasants in which sexual dimorphism is well developed and in which the tail is greatly elongated and strongly barred (Johnsgard, 1999). In general, adult males are easily identified for their colorful plumage, while females are cryptically colored with brown or tan plumage with white extensive on the sides and flanks and as shaft-streaks dorsally. The rectrices of both sexes can spread laterally in courtship display when being excited (Cheng *et al.*, 1978; Johnsgard 1999).

The five extant species in the genus are all distributed in the natural habitats of eastern Asia. Reevess pheasant (*Syrmaticus reevesii*) is a native species of central of China. Elliot's pheasant (*S. ellioti*) occurs in central and southern China and overlaps with Reeves's pheasant in parts of its range. Hume's pheasant (*S. humiae*) lives in Yunnan and Guangxi of China, Thailand, Myanmar and India. Mikado pheasant (*S. mikado*) is restricted to Taiwan and the copper pheasant (*S. soemmerringii*) to Japan (Cheng *et al.*, 1978; Zheng and Wang, 1998; Johnsgard, 1999; BirdLife International, 2001) (Fig. 1).

There is considerable disagreement concerning the taxonomy and phylogenetics of the genus *Syrmaticus*. *Syrmaticus* was even once subsumed to be within genus *Phasianus* (The Ornithological Society of Japan, 1974). Yamashina (1976) suggested that the *Syrmaticus* was an independent genus with four species, namely, Reeves's pheasant, Hume's pheasant, Elliot's pheasant and Mikado pheasant. He placed the copper pheasant within the genus *Phasianus*. Recently, Johnsgard (1999) concluded that the genus *Syrmaticus* should encompass all five above-mentioned species, which was accepted by most ornithologists. However, some Chinese researchers also suggested the Reeves's pheasant should be classified as an independent genus because of its distinct morphological traits such as the extreme long tail and lack of facial skins.

The phylogenetic relationships within the genus *Syrmaticus* are also unclear at present. Based on morphological traits, individual development, behavior and physiological characteristics, Johnsgard (1986) suggested that there were two branches within the genus, one of which was composed of Reeves's pheasant and copper pheasant, and the other including Elliot's, Hume's and Mikado pheasant with the Mikado as basal (Fig. 2A). Ding (1998) also accepted

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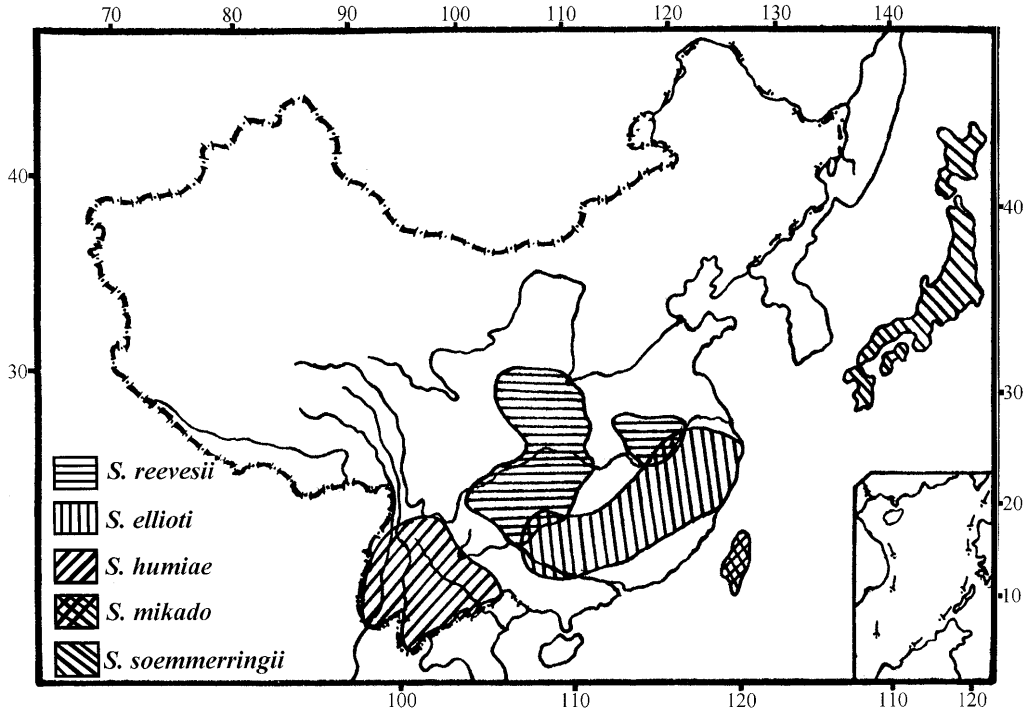

Fig. 1. Distribution of the long-tailed pheasants.

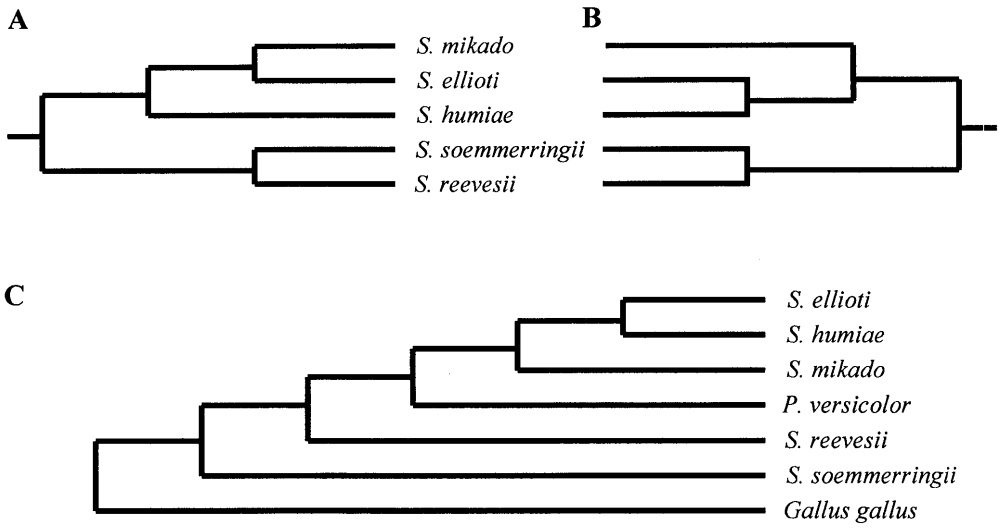

Fig. 2. Phylogentic hypothesis proposed for *Syrmaticus*: (A) from Johnsgard (1986). (B) from Ding (1998). (C) from Munechika *et al.* (1999).

the classification of the two branches within this genus, but suggested that Hume's pheasant diverged first from the latter branch (Fig. 2B). Whereas, Munechika *et al.* (1999) gave a very different evolution order of the genus: the copper pheasant diverged first, then followed by Reeves's pheasant and the other three long-tailed pheasants based on mitochondrial DNA cytochrome *b* (cyt *b*) analysis. Surprisingly, their results indicated that green pheasant (*Phasianus versicolor*) was within the genus *Syrmaticus* and diverged just after Reeves's pheasant (Fig. 2C).

Bearing in mind with the turbulent history and controversial issues related to the genus *Syrmaticus*, it is necessary to further investigate the classification and phylogeny of this genus. Mitochondrial phylogenetic studies on several genera within the Phasianidae have been conducted recently (Randi, 1996, 2000), but there are few studies on the molecular phylogeny of the long-tailed pheasants. Therefore, the aims of this study are: 1) to analyze mitochondrial DNA cyt *b* and Control Region (CR) sequences extracted from the five species of long-tailed pheasants in order to clarify their taxonomy and phylogeny; and 2) to propose the evolution pattern of the long-tailed pheasants from molecular data and biogeography evidences.

MATERIALS AND METHODS

Taxon Sampling

In this study, 15 samples (blood and feather) of all five extant species in the genus *Syrmaticus* (*S. reevesii, S. humiae, S. ellioti, S. mikado and S. soemmerringii*) and several species from other genera (*Phasianus colchicus and Chrysolophus amherstiae*) were obtained from the field or wild individuals reared in zoos. Our analysis also included some published cyt *b* and CR sequences in the genus *Syrmaticus* from GenBank (Table 1).

DNA Extraction, Amplification and Sequencing

Total DNA was extracted from the blood samples based on the protocol of Han *et al.* (1999). The feather DNA was extracted according to protocol of Taberlet and Bouvet (1991) with some modifications: fragments of feather tip were digested over night at 55°C in the system containing 10 mM Tris-HCl (pH7.5), 10 mM EDTA (pH8.0), 100 mM NaCl, 1mg/ml Proteinase K, 0.5 M DTT, 1% SDS; DNA then was extracted by the conventional Chloroform/Phenol method (Sambrook *et al.* 1989). Amplification and sequencing of the cyt *b* and CR genes were conducted using the protocol described by Zhan *et al.* (2003). The primers used for both the amplification and sequencing are listed in Table 2.

Phylogenetic Analyses

The whole cyt *b* and CR sequences were obtained by overlapping the partial sequences with the software SeqEdit (Applied Bio-

Table 1. The species, their sources and Accession No. of sequence data in GenBank used in this study

Species	Sources	Cyt b	CR	Reference
Syrmaticus				
S. reevesii (Reeves's pheasant)	Shaanxi, Henan, China	AY368059, AF028801	AY368063	This study, Kimball *et al.* 1999
S. soemmerringii (copper pheasant)	The aviary in England[a]	AY172840	AY368068	This study
S. mikado (Mikado pheasant)	The aviary in England[a]	AY368056, AF534561	AY368070	This study, Bush & Strobeck, 2003
S. humiae (Hume's pheasant)	Beijing Zoo, China	AF534560, AF534706, AY368055	AY368069	This study, Bush & Strobeck, 2003
S. ellioti (Elliot's pheasant)	Beijing Zoo, Beijing Breeding Center for Endangered Animals, China	AF534705, AY534753, AY534757, AY368061	AY368062 AY368064	This study
Phasianus				
P. colchicus (Ring-necked pheasant)	Shaanxi and Henan, China	AY368054, AY368060	AY874873	This study
Chrysolophus				
C. amherstiae (Lady Amherst's pheasant)	Hunan, China	AY368051, AY368052	AY368067	This study
Crossoptilon				
C. crossoptilon (White Eared pheasant)	Beijing Zoo, China	AF028794	AF343525	Kimball *et al.* 1999, Ding & Zhang,
C. mantchuricum (Brown Eared pheasant)	Beijing, China	AF534553	AF343522	unpublished GenBank submission
Lophura				
L. bulweri (Bulwer's Phesant)	Antwerp Zoological Garden, Antwerp, The Netherland	AF314637	AJ300146	Randi *et al.* 2001
L. swinhoii (Swinhoe's pheasant)	Parc Zoologique de Cle'res, Cle'res, France	AF314644	AJ300155	Randi *et al.* 2001

[a] Private Collection of Mr J. Corder in England

Table 2. Amplification and sequencing primers for cytochrome *b* and control region

	Name[a]	Sequence (5' → 3')	Source
Cyt *b*	L14731	ATCGCCTCCCACCT(AG)AT(CG)GA	Kimball *et al.*, 1999
	L14788	TGCCAACCTTCATCTTATTAT	This study
	L14851	TACCTGGGTTCCTTCGCCCT	Kornegay *et al.*, 1993
	L14932	AAAGTCCCACCCCCTGCTAAAAA	This study
	H15586	TGAGTATGAGTGCTAGGC	This study
	H15826	CGGAAGGTTATGGTTCGTTGTTT	Kimball *et al.*, 1999
	H15979	GATTGCGGGGAAGAGGATGAGTA	This study
	H16065	TTCAGTTTTTGGTTTACAAGAC	Kimball *et al.*, 1999
	H16076	GGGGGGCCTTCAGTTTTT	This study
CR	L16757	AGGACTACGGCTTGAAAAGC	Randi and Lucchini, 1998
	L426	ATTTATTGATCGTCCACCTCACG	Randi and Lucchini, 1998
	L797	GACGGTTTGCGTGTATGTGG	This study
	H1259	CATCTTGGCATCTTCAGTGCC	Randi and Lucchini, 1998

[a]Name refers to the position of the 3' end of the primers sequences in the Light (L) and Heavy (H) strand in the chicken mitochondrial genome (Desjardins and Morais 1990).

systems Inc., USA). This study provided 9 new complete cyt *b* and 5 CR sequences representing five extant long-tailed pheasants, all of which were deposited in GenBank. At least two specimens were sequenced for each species in most species (Table 1). As the cyt *b* sequences are uniform in length, the alignment was straightforward. CR sequences were aligned with Clustal X (Jeanmougin *et al.*, 1998) and edited with Se-Al (Rambaut, <http:// evolve.zoo.ox.ac. uk/Se-Al/Se-Al.html>). Nucleotide composition, variation at different positions and domains, and genetic distances were estimated using MEGA2 (Kumar *et al.*, 2001). K independent sample test was conducted in SPSS10.0.

Phylogenetic trees were constructed using the parsimony (MP) and maximum likelihood (ML) methods in PAUP* 4.0 (Swofford, 1998) based on the sequences of cyt *b*, CR and cyt *b*+CR, respectively. Sequences of *P. colchicus, C. amherstiae, Crossoptilon mantchuricum, C. crossoptilon, Lophura bulweri and L. swinhoii* from our lab and GenBank (Table 1) were used as outgroups. All MP and ML trees were constructed via randomized, 1000 replicates, heuristic searches, with TBR branch-swapping and MULPARS options in effect. The best-fit models for ML analysis were selected by Modeltest 3.06 (Posada and Crandall, 1998). The bootstrap values (BP, Felsenstein, 1985) were derived from 1000 replicates with 10 random additional sequencing replicates for each bootstrap replicate in ML and MP analyses, and the clades were considered to be well resolved when the BP≥70% (Hills and Bull 1993; Kimball *et al.*, 1999). Furthermore, the Bayesian tree was constructed by executing the MCMC (Markov Chain Monte Carlo) procedure in the software MrBayes (Huelsenbeck and Ronquist, 2001) with 3,000,000 generations. 4 chains were used. Sampling frequnency was once every 1000 generations. The lset parameters for the analysis were derived from ML analyses. In the end, initial 22 trees that were written before ln likelhoods sum converges on a stable value were discarded. The reliability of Bayesian trees was assessed by the posterior probability (PP), which was considered to be more advantages in estimating the phylogenetic relationships (Zhang *et al.*, 2003).

RESULTS

Authenticity of the mitochondrial sequences

The nonspecific amplification of the long-tailed pheasants genes with published avian primers may be the result of nuclear integration (Zhang and Hewitt, 1996). Therefore, some methods were used to determine that our sequences were mitochondrial DNA gene instead of the nuclear pseudogenes (numt): (1) Numt and mitochondrial counterparts, co-amplified by PCR, constantly result in more than one band in the PCR amplification or double peaks and ambiguities at some positions in the sequencing reactions (Zhang and Hewitt, 1996). In our study, the cyt *b* and CR PCR produced clean single bands for each sample and no multiple peaks during the sequencing reaction. (2) Blood, muscle and feather samples are different in the ratio of mtDNA/nDNA (nuclear DNA). We used all the three types of samples of one individual of Reeves's pheasant to sequence and found the three sequences were exactly the same, suggesting that only one gene was amplified and sequenced. (3) All cyt *b* sequences had an open reading frame without indels or internal stop codons and the proteins encoded by these sequences were significantly identical to other reported cytochrome *b* proteins. The hemeligating histidines and other conserved residues described as Howell (1989) could be identified, which was the symbol of the functional cyt *b* gene. (4) A genotype of Reevess pheasant is the same as the sequence published in GenBank (AF028801), which was proven to be the functional gene using Southern Blotting analysis (Kimball *et al.*, 1999). (5) All CR sequences in the study were easily aligned with the homologous CR sequences from *Gallus gallus* and *Coturnix japonica*, which were not PCR-amplified (Desjardins and Morais, 1990, 1991). Moreover, we found a lot of structure motifs in the CR sequences of the long-tailed pheasants, most of which were conserved across all studied birds.

Molecular Dynamics of the cyt *b* and CR of the *Syrmaticus*

All the cyt *b* sequences of the *Syrmaticus* were 1143bp. Although the substitution rates among the three codon positions of cyt *b* were heterogeneous, e.g. the third codon positions showed the greatest proportion of variable sites (251/310), nucleotide frequencies of most bases were not significantly different among species (Table 3). The average number of transitions (ts) of cyt *b* was 6.8 times higher than that of transversion (tv) among *Syrmaticus* and it was 4.8 times between *Syrmaticus* and outgroups. As shown in Fig. 3A, the regression of ts vs tv was nonlinear: ts increased rapidly among the *Syrmaticus* species and reached a plateau before 11 tv difference. Thus, there was an apparent trend towards saturation of ts in the pairwise comparisons between either Reeves's or copper pheasant and other *Syrmaticus spp.* and between the genus and outgroups. Since the substitution rates among sites were heterogeneous and the ts:tv ratio was highly biased, the Tamura-Nei (Gamma) model was an appropriate estimator of genetic distances with α=0.15 computed from Modeltest3.06. Genetic distances within species, among species and between the *Syr-*

Table 3. Base compositions of the whole sequence and at the first, second and third codon positions for the *Syrmaticus* cyt *b* sequences (k independent sample test value among species in the parentheses)

	Whole sequence	First	Second	Third
T	25.0(0.073)	22.3(0.064)	39.3(0.038[a])	13.4(0.058)
C	28.1(0.047[a])	26.6(0.027[a])	20.7(1.000)	37.0(0.055)
A	34.8(0.086)	30.2(0.106)	27.6(0.038[a])	46.7(0.041[a])
G	12.1(0.068)	21.0(0.055)	12.3(1.000)	2.9(0.041[a])

[a]P<0.05, significantly different

maticus and outgroups were 0.1–0.8%, 1–24.7% and 18.1–39.5% respectively.

The length of the CR sequences of the *Syrmaticus spp.* was relatively conserved (1151–1154bp). Like other reported birds (Baker and Marshall, 1997; Randi *et al.*, 1998), most variable sites were distributed in the peripheral regions of CR sequences of the *Syrmaticus*. The genetic distances (TN, α=0.89) of CR sequences within, among species and between the *Syrmaticus* and outgroups respectively were 0.6% (only *S. ellioti* was analyzed with two genotypes in the study), 2%–12% and 9.2–14.5%. In contrast to cyt *b*, there was no "ts saturation" in the pairwise comparisons of CR sequences (Fig. 3B). Moreover, on average, the CR accumulated comparatively more tv but fewer ts than the cyt *b* (Fig. 3C).

Models of DNA substitution

In the ML analyses, the best-fit models were K81uf+G, TVM+I+G and TVM+I+G for cyt *b*, CR and cyt *b*+CR, respectively, by Modeltest 3.06. In the Baysian analyses, the best-fit model was the general time-reversible model (GTR) for all three types of sequences, which is set according to the parameters used in the ML analyses.

Molecular phylogeny of the *Syrmaticus*

All phylogenetic trees (Figs. 4A, B, C) showed the five *Syrmaticus* species formed a monophyletic group. Within that group, phylogenetic trees based on the cyt *b*, CR or cyt *b*+CR sequences were well resolved. All phylogenetic trees indicated that Reevess pheasant diverged first with high BP (>70%) and PP (100%). copper pheasant diverged second,

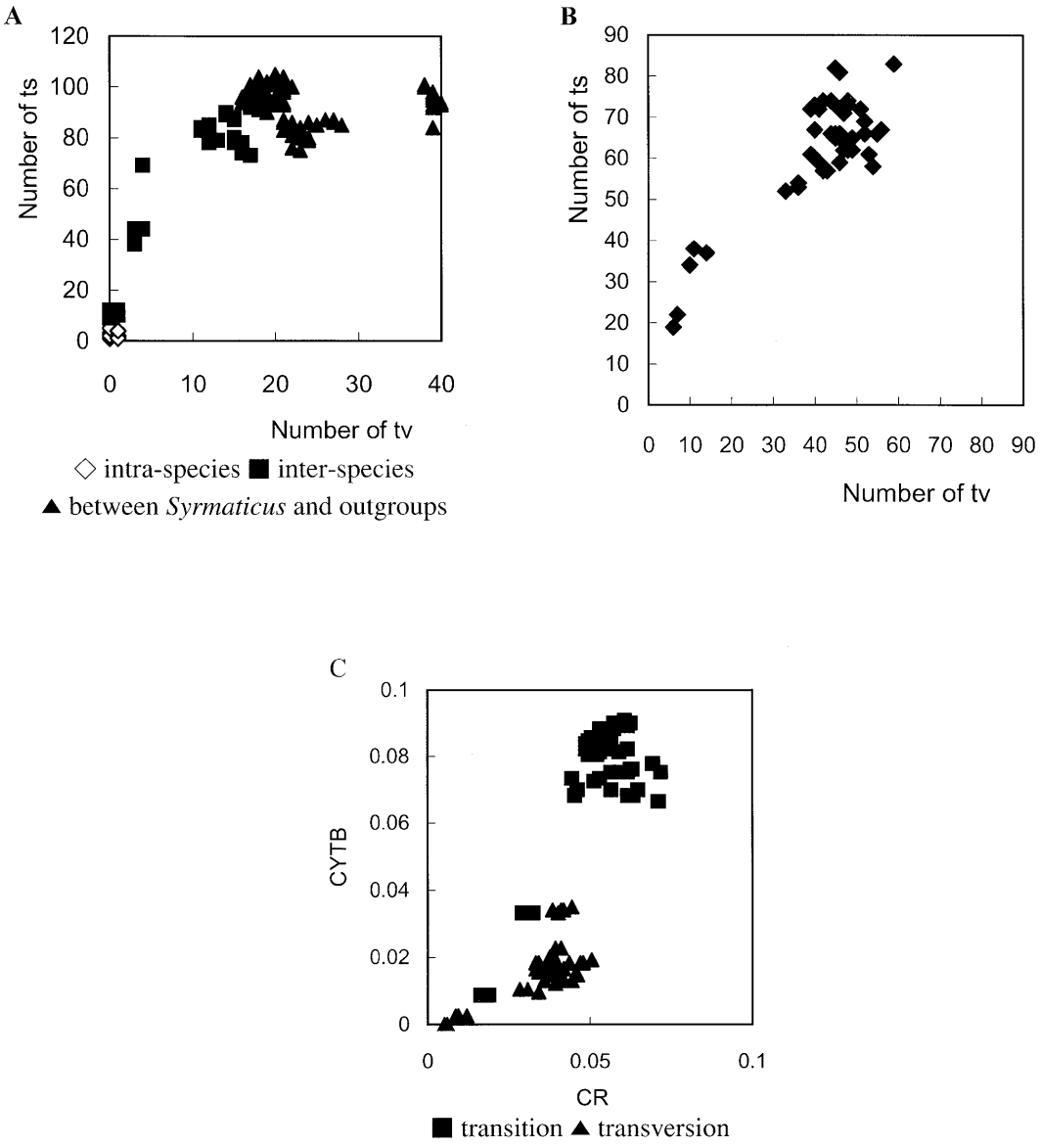

Fig. 3. (A) Relationships of number of ts vs tv among cyt *b* sequences of the *Syrmaticus* and between *Syrmaticus* and outgroups. (B) Relationships of number of ts vs tv among CR. Sequences of the *Syrmaticus* and between *Syrmaticus* and outgroups. (C) Cyt *b* vs CR percent differences among *Syrmaticus* and between *Syramticus* and outgroups.

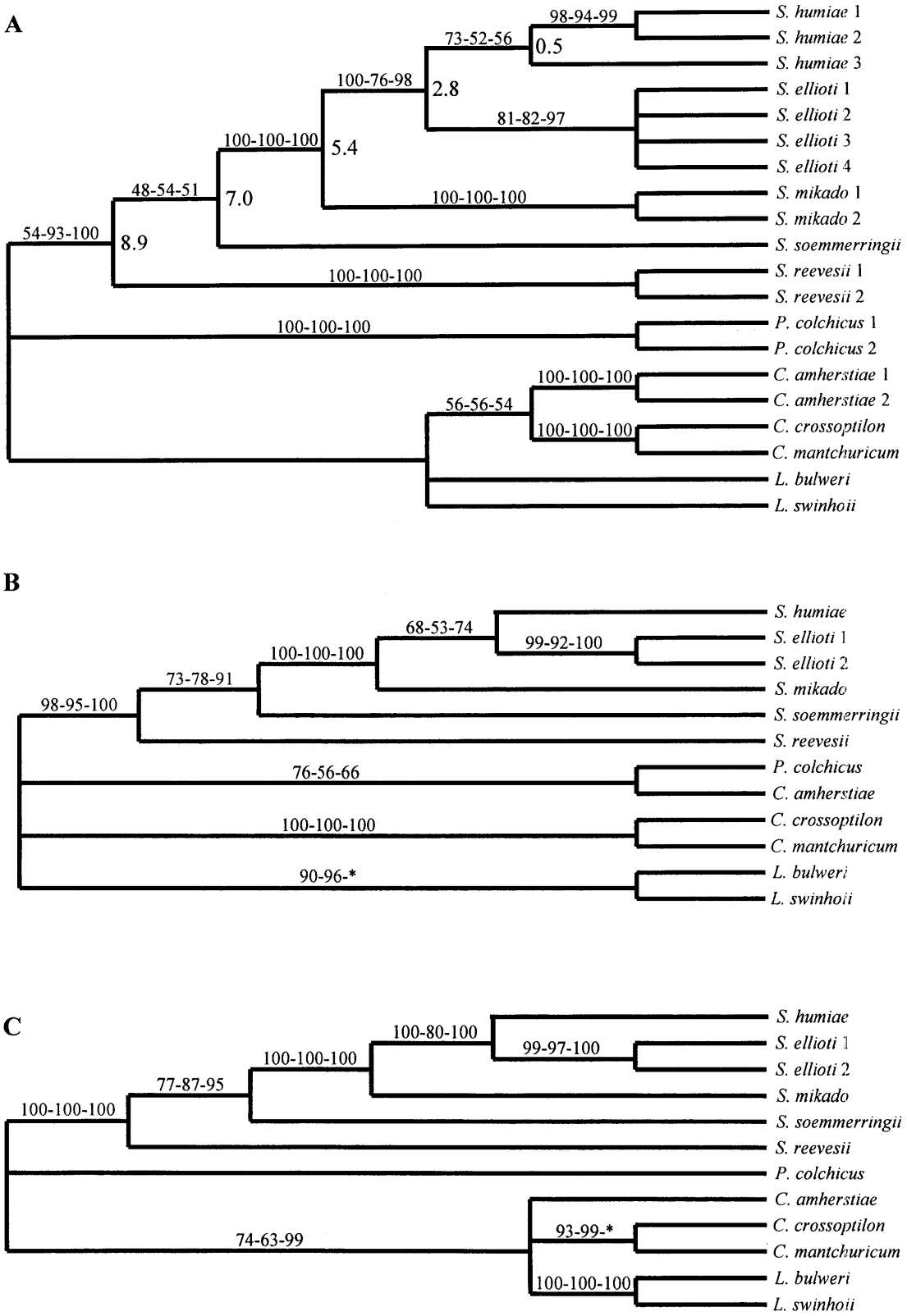

Fig. 4. Molecular phylogenetic trees of the *Syrmaticus*: (A) phylogenetic trees of fifteen individual cyt *b* sequences from *Syrmaticus* pheasants and eight phasianid outgroups; (B) phylogenetic trees of six individual CR sequences from *Syrmaticus* pheasants and six phasianid outgroups; (C) phylogenetic trees inferred from the combined CR + cyt *b* sequences. Identical topologies were obtained using MP, ML and Baysian analyses based on each set of data. Values of bootstrap support (BP) in MP and ML analyses and posterior possibility (PP) computed using MrBayes were showed above internodes of tree in turn. * The PP values <50% weren't shown. Numbers at the internode of Fig. 4A were approximate time of divergence (myr).

but the branch support was low in the BP and PP in cyt *b* trees in contrast to either CR or cyt *b*+CR trees. The phylogenetic position of Mikado pheasant was well resolved and it diverged just following copper pheasant. At last, Elliot's pheasant and Hume's pheasant diverged from each other, which was supported by either BP or PP.

DISCUSSION

Phylogeny of the *Syrmaticus*

Based on Figs. 4(A, B and C), all five species of the long-tailed pheasants formed a monophyletic group. Yamashina (1976) considered copper pheasant to be within the genus *Phasianus* because it could be hybridized with green pheasant in captivity (Hachisuka, 1953) and in the wild (Yamashina, 1976). But these hybrids between the two species were infertile, whereas male hybrids between Copper and another species of long-tailed pheasants, Reeves's pheasant, were fertile (Johnsgard, 1986). From both reproduction data and molecular evidence, we believed that copper pheasant should be classified into the genus *Syrmaticus*. Thus, we might draw a conclusion that the *Syrmaticus* was an independent genus with five species, which was in agreement with the results based on morphological characters, breeding ecology, etc (Delacour, 1977; Johnsgard 1999). However, it can not exclude the possibility that Reeves's pheasant, even copper pheasant, warrant generic status. There are two reasons: firstly, the genetic distance of cyt *b* sequences between either Reeves or copper pheasant and other long-tailed pheasants is more than 0.175, which far surpasses the average distance between confamilial avian genera (Johns and Avise, 1998) and galliform genera (Glaus *et al.*, 1980); secondly, Reeves's pheasant has many distinct morphological characters, such as the narrowest facial protrusion, the most extensive white portion on the head and the most highly developed tail feathers of all of the species. Therefore, more evidence should be collected in the future for defining the classification of the long-tailed pheasants.

However, whether the *Syrmaticus* is a genus or not does not affect the phylogenetic relationships among the long-tailed pheasants. By comparing Fig. 4 with Fig. 2, our result is evidently different from all previous reports on the evolution of the *Syrmaticus* (Johnsgard, 1986; Ding, 1998; Munechika *et al.*, 1999). As to the former two species, Johnsgard (1986) and Ding (1998) thought that the two species formed a separate clade (Fig. 2A and B). But their conclusions were inferred only from successful hybridization between the two species and they didn't show whether copper pheasant could hybridize with other three species of *Syrmaticus*. Using cyt *b* sequences, Munechika *et al.* (1999) suggested that copper pheasant diverged first, followed by Reeves's pheasant and other three long-tailed pheasants (Fig. 2C). However, this conclusion may be doubtful because they only sequenced 269bp of cyt *b* gene, which provided limited informative sites. Moreover, they only used NJ procedures to construct the phylogenetic tree, which might not enough for reflecting the evolutionary history of the *Syrmaticus*. Improper outgroup could also affect their topology because the outgroup (*Gallus gallus*) that they used was fairly far from the genus *Syrmaticus* in evolutionary history. Thus, it was not surprising that they also included green pheasant into the genus *Syrmaticus.* In our study, although the node support for copper pheasant was low in cyt *b* trees, it might be caused by the "ts" saturation in the cyt *b* alignment because supporting values inferred from CR and cyt *b*+CR without "ts" saturation were high. Another reason maybe was that only one individual of the copper pheasant were sampled.

Mikado pheasant diverged third and Elliot's and Hume's pheasant diverged at last. This result is in agreement with Johnsgard (1986) and we can find many morphological and hybridization evidence to support this conclusion: Basically, the three species should have close relationship, not only because either Elliot's and Hume's or Mikado and adult females of Hume's is similar in morphology, but only because hybrids between Elliot's and Mikado pheasants were fertile (Johnsgard, 1986). However, hybrids between Elliot's and Mikado pheasants were fertile only among males, which suggested a fairly prolonged separation between them (Delacour, 1977). In contrast, Elliot's pheasant was considered to be closely related to Hume's pheasant in that they were similar in the body form and pattern of feather colors (Ding, 1998). Therefore, among the three species, Elliot's pheasant should be closer to Hume's than to the Mikado pheasant.

Approximate times of divergence

Although there are many controversies about the application of the molecular clock (e.g. Klica and Zink 1997), we are aware of the heuristic value of assigning tentative divergence time to phylogenetic branches event. TN genetic distances of cyt *b* sequences were taken to estimate the divergence among species because they had no additional indels in the alignment, which were also used in many studies of birds (e.g. Randi *et al.*, 2000; Sato *et al.,* 1999; Klicka *et al.*, 2001). Since there is no fossil record of the *Syrmaticus* available to calibrate the molecular clock, the commonly used rate of 2% divergence per million years (myr) (Shileds and Wilson, 1987) was used in our study to estimate the divergence time of Elliot's pheasant; Hume's pheasant and Mikado pheasant. Considering the 2% molecular clock usually underestimates the divergence time in case of ts saturation (Burns, 1997), another clock, 2.2 transversions at the third codon positions per myr (Randi, 1996), was used to estimate the divergence time of the Reeves's pheasant, copper pheasant and the *Syrmaticus* genus. If the genus *Phasianus* is the sister-taxon of the *Syrmaticus* group (Johnsgard, 1999), the genus *Syrmaticus* should originate 8.9±1.8 myr ago, which is very similar to the divergence time of the genus *Gallus* (Tuinen and Dyke, 2004). The divergence time of the five species within the genus *Syrmaticus*

is 7.0±1.7 myr ago for the Reevess pheasant, 5.4±1.5 myr ago for the copper pheasant, 2.8±1.8 myr ago for the Mikado pheasant and 0.5±0.2 myr ago between the Elliot's and Hume's pheasant.

Biogeography and Speciation of the *Syrmaticus*

The molecular evolution pattern and divergence dates of the *Syrmaticus* are well congruent with historical geological events, which make it possible to study the origin and speciation process of the *Syrmaticus* based on the combinations of molecular data, geographic distribution and geological scenarios. According to the rule that the taxon diversity of the origin center is the richest (Nelson and Platnick, 1984), the major origin center of pheasant diversity is in the eastern Himalayas and across northern Burma and Yunnan (Johnsgard, 1999). Moreover, the genus *Phasianus*, thought to be the nearest genus to *Syrmaticus* group, was thought to have originated in the Hengduan Mts. of southwestern China (Cheng *et al.*, 1978; Johnsgard, 1999). Therefore, the *Syrmaticus* group might originate in the mountains of southwestern China. From the distribution of the *Syrmaticus* (Fig. 1), we found that three species of *Syrmaticus*, Reeves's, Elliot's and Hume's living in southwestern China, additional evidence for the origin center. As stated before, the ancestor of *Syrmaticus* originated 8.9±1.8 myr ago, and the climate of the origin center during that time was mid-subtropical. Notably, at that time the rising of Himalayas had nearly completed in this region (The Editor Committee of Physical Geography of China, 1978).

The rising of Himalayas and the uplifting of the fault-blocks in the Miocene and Pliocene not only changed the local terrain, but also induced some major climatic consequences (The Editor Committee of Physical Geography of China, 1978). For example, the monsoon circulation was found, the temperature and humidity were decreased, and the horizontal composition of subtropical was destroyed. While influenced by the southwest monsoon, vertically distributing landscapes were formed in eastern Himalayas and Hengduan Mts. Regions. The ancestor of the *Syrmaticus* might have evolved in these landscapes and dispersed across the mainland of China. In the subtropical forest, the ancestor of *Syrmaticus* was able for dispersing, and even reached Japan. It had been proven that the communication of faunas between east and west was the most frequent in the mid-Miocene. eg. the *Bunolophodon*, *Stegolophodon* and *Rhinocerotoid* fossils found in China mainland were fairly close to the fossil faunas found in Japan (The Editor Committee of Physical Geography of China, 1978).

If the above-estimated divergence time was taken, it was in the late Miocene that Reeves's pheasant diverged from the ancestor of other *Syrmaticus*, maybe also in the mountains of southwestern China. Whether Reeves's pheasant had stayed and lived in the regions for some time or progressively dispersed towards east and north still needs further studies. The speciation time of copper pheasant is largely in agreement with the isolation of Japan islands from China mainland, which suggested that the speciation of copper pheasant might be triggered by the geographic isolation occurred in the early Pliocene. Taiwan Island was formed in the Pliocene but connected with the mainland for several times since that time, which was supported by the similar mammal faunas shared by both sides. Therefore, the ancestor of Mikado, Elliot's and Hume's pheasant might have dispersed into Taiwan in the Pliocene. In the early Pleistocene, the sea isolated Taiwan from the mainland and seperated the long-tailed pheasants in Taiwan from those in China mainland, which should have strong influence on the speciation of Mikado pheasant. Elliot's pheasant diverged from Hume's pheasant about 0.5 myr ago, which was just in the so-called Mindel (750,000 before) and Riss (350,000) interglacial period. Pleistocene glaciation and isolation of the high north-to-south mountains in southwestern China must have played roles in the divergence and speciation of the two species, and producing the distribution patterns of the Elliot's (east) and the Hume's (west).

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