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Taxonomic status of eight Asian shrike species (*Lanius*): phylogenetic analysis based on Cyt *b* and CoI gene sequences

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Abstract. Complete Cyt *b* gene sequences (1143bp), partial CoI gene sequences (1176bp) and Cyt *b* gene sequences combined with CoI gene sequences (2319bp) from 22 samples of 8 *Lanius* species were analysed using the phylogenetic method. Molecular phylogenetic trees were reconstructed using the Maximum Parsimony (MP), Maximum Likelihood (ML), Neighbour-joining (NJ) and MrBayesV3.1 (BI) methods. 228 and 216 nucleotide sites were found to be substituted in the Cyt *b* gene and CoI gene sequences respectively, accounting for 19.5% and 18.4% of the total nucleotide sites in the Cyt *b* gene and CoI gene sequences. In the phylogenetic trees, *L. minor* and *L. tigrinus* were the first to diverge. Then, a parallel clade diverged: one was clustered with *L. isabellinus* and *L. collurio*, which formed a sister group; the other was clustered with *L. schach* and *L. cristatus*, which was parallel to the cluster of *L. tephronotus* and *L. bucephalus*. Shrikes *L. isabellinus*, *L. collurio*, *L. schach* and *L. tephronotus* were independent species. The melanistic form of *L. schach* is a variation group of *L. schach*.

Key words: *Lanius*, Cyt *b* gene, CoI gene, taxonomy, phylogeny, sequence characteristic

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INTRODUCTION

Shrikes (Laniidae) are a peculiar group of small or medium-sized passerines, mostly insectivourous. The family includes 31 species of 3 genera, distributed in Asia, Africa, Europe and North America. Altogether 27 species of the genus *Lanius* are variable in their size and morphology (Panov 1995), their taxonomic status is not firmly established (Harris & Franklin 2000). The taxonomic status and phylogeny of this genus were mainly based on the study of their morphological parameters, behavior, ecology and distribution (Biswas 1950, Vaurie 1955, Mayr & Greenway 1960, Panov et al. 1972, Lefranc & Worfolk 1997, Chen et al. 1998). More recent studies used tandem repeats in the mitochondrial DNA control region (Yosef & Lohrer 1995, Hernández et al. 2004, Mundy & Helbig 2004). The taxonomic status of some shrike species is still disputable (Biswas 1950, Vaurie 1955, Mayr & Greenway 1960, Chen et al. 1998, Cheng 2000). Cheng (1976) had suggested that *L. collurio* and *L. cristatus* had belonged to the same species, but later separated *L. collurio* from *L. cristatus* (Cheng 2000). Furthermore Cheng (2000) thought that *L. isabellinus* was a Xinjiang-subspecies of *L. collurio*. Different authors suggested that *L. isabellinus* distributed in Europe and Middle Asia belonged to *L. collurio*, while *L. isabellinus* distributed in eastern Asia belonged to *L. cristatus* (Vaurie 1955, Mayr & Greenway 1960). Since the 1960s, the view that *L. isabellinus* and *L. collurio* are two independent species has been accepted by most taxonomists (Chen et al. 1998, MacKinnon et al. 2000). The exact number of *Lanius* species in China is inconsistent. Chen et al. (1998) recognize 12 species, but Cheng (2000) reports that there are only 10 — the discrepancy

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results from different opinions on taxonomical status in the case of the *L. isabellinus*, *L. collurio* and *L. cristatus* complex, the *L. schach* and *L. tephronotus* complex, and *L. schach* whose melanistic form was previously considered as *L. fuscatus*.

Molecular techniques have been used with profit to construct the phylogeny and in species identification. Fast evolving mitochondrial DNA, especially the cytochrome *b* (Cyt *b*) and cytochrome *c* oxidase subunit I (CoI) gene sequences with both variable and conventional sites, were suitable tools for bird phylogeny and identification (Zhang & Shi 1992, Hebert et al. 2004).

The aim of this paper is mainly to resolve the taxonomical dispute and identify a preliminary phylogeny of eight shrike species in China using Cyt *b* and CoI gene sequences.

MATERIAL AND METHODS

A total of 22 individuals from 8 well-defined species of *Lanius* and melanistic form *L. schach* were examined (Table 1). Samples were taken from the field-collected tissues, blood and feathers. Except for *L. minor* and *L. tigrinus*, every sample comprised at least two individuals. For other species, individuals from different localities were sampled to increase the reliability of the phylogenetic analysis.

All DNA was extracted from most tissues, using SK1205 tissue kits (Sangon, Shanghai). The course of extraction followed the manufacturer's protocol. In order to examine whether it was polluted by foreign DNA, the negative control sample (extract without tissues) was treated identically through both the extraction procedure and following PCR amplification. Their PCR products were examined through electrophoresis in 1.2% gelose. If there was no light stripe, it meant that the sequence was not contaminated by other DNA.

Cyt *b* gene was amplified with two pairs of recognized primers: L14731-H16064 (Sorenson 1999, Saetre et al. 2001), L14770-H16064 (Sorenson 1999, 2003). For CoI gene, PCR amplifications were accomplished with three primers: L6615-H7539, H7956 (Mindell et al. 1991, Miranda et al. 1997). Reactions were performed in a total volume of 50 μl, which contained 10 $\times$ buffer 5 μl, 25 mmol/L $MgCl_2$ 25 μl, *Taq* DNA polymerase 0.5 U, 2 mmol/L dNTP 5 μl, 10 mol/L each primer 2 μl and 100 ng genomic DNA. After a pre-denaturing step representing exposure to a temperature of 94°C for 4 min, each cycle comprised a 45-second denaturing step at 95°C. Annealing lasted 1 min per cycle, with a touchdown step from 44°C to 52°C. Extension was at 72°C for 1 min per cycle. Total number of cycles was 36. Post-extension at 72°C was performed for 10 min. We set a blank contrast in the course of PCR amplification to examine whether they were contaminated.

The PCR products were gel-purified using H.Q. & .Q Gel Extraction Kit II (Anhui U-Gene Biotechnology, China) following manufacturer's protocols. After purifying, the products were the sequencing-ready DNA. We sent the sequenced DNA to Sangon (Shanghai, China), and analyzed it on an ABI Model 3730XL Automated Sequencer (PE Biosystems). All PCR products were sequenced from both directions. Sequences of both Cyt *b* gene and CoI gene were submitted to GenBank under accession numbers from EF621572 to EF621619 (Table 1).

Data analysis

DNA sequences were checked manually using a Chromas (V1145), edited using SeqEdit (Applied Biosystems Inc1., USA) and aligned using CLUSTAL X (Thompson et al. 1997). We calculated P-distances, the value of Transversion/Transition, the number of variable sites and parsimony information sites of different sequences with using MEGA 3.0 (Kumar et al. 2004) with Kumar-2 Paremants (Kimura 1980). In order to examine whether the sequences had the strong phylogenetic information, we carried out PTP (Permutation tail probability) examination before reconstructing phylogeny. We have selected *Ficedula hyperythra* (Muscicapinae) and *Cinclus pallasii* (Cinclidae) as outgroups. Because of the relation between Laniidae and the ones of Muscicapinae and Cinclidae was neither close nor far, to use them as out groups could help us to clearly explain the phylogenetic relationship (Sibley & Monroe 1993). Phylogenetic analyses were performed by means of PAUP 4.0 (Swofford 2000). Data from the two genes were initially analyzed separately using the Maximum-parsimony (MP), Maximum-likelihood (ML), Neighbor-joining (NJ) and MrBayesV3.1(BI) (Ronquist & Huelsenbeck 2003) methods, then the two datasets were combined, congruence between them was examined with a partition-homogeneity test (Farris et al. 1994), and analyzed in the same way. Each nucleotide was treated as an unordered character with four alternative states, and gaps were considered as missing data in all analyses.

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The data were treated with the equal weight for all analyses. We reconstructed the MP tree, ML tree, NJ tree and BI tree based on Cyt *b* gene, CoI gene and both sequences combined, respectively. Bootstraps of MP trees and NJ trees were obtained from 1000 replications, and the bootstrapping for ML trees (200 replications) were performed using SPR branch swapping (which is computationally faster than TBR branch swapping) on initiating trees obtained by NJ, with each replicate being limited to 250 rearrangements. Bayesian inference (BI) was carried out using MrBayes 3.0 (Ronquist & Huelsenbeck 2003). Two separate runs were performed with four Markov chains, one starting from a random tree and another from the ML tree. The Markov chains were run for 500 000 generations. Sampling every 100 generations thinned the data to 5 000 sample points each run. The first 1000 samples from each run were discarded as burn-in, and the remaining samples analyzed using the "sumt" command (contype = allcompat) in MrBayes. Both independent runs found essentially identical tree topologies and posterior probabilities, indicating that the sample number was sufficient to permit the algorithm to converge on a global solution.

RESULTS

DNA sequences and sequence characteristics

Altogether 48 DNA sequences were obtained. Cyt *b* and CoI gene sequences were obtained for every sample (there are 24 samples including the outgroup; see Table 1).

Two protein-coding genes (Cyt *b* and CoI) showed neither deletion nor insertion. After examining the congruence through a partition-homogeneity test between Cyt *b* and CoI, we found that they could be combined for analysis.

Except for the outgroups, Cyt *b* and CoI, combined data (2319bp) had 444 variable sites, which made up 19.2% of the total database, and 285 parsimony information sites, which made up 64.2% of the variable sites. Transversion played the key role in substitution. The average value of transversion/transition was 8.08. The average values of A, T, C and G respectively were 28.7%, 26.9%, 29.2%

Table 1. The samples used in this study (including outgroups).

Family/Genus/Species name	Code of sample	Type of sample	Locality of samples	GenBank accession number	
				Cyt *b*	CoI
Laniidae					
Lanius					
L. schach	*L. schach1*	Muscle	Chao'an, Guangdong Prov.	EF621576	EF621600
	L. schach2	Muscle	Chao'an, Guangdong Prov.	EF621581	EF621605
	L. schach3	Muscle	Honghu, Hubei Prov.	EF621584	EF621608
	L. schach5	Muscle	Jiedong, Guangdong Prov.	EF621591	EF621615
	L. schach7	Muscle	Wenxian, Gansu Prov.	EF621593	EF621617
L. schach "melanistic form"	*L. schach4*	Muscle	Haifeng, Guangdong Prov.	EF621585	EF621609
	L. schach6	Muscle	Chao'an, Guangdong Prov.	EF621592	EF621616
L. collurio	*L. collurio1*	Muscle	Hejing, Xinjiang Uygur Aut. Reg.	EF621572	EF621596
	L. collurio2	Feather	Slovakia	EF621589	EF621613
	L. collurio3	Muscle	150 Corps, Xinjiang Uygur Aut. Reg.	EF621590	EF621614
L. tephronotus	*L. tephronotus1*	Muscle	Yanbian, Sichuan Prov.	EF621573	EF621597
	L. tephronotus2	Muscle	Wenxian, Gansu Prov.	EF621574	EF621598
L. bucephalus	*L. bucephalus1*	Blood	Wenxian, Gansu Prov.	EF621577	EF621601
	L. bucephalus2	Blood	Wenxian, Gansu Prov.	EF621583	EF621607
L. cristatus	*L. cristatus1*	Muscle	Xi'an, Shaanxi Prov.	EF621578	EF621602
	L. cristatus2	Muscle	Shanbei, Shaanxi Prov.	EF621579	EF621603
L. cristatus	*L. cristatus3*	Muscle	Dunhua, Liaoning Prov.	EF621580	EF621604
L. isabellinus	*L. isabellinus1*	Muscle	150 Corps, Xinjiang Uygur Aut. Reg.	EF621586	EF621610
	L. isabellinus2	Muscle	150 Corps, Xinjiang Uygur Aut. Reg.	EF621587	EF621611
	L. isabellinus3	Muscle	150 Corps, Xinjiang Uygur Aut. Reg.	EF621588	EF621612
L. minor	*L. minor*	Blood	Slovakia	EF621575	EF621599
L. tigrinus	*L. tigrinus*	Blood	Beijing	EF621582	EF621606
Cinclidae					
Cinclus pallasii	*C. pallasii*	Muscle	Wenxian, Gansu Prov.	EF621594	EF621618
Muscicapinae					
Ficedula hyperythra	*F. hyperythra*	Muscle	Yanbian, Sichuan Prov.	EF621595	EF621619

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and 15.2%. There were 228 variable sites and 153 parsimony information sites in the Cyt *b* datasets. The average values of A, T, C and G from Cyt *b* datasets were 29.3%, 26.4%, 31.1% and 13.2%, and their average value of transversion/transition was 12.9. There were 216 variable sites and 132 parsimony information sites in the CoI datasets. The average value of A, T, C and G from CoI datasets were 28.2%, 27.4%, 27.2% and 17.2%, and their average value of transversion/transition was 6.12. The base compositions for three groups of data cyt *b* and CoI sequences for each codon position. The values of the nucleotide compositional bias index are very similar to the values reported for mammalian cyt *b* sequences (Irwin et al. 1991), and they reflect previous observations for mtDNA base composition (Kocher et al. 1989, Edwards et al. 1991).

A comparison of three groups of data showed that the percentage of variable sites (19.5%) and parsimony information sites (67.1%) in the Cyt *b* datasets were higher than those in the CoI datasets (18.4%, 61.1%). The database of G was poorer than others databases (A, T, C) in Cyt *b* and CoI datasets, and the database of C in the Cyt *b* datasets and the database of A in the CoI datasets were comparatively rich. Transversion was the main part in substitution of Cyt *b*, CoI and their combined datasets, and the number of transitions was obviously higher than that of transversion.

Phylogenetic analysis

In general, if the ratio of transversion/transition is less than 2.0, the mutation of the gene is already saturated. When reconstructing the phylogeny, if the sequences do not have extra weight, the results are likely to be affected by the evolutionarily random noise and non-reliable. The ratios of transversion/transition of three datasets in this study were higher than 2.0. Therefore, when we reconstructed the phylogenetic tree by the MP method, they had no extra weight for any of the three datasets. In order to analyze the saturation of transversion and transition, we used the uncorrected P-distances as the abscissa, and transversion and transition as the y-axis, respectively (Fig. 1). We found that the number of transitions was evidently higher than that of transversions in the Cyt *b* and CoI datasets (Fig. 1). The uncorrected P-distances of all sequences ranged from 0 to 0.1, from 0.15 to 0.2, but no uncorrected P-distance was within 0.10 to 0.15. With increasing differentiation from sequences, the number of transversions and transitions increased linearly, which showed that transversion and transition were not saturated either.

We did the PTP (Permutation tail probability) examination of Cyt *b*, CoI and their combined datasets by using PAUP 4.0. The P-values were all 0.002. It indicated that all datasets provided quite good information about phylogeny, that they were not random datasets and therefore could be used in phylogeny. In PAUP 4.0, we reconstructed the NJ tree (Fig. 2B) of three datasets with using Neighbor-join, the MP tree (Fig. 2C) with Maximum-parsimony (Table 2) and the ML tree (Fig. 2A) with Maximum-likelihood, the BI tree with MrBayes3.0. When we reconstructed ML tree, the Models for Cyt *b*, CoI and their combined datasets were GTR + I + G, TrN + G and GTR + I + G, the models being selected from Modeltest 3.7 (for parameters of all models see Table 3).

We found that the topologies of four-type tree based on Cyt *b* datasets were approximately identical (Fig. 2A). At first, *L. tephronotus* and *L. bucephalus* were clustered at the terminal clade, then they clustered with *L. cristatus*, finally the clade was grouped with *L. schach*. Furthermore, *L. isabellinus* and *L. collurio* clustered in another clade. *L. minor* and *L. tigrinus* clustered forming their own clade. The main differences of three types of tree were presented in NJ tree from Cyt *b* gene, firstly *L. minor* and *L. tigrinus* diverged, then *L. isabellinus* and *L. collurio*, finally a clade of *L. schach* and a clade from *L. tephronotus*, *L. bucephalus* and *L. cristatus* were divergent. In MP tree based from Cyt *b* gene, clades of most species were gathered into a parallel clade, and the bootstraps values were low. In ML and BI tree from Cyt *b* gene, the clade of a *L. collurio* was clustered with the

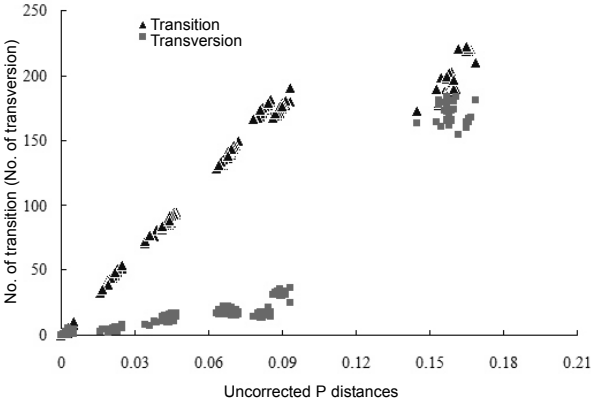


Fig. 1. Variation of substitution versus P-distance of CoI and Cyt *b* sequences fragment of 8 shrike species (n = 22).

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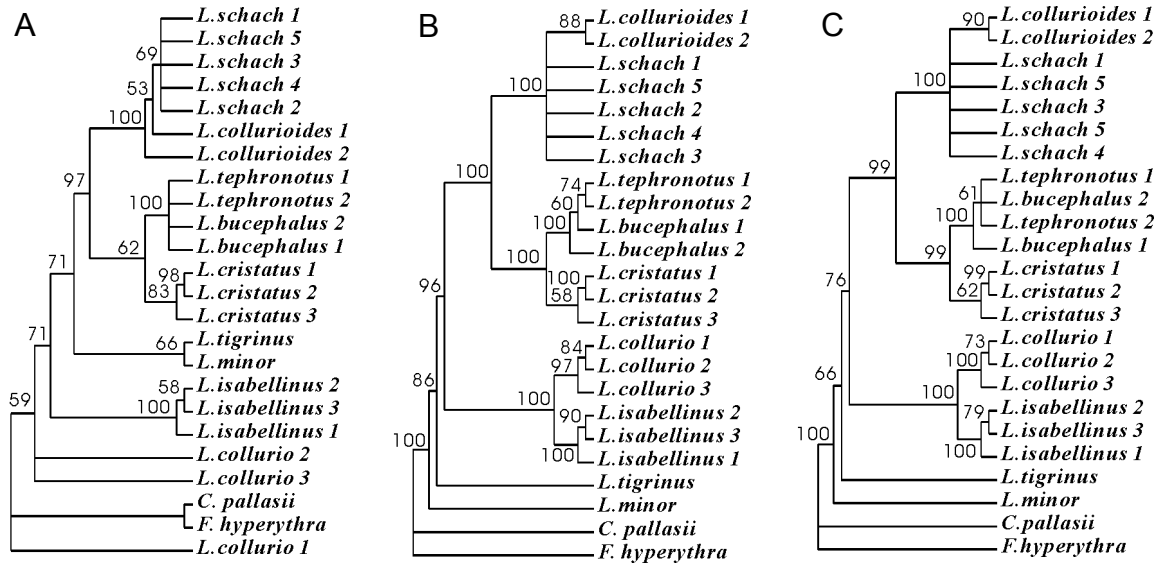


Fig. 2. Phylogenetic trees of 8 shrike species resulting from separately analysis of the Cyt b gene and CoI gene sequences and their combined data. A — ML tree of Cyt b gene; B — NJ tree of CoI gene; C — MP tree based on Cyt b gene combined with CoI gene sequences data. Bootstrap (only 50 % or higher) values are shown above nodes on trees.

outgroup, and *L. isabellinus* and *L. collurio* were not a sister group, but they were most divergent in order, followed by *L. minor* and *L. tigrinus*.

The topology of eight trees based on CoI and combined datasets was very similar; therefore only one was presented (Fig. 2B). *L. minor* and *L. tigrinus* diverged firstly. Then, a parallel clade was divergent: one branch was clustered with the *L. isabellinus* and *L. collurio*, which forming a sister group; the other was clustered with *L. schach* and *L. cristatus*, which was parallel to the cluster of *L. tephronotus* and *L. bucephalus*. Therefore, *L. tephronotus* and *L. bucephalus* diverged later than others.

Comparing all these trees shows that the relations of clusters of species from NJ tree, MP tree, ML tree and BI tree have nearly the same topologies, but there are still some differences. On the basis of species relationships, *L. tephronotus* and *L. bucephalus* were joined; then they were clustered with *L. cristatus* to form a clade; finally the clade became a parallel clade to *L. schach*. *L. isabellinus* and *L. collurio* formed another clade. *L. minor* and *L. tigrinus* became two independent clades. As in the case of the phylogenetic tree topology, except that *L. minor* and *L. tigrinus* were clustered in a clade and diverged later than *L. isabellinus* and *L. collurio* in MP tree, ML tree and BI tree obtained from the Cyt *b* dataset, the phylogeny of ingroups discussed in this paper was distinct from other phylogenetic trees. The clade of *L. minor* and *L. tigrinus* diverged earlier.

DISCUSSION

Taxonomical status of *Lanius isabellinus*, *L. collurio* and *L. cristatus*

In the molecular phylogenetic trees *Lanius isabellinus* and *L. collurio* form a sister group, and the lower bootstrap value of the clade is 83%; Kimball et al. (1999) pointed out that values higher than 70% are reliable. Although the phylogeny of *L. isabellinus* and *L. collurio* disturbed

Table 2. Separately heuristic search results of Cyt *b* and CoI gene sequences and their combined sequences using MP method.

MP tree based on sequences	Tree number	Tree length	Consistency index (CI)	Retention index (RI)	Re-modulatory retention index (RCI)
Cyt *b* gene	4	610	0.718	0.801	0.575
CoI gene	20	566	0.707	0.788	0.557
Cyt *b* and CoI gene combined	9	1178	0.711	0.793	0.564

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Table 3. Nucleotide substitution model parameter estimates of Cyt *b* and CoI gene sequences and their combined sequences for hLRTs (the standard for model of ML searching). -ln*L* — likelihoods, *f*(M) — assumed nucleotide frequencies, R[M-N] — rate of substitution from M to N.

Data name	Model name	-lnL	f(A)	f(C)	f(G)	f(T)	R[A-C]	R[A-G]	R[A-T]	R[C-G]	R[C-T]	R[G-T]
Cyt *b*	GTR+I+G	4320.4604	0.3140	0.3677	0.1035	0.2148	0.4475	9.4067	0.5515	0.0044	12.4779	1.0000
CoI	TrN+G	4218.8345	0.3156	0.3180	0.1315	0.2348	1.0000	17.9454	1.0000	1.0000	18.4664	1.0000
Combined sequences	GTR+I+G	8573.3711	0.3150	0.3440	0.1174	0.2237	0.5635	12.1245	0.7981	0.1673	13.5474	1.0000

phylogenetic trees, their clustered relationship was very clear and steady. Three *L. isabellinus* and three *L. collurio* samples were divided into two groups, respectively. The analysis of the three datasets (Cyt *b*, CoI gene and their combined datasets) showed the P-distances of *L. isabellinus* and *L. collurio* were 4.2%, 3.3%, 3.7%, respectively (see Appendix). They comply with the rule that the P-distance between species of birds is more than 1% (Xiangyu et al. 2000). Most of *L. isabellinus* have patches on their wings and elongated lateral tail feathers, and they inhabit dry and thin trees, while *L. collurio* have no such wing and tail features. They were obviously different during both molting and breeding seasons (Chen et al. 1998). Based on these analyses, it is confirmed that *L. isabellinus* and *L. collurio* are two independent species.

Are *Lanius schach* and *L. tephronotus* conspecific?

Mayr & Greenway (1960) suggested that *L. schach* was a subspecies of *L. tephronotus*, while Biswas (1950) thought it was a valid species. Investigating their taxonomic position, we firstly analyzed their phylogenetic trees. *L. tephronotus* and *L. bucephalus* were gathered at a terminal branch, they were clustered with *L. cristatus* to form a clade, and finally the clade was assembled with *L. schach*. This suggested that *L. schach* and *L. tephronotus* were not closely related. Secondly, we analyzed their P-distances. The P-distances in *L. schach* and *L. tephronotus* from our three datasets were 7.4%, 6.7%, 7.1% (Appendix), and they fit to the rule of distance according to which the P-distance is more than 1% between the species. Thirdly, we analyzed their distribution and morphological characteristics. Their breeding territories overlap in central and south-western China but no hybrids have been found (Chen et al. 1998). Species of *L. tephronotus* has wings lacking speckle and it breeds mostly in the Palearctic, while *L. schach* has speckled wings and breeds mostly in the Oriental realm (Chen et al. 1998). Therefore, our study supported viewpoint of Biswas (1950) and Harris & Franklin (2000) concerning the taxonomic status of *L. schach* and *L. tephronotus* as two distinct species.

Taxonomic status of *Lanius schach* melanistic form

MacKinnon et al. (2000) and Cheng (2002) et al. suggested that *L. schach*, "melanistic form" was only a colour morph of *L. schach* (because their

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melanin is especially well developed) and not a distinctive species. La Touche (1930, see Chen et al., 1998) thought that the melanistic form of *L. schach* was a valid species as *L. fuscatus* (Chen et al. 1998, Zheng 2005).

In order to examine the taxonomic status of the melanistic form of *L. schach*, we selected two birds of this form and five "normal colored" *L. schach*, reconstructed their phylogenic trees and calculated their P-distances from Cyt *b*, CoI gene and their combined datasets. In phylogenic trees (Fig. 2), all these seven samples were clustered in one clade. Amongst others, one *L. schach* (melanistic form) (from Chao'an, Guangdong Prov.) and one *L. schach* (from Wenxian, Gansu Prov.) were gathered in a terminal branch, and the others were gathered together randomly. Still, melanistic and "normal" forms of *L. schach* did not become a clade among species, which means that their differentiation has not reached the species level. The P-distances of both forms from three datasets in this study were all 0.3%, and between the melanistic form of *L. schach* they were 0.2%, 0.3% and 0.2%, respectively (see Appendix). Thus, the P-distances of both forms were not beyond the species threshold. Thus differentiation of the *L. schach* melanistic form is a intraspecies color plumage variation.

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STRESZCZENIE

[Status taksonomiczny ośmiu gatunków dzierzb występujących w Chinach]

Przeprowadzono analizy filogenetyczne 8 gatunków dzierzb z rodzaju *Lanius* (Tab. 1). Osobno analizowano melanistyczne osobniki *L. schach*, uważane wcześniej za osobny gatunek *L. fuscatus*. Analizy oparto o sekwencje mitochondrialnego DNA — cytochromu b (cyt b, 1143 par zasad) oraz podjednostki I oksydazy cytochromowej (CoI, 1176 par zasad), oraz obu sekwencji łącznie. Jako grupy zewnętrzne użyto sekwencji uzyskanych dla *Cinclus pallasi* oraz *Muscicapa hyperythra*. Zastosowano 4 metody rekonstrukcji drzewa filogenetycznego: Maximum Parsimony, Maximum Likelihood, Neighbour-joining and MrBayes (Tab. 2 i 3 oraz Appendix). W drzewie filogenetycznym pierwsze wyodrębniały się *L. minor* oraz *L. tigrinus*. *L. collurio* i *L. isabelinus* tworzyły grupę siostrzaną, zaś *L. schach* grupował się z *L. cristatus*. (Fig. 2). Potwierdzono, że forma melanistyczna *L. schach* jest odmianą barwną.

Appendix. Pairwise uncorrected P distance of CoI (right of below diagonal) and Cyt *b* combined sequences (left of below diagonal) and the numbers of nucleotide transition/transversions (above diagonal) of 8 Lanius species. Group 1 — *L. collurio1*, *L. collurio2*, *L. collurio3*; Group 2 — *L. isabellinus1*, *L. isabellinus2*, *L. isabellinus3*; Group 3 — *L. schach4*, *L. schach6*; Group 4 — *L. schach1*, *L. schach2*, *L. schach3*, *L. schach5*, *L. schach7*; Group 5 — *L. tephronotus1*, *L. tephronotus2*; Group 6 — *L. bucephalus1*, *L. bucephalus2*; Group 7 — *L. cristatus1*, *L. cristatus2*, *L. cristatus3*; Group 8 — *L. tigrinus*; Group 9 — *L. minor*.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Group 1		0.037	0.075	0.074	0.071	0.070	0.072	0.089	0.100
Group 2	0.042 0.033		0.073	0.073	0.071	0.069	0.069	0.088	0.095
Group 3	0.086 0.064	0.081 0.065		0.003	0.048	0.046	0.044	0.092	0.095
Group 4	0.085 0.064	0.080 0.066	0.003 0.003		0.047	0.046	0.044	0.092	0.095
Group 5	0.074 0.068	0.074 0.067	0.047 0.048	0.047 0.047		0.002	0.024	0.086	0.099
Group 6	0.072 0.068	0.072 0.066	0.045 0.048	0.045 0.047	0.003 0.002		0.023	0.085	0.099
Group 7	0.078 0.066	0.078 0.061	0.044 0.044	0.044 0.044	0.027 0.020	0.026 0.021		0.089	0.098
Group 8	0.090 0.089	0.090 0.087	0.097 0.087	0.097 0.087	0.088 0.083	0.086 0.083	0.088 0.090		0.102
Group 9	0.099 0.098	0.100 0.091	0.100 0.090	0.100 0.090	0.103 0.096	0.102 0.096	0.102 0.094	0.104 0.100	

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