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Molecular phylogenetic relationship of snow finch complex (genera *Montifringilla*, *Pyrgilauda*, and *Onychostruthus*) from the Tibetan plateau

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Abstract

The snow finch complex (*Montifringilla*, *Pyrgilauda*, and *Onychostruthus*) has its center of distribution on the Tibetan plateau, with six out of seven species in the genera occurring there. Phylogenetic relationships among these six species of three genera have been studied based on DNA sequence data obtained from the mitochondrial cytochrome *b* gene and the nuclear myoglobin gene. The results support monophyly of the snow finch complex group and three major evolutionary lineages are recognized. The first clade consists of *ruficollis*, *blanfordi*, and *davidiana*. These three taxa are sometimes placed in their own genus, *Pyrgilauda*, and the DNA data supports this. The three taxa *nivalis*, *henrici*, and *adamsi* have traditionally been placed in the genus *Montifringilla*, and they group together strongly in the present analysis. The results further suggest that *nivalis* and *adamsi* are more closely related to each other than are *nivalis* and *henrici*, despite that the latter two are often regarded as conspecific. The third distinct lineage within the snow finch complex consists of *taczanowskii*, which has been placed its own genus, *Onychostruthus*. This taxon has a basal position in the phylogenetic tree and is sister to all other snow finches. We estimated that *taczanowskii* split from the other taxa between 2 and 2.5 mya, i.e., about the time for the most recent uplift of the Tibetan plateau, "the Tibet movement", 3.6–1.7 mya. Cladogenesis within the *Montifringilla* and *Pyrgilauda* clades seems to be contemporary with the second phase of "Tibet movement" at 2.5 mya and the third phase at 1.7 mya and "Kunhuang movement" in 1.5–0.6 mya. The dramatic climatic and ecological changes following from the uplift of the Tibetan plateau, together with the cyclic contraction and expansion of suitable habitats during the Pleistocene, are probably the most important factors for the cladogenesis in snow finch complex.

Keywords: Molecular phylogenetic relationship; Snow finch complex; Tibetan plateau

1. Introduction

The snow finch complex (Passeridae: *Montifringilla*, *Pyrgilauda*, and *Onychostruthus*, see, e.g., Gebauer et al., 2003; Qu et al., 2004) is an unusually poorly known group of passerines. Probably, this is primarily due to the fact that the snow finches spend most of their lives in remote places, far away from human. All taxa except one in this group, are distributed sympatrically on the Tibetan plateau and surrounding areas. The last taxon, *nivalis*, is disjunctly distributed along a chain of mountains from Asia to Europe. The snow finches are among the highest-living of all birds, nesting

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at latitudes above 2300 m in the Abruzzi mountains in Italy, between 1900 and 3000 m in the Swiss Alps, normally between 2750 and 3160 m in Caucasus, and up to 5300 m in Tibet (Cramp and Perrins, 1994; Dementiev and Gladkov, 1954). They inhabit the rocky regions characterized by crags, stony flat areas, scree-covered slopes and meadows of short grass in the high alpine peak zone (Cheng, 1981), and they are mainly sedentary, only making irregular altitudinal movements in response to particularly bad weather conditions (Fu, 1998; Jochen, 1993). Like some sparrows (e.g., *Passer*), the snow finches often stay close to human surroundings when present, e.g., cowhides, structures protecting against avalanches, timber stacks, and even busy hotels where they may find food in the refuge (Cramp and Perrins, 1994; Heiniger, 1991).

Conventionally all seven species of snow finch complex are grouped in one genus, *Montifringilla* (e.g., Fu, 1998; Mlikovsky, 1998; Sibley and Monroe, 1990). However, from that the genus *Montifringilla* was first described in 1828 by Brehm, it has been much discussed how many species that should be recognized in the genus, as well as their interrelationships. The taxa have in common some morphological traits, for example the white spots in the wings and tails (Cheng, 1976, 2000). They also share certain ecological characters as their seasonal altitudinal movements, an alpine and subalpine distribution, and a preference to breed in rocky semi-arid, open countries and stony hillsides. They also have evolved a higher rate of metabolism than most other birds, a better tolerance of low temperatures, and a greater capacity for moving (Deng and Zhang, 1990). Due to that, many systematists have agree that genus *Montifringilla* consists of all seven species: *nivalis*, *adamsi*, *taczanowskii*, *ruficollis*, *blanfordi*, *davidiana*, and *therease* (e.g., Fu, 1998; Mlikovsky, 1998; Sibley and Monroe, 1990).

The snow finch complex is sometimes divided into two genera: *Montifringilla* (including *nivalis* and *adamsi*) and *Pyrgilauda* (with *taczanowskii*, *ruficollis*, *blanfordi*, *davidiana*, and *therease*) (Gebauer and Kaiser, 1994; Ivanitskii, 1991). The differences between the two genera involve some ecological characteristics: *nivalis* and *adamsi* are active in rocky habits during both the breeding and wintering seasons, and they both use rock crevices for roosting and nesting. In contrast, *taczanowskii*, *ruficollis*, *blanfordi*, and *davidiana* prefer short grass, and flatter, steppe-like habitats. They normally select burrows of pikas (*Ochotona curzoniae*, *O. ladacensis*, *O. alpina*, and *O. daurica*) or other small rodents, as nest place, and these burrows are also used for roosting and hiding. Moreover, *nivalis* and *adamsi* are not as well adapted to ground living as the *Pyrgilauda* species, they often fly around more and seem to be better flyer. *Nivalis* and *adamsi* are also more social, defending relatively small territories with a few specialized social display movements. They sing relatively seldom, but have a rich call repertoire that differs in function and structure from that in the *Pyrgilauda* species. Based on these differences, Ivanitskii (1991), Gebauer and Kaiser (1994) suggested the "snow finch complex" should be divided into two genera, *Montifringilla* and *Pyrgilauda*. Also morphology supports this, as evident from cluster and cladistic analyses of various anatomical characters (Lei et al., 2000, 2001).

The singularity of *taczanowskii* has often been discussed and it has sometimes been put in its own genus, *Onychostruthus* (Eck, 1996; Mayr, 1927; Qu et al., 2004). With its large body size and in the tail morphology *taczanowskii* resembles *nivalis* and *adamsi*, but in the ecology and wing morphology it is more similar to the *Pyrgilauda* species (Lei et al., 2001).

The intergeneric relationship in snow finch complex is a much discussed issue (Dementiev and Gladkov, 1954; Gebauer and Kaiser, 1994; Inskipp et al., 1996; Mlikovsky, 1998). A generally accepted view is that the species of the genus *Pyrgilauda* are more "primitive" than the more "advanced" species in *Montifringilla* (Lei et al., 2001; Voous, 1977). From the phylogenetic point of view, *Pyrgilauda* thus seems to be an ancestral group from which *Montifringilla* has evolved probably during Pliocene orogenesis (Ivanitskii, 1991).

A phylogenetic study based on random amplified polymorphic DNA (RAPD), showed that the genetic distances between *taczanowskii* and *ruficollis*, *blanfordi* and *davidiana* were almost as large as between each of these taxa and *Petronia*, which served as outgroup (Qu et al., 2004). However, it was not possible to determine the phylogenetic relationships among the snow finch complex by this RAPD analysis in absence of the data of *nivalis* and *adamsi*.

In this paper, we aim at outlining the phylogenetic relationships of the snow finch complex by comparing DNA nucleotide sequences obtained from intron II in the nuclear myoglobin gene, and a segment of the mitochondrial cytochrome *b* gene. These two genes have been extensively used in phylogenetic analyses within passerine birds and have proven useful in producing reliable phylogenetic relationship (see e.g. Allenda et al., 2001; Ericson et al., 2003, 2002; Filardi and Moyle, 2005).

2. Methods

2.1. Taxon selection and choice of outgroups

Seven taxa of snow finch complex (*Montifringilla*, *Pyrgilauda*, and *Onychostruthus*, see, e.g., Gebauer et al., 2003; Qu et al., 2004) were included in the study (Table 1). The sample represents all currently recognized species in the group except *therease*, which was unavailable to us. We included one individual of *nivalis* from the European Alps and one of *henrici* from Tibet, as these taxa often are treated taxonomically as different subspecies of *Montifringilla nivalis*. According to Bock and Morony (1978), the snow finch complex (*Montifringilla*, *Pyrgilauda*, and *Onychostruthus*), *Petronia* and *Passer* constitute a monophyletic group and *Petronia petronia* and *Passer montanus* were also included in the analyses in order to ascertain the monophyly of snow finch complex. We used *Aethopyga*

Table 1
Samples used in the study and list species, mitchondrial DNA cytochrome *b*, and nuclear myoglobin intron II gene sequences

Genus	Taxon	GenBank Accession No.		Collection sites
		Cytochrome *b*	Myoglobin intron II	
Montifringilla	*nivalis nivalis*	DQ244058	DQ244066	Germany
Montifirniglla	*nivalis henrici*	DQ244059	DQ244067	China, Qinghai, Huashixia
Montifringilla	*adamsi*	DG244060	DQ244068	China, Qinghai, Heimahe
Onychostruthhus	*taczanowskii*	DQ244061	DQ244069	Chian, Tibet, Tanggula mountains
Pyrgilauda	*ruficollis*	DQ244062	DQ244070	China, Tibet, Nanmulin
Pyrgilauda	*blanfordi*	DQ244063	DQ244071	China, Tibet, Tanggula mountains
Pyrgilauda	*davidiana*	DQ244064	DQ244072	China, Qinghai, Tianjun
Petronia	*petronia*	DQ244065	DQ244073	China, Qinghai, Tianjun
Passer	*montanus*	AY228073	AY228311	Sweden
Ploceus	*velatus*	AY228078	AY228316	Kenya
Coccothraustes	*coccothraustes*	AY228055	AY228292	Sweden
Aethopyga	*flagrans*	AY228045	AY228282	

flagrans (Nectariniidae), *Coccothraustes coccothraustes* (Fringillidae), and *Ploceus velatus* (Ploceidae) as outgroups.

2.2. Extraction, amplification, and sequencing

Genomic DNA was extracted from blood or tissue specimens using the QIAamp™ DNA Mini Kit (QIAGEN®) as per manufacturer's instructions. Nucleotide sequence data were obtained from the nuclear gene myoglobin and from the mitochondrial cytochrome *b* gene. PCR amplification and sequencing of myoglobin (intron II) follow Irestedt et al. (2002), while Ericson et al. (2002) describe protocols for cytochrome *b*.

2.3. Alignment and sequence properties

For each gene and sample, multiple sequence fragments were obtained by sequencing with different primers. They were assembled to complete sequences with SeqMan II™ (DNASTAR®). Positions where the nucleotide could not be determined with certainty were coded with the appropriate IUPAC code. The sequences of the two gene regions were aligned by eye and analyzed both separately and combined. To investigate sequence saturation, the numbers of transition and transversion substitutions were plotted against genetic distance for each sequence pair. Statistics for nucleotide variation were computed with MEGA 3.1 (Kumar et al., 2004).

Saturation was considered to have occurred in any of the data partitions if the scatter of points shows a leveling off mutations as sequence divergence increased.

2.4. Phylogenetic analyses

The phylogenetic analyses were performed on the combined sequences from the myoglobin intron II and cytochrome *b* genes. In addition, the phylogenetic signals in the two datasets were compared by analyzing each gene region separately.

Maximum parsimony and maximum-likelihood analyses were performed in Paup* 4.0b10 (Swofford, 2002). The parsimony analyses were performed under the heuristic search option with all characters coded as unordered. To reduce the risk of finding local optima only, all analyses were per-

Table 2
Parameters for the maximum-likelihood analyses

	Cytochrome *b* gene	Myoglobin gene	Combined gene
No. of sites (aligned sequence)	879	724	1603
No. of variable sites (%)	300 (34.1)	88 (12.1)	388 (24.2)
No. of informative sites (%)	162 (18.4)	14 (1.9)	176 (11.0)
MP tree length (No. of steps)	579	98	670
No. of MP trees	3	2	1
CI (uninformative sites excluded)	0.6942	0.949	0.5797
RI	0.4405	0.8077	0.4599
ML model selected	K81uf + I + G	TrN + I	TVM + I + G
$r_{(AC)}$	1	1	2.4566
$r_{(AG)}$	5.5504	5.0866	8.1663
$r_{(AT)}$	0.5642	1	1.1026
$r_{(CG)}$	0.5642	1	1.0132
$r_{(CT)}$	5.5504	4.3525	8.1663
$r_{(GT)}$	1	1	1
Shape	1.1227	N/A	0.7057
Proportion of invariant sites	0.5077	0.4899	0.4866
ML tree fit (−ln likelihood)	3817.44829	1613.19506	5569.40679

formed with 10 random additions of taxa. Nodal supports were estimated by bootstrapping the data (1000 replicates with 10 random additions of taxa).

Parameters for the maximum-likelihood analyses were estimated from the data (Table 2). The models for nucleotide substitutions used in the analyses of the genes separately and combined, were selected using the likelihood-ratio test implemented in Modeltest 3.06 (Posada and Crandall, 1998). With this program, the simplest model that cannot be rejected in favor of a more complex model is chosen. Bootstrap support values are based on 100 replicate, maximum-likelihood analyses.

The data set was also analysed by Bayesian inference. The models for nucleotide substitutions were selected for the two genes individually using the Akaike Information Criterion (Akaike, 1973). The program MrModeltest 2.2 (Nylander, 2002) in conjunction with PAUP* (Swofford, 1998) was used to evaluate the fit of the data to different models for nucleotide substitutions. The HKY + I + G model for nucleotide substitutions had the best fit for cytochrome *b* data, while the HKY + G model was selected for myoglobin. The models and parameter settings chosen for the individual genes were also used in the analysis of the combined data set. The posterior probabilities of trees and parameters were approximated with Markov chain Monte Carlo and Metropolis coupling using the program MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003).

For the two genes separately and for the combined data set we ran two analyses of four million generations each with trees sampled every 100 generation. The trees saved during the "burn-in phase" (the first 100,000 generations in each analysis) were discarded. The posterior probabilities were then calculated from the remaining 80,000 saved trees.

3. Results

3.1. Pairwise sequence divergences and saturation analysis

In cytochrome *b* 300 out of 879 sites varied among taxa. Of these, 162 sites (18%) were parsimony informative. The corresponding figures for the myoglobin intron II are much lower: 74 out of 724 sites vary among taxa, and only 14 sites (2%) are parsimony informative. Based on comparisons between pairwise sequence divergences (Tables 3 and 4) it is estimated that cytochrome *b* gene on average has evolved six times faster than myoglobin intron II. In the myoglobin intron II gene, the observed sequence divergence within snow finch complex varies from 0.3% (*nivalis* vs. *adamis*) to 2.3% (*taczanowskii* vs. *henrici*). The smallest divergence in myoglobin between the ingroup and the other two representatives of the family Passeridae is 1.7% (*adamsi* vs.

Table 3
Pairwise sequence divergence (uncorrected distances in percent) below the diagonal, and the observed numbers of transitions and tranversions in myoglobin intron II (ts/tv) above the diagonal

	adamis	*blanfordi*	*davidiana*	*nivalis*	*henrici*	*ruficollis*	*taczanowskii*	*Petronia*	*Passer*	*Ploceus*	*Coccothraustes*	*Aethopyga*
adamsi		02/01	06/01	01/01	08/00	02/02	06/03	09/03	08/06	06/06	13/07	15/09
blanfordi	0.56		04/00	03/02	08/01	01/03	06/04	09/04	08/07	06/07	13/08	15/10
davidiana	0.98	0.69		07/02	12/01	05/03	10/04	13/04	12/07	10/07	17/08	19/10
nivalis	0.28	0.98	1.4		09/01	03/03	07/04	10/04	09/07	07/07	14/08	16/10
henrici	1.12	1.54	1.96	1.40		04/01	12/03	15/03	14/06	12/06	19/07	21/09
ruficollis	0.56	0.83	1.25	0.84	1.40		06/05	09/05	09/06	06/08	13/09	15/11
taczanowskii	1.41	1.83	2.25	1.69	2.26	1.69		13/06	12/09	10/09	17/10	19/12
Petronia	1.70	2.12	2.54	1.98	2.55	1.97	2.69		13/05	13/05	18/06	22/08
Passer	2.11	2.53	2.95	2.39	2.95	2.24	2.96	2.54		10/08	17/09	16/11
Ploceus	1.97	2.24	2.80	2.24	2.81	2.24	2.81	2.54	2.66		16/07	16/09
Coccothraustes	2.94	3.22	3.63	3.36	3.92	3.35	3.93	3.52	3.78	3.49		22/10
Aethopyga	3.78	4.20	4.61	3.92	4.62	4.05	4.63	4.36	4.06	3.91	4.87	

Table 4
Pairwise sequence divergence (uncorrected distances in percent) below the diagonal, and the observed numbers of transitions and tranversions in cytochrome *b* (ts/tv) above the diagonal

	adamis	*blanfordi*	*davidiana*	*nivalis*	*henrici*	*ruficollis*	*taczanowskii*	*Petronia*	*Passer*	*Ploceus*	*Coccothraustes*	*Aethopyga*
adamsi		42/10	53/09	33/01	33/01	49/12	59/08	47/19	50/28	66/45	45/42	63/49
blanfordi	7.25		23/09	51/11	46/10	51/12	60/12	41/25	47/34	67/49	44/48	66/55
davidiana	7.71	3.87		61/10	59/09	52/17	67/13	46/24	59/35	74/50	54/47	74/52
nivalis	4.34	8.27	8.86		47/01	59/13	69/09	57/20	57/29	69/44	51/43	66/50
henrici	4.57	7.80	8.50	6.04		55/12	65/08	54/19	56/28	69/45	51/42	65/49
ruficollis	7.87	8.26	4.95	9.58	8.54		65/14	44/27	54/34	64/47	51/48	62/47
taczanowskii	8.74	9.24	9.72	9.75	9.05	9.99		55/19	62/28	81/47	60/44	71/53
Petronia	8.56	9.06	9.08	9.68	9.56	8.65	9.28		52/33	66/48	49/47	61/54
Passer	9.82	10.78	11.87	10.71	10.81	11.53	11.34	10.58		69/53	51/50	61/55
Ploceus	13.81	14.79	15.25	14.01	14.33	14.19	15.92	14.33	14.79		67/57	57/54
Coccothraustes	10.84	11.93	12.46	11.50	11.49	12.92	12.83	11.95	12.51	15.02		58/55
Aethopyga	14.27	15.25	15.37	14.58	14.11	14.42	15.58	14.79	14.45	13.54	13.88	

Petronia), and the largest 3.0% (*taczanowskii* and *Passer*). In cytochrome *b* the smallest sequence divergence within the ingroup is 4.3% (*nivalis* vs. *adamsi*), and the largest is 10.0% (*ruficollis* vs. *taczanowskii*). The smallest divergence observed between the ingroup and the two other sparrows is 8.6% (*adamsi* and *Petronia*) while the largest divergence is 11.9 % (*taczanowskii* vs. *Passer*).

The saturation plots for both genes show that transitions and tranversions were roughly linearly correlated with the uncorrected pairwise distances with no obvious tendency to level off (Fig. 1). This indicates that neither gene is saturated.

Nucleotide biases, transition/tranversions ratios, and rate heterogeneities among sites in the mitochondrial cytochrome *b* gene of snow finch complex species were similar to what previously have been observed in birds and mammals (e.g., Allenda et al., 2001; Edwards et al., 1991; Kornegay et al., 1993). At first codon position, the four bases were equally distributed. At second position, fewer G residues and a higher amount of T were seen, while the bias against G and T was strong at third codon position.

Transition/transversion ratios calculated between pairs of taxa ranged between 1.0 and 6.0 in myoglobin intron II, and between 1.8 and 6.8 in cytochrome *b* (Tables 3 and 4). The two loci show no difference in their ratios of transitions/transversions despite the much faster rate of nucleotide substitution observed in cytochrome *b*.

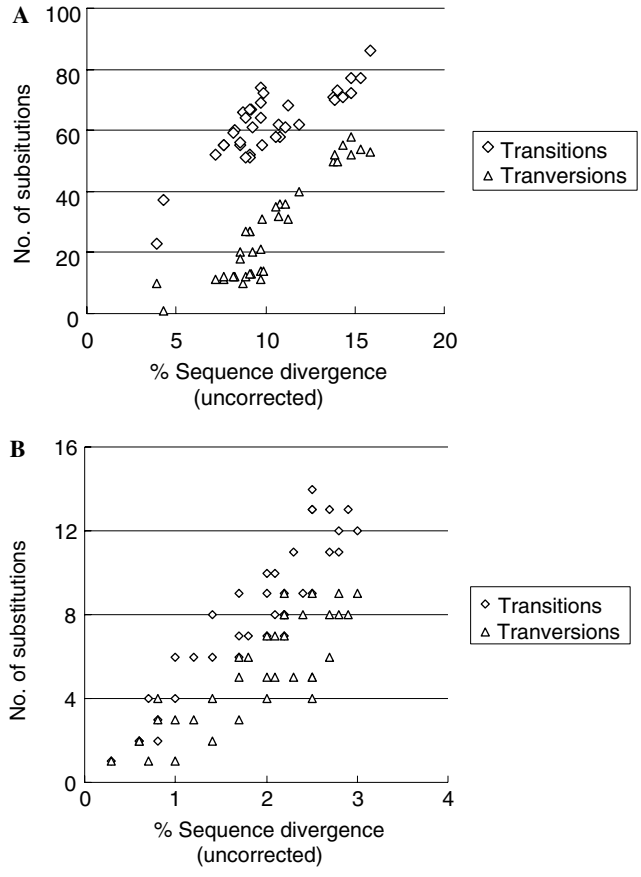

Fig. 1. Saturation plots for the mitochondrial DNA cytochrome *b* gene and nuclear myoglobin intron II gene. The number of transitions and transversions of each pairwise comparison of taxa are plotted against the pairwise, uncorrected sequence divergence. (A) Cytochrome *b* gene. (B) Myoglobin intron II gene.

3.2. Phylogenetic analysis

We first analyzed the aligned sequences of the two gene segments independently in order to compare the phylogenetic signals in the two datasets. For myoglobin intron II the parsimony, maximum-likelihood and Bayesian analyses resulted in the same phylogenetic tree (Fig. 2). Monophyly for the snow finch complex was well supported (with 83% bootstrap support value in the MP analysis, 81% in the ML analysis, and 100% posterior probability in Bayesian analysis). Within the ingroup, *taczanowskii* forms the sister to the other taxa (MP: 62%, ML: 60%, and B: 95%). The remaining taxa are poorly resolved and the only groups that receive bootstrap support are *adamsi* and *nivalis* (MP: 62%, ML: 60%, and B: 96%), *blanfordi* and *davidiana* (MP: 91%, ML: 93%, 100%). Monophyly for all ingroup taxa except *taczanowskii* is further supported by a loss of three base pairs (AGG) at position 986–988 in these taxa. Another, presumably synapomorphic insertion of four base pairs (CATA) at position 1225–1228 is shared by *blanfordi*, *davidiana*, and *ruficollis*.

The analyses of the cytochrome *b* dataset yielded an overall topology that is similar to that based on the myoglobin gene (Fig. 3). Snow finch complex is monophyletic (but with no bootstrap support) and *taczanowskii* is basal to all other taxa. The remaining taxa fall into two clades with good nodal supports: one with *adamsi*, *henrici*, and *nivalis* (MP: 97%, ML: 94%, and B: 100%) and another with *blanfordi*, *davidiana*, and *ruficollis* (MP: 72%, ML:

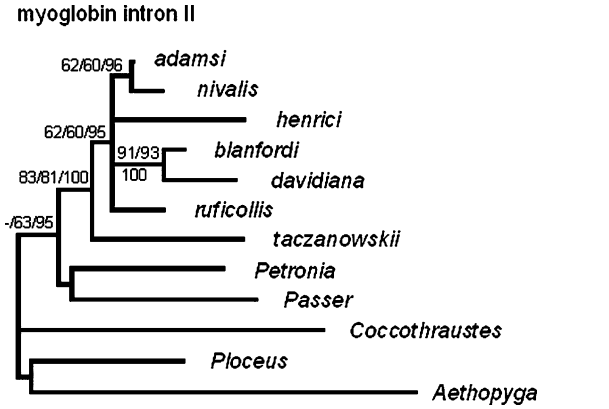

Fig. 2. Maximum-likelihood tree estimated from DNA sequences of intron II of the myoglobin gene (see Table 2 for the chosen model of nucleotide substitutions and parameter settings used in the analysis). The topologies of the two most parsimonious trees are identical to this ML tree, and the topology shown in the tree is identical to the tree resulted from Bayesian analysis. Branch lengths are proportional to the estimated genetic distances. Bootstrap values for the branches in the tree are given for (right) the parsimony analysis (1000 replicates), and (middle) the maximum-likelihood analysis (100 replicates). The posterior probabilities in Bayesian analysis are given (left).

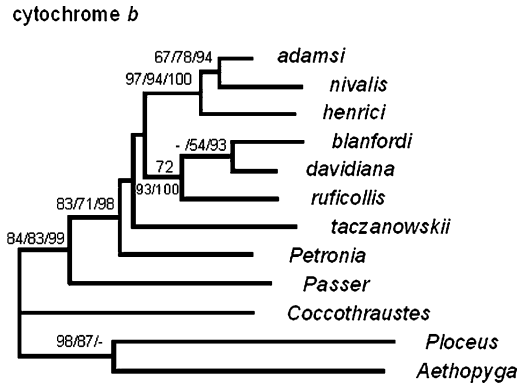

Fig. 3. Maximum-likelihood tree estimated from DNA sequences of the cytochrome *b* gene (see Table 2 for the chosen model of nucleotide substitutions and parameter settings used in the analysis). The topologies of the three most parsimonious trees differ from this tree in that *tazcanowskii* groups with *Petronia* (with no bootstrap support), and the topology shown in the tree is identical to the tree resulted from Bayesian analysis. Branch lengths are proportional to the estimated genetic distances. Bootstrap values for the branches in the tree are given for (right) the parsimony analysis (1000 replicates), and (middle) the maximum-likelihood analysis (100 replicates). The posterior probabilities in Bayesian analysis are given (left).

93%, and B: 100%). Also cytochrome *b* data support the sister group relationships of *adamsi* and *nivalis*, and *blanfordi* and *davidiana*, respectively, albeit with rather low support values.

The analyses of the combined data set yielded a phylogenetic tree contained all clades that received bootstrap support in the analysis of the individual genes (Fig. 4). The monophyly of snow finch complex received a 78% support in the maximum-likelihood analysis and 100% posterior probability in Bayesian analysis but no bootstrap support in the

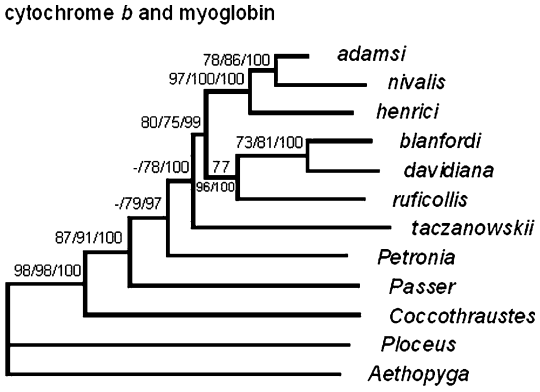

Fig. 4. Maximum-likelihood tree estimated from the combined data set consisting of DNA sequences of the cytochrome *b* and myoglobin (intron II) genes (see Table 2 for the chosen model of nucleotide substitutions and parameter settings used in the analysis). The topology of the single most parsimonious tree differs from this tree in that *tazcanowskii* groups with *Petronia* (with no bootstrap support), and the topology shown in the tree is identical to the tree resulted from Bayesian analysis. Branch lengths are proportional to the estimated genetic distances. Bootstrap values for the branches in the tree are given for (right) the parsimony analysis (1000 replicates), and (middle) the maximum-likelihood analysis (100 replicates). The posterior probabilities in Bayesian analysis are given (left).

parsimony analysis. Within snow finch complex, a basal position of *taczanowskii* was rather well-supported (MP: 80%, ML: 75%, and B: 99%). The clade with *nivalis*, *henrici*, and *adamsi* was strongly supported (MP: 97%, ML: 100%, and B: 100%), as was that with *ruficollis*, *blanfordi*, and *davidiana* (MP: 77%, ML: 96%, and B: 100%). Also the combined data set supported strongly the close relationships between *adamsi* and *nivalis*, and *blanfordi* and *davidiana*, respectively.

4. Discussion

The deeper splits of lineages within snow finch complex are generally congruent with the division of the taxa into two clades, corresponding to the genera *Montifringilla* and *Pyrgilauda*. However, *taczanowskii* does not group with any of these but constitutes the sister group to these clades.

The *Pyrgilauda* clade was well supported with a synapomorphic insertion of four basepairs in the myoglobin gene. Monophyly of the *Pyrgilauda* clade, including all southern snow finches, supports previous ecological observations (Gebauer and Kaiser, 1994; Ivanitskii, 1991) and morphology (Lei et al., 2001), although these studies suggested *taczanowskii* to be part of this clade. However, Mayr (1927), Eck (1996), Gebauer et al. (2002, 2003), and Qu et al. (2004) placed *taczanowskii* into it own genus, *Onychostruthus*, and Lei et al. (2001) emphasized the distinctiveness of *taczanowskii* by elevating it to subgenus rank in their morphological study.

The parsimony, maximum-likelihood, and Bayesian analyses suggest that *taczanowskii* is sister to all other species in snow finch complex. The monophyly of this more inclusive clade is further supported by a synapomorphic insertion of three basepairs in myoglobin in all investigated taxa in *Montifringilla* and *Pyrgilauda*.

The clade with *nivalis*, *henrici*, and *adamsi* is strongly supported in the analyses in agreement with their traditional placement in the genus *Montifringilla* (Gebauer and Kaiser, 1994; Ivanitskii, 1991). Interestingly, *nivalis* does not group with *henrici* although they often have been regarded to be conspecific. Instead, *nivalis* and *adamsi* group together. *Henrici* is distributed in the Tibetan plateau and has traditionally been considered as a subspecies of *Montifringilla nivalis* distributed in mountains from China to Europe. Compared to *nivalis* of the European Alps, *henrici* has a darker and browner body colour and is larger in size. Moreover, *henrici* has a stronger, larger, and more curved bill. Some researchers, as, e.g., Gebauer et al. (2002, 2003) recognize the distinctiveness of *henrici* compared to *nivalis* and regards it as a separate species. They divided these three taxa into two geospecies, namely the isospecies *henrici* (monotypic) and the superspecies *nivalis*, which includes polytypic allospecies *nivalis* and *adamsi*. Such a taxonomic treatment is supported by the DNA sequence data, both by the phylogenetic relationships taken at face value, and by comparisons of the pairwise sequence divergences in cytochrome *b*. Given that *adamsi* are closer to *nivalis* in the phylogenetic tree than is *henrici*, and that

henrici and *adamsi* live in sympatry in parts of their distribution, it follows that *henrici* cannot be conspecific with *nivalis*. Furthermore, the observed sequence divergence in cytochrome *b* between *henrici* and *nivalis* is 4.6%, which is of the same magnitude as between *adamsi* and *nivalis* (4.3%) and *blanfordi* and *davidiana* (3.9%).

By applying the estimated rate of 2% sequence divergence per million years in cytochrome *b* gene in many passerine birds (Avis and Walker, 1998; Crochet and Desmarais, 2000; Fleischer et al., 1998; Klicka and Zink, 1997, 1999; Kidd and Friesen, 1998; Kvist et al., 1999, 2001; Peck and Congdon, 2004), we estimate that the ancestor of the snow finch complex clade might split from the ancestor of *Petronia* between 2.5 and 3 mya. The subsequent split of snow finch complex into three clades (*Montifringilla*, *Pyrgilauda*, and *Onychostruthus*) occurred around 2.5–2 mya. Rapid cladogeneses within *Montifringilla* and *Pyrgilauda* seem to have occurred about 1.5–2 mya. The similar age of the speciation events in *Montifringilla*, *Pyrgilauda* may be explained as resulting from a single strong external signal in the environment (Pregill and Olson, 1991). As most snow finch complex species are distributed in the Tibetan plateau and surrounding areas, it is likely that the causal factor may be found in the geological history of this region. However, when we used a standard divergence rate of cytochrome *b* gene, the estimate might underestimate the actual divergence time more or less (Allenda et al., 2001). Thus, here presented only a rough estimate of divergence time.

The uplift of the Himalaya and Tibetan plateau is assumed to be the result of the collision of the Indian subcontinent with the Eurasian plate (Le Pichon and Heirtzler, 1968; McKenzie and Sclater, 1971). Compressed by the Indian and Euransian plates, the rim of the Tibetan plateau rose, while intensive tectonisms occurred in West China in the Late Neogene–Early Quaternary (Li and Fang, 1999). The most recent uplift of Tibetan plateau, "the Tibet movement," occurred between 3.6 and 1.7 mya and included three phases commencing at 3.6, 2.5, and 1.7 mya, respectively (Li et al., 1996). Before that, there was a relatively warm and humid environment in the region: a subtropical mixed forest of evergreen broadleaf and broadleaf deciduous woodland (Kong et al., 1981; Wu et al., 2001). The second phase of the "Tibetan movement" at 2.6 mya raised the plateau to the critical height of 2000 m, intensifying the Siberian-Mongolian High and triggering the onset of winter monsoons. The following third phase of the Tibet movement at 1.7 caused a large geomorphological, hydrologic, sedimentologic, and tectonic configurations. The "Kunhuang movement" at 1.5–0.6 mya uplifted the plateau to an average height of 3000 m with mountains up to over 4000 m (Li and Fang, 1999). The uplift of the Tibetan plateau and surrounding areas caused a climatic and ecological shift: forests were replaced by grasslands while the climate gradually became drier, colder, and more windy—glaciers started to develop and deserts were formed (Wu et al., 2001). The dramatic climatic and environmental changes caused by the uplift of the Tibetan plateau in the Pliocene resulted in new habitats that in turn facilitated the evolution of new groups of birds (Curray et al., 1982; Gansser, 1964).

When comparing these geological events of the Tibetan plateau with the assumed times for cladogeneses within snow finch complex, we observe a general agreement between them. The separation of snow finch complex from the *Petronia* lineage was assumed to occur 2.5–3 mya, following the second phase of the "Tibet movement." Speciation within *Montifringilla* and *Pyrgilauda* is estimated to have occurred 1.5–2.0 mya. This is about the time for the third phase of "Tibet movement" and the "Kunhuang movement" when the plateau was uplifted to an average height of 3000 m with mountains up to over 4000 m, a critical height for glacial development on a large scale. Since then, the plateau has undergone several glaciations (Shi et al., 1995), which certainly have influenced the further cladogenesis within *Montifringilla* and *Pyrgilauda*. When this study strongly suggests that split of snow finch complex started after "Tibet movement," we cannot exclude a probably earlier origin because currently divergence rate might underestimate the divergence time.

In contrast Asia, Africa (flycatchers, thrushes, warblers, and allies) and Australia (subocine birds, see Boles, 1993) have been considered as the center of the development of the Passeridae (Ericson et al., 2003), snow finch complex provides the new paradigm in evolution of alpine birds. Diesselhorst (1968) and Vaurie (1972) have already mentioned that the Sino-Himalayan region is a refuge for Asian bird species as well as a center of their evolution. Our data shows that especially in this period (3–1.5 million years ago)—before the glaciation of Pleistocene—most of the currently known taxa of snow finches evolved. Therefore the Pliocene might be the period in which the adaptive radiation of snow finch complex reached its peak. The pronounced mountains formation at that time led to an isolation of populations and a forming of "mountain-island" species.

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