A Tibetan Cradle of Evolution – in-situ speciation and Out-of-Tibet radiations in passerine model species

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List of Figures

Abbreviations List

01 QTP: Qinghai-Tibet Plateau

02 THR: Tibeto-Himalayan Region

03 mya: Million Years Ago

04 IOC: International Ornithological Congress

05 NGS: Next-Generation Sequencing

06 SNP: Single Nucleotide Polymorphism

07 DNA: Deoxyribonucleic Acid

08 HBW: Handbook of the Birds of the World

09 ddRAD: Double digest restriction-site associated DNA

10 ESU: Evolutionary Significant Unit

General Summary

This work tackles the evolutionary biology and phylogenetic relationships of Old World sparrows, particularly snowfinches within the Passeridae family. The thesis also underscores the Qinghai-Tibet Plateau's (QTP) pivotal role in shaping the diversification and distribution of these birds.

The phylogenetic relations of Old World sparrows

The first paper (Päckert et al., 2021) of this thesis studies the diversity and adaptation of Old World sparrows, highlighting their ability to thrive in diverse environments across the Palearctic, Afrotropics, and parts of the Oriental Region. This work extends our phylogenetic understanding of the Passeridae family by integrating a more comprehensive taxon sampling (18 previously not genotyped species of genus *Passer* and 11 species from other related genera), primarily from underexplored and previously poorly researched areas like the Afrotropics, resolving ongoing questions regarding the evolutionary relations within the family (Jønsson and Fjeldså, 2006). It also investigates the complex evolutionary history of snowfinches, which have mainly adapted to extreme alpine ecosystems, primarily on the QTP. Overall, the inclusion of these formerly unsampled species has refined our understanding of how different species within Passeridae have diverged and adapted to different environments and prompts a re-evaluation of the family's biogeographic diversification and speciation processes.

Evolutionary history and dispersal of snowfinches:

The second paper (Islam et al., 2024) of this thesis discusses the evolutionary history of snowfinches, tracing their origins back to in-situ speciation events on the QTP during the Miocene. This study incorporates genome-wide SNP data with mitochondrial sequences obtained from next-generation sequencing (NGS), improving previous studies which relied only on mitochondrial markers, frequently resulting in contradictory tree topologies due to the limited resolution of these markers (Cobos et al., 2021; Lei et al., 2014). The results illuminated the evolutionary relationships of the snowfinches (genera *Montifringilla*, *Pyrgilauda* and *Onychostruthus*), resolving longstanding phylogenetic uncertainties in the group. In this project, we included the first genetic analysis of the Afghan snowfinch (*Pyrgilauda theresae*), an endemic species with a very limited range within Afghanistan. Due to fieldwork restrictions, sampling in this region is impossible today. Therefore, we relied to the historical museum collections, highlighting the important role of natural history collections in modern biodiversity research. This chapter also explores the dispersal patterns of these

birds from the QTP to other regions, highlighting the plateau's role as a centre of biodiversity and a source region of cold-adapted Palearctic species. Overall, this work increases our understanding of the intraspecific diversification of the white-winged snowfinch (*Montifringilla nivalis*) and snowfinch phylogeny, providing a methodological framework for addressing similar questions in other avian taxa.

Genomic Insights into Intraspecific Diversification in the species *Montifringilla nivalis*

The third paper (Islam et al., in prep.) explores the intraspecific diversification *in Montifringilla nivalis*. The work uses an integrative taxonomy method, combining population-wide analysis using genome-wide SNPs and detailed morphological assessments and species distribution modelling. This paper reveals that environmental factors and historical biogeography have driven the diversification within *M. nivalis*. This research also highlights the Qinghai-Tibet Plateau as a crucial centre of diversification, where fluctuating environmental conditions across altitudinal gradients have driven genetic and phenotypic differences within *M. nivalis* populations and other bird species. This work sheds light on the mechanisms of speciation within *M. nivalis* population across Eurasia; it discloses the complexity of the evolution of this species, highlighting the need for more detailed phylogenetic and population studies of *M. nivalis* and a better understanding of their population structure. Overall, this chapter underscores the importance of integrating genomic, morphological data and phylogenetic analysis to study intraspecific diversification, and offers a broader understanding of the evolutionary processes that shape speciation in mountainous environments.

Aims of the Thesis

The objective of this thesis is to understand the phylogenetic relationships of the Passeridae (Aves) family, with the focus on the snowfinches' (genera *Montifringilla*, *Pyrgilauda* and *Onychostruthus*) historical biogeography across the Eurasian mountain systems. We address the following key objectives:

a). Firstly, we wanted to include more species in the phylogeny of the Passeridae family.

b). Secondly, we wanted to utilize mitogenome markers and genome-wide single nucleotide polymorphisms (SNPs) to generate the first taxon-complete phylogeny of snowfinches (genera *Montifringilla*, *Pyrgilauda* and *Onychostruthus*) at the species-level to resolve taxonomic inconsistencies connected to their position in the phylogenetic tree. Moreover, this research explains the influence of the Qinghai-Tibet Plateau (QTP) on the diversification of snowfinches.

c). Finally, we wanted to explain the intraspecific diversification of the white-winged snowfinch (*Montifringilla nivalis*), with a focus on the historical diversification across Eurasian mountain systems. Moreover, we explained the divergence in phenotypic traits between European and Asian clades and tried to explain evolutionary history of the species *Montifringilla nivalis* by modelling past and present distributions, along with morphological analyses.

Introduction

The Qinghai-Tibet Plateau (QTP), the highest and youngest plateau on Earth (Deng et al., 2020), is commonly documented as the "cradle of evolution" (Wu et al., 2022; Wen et al., 2014) and serves as a natural laboratory for observing environmental fluctuations and the evolutionary history of many organisms (Zheng & Yao, 2006; Chang & Miao, 2016; Yao et al., 2017). Its unique climatic conditions driven by extreme elevation, irregular geography, and harsh climate—have adopted the diversification of countless lineages (Liu et al., 2021; Favre et al., 2015). Geological and paleoenvironmental reconstructions suggested that over geological time, the QTP has undergone complex and large-scale environmental changes (An et al., 2001; Royden et al., 2008; Wang et al., 2008; Zhu et al., 2013; Spicer, 2017; Su et al., 2019a, 2019b), which have reformed regional climate regimes and shaped isolated refugia, particularly since the mid-Miocene (Spicer et al., 2003; Favre et al., 2015). Mountains are crucial ecosystems for global biodiversity, accommodating over 85% of the world's species of amphibians, birds, and mammals (Rahbek et al., 2019). These regions harbour numerous endemic plants and animals, many of which are considered biodiversity hotspots. There are 36 globally recognized biodiversity hotspots located in tropical regions (Myers et al., 2000), and many are allied with large mountain systems such as the Andes, the East African Arc, and the Himalayas (Marchese, 2015).

The Qinghai-Tibet Plateau (QTP) recognized as one of the areas of the Eurasian mountain systems with the highest species richness both of plants and of animals (Fjeldså et al., 2012; Li et al., 2022). The QTP boundaries are connected to the four different hotspots of biodiversity (Fig. 1) which are listed among the main biodiversity hotspots of the Northern Hemisphere (Tang et al., 2006). The organismic biodiversity of the QTP is highest along its forested boundaries (Favre et al., 2015), southwestern China, the eastern Himalayas, and the Central Asian mountains, representing an important richness in biodiversity (Myers et al., 2000). Geological fluctuations on the QTP have designed climate patterns and facilitated species dispersal (Jacques et al., 2011). Although the high alpine environments of the QTP harbour comparatively few species, this region has played an important role in species origin, diversification and global dispersal of species, making it a key centre for global species formation and dispersal (Fjeldså et al., 2012; Hoorn et al., 2018; Muellner-Riehl et al., 2019). Many

cold-tolerant species that originated in the QTP, for example, have spread widely from the plateau, achieving Holarctic distribution (Jones et al. 2017).

The Qinghai-Tibet Plateau is also home to numerous endemic species (Wen et al., 2014; Sun et al., 2017). The snow leopard (*Panthera uncia*), for example, the high-altitude cat species (Janečka et al., 2008) endemic to the QTP and adjacent areas, and in fishes an entire family, the snow carps (*Schizothoracinae*), diversified in this region and many species adapted to high-alpine environments of the plateau (Qi et al. 2015; Zhou et al., 2020). Evolutionary research suggested that adaptations during ice age events were crucial for biological community survival and they were important to the Tibetan Plateau ecosystem for millions of years (Wang et al., 2015), illustrating the plateau's role as an evolutionary hub. The uplift of the plateau created an isolated ecological environment that facilitated speciation, but many species originating here spread globally afterwards (Deng *et al.,* 2015). For instance, the seven species plant genus *Hippophae* originated in the Qinghai- Tibetan Plateau (Gu et al. 2024) and near high elevation area and later migrated to across Eurasia, in which six species (*H. goniocarpa, H. litangensis, H. gyantsensis, H. neurocarpa, H. salicifolia and H. tibetana*) are only distributed in the QTP and the adjacent Himalaya region and the one species

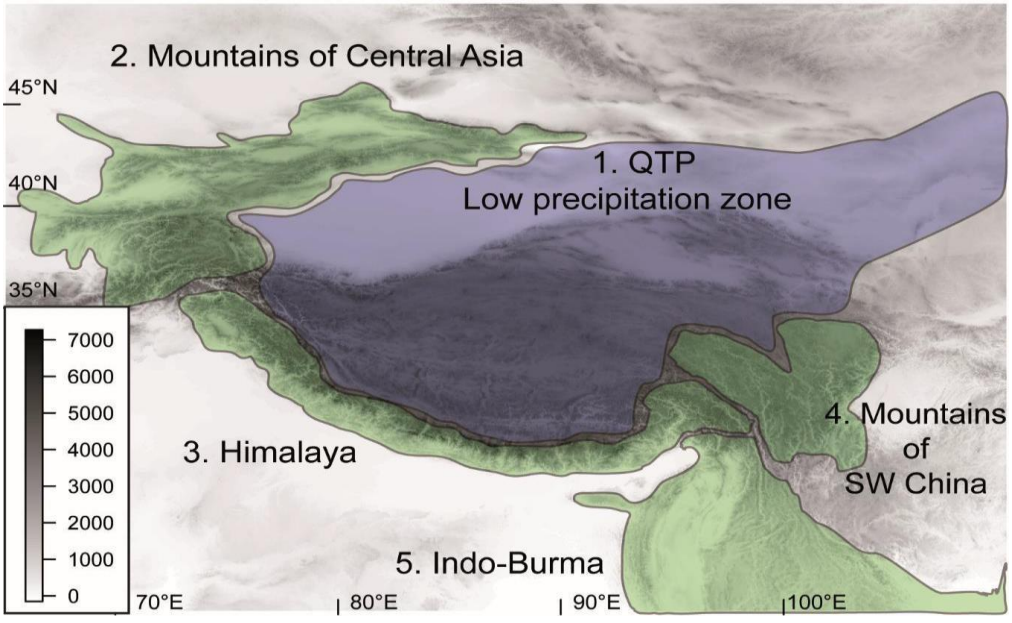

Fig 1: The Qinghai-Tibet Plateau and four biodiversity hotspots areas (modified from Favre et al., 2015).

H. rhamnoides distributed across Eurasia occurring in the QTP, northern China, Mongolia, Siberia, central Asia, Asia Minor and Europe (Jia et al., 2012). The southeastern Tibetan Plateau is recognized as the modern center of diversification snowfinch species (Päckert et al., 2020). In fact, the biodiversity and evolutionary history of forest-bird communities in the Himalayas have been widely studied (Price et al., 2014), but the alpine accumulations from the high plateau region of the QTP remain poorly understood.

Recent studies have highlighted bidirectional faunal exchange between the QTP and adjacent regions, emphasizing the complex interplay of *in-situ* diversification, dispersal and immigration processes in this area (Päckert et al., 2020). This exchange has been observed across multiple clades of passerine birds, paralleling findings observed in evolutionary studies of alpine plants (Ebersbach et al., 2017). For example, rosy-finches (*Leucosticte*) are distributed in North America, but likely trace their ancestries to the QTP (Funk et al., 2021). From this area, they dispersed and successfully colonized North America. Key to this expansion is probably their adaptation to the varied and often harsh high alpine environments (Favre et al., 2015), as recovered for many other species (Mosbrugger et al., 2018; Wang et al., 2015; Wen et al., 2014).

Phylogenetic studies suggested that the white-winged snowfinch (*Montifringilla nivalis*) (Fig. 2.A) originated from QTP (Qu et al. 2006; Islam et al. 2024) and later migrated to Eurasian alpine habitat. The Afghan snowfinch (*Pyrgilauda theresae*) also originated from QTP but presently only distributed northern parts of the Hindu Kush mountains in Afganisthan. Similarly, the Red-fronted and Great Rosefinch (*Carpodacus puniceus*) and Great Rosefinch (*Carpodacus rubicilla*) originated from QTP, later migrated across Asia, supporting the "Out-of-Tibet" hypothesis (Päckert et al., 2020). On the contrary, the "Into-Tibet" hypothesis suggested that the Ground Tit (*Pseudopodoces humilis*) and Himalayan Vulture (*Gyps himalayensis*) species originated in surrounding lower-altitude areas and later migrated into QTP (Favre et al., 2015; Johnson et al., 2006). The "Out-of-Himalayas" hypothesis explain that the Himalayas served as a source for species like rosefinches (*Carpodacus* spp.) which originated in the Himalayas and migrated into Asia and Europe (Fig. 2B) and similarly the mountain finches (genus *Leucosticte*), which originated in the Himalayas later migrated in the Nearctic area (Fig .2.C). The "multidirectional" hypothesis explains the faunal exchange events between the QTP and

its near mountains. The ancestors of the superfamily Passeroidea shows the multidirectional exchange events between the QTP and peripheral region. (Fig.2.E)

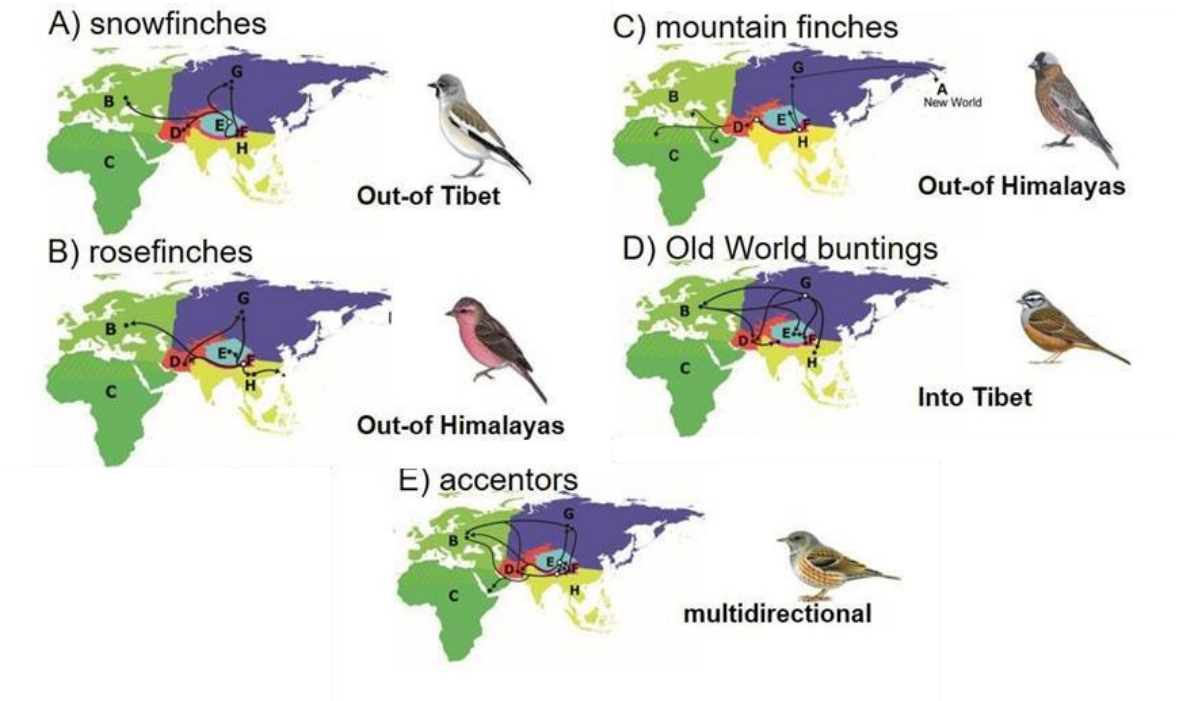

Fig 2. Multidirectional faunal exchange events between the QTP and outer regions for different bird families of the superfamily Passeroidea (modified from Päckert et al., 2020).

Our phylogenetic studies, mainly those involving Passeroidea, disclose that the oldest endemic species on the QTP date back to the early Miocene (Päckert et al., 2020). The Himalayas appeared to have played a significant role in the diversification phase of passerines from 10 to 5 million years ago, especially throughout the late Pliocene, by promoting niche divergence through the fast formation of altitudinal differences (Jønsson and Fjeldså, 2006). Päckert et al., (2020) also suggested that the QTP has represented a biogeographic source region, facilitating multiple colonization events in adjacent regions and suggestively influencing local faunal and floral communities (Favre et al., 2015).

In fact, the QTP appears to have played a central role in the evolutionary history of superfamily Passeroidea (Favre et al., 2015; Päckert et al., 2020). The oldest known Tibetan endemic passerine is Przevalski's Finch (*Urocynchramus pylzowi*), whose ancestors

diverged from their closest relatives in the late Oligocene, around 25 million years ago (Päckert et al., 2016). Several other alpine QTP endemics originated from lineage splits during the Late Miocene, approximately 10 to 7 million years ago, such as the Tibetan bunting (Päckert et al., 2015b) and the Tibetan rosefinch (Tietze et al., 2013).

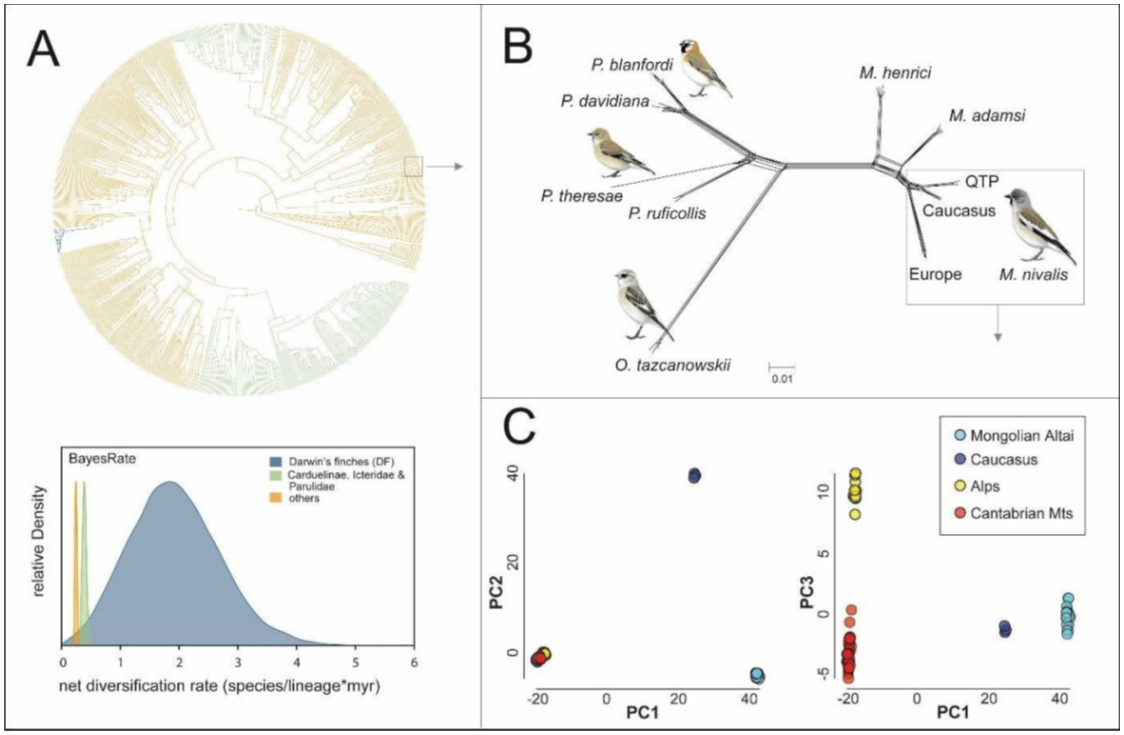

Fig. 3: The phylogeny of Passeroidea and phylogenetic network of Passeridae. A). The phylogeny of Passeroidea illustrates long evolutionary time spans and multiple, parallel radiations across various songbird families. A) Phylogeny of Passeroidea (above, n= 660 species, four markers, total alignment of 4,813 bp) and three regimes of net-diversification rates (below); the snowfinch radiation that started at approximately 8 Mya; B) neigbor network for in particular in one subclade of Passeridae, namely the snowfinches. This includes all snowfinches including Afghan *Pyrgilauda theresae* (specimen from 1968), genome-wide SNP data set, > 100,000 SNPs; C) PCoa explains the trans-Eurasian diversification of a snowfinch species namely, the white-winged snowfinch (*Montifringilla nivalis*; n= 67) started at about 2.3 to 2.7 mya, scatterplots of principal components 1, 2 and 3 from PCA based on genome-wide SNPs.

The order Passeriformes, frequently known as perching birds or songbirds, is the largest and most diverse group of birds, covering over 140 families and 6,533 species which

are enlist about 60% of all bird species worldwide (Gill et al., 2020; Ericson et al., 2003; Selvatti et al., 2015). Within this order, the family Passeridae (Old World sparrows and Snowfinches) is widely distributed across the Afrotropical, Palearctic, and parts of the Oriental regions. Today, the Passeridae family (Old World Sparrows), consists of eight genera: *Hypocryptadius, Carpospiza, Petronia, Onychostruthus, Montifringilla, Pyrgilauda, Gymnoris*, and *Passer*, encompassing a total of 43 identified species (Gill et al., 2020). These birds occupy diverse landscapes, across a wide range of territories, from the Palearctic to the Afrotropics and parts of the Oriental Region. Their ability to adapt to different environments is apparent in their wide distribution, as they have accomplished to thrive in nearly all regions of the Old World, with the exclusion of the Australian Region and Madagascar. Especially striking is the genus *Passer*, the most diverse, containing 28 species. Among these, the house sparrow (*Passer domesticus*), is a species that, unlike its relatives, has spread globally as a commensal of human civilization, largely due to human introduction (Päckert et al., 2021), and has been therefore well-studied as a case study of close association with human habitats (del Hoyo & Collar, 2016).

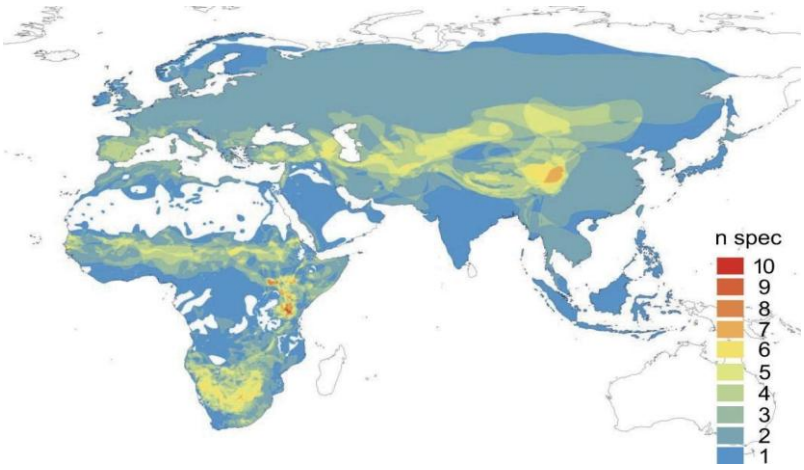

Fig. 4: Distribution of Passeridae species (Old World sparrows): Figure from Päckert et al., (2020).

Despite their global importance, phylogenetic relationships within the Passeridae family had not been thoroughly explored mainly due to a lack of complete data, particularly conflicts areas such as Afganisthan. The earliest phylogenetic hypothesis for the Passeridae, based on a single mitochondrial gene, comprised only of eleven species

(Allende et al., 2001). While Jønsson and Fjeldså (2006) published a more extensive phylogenetic hypothesis including 14 species from three genera, no multi-locus analysis had been conducted across a wider taxon sampling of Passeridae. This has hindered our efforts to understand diversification patterns across the Passeridae family, which features two key diversity hotspots: the African Rift Valley and the eastern edge of the Qinghai-Tibetan Plateau (QTP) (Fig. 3). The QTP, in precise, stands out for its species richness among snowfinches. By carefully analyzing the role of the QTP, we aim to highlight its importance of the QTP in their Eurasian early diversification and subsequent role as a centre of origin and destination for many Passeridae species.

In my PhD projects, we explored the multilocus phylogeny of the Passeridae (chapter 1) family and added unexplored species. We used genome-wide single nucleotide polymorphisms (SNPs) to construct the first taxon-complete phylogeny of snowfinches (chapter 2). We analysed the intraspecific diversification of the white-winged snowfinch (*Montifringilla nivalis*), focusing on its historical spread across Eurasian mountain systems (chapter 3). Additionally, we investigated divergence in phenotypic traits between European and Asian clades, modeling both past and present distributions to understand the evolutionary history of *Montifringilla nivalis*.

Chapter 1: A revised multilocus phylogeny of Old World sparrows (Aves: Passeridae)

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A revised multilocus phylogeny of Old World sparrows (Aves: Passeridae)

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Abstract

The Old World sparrows include some of the best-studied passerine species, such as the cosmopolitan human commensal, the house sparrow (*Passer domesticus*) as well as poorly studied narrow-range endemics like the Iago sparrow (*P. iagoensis*) from the Cape Verde Archipelago or specialists from extreme environments like the desert sparrow (*P. simplex*). It is therefore notable that to date the most complete phylogenetic hypothesis for the Old World sparrows comprised only ten of 43 currently accepted species. With this study we provide an updated phylogeny of Passeridae covering about two third of the family's species richness. Though still being far from taxon-complete, this new phylogenetic hypothesis provides firm evidence to clarify some open taxonomic questions. All genus-level taxa were reciprocally monophyletic with strong support. Contrary to previous classifications, bush sparrows and rock sparrows were not sister taxa, and therefore their classification in separate genera *Gymnoris* and *Petronia* is justified. Plumage color traits like the yellow throat patch of the latter two genera or head color pattern in *Passer* species do not provide reliable phylogenetic information, except for the large-sized African grey-headed sparrows that resulted as a monophyletic group (*P. diffusus*, *P. griseus*, *P. gongoensis*). Unexpectedly, two small-sized species, *P. eminibey* and *P. luteus* that to date are regarded as close relatives were firmly nested in two separate clades of *Passer* sparrows. Therefore, their separate generic treatment under *Sorella eminibey* and *Auripasser luteus* (together with *A. euchlorus*) does not seem justified.

Keywords

bush sparrows, introns, mitochondrial DNA, snowfinches, systematics, taxonomy

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Introduction

The Old World sparrows, Passeridae, are a speciose passerine family distributed all over the Afrotropics, the Palearctic and parts of the Oriental Region. Throughout the entire Old World, only the Australian Region and Madagascar are not inhabited by any species of the family – except the human-introduced house sparrow. Several species are highly adapted to extreme environments such as the snowfinches (*Montifringilla*, *Pyrgilauda* and *Onychostruthus*) from the high alpine ecosystems of Eurasian mountain systems (Lei et al. 2014; Päckert et al. 2020). Recent comparison of high-quality genomes provided evidence of divergent adaptation to local selective pressures in each of the three snowfinch genera (Qu et al. 2021). Also, the extremely hot and dry Sahara harbors suitable habitat for specialists like the Desert sparrow, *Passer simplex*. Areas of highest species richness are located in the African Rift Valley and at the eastern margin of the Qinghai-Tibet Plateau (QTP) (Fig. 1).

Though formerly included in Passeridae (e.g. Dickinson 2003), the sparrow-weavers (genera *Plocepasser*, *Histurgops*, *Pseudonigrita* and *Philetarius*) had often been affiliated to the Ploceidae based on morphological features like tongue musculature (Bock and Morony Jr 1978; Summers-Smith 2010). Recent phylogenies by de Silva et al. (2017, 2019) confirmed the inclusion of sparrow-weavers in Ploceidae (compare also Jønsson and Fjeldså 2006) in accordance with most taxonomic authorities (Dickinson and Christidis 2014; del Hoyo and Collar 2016; Clements et al. 2019; Gill et al. 2020). The Passeridae are characterized by several synapomorphies of tongue morphology, too (Bock and Morony Jr 1978) and representatives of major genera (*Montifringilla*, *Passer* and *Petronia*) belong to a monophyletic group that was consistent across several recently published phylogenies (e.g. Ericson and Johansson 2003; Zuccon et al. 2012).

To date, the Passeridae are generally classified into eight genera, four of them monotypic (*Hypocryptadius*, *Carpospiza*, *Petronia* and *Onychostruthus*), with a total number of 43 currently accepted species (according to the IOC World Bird List by Gill et al. 2020). Among these, *Passer* is the most diverse genus with 28 currently recognized species (del Hoyo and Collar 2016; Gill et al. 2020), of which the house sparrow, *Passer domesticus* (Fig. 2C), is probably one of the best studied species (reviews in Anderson 2006; Liebl et al. 2015), not least because as a commensal of human civilization it is fairly common all over its range (Sætre et al. 2012). Moreover, past and extant hybridization of the house sparrow with other conspecifics has been intensively studied on a genetic basis with respect to the stabilized hybrid form *Passer italiae* (Elgvin et al. 2011, 2017; Hermansen et al. 2011, 2014; Eroukhmanoff 2013, 2017; Sætre et al. 2017; Runemark et al. 2018), to distinct genetic lineages in Asia (Ravinet et al. 2018) and to the mosaic hybrid zone with the Spanish sparrow, *P. hispaniolensis*, in North Africa (Belkacem et al. 2016; Päckert et al. 2019).

In contrast, the phylogenetic relationships among genera and species of Passeridae are poorly studied to date, which is mainly due to a lack of data from the Afrotropics. Recently, it came out as a rather surprising finding, that the Philippine endemic cinnamon ibon, *Hypocryptadius cinnamomeus*, was sister to a clade of Passeridae species (Fjeldså et al. 2010). Previously, that Philippine endemic had been included in the white-eyes (Zosteropidae), however based on molecular phylogenetic evidence this species is included in Passeridae by several taxonomic authorities today (del Hoyo and Collar 2016; Gill et al. 2020).

A first phylogenetic hypothesis for Passeridae was based on a single mitochondrial gene (Allende et al. 2001) and included only eleven species. Since then, a few molecular studies focused on the phylogenetic relationships of snowfinches (*Onychostruthus*, *Pyrgilauda*, *Montifringilla* [Fig. 2A]), a group of eight high alpine endemic species from the QTP and from other Palearctic mountain systems (Qu et al. 2006; Gebauer et al. 2006; Lei et al. 2014; del Mar Delgado et al. 2019; Päckert et al. 2020). However, to date no multi-locus analysis has ever been performed for a broader taxon sampling across different genera of Passeridae. **T**he most comprehensive phylogenetic hypothesis available for Passeridae by Jønsson and Fjeldså (2006; their Passeroidea clade 8) included 14 species from three genera.

As a contribution to the current discussion on phylogenetic relationships within Passeridae, we provide a new phylogenetic hypothesis for 18 species of Old World sparrows (*Passer*) and another 11 species of African bush-sparrows (*Gymnoris*), rock sparrows (*Petronia,* [Fig. 2B]) and snowfinches (*Onychostruthus*, *Pyrgilauda*, *Montifringilla*) from the Qinghai-Tibet Plateau and other Palearctic mountain systems.

Methods

We amplified and sequenced four molecular markers using 65 samples from 22 species of the Passeridae genera *Passer*, *Petronia*, *Gymnoris*, *Montifringilla*, *Pyrgilauda* and *Onychostruthus*. Based on previous evidence of intraspecific diversification from Päckert et al. (2020) we included some additional subspecific taxa of *Montifringilla nivalis* and *Petronia petronia* (Table 1), further samples from different island populations of the Cape Verde endemic Iago sparrow (*Passer iagoensis*; Fig. 2D) and from the range of overlap of two of the smaller snowfinch species (*Pyrgilauda blanfordi* and *P. davidiana*; further samples for intraspecific comparison, see supplementary Table S1).

We extracted DNA from frozen blood or tissue samples using the innuPREP DNA Mini Kit (for muscle tissue) or the innuPREP BloodDNA Mini Kit (for blood), respectively (both Analytik Jena AG, Germany) accord-

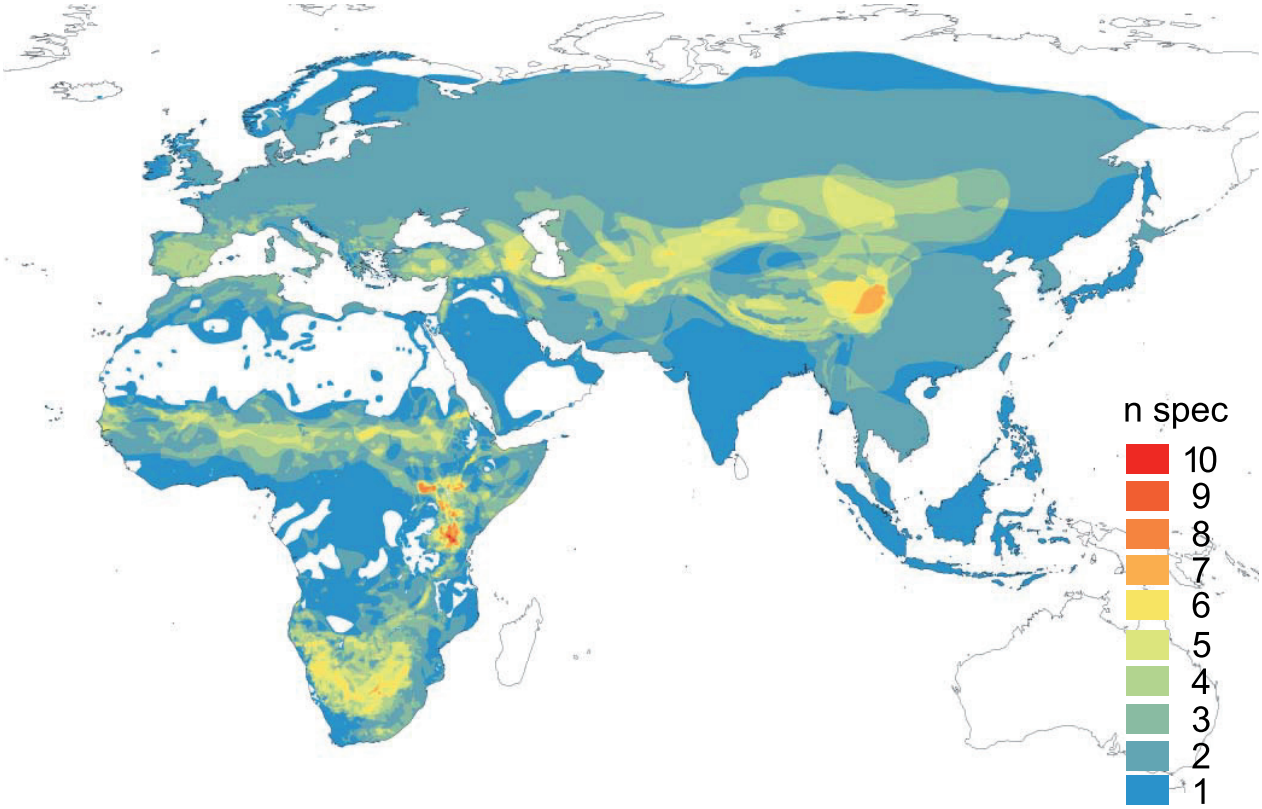

Figure 1. Diversity heat map of Old World sparrows (Passeridae) with two hotspots of diversity in the African Rift Valley and at the eastern margin of the Qinghai-Tibet Plateau; modified from Päckert et al. (2020).

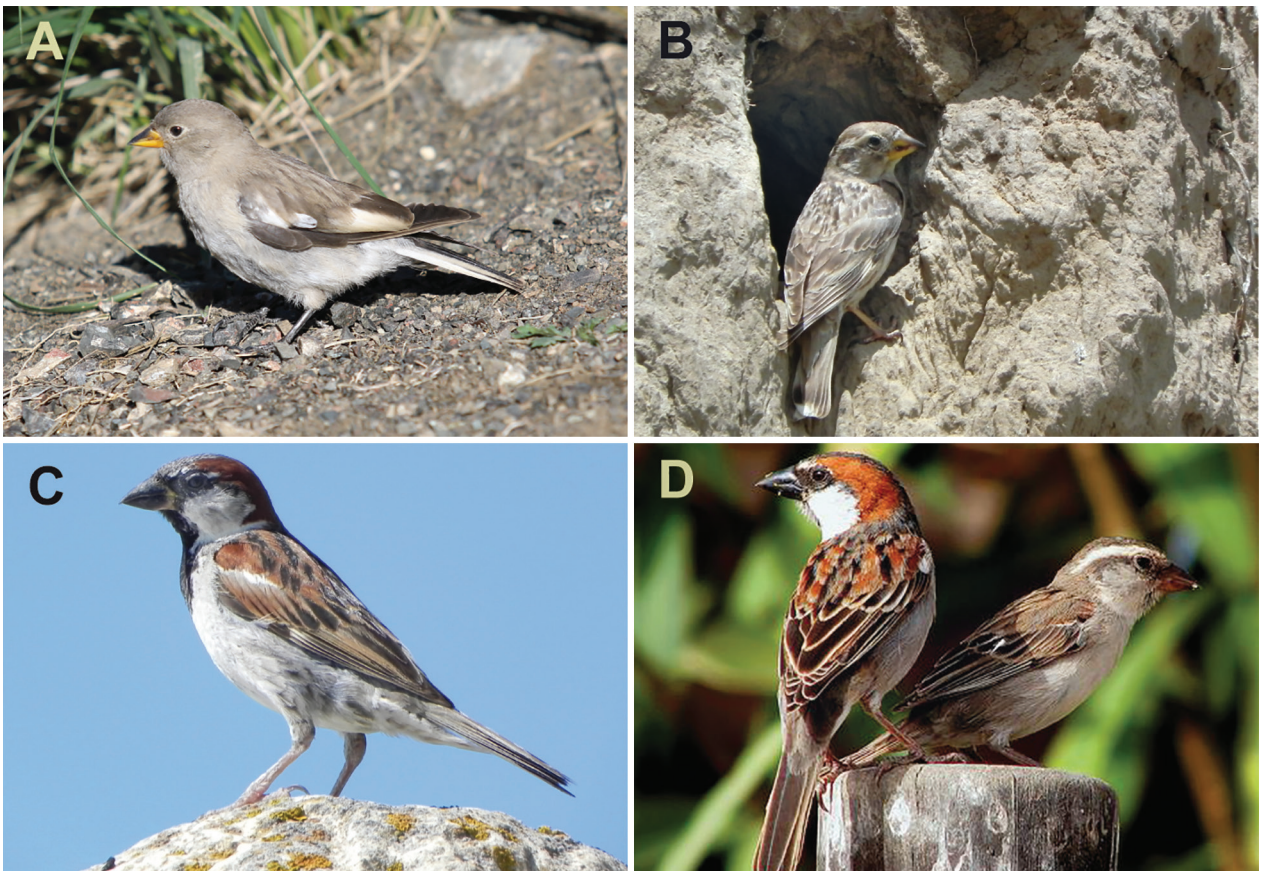

Figure 2. Selected study species of Old World sparrows, Passeridae; A) white-winged snowfinch, *Montifringilla nivalis* (photo: DL, Gobi Altai, Mongolia); B) rock sparrow, *Petronia petronia*, at nesting hole (photo: MP, China Qinghai); C) house sparrow, *Passer domesticus* (photo: MP, Greece, Santorini); D) Iago sparrow, *Passer iagoensis*; (photo: SH, Cape Verde Islands).

Table 1. Samples and sequences used for phylogenetic reconstruction; collections who donated samples for this study: MTD = Senckenberg Natural History Collections Dresden (SNSD), Museum of Zoology, Germany (MAR= tissue sample collection J. Martens at SNSD); IPMB= Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany; ZMUC = Zoological Museum of the University of Copenhagen, Denmark (NHMD = Natural History Museum of Denmark); UWBM = Burke Museum of Natural History and Culture, Seattle, USA– further collection acronyms (GenBank sequences; sample numbers marked with an asterisk): NRM = Natural History Museum of Stockholn, Sweden; ANSP = The Academy of Natural Sciences of Drexel University, USA; FMNH = The Field Museum of Natural History, Chicago, USA; CAS = Chinese Academy of Sciences, Institute of Zoology, Beijing, China.

sample no	species (Gill et al. 2020)	family	country	location	cytb	ND2	myo2	ODC
NRM 986044*	*Bombycilla garrulus*	Bombycillidae	Sweden	—	AY228049	DQ466855	AY228286	EU680709
NHMD135615*	*Amandava amandava*	Estrildidae	Captivity	—	KJ456191	KJ455319	KJ454750	KJ455720
ZMUC0785*	*Cryptospiza reichenovii*	Estrildidae	—	—	AY228056	GU816843	AY228293	EU680719
GenBank*	*Erythrura gouldiae*	Estrildidae	—	—	AY495403	AF407030	U40496	—
GenBank*	*Lonchura malacca*	Estrildidae	India	—	KJ456324	KJ455481	KJ454825	—
CAS:92755*	*Lonchura punctulata*	Estrildidae	China	—	KJ456325	KJ455482	KJ454826	KJ455824
ZMUC1425*	*Hypocryptadius cinnamomeus*	Passeridae	Philippines	Mindanao, Katanglad Volcano	—	FJ460769	GU816939	GU816916
MAR2212	*Montifringilla adamsi*	Passeridae	China	Qinghai, Huashixia	MN337349	MN337357	MN337368	MN337374
MAR2004	*Montifringilla henrici*	Passeridae	China	Qinghai, Nanshan	DQ244059	MN337360	MN337369	MN337376
MTD C64406	*Montifringilla nivalis nivalis*	Passeridae	Italy	Dolomites	KX109628	KX109703	KX109668	KX109742
MAR3111	*Montifringilla nivalis groumgrzimaili*	Passeridae	Mongolia	Bondoch Gol, Altai	MN337353	MN337362	MN337371	MN337378
MAR1532	*Montifringilla nivalis alpicola*	Passeridae	Russia	Dagestan, Kurush	MN337352	MN337361	MN337370	MN337377
MAR1775	*Pyrgilauda blanfordi*	Passeridae	China	Qinghai, highlands near Madoi	MN337350	MN337358	MN337366	—
MAR2093	*Pyrgilauda davidiana*	Passeridae	China	Qinghai, Heimahe	MN337351	MN337359	MN337367	MN337375
NC_25915	*Pyrgilauda davidiana*	Passeridae	China	—	NC_25915	NC_25915	—	—
MAR2206	*Pyrgilauda ruficollis*	Passeridae	China	Qinghai, Heimahe	MN337354	MN337363	AY228306	GU816915
MAR426	*Onychostruthus tazcanowskii*	Passeridae	China	Qinghai, Heimahe	MN337355	MN337364	MN337372	MN337380
MAR8787	*Passer ammodendri*	Passeridae	Mongolia	Gobi Altai, Echin Gol oasis	MT210107	MT210145	MT277434	MT336206
UWBM95153	*Passer diffusus*	Passeridae	South Africa	Vorstershoop, 10 km W	MT210109	MT210144	MT277435	MT336207
MTD C64358	*Passer domesticus*	Passeridae	Germany	Saxony, Dresden	KX109629	KX109704	KX109669	KX109743
MTD 2012-202	*Passer eminibey*	Passeridae	captivity	—	MT210111	—	MT277436	MT336208
GenBank*	*Passer flaveolus*	Passeridae	Vietnam	—	AF230907	—	—	—
ZMUC117473	*Passer gongoensis*	Passeridae	Kenya	Samburu Serena Lodge	MT210112	MT210140	MT277437	MT336209
GenBank*	*Passer griseus*	Passeridae	Senegal	—	AF230908	—	—	—
IPMB9505	*Passer hispaniolensis*	Passeridae	Spain	Lanzarote	MT210113	MN488960	MT277438	MT336210
MAR4076	*Passer iagoensis*	Passeridae	Cape Verde	Sal, Buracona	MT210104	MT210136	MT277439	MT336211
SOC4*	*Passer insularis*	Passeridae	Yemen	Socotra	EU478434	—	—	—
ITA1	*Passer italiae*	Passeridae	Italy	Pantelleria	MT210114	KX370756	MT277440	MT336212
NRM20106041*	*Passer luteus*	Passeridae	Nigeria	—	AY495394	GU816846	GU816938	GU816913
MAR7031	*Passer melanurus*	Passeridae	Namibia	Sossusvlei-Namtip	MT210106	MT210142	MT277441	MT336213
ISR237192*	*Passer moabiticus*	Passeridae	Israel	—	MF767302	—	—	—
UWBM95160	*Passer motitensis*	Passeridae	South Africa	Vorstershoop, 10 km W	MT210110	MT210147	MT277442	MT336214

sample no	species (Gill et al. 2020)	family	country	location	cytb	ND2	myo2	ODC
NRM976359*	*Passer montanus*	Passeridae	Sweden	—	AY228073	GU816845	AY228311	DQ785937
MAR6957	*Passer cinnamomeus*	Passeridae	China	Sichuan, Mamize Nat. Res.	MT210105	MT210143	MT277443	MT336215
PS3	*Passer simplex*	Passeridae	Algeria	—	MZ005607	MZ005629	MZ054179	MZ054180
UWBM66486	*Petronia petronia brevirostris*	Passeridae	Russia	Mongun-Taiginskiy Kozhuun	MT210108	MT210141	MN337373	MN337381
GenBank*	*Petronia petronia petronia*	Passeridae	Spain	Madrid	AF230914	—	—	—
ANSP:25010*	*Gymnoris superciliaris*	Passeridae	—	—	KJ456382	KJ455547	KJ454861	KJ455859
AV20*	*Gymnoris xanthocollis*	Passeridae	India	—	KF289836	—	—	—
GenBank*	*Gymnoris dentata*	Passeridae	Ghana	Gbele Resource Reserve	—	KY120916	KY201280	—
NRM20076168*	*Dinemellia dinemelli*	Ploceidae	—	—	—	GU816840	GU816935	GU816908
ZMUC O1706*	*Euplectes ardens*	Ploceidae	—	—	—	GU816841	KY201263	GU816909
GenBank*	*Euplectes progne*	Ploceidae	—	—	AY228061	—	AY228299	—
FMNH357374*	*Ploceus cucullatus*	Ploceidae	DR Congo	—	AF290141	AF290104	EU740022	—
NHMD 118547*	*Ploceus manyar*	Ploceidae	—	—	KJ456410	KJ455581	KJ454869	KJ455877
UWBM83556*	*Ploceus philippinus*	Ploceidae	Singapore	Pasir Ris, 1 km NW	KJ456411	KJ455583	KJ454870	KJ455878
MTD C64770	*Urocynchramus pylzowi*	Urocynchramidae	China	Qinghai, near Heimahe	KX109639	KX109715	KX109682	KX109758
GenBank*	*Vidua chalybeata*	Viduidae	—	—	NC_000880	NC_000880	EU740058	—
NRM20026168*	*Vidua macroura*	Viduidae	—	—	DQ270405	GU816842	GU816936	GU816910

ing to the manufacturer's instructions except for overnight incubation of tissue with proteinase K (instead of one hour).

We amplified and sequenced the mitochondrial cytochrome-*b* (cyt-*b*) for all samples available for comparison with the *Passer* phylogeny by Allende et al. (2001). For multi-locus reconstruction we sequenced one further mitochondrial gene, NADH-dehydrogenase subunit2 (ND2) and two nuclear introns, myoglobin-intron2 (myo) and ornithine-decarboxylase intron7 (ODC). Primers and PCR protocols are documented in Päckert et al. (2020). PCR products were purified using ExoSap-IT (GE Healthcare; adding 0.1 mL ExoSap-IT solution in 4 mL H2O to each sample; 37 °C for 30 min, 94 °C for 15 min). The sequencing of the PCR products was performed with BigDyeTM 3.1 Dye Terminator Cycle Sequencing Kits (Applied Biosystems), according to the manufacturers' instructions. Cycle sequencing products were purified by salt/ethanol precipitation or by using Sephadex (GE Healthcare, Munich, Germany), and sequenced in both directions on an ABI 3130xl DNA sequencer.

We aligned forward and reverse Sanger sequences for each gene by ClustalW using MEGA 5.1 (Tamura et al. 2011) and we cross-checked the respective electropherograms with Chromas v.2.6.5 (Technelysium Pty Ltd) for possible inaccuracies due to sequencing or reading errors. For each marker per sample, we manually combined sequences of both reading directions to a single consensus sequence. All sequences used for analysis were deposited at GenBank (Table 1).

Newly generated sequences were incorporated in a sequence alignment for Passeroidea from Päckert et al. (2016, 2020), including outgroup taxa from closely related families Ploceidae, Viduidae, Estrildidae and Urocynchramidae (Table 1). The final alignment comprised 3485 base pairs (cyt-*b*: 1041 bp; ND2: 1041 bp; myo: 732 bp; ODC: 671 bp). We complemented our sequence data set for Passeridae with sequence data from GenBank for eight species missing from our sampling including the cinnamon ibon, *Hypocryptadius cinnamomeus* (Table 1). Altogether, our final data set comprised 30 species of Passeridae among these 18 out of 28 currently recognized species from genus *Passer* (del Hoyo and Collar 2016). These are more than two third of all species from this genus (see Table 1) and twice as many species-level taxa compared to the most recent phylogenetic hypothesis for Passeridae (Jønsson and Fjeldså 2006). For hierarchical outgroup rooting we used the waxwing, *Bombycilla garrulus* (compare Päckert et al. 2020).

We reconstructed multi-locus phylogenies using Bayesian inference of phylogeny BEAST vers. 1.8.1 (Drummond et al. 2012) and Maximum Likelihood (ML) using RAXML (Stamakakis 2006, 2014). We relied on the partitioning scheme applied to the Passeroidea data set by Päckert et al. (2020) who included Passeridae with 26 species. According to their estimates using PARTITIONFINDER (Lanfear et al. 2012) the best-fit partition scheme was a nine-partition scheme by gene and codon: ND2, 1041 bp, three partitions by codon position, GTR +Γ+I model; cytochrome-*b*, 1041 bp, three partitions by codon position,

GTR +Γ+I model; myo, 730 bp, one partition, HKY+Γ model; ODC, 643 bp, one partition, GTR+Γ model.

For inference of divergence times estimates, we applied a molecular clock calibration using mean substitution rate estimates for the two mtDNA markers estimated by Lerner et al. (2011) for Hawaiian honeycreepers (Drepanidinae): cyt-*b*= 0.014; ND2= 0.029 (both in in substitutions per site per lineage per million years). The cyt-*b* rate applied here ranges at a similar dimension like the empirical cyt-*b* rate of 0.0105 evaluated by Weir and Schluter (2008).

We performed three independent runs with BEAST for 30,000,000 generations (parameters were logged and trees sampled every 3,000 generations) under the uncorrelated lognormal clock model for all loci with the "auto-optimize" option activated and a birth-death process prior applied to the tree. We combined log files and tree files from independent BEAST runs with used LOGCOMBINER v.1.8.1 and checked the combined log file in TRACER v. 1.4 (Rambaut and Drummond 2007) to ensure adequate ESS files for all parameters (all ESS > 200). All obtained phylograms were edited in FIGTREE vers. 1.4.2 (Rambaut 2009).

For illustration of intra- and interspecific genetic variation and divergence of selected species, we reconstructed unrooted minimum parsimony networks with PopART (http://popart.otago.ac.nz) using the "tcs network" algorithm (Clement et al. 2000). We calculated uncorrected pairwise p-distances (based on cytochrome-*b* sequences) using MEGA 5.1.

Results

The Old World sparrows resulted as a strongly supported monophyletic group from all analyses and were sister to another well supported clade including weavers (Ploceidae), Przewalski's finch (*Urocynchramus pylzowi*), estrildid finches and wydahs (Estrildidae and Viduidae; Fig. 3). The subclade of Passeridae is shown in Fig. 4. The basal split in Old World sparrows was dated to approximately 17.5 mya and separated the cinnamon ibon (*Hypocryptadius cinnamomeus*) from all other Passeridae. These were divided into two major clades.

Clade I showed a deep split at about 10 mya between the rock sparrows (genus *Petronia*; clade Ia) and the snowfinches (*Montifringilla*, *Pyrgilauda*, *Onychostruthus*; clade Ib, Fig. 4). The latter three snowfinch genera started diversifying at about 6.9 mya, a sister-group relationship between *Montifringilla* and *Pyrgilauda* (with *Onychostruthus* as the earliest offshoot) received only poor support (Fig. 4; clade Ib). However, the clade uniting *Montifringilla* and *Pyrgilauda* was characterized by a shared 3-bp deletion in myoglobin intron 2, whereas the *Pyrgilauda* clade was characterized by another 4-bp insertion in the same intron marker (Fig. 4).

Clade II includes the sister genera *Passer* (IIa) and *Gymnoris* (IIb) and each of them with strong node support. Members of Clade II shared a 23-bp deletion in ODC intron 7 (Fig. 4) and all members of *Passer* shared another 4-bp deletion in myo intron 2 (Fig. 4). Contrary to traditional systematic classification, the yellow-throated species of bush sparrows (*Gymnoris*) and rock sparrows (*Petronia*) were not closest relatives in the Passeridae phylogeny (Fig. 4).

Old World sparrows of genus *Passer* were also divided into two strongly supported clades. One entirely Afrotropical clade included seven species from Sub-Saharan Africa. One Sub-Saharan subclade included three species of grey-headed sparrows (Fig. 4: *P. griseus*, *P. diffusus* and *P. gongoensis*). Except for that monophyletic group of grey-headed sparrows, head color pattern does not reflect monophyletic units in the *Passer* clade (Fig. 4), which is once more in contrast to previous superspecific classifications. The second subclade united two species from South Africa (*P. melanurus*, *P. motitensis*) with the small-sized chestnut sparrow (*P. eminibey*) from East Africa. The Afrotropical *Passer* clade was sister to a second moderately supported clade that comprised 12 species from the Palearctic and the Oriental Region that started diversifying at about 5.5 mya (Fig. 4). Phylogenetic relationships among members of that clade were ambiguous because of poor support values for many nodes. A basal split separated the Asian russet sparrow (*P. cinnamomeus*) from the remaining *Passer* species. Two further ancient offshoots of the Palearctic/Oriental clade, the widespread tree sparrow (*P. montanus*) and the Central Asian Saxaul sparrow (*P. ammodendri*) received moderate and poor support, respectively. Another poorly supported Afro-Arabian clade of four sparrow species united the Saharan desert sparrow (*P. simplex*), the Sudan golden sparrow (*P. luteus*) from the Sahel Region, the Dead Sea sparrow (*P. moabiticus*) from the Near East and the Middle East and the Cape Verde endemic Iago sparrow (*P. iagoensis*)(Fig. 4). In the latter, no clear phylogeographic structure among island populations could be observed in the maximum parsimony network of five cyt-*b* haplotypes (Fig. 5C). Finally, a well-supported terminal clade united four closely related species that started diversifying in the early Pleistocene: the house sparrow (*P. domesticus*), the Spanish sparrow (*P. hispaniolensis*), the Italian sparrow (*P. italiae*) and the Socotra sparrow (*P. insularis*) (Fig. 4). The sister-group relationship of the Southeast Asian plain-backed sparrow (*P. flaveolus*) to that terminal clade received moderate support.

High intraspecific differentiation with split ages estimated at 2.3–2.7 Ma was found in two species of clade I: Both *Petronia petronia* and *Montifringilla nivalis* showed a deep split between European and Asian lineages (Fig. 4). Paraphyly of *M. nivalis* with respect to its Tibetan congener *M. adamsi* was only poorly supported. European and Asian populations of the white-winged snowfinch (*M. nivalis*) appeared as two distinct clusters in the cyt-*b* haplotype network separated by a minimum of 22 substitutions (Fig. 5A). Uncorrected pairwise distances between the European and the Asian mitochondrial lineage ranged between 4.9–5.1% (cyt-*b*) at the same p-distance level like interspecific comparison between *M. nivalis* and *M. ad-*

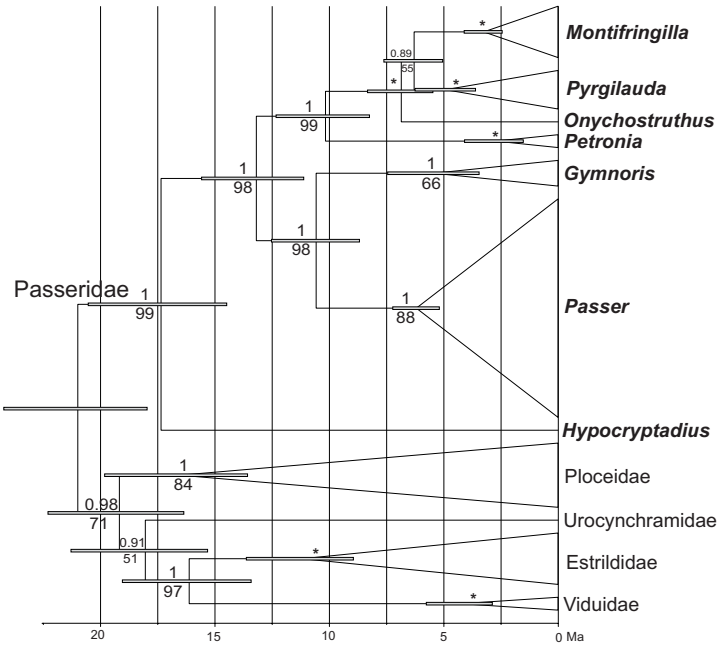

Figure 3. Phylogenetic relationships of Old World sparrows (Passeridae) and closely related outgroups weaverbirds (Ploceidae), estrildid finches (Estrildidae), indigobirds and wydahs (Viduidae) and Przewalski's finch (Urocynchramidae, monotypic: *Urocynchramus pylzowi*); combined MCMC tree from three runs with BEAST 30 Million generations each, burning 3000 trees (of 30.000 sampled trees); node support: Bayesian posterior probabilities above nodes, thorough bootstrap from RAXML below nodes.; full node support from both analyses (BI: 1.00, ML:100) indicated by an asterisk.

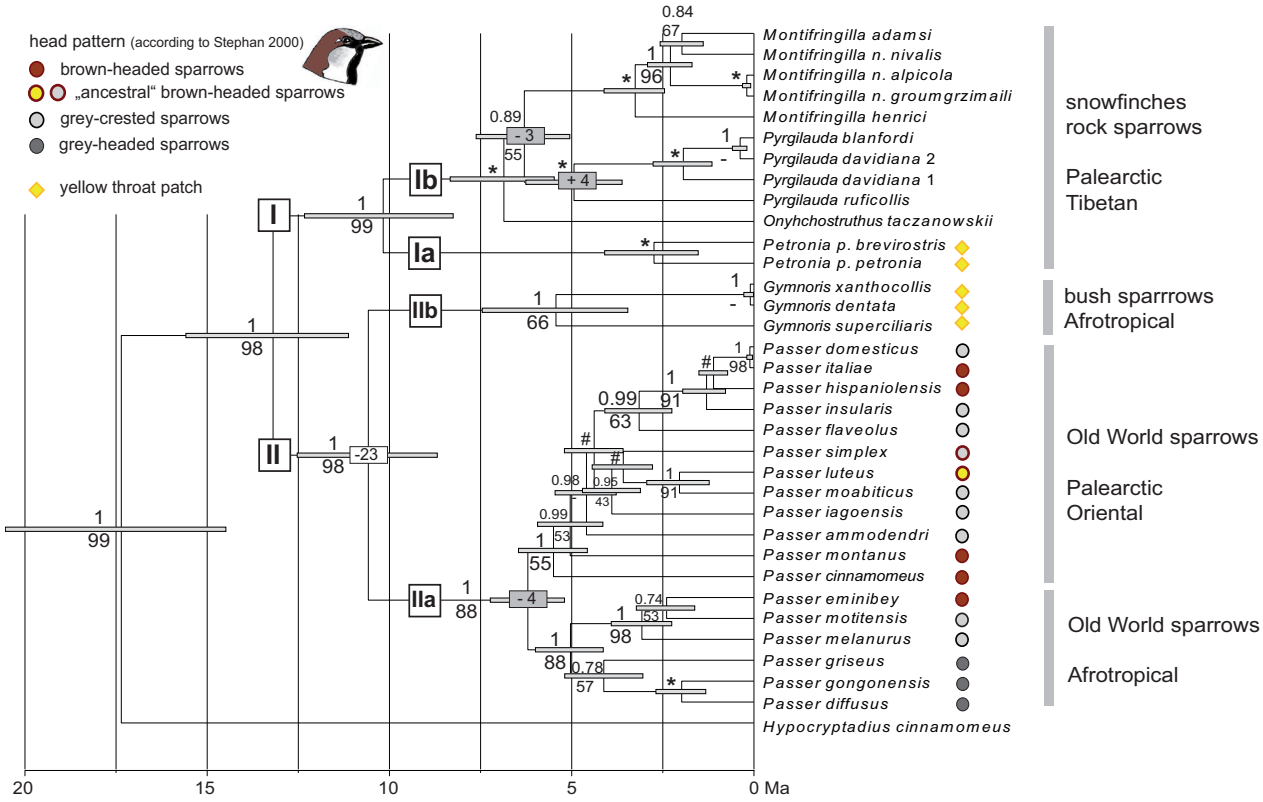

Figure 4. Inter- and intrageneric phylogenetic relationships of Passeridae; zoom on the Old World sparrow clade of the combined MCMC tree from three runs with BEAST 30 Million generations each, burning 3000 trees (of 30.000 sampled trees); node support: Bayesian posterior probabilities above nodes, thorough bootstrap from RAXML below nodes; full node support from both analyses (BI: 1.00, ML:100) indicated by an asterisk; conflicting topology in the RAXML tree indicated by "-";# = poor node support values below 0.5 (BI) and 50 (ML); bars with numbers indicate indels of nuclear introns shared by all members of the respective clade (grey= myoglobin; white= ODC; += insertion; -= deletion); variation of two male plumage color traits indicated at tip clades for species of *Passer*, *Petronia* and *Gymnoris*; head pattern according to Stephan (2000) who classified the grey-headed *P. simplex* and the Sudan golden sparrow (*P. luteus*) as ancestral forms of brown-headed sparrows.

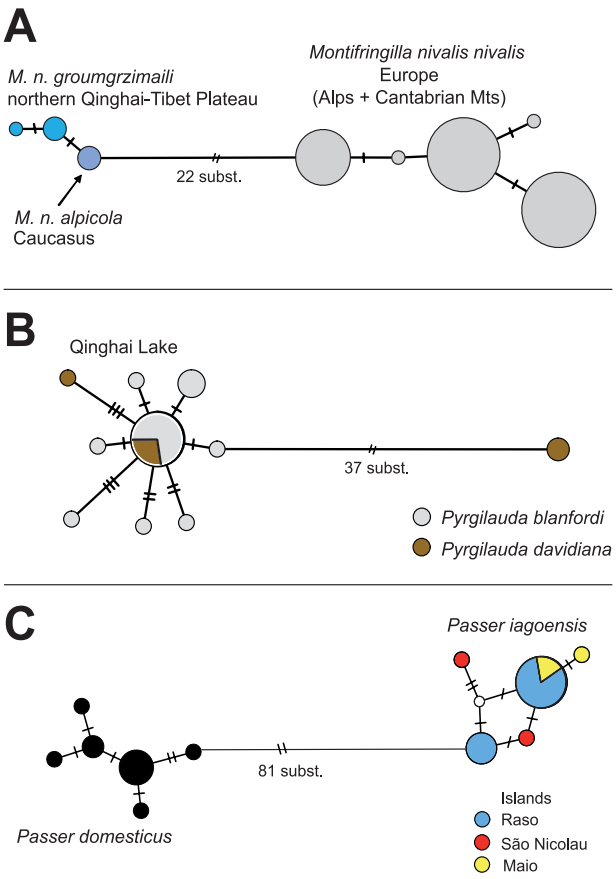

Figure 5. Haplotype networks showing intra- and interspecific variation of A) the white-winged snowfinch, *Montifringilla nivalis* (including the sequence data set by Resano-Mayor et al. 2016) based on 341 bp cytochrome-*b* (n= 87); B) Blanford's snowfinch and Père David's snowfinch (*Pyrgilauda blanfordi*, *P. davidiana*), based on 819 bp cytochrome-*b* (n=23); C) the Cape Verde endemic Iago sparrow (*Passer iagoensis*) compared to the house sparrow (*P. domesticus*; population from eastern Germany from Päckert et al. 2019) based on 773 bp ND2 (n=29).

amsi (4.5–4.8%; cyt-*b*). Similarly, uncorrected p-distances between rock sparrow populations from Spain (*P. p. petronia*) and from China (*P. p. brevirostris*) were as high as 4.8% (cyt-*b*; compare the deep split in Fig. 4). In the small-sized species of genus *Pyrgilauda*, one specimen of Père David's snowfinch, *P. davidiana*, was sister to a syntopic *P. blanfordi* specimen instead to a conspecific specimen from the northern allopatric part of the breeding range (Fig. 4; however this grouping was not supported in the RAXML tree that united both *P. davidiana* sequences in a poorly supported clade). The haplotype network for a larger set of *Pyrgilauda* samples showed that regardless of phenotypic species identification all specimens from the region of sympatry at Koko Nor in northern Qinghai belonged to one haplotype cluster that was separated from another distantly related *P. davidiana* haplotype (shared by two specimens of unknown origin) by 37 substitutions (Fig. 5B). The Koko Nor cluster had a star-like structure with eight tip haplotypes and a central haplotype shared by eleven individuals of both species (*P. davidiana* and *P. blanfordi*).

Discussion

To date, there is no comprehensive phylogeny of Old World sparrows (Passeridae) available except for a single-locus tree covering about 40% of the currently accepted species (Allende et al. 2001) and a Passeridae clade from a supertree by Jønsson and Fjeldså (2006) which was largely based on the same sequence information (see below). Though our new phylogeny still misses ten out of 28 *Passer* species we are covering 30 of 43 currently accepted species of Passeridae (about 70%) and several clear conclusions can be drawn from this new (though still incomplete) phylogenetic hypothesis. Most importantly, our results confirm the monophyly of the genera *Gymnoris*, *Passer*, *Montifringilla* and *Pyrgilauda* (the remaining genera are monotypic). This is particularly relevant with respect to the taxonomic treatment of bush sparrows and rock sparrows.

Bush sparrows and rock sparrows

Bush sparrows (*Gymnoris*) have long been merged in one genus *Petronia* together with rock sparrows (Wolters 1952; Vaurie 1956; Stephan 2000; Summers-Smith 2010: fig. 113). In their Illustrated Checklist of the Birds of the World del Hoyo and Collar (2016) classified bush sparrows in a separate genus *Gymnoris* but added a side remark that these species were "often merged into *Petronia*". Until recently, congeneric treatment of these species was even reflected by vernacular names, such as "bush petronia" and "rock petronia" (*P. dentata* and *P. petronia*, in Clements et al. 2017, 2019, with reference to Rasmussen and Anderton 2005 and to Praveen et al. 2016). A distinctive yellow throat patch that is shared by bush sparrows and rock sparrows might have been the major common trait to mislead taxonomists and to treat those species under the same genus name (Fig. 4). However, Roselaar (1995) suggested a recognition of *Gymnoris* as a genus of its own for major differences from *Petronia petronia* in other plumage traits, habitat preferences and behavior (see also Summers-Smith 2010). This recommendation was discussed by the Taxonomic Advisory Committee of the Association of European Records and Rarities (AERC TAC 2003), however, they stressed the need of a reliable phylogenetic framework and postponed a decision on this "pending category". Despite this lack of evidence from phylogenetic studies, several taxonomic authorities later restricted *Petronia* to the type species (the rock sparrow, *P. petronia*) and subsumed bush sparrows under *Gymnoris* (del Hoyo and Collar 2016; Gill et al. 2020). Jønsson and Fjeldså (2006) who to date provided the most complete phylogenetic hypothesis for Passeridae [their Passeroidea clade 8] could not show the paraphyly of *Petronia* sensu lato because their tree included only two species from this group, *Petronia petronia* and *Gymnoris pyrgita*. These two formed a monophyletic group of the Passeroidea tree (Jønsson and Fjeldså, 2008) which might be an effect of incomplete taxon sampling. Density of taxon

sampling has been repeatedly evaluated as one of the crucial factors affecting the accuracy of phylogenetic analyses and the resulting topologies (Zwickl and Hillis 2002; Heath et al. 2008; Albert et al. 2009; Nabhan and Sarkar 2011; Wiens and Tiu 2012; Tritsch et al. 2017). Though important for our phylogeny we still failed to include the yellow-spotted bush sparrow (*G. pyrgita*) from the Sahel Region, however, our tree topology clearly rejects a sister group relationship of the three remaining *Gymnoris* species and *Petronia petronia* and therefore supports their taxonomic treatment in different genera. Jønsson and Fjeldså (2006) had apparently included *G. pyrgita* as the sole bush sparrow species in their supertree (see above), however, the source of sequence information could not be inferred from the documentation in their paper. To date, there is no sequence data available for this species at Genbank.

Rock sparrows (*Petronia*) were consistently revealed as sister to snowfinches (*Montifringilla* and allies) and are therefore part of a trans-Eurasian alpine radiation (Päckert et al. 2020; this study) whereas bush sparrows (*Gymnoris*) represent a subtropical/tropical radiation across the Afrotropics, the Middle East and southern Asia (this study).

Snowfinches

Snowfinches were shown to represent a monophyletic group in previous phylogenetic studies (Qu et al. 2006; Lei et al. 2014; both based on cyt-*b* and myoglobin intron 2). Like the previous studies, our four-gene phylogeny did not fully resolve their intergeneric relationships and provided only poor support of a sister-group relationship of *Pyrgilauda* and *Montifringilla*. Future studies based on genome-wide SNPs may shed light on this. All snowfinch species except *M. nivalis* are endemics of the Qinghai-Tibet Plateau with a large area of sympatry at its eastern margin (Fig. 1). In this region, in the vicinity of Qinghai Lake we found indications of mitochondrial introgression of *P. blanfordi* haplotypes into phenotypic *P. davidiana*. Though this conclusion certainly needs further support from population genetic analyses based on nuclear markers, introgression and gene flow was documented for several regions where two sparrow species come into secondary contact (Elgvin et al. 2011; Hermansen et al. 2011, 2014; Belkacem et al. 2015; Gedeon et al. 2015; Päckert et al. 2019).

Since long, there is firm evidence from previous phylogenies of a placement of snowfinches in sparrows (Passeridae) rather than in finches (Fringillidae) – unlike for example other high-alpine specialists from the same region, the mountain finches *(Leucosticte*). These are indeed members of Fringillidae (Zuccon et al. 2010) and represent a recent radiation of East Asian faunal elements to the Nearctic (Päckert et al. 2020). Despite many recent changes of vernacular names, Gebauer et al. (2006) were the only authorities who used the names "mountain-steppe sparrows" (for *Pyrgilauda*) and "snow sparrows" (for *Montifringilla*), which is in good accordance with their sister clade, the rock sparrows (for *Petronia*). However, since the terms "sparrows" and "finches" in particular are in use for completely different bird families without any closer relationships (e.g. New World sparrows, Passerellidae, are indeed the closest relatives to buntings, Emberizidae, and were previously included in this family), a correction of vernacular names for snowfinches might not be recommendable.

Although paraphyly of the white-winged snowfinch, *Montifringilla nivalis*, did not receive strong support, divergence times between the nominate form *M. n. nivalis* and Asian subspecies (*M. n. alpicola* and *M. n. groumgrzimaili*) equal (and even exceed) those between several currently accepted *Passer* species. In fact, there has been a long debate on species-level taxa in *Montifringilla*: Both the black-winged snowfinch and the Tibetan snowfinch have been previously included in *M. nivalis* at the subspecies level (*M. nivalis adamsi*: Cramp and Perrins 1994; *M. nivalis henrici*: Vaurie 1956; Moreau and Greenway Jr 1962; Portenko and Vietinghoff-Scheel 1974; Cheng 1987). A closer relationship among *M. nivalis* and *M. adamsi* than among *M. henrici* and each of the latter two was already suggested based on morphological traits (Eck 1996; Martens and Eck 1995) and was confirmed by our phylogeny. Based on the criterion of diagnosability (Sangster 2014) with respect to phenotypes (del Hoyo and Collar 2016), vocalizations and ecology (Gebauer and Kaiser 1994; Gebauer et al. 2006) and mitochondrial lineages (Qu et al. 2006; Lei et al. 2014; Päckert et al. 2020) the three currently accepted *Montifringilla* species are currently separated at the species-level (e.g. Gill et al. 2020).

For the time being, we refrain from making any taxonomic recommendations for *M. nivalis* until further evidence for another species-level split can be inferred from population genetic studies based on a range-wide sampling (including missing *M. n. leucura* from the Near East, *M. n. gaddi* from Iran, *M. n. tianshanica* from the Central Asian mountains and *M. n. kwenluensis* from the Kunlun Shan in southwestern China; del Hoyo and Collar 2016).

Old World sparrows – the genus *Passer*

To date, phylogenetic relationships among members of the most diverse genus of Passeridae are insufficiently resolved and our study can only be considered another step further towards a taxon-complete *Passer* sparrow tree. The Passeroidea tree by Jønsson and Fjeldså (2006) is a supertree inferred from sequence data from 99 independent studies of which Allende et al. (2001) provided single-locus data (cytochrome-*b*) for all ten *Passer* species included in the final supertree. Thus, the phylogenetic hypothesis by Jønsson and Fjeldså (2006) is largely based on the cytochrome-*b*-based tree by Allende et al. (2001), and since node support values were not provided for their supertree, these phylogenetic relationships have to be interpreted with maximum caution. Except for the grey-headed sparrows, none of the major superspecific

classifications in *Passer* based on phenotypic traits is reflected by monophyletic groups in our phylogeny, neither the "grey-crested" nor the "brown-headed" sparrows, two groups classified by Stephan (2000: "Grauscheitelsperlinge" and "Braunkopfsperlinge"). His classification was based on a combination of plumage color traits (i.e. 10 traits of the facial color pattern and 17 gradually varying color patterns of single contour feathers; Figs 1, 2 and 3 in Stephan 2000). Based on this combination of traits Stephan (2000) came to some rather striking conclusions, e.g. he classified the grey-headed *P. simplex* and the entirely yellow-headed "golden-sparrows" (*P. luteus* and *P. euchlorus*) as ancestral forms of his "brown-headed sparrows" (Fig. 4). However, our phylogeny does not support Stephan's (2000) classification: Members of "brown-headed sparrows" and "grey-crested sparrows" are scattered across the two major subclades of the *Passer* clade, thus this phenotypic trait is not really informative as concerns phylogenetic relationships – as could have been expected due to a low phylogenetic signal of many morphological traits compared for example to behavioral traits, such as bird song (Cicero et al. 2020).

According to our multi-locus phylogeny, two major radiations of Old World sparrows started during the late Miocene at about 6 Mya. Six species united in a monophyletic group represent a Subsaharan radiation south of the equator. The large-sized grey-headed sparrows (*P. griseus*, *P. diffusus*, *P. gongoensis*) were often lumped in one species, Wolters (1979), however even placed them in a separate genus *Pyrgitopsis*, whereas Summers-Smith (2010) united them in one superspecies (Amadon 1964). Dickinson and Christidis (2014) treated *gongoensis* as a subspecies of the northern grey-headed sparrow, *P. griseus*, and separated *P. diffusus* at the species level (compare Dickinson 2003). However, because in our tree *P. gongoensis* was sister to the southern grey-headed sparrow, *P. diffusus*, with strong support (Fig. 4), our phylogenetic hypothesis does not support this classification.

The sister clade of the grey-headed sparrows united two representatives of the Cape fauna, *P. motitensis* and *P. melanurus*, with a small-sized East-African species, the chestnut sparrow, *P. eminibey*. This grouping is instantly surprising, because the latter species was regularly affiliated with two other small-sized 'golden sparrows', *P. luteus* and *P. euchlorus*. These three have long been regarded as rather ancient lineages of Old World sparrows without any closer relationships to other *Passer* species (Summers-Smith 2010). Our tree topology does neither support a placement of *P. eminibey* and *P. luteus* outside *Passer* nor a placement of the chestnut sparrow in a monotypic genus *Sorella* Hartlaub, 1880 (Wolters 1979). The great sparrow, *P. motitensis*, from the Cape Region was traditionally affiliated with further Subsaharan sparrow taxa. Summers-Smith (2010) distinguished "five allopatric populations" of *P. motitensis*, Dickinson and Christidis (2014) included three of them in *P. motitensis*: *P. m. chordofanicus*, *P. m. shelleyi* and *P. m. rufocinctus*. Today, they are all separated at the species-level (del Hoyo and Collar 2016; Gill et al. 2020) and their phylogenetic relationships will remain subject to future studies.

The second major clade including twelve *Passer* species represents a larger radiation across the Palearctic and the Oriental Region with an early Pliocene onset at about 5.5. Mya. The East Asian russet sparrow as the earliest offshoot from this clade was traditionally known under the scientific name *Passer rutilans* (as such included in the phylogenies by Allende et al. 2001 and by Jønsson and Fjeldså 2006; see also Clements et al. 2017). However, a recent debate on the correct dates of two competing original descriptions by C. J. Temminck and J. Gould ended up in a broad consent on the priority of the name *Passer cinnamomeus* Gould, 1835 (based on Mlíkovský 2011). Except that basal split, the position of the tree sparrow, *P. montanus*, as the second oldest offshoot and further phylogenetic relationships in this Eurasian/Oriental clade are poorly to moderately supported or even conflicting between the Bayesian and the maximum likelihood tree. For the Cape Verde endemic, *P. iagoensis*, a close relationship with Afrotropical species (*P. motitensis* and *P. melanurus*) was previously assumed (Stephan 2000), conspecific classification with *P. motitensis* was even advocated by Wolters (1979) and by Summers-Smith (2010). Our phylogenetic hypothesis clearly rejects any closer relationship of *P. iagoensis* with these two representatives of the Cape fauna, but suggests a closer relationship with *P. moabiticus* from the eastern Mediterranean and the Middle East and two Afrotropcial species: *P. simplex*, a desert-dwelling specialist from the Sahara and *P. luteus* from the Sahel Region (however with poor node support). The firm placement of the latter in the *Passer* clade is as unexpected as that of *P. eminibey* (see above), and does not support a classification of golden sparrows in a separate genus *Auripasser* (Wolters 1979; Summers-Smith 2010). Contrary to the traditional classification, our tree topology clearly rejected a closer relationship of *P. eminibey* with the Sudan golden sparrow, *P. luteus*, whereas phylogenetic relationships of the Arabian golden sparrow, *P. euchlorus*, remain an open question due to data deficiency (Summers-Smith 2010 included it in the Sudan golden sparrow as subspecies *P. l. euchlorus*). A zone of sympatry in western Sudan without evidence of interbreeding between *P. eminibey* and *P. luteus* also justifies their treatment as separate species (Summers-Smith 2010).

Finally, a well-supported terminal clade represents a very recent circum-Mediterranean/ Eurasian radiation of the house sparrow, *P. domesticus*, the Spanish sparrow, *P. hispaniolensis*, the stabilized hybrid form *P. italiae* and the Socotran endemic, *P. insularis*. According to our divergence time estimates, this radiation started during the mid-Pleistocene and according to population genetic analyses lineage separation went along with multiple independent events of horizontal gene flow between the house sparrow and the Spanish sparrow that gave rise to several hybrid lineages in the Mediterranean of different age and origin (Runemark et al. 2018; Päckert et al. 2019). A sister-group relationship of the Southeast Asian *P. flaveolus* with the latter circum-Mediterranean quartet was only poorly supported. A putative closer relationship of *P. insularis* and *P. motitensis* as suggested by Summers-Smith (2010) could be rejected by our phylogeny.

Conclusions and perspectives

Despite from being far from taxon-complete, this updated phylogeny contributed further evidence for clarification of taxonomic controversy, e.g. the status of *Petronia* and *Gymnoris* as separate genera, the monophyly of grey-headed sparrows (but not of all grey-crested *Passer* species) or a lack of phylogenetic justification for recognizing the genera *Sorella* and *Auripasser*. We failed to include the enigmatic pale rock sparrow, *Carpospiza brachydactyla*, from the Middle East and Central Asia that was long regarded as a member of Fringillidae. Based on shared traits of tongue morphology inclusion in Passeridae was recommended by Bock (2004), generally *Carpospiza* has been affiliated with *Petronia* sensu lato (including *Gymnoris*), however, it lacks the yellow throat patch (being a rather uninformative trait as shown in our phylogeny). Also, phylogenetic relationships of missing *Passer* species from India (*P. pyrrhonotus*), Central Asia (*P. zarudny*), the Socotra archipelago (*P. hemileucus*) and East Africa (*P. chordofanicus*, *P. euchlorus*, *P. rufocinctus*, *P. shelleyi*, *P. suahelicus*, *P. swainsoni*, *P. castanopterus*) will remain unresolved so far. Recently, the narrow-range endemic Somali sparrow, *P. castanopterus*, has attracted ornithologists' attention for its putative hybridization with the house sparrow, *P. domesticus*, in the areas of range overlap in Somalia (Summers-Smith 2020), Ethiopia (Gedeon et al. 2015), Kenya (Turner 2016) and Djibouti (Cohen et al. 2011; Hering et al. 2020).

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References

AERC TAC (2003) Association of European Records and Rarities Committees TAC's Taxonomic Recommendations 1st December 2003. Online version under http://www.aerc.eu/tac.html

Albert EM, San Mauro D, García-París M, Rüber L, Zardoya R (2009) Effect of taxon sampling on recovering the phylogeny of squamate reptiles based on complete mitochondrial genome and nuclear gene sequence data. Gene 441: 12–21. https://doi.org/10.1016/j.gene.2008.05.014

Allende LM, Rubio I, Ruíz-del-Valle V, Guillén J, Martínez-Laso J, Lowy E, Varela P, Zamora J, Arnaiz-Villena A (2001) The Old World sparrows (Genus *Passer*). Phylogeography and their relative abundance of nuclear mtDNA pseudogenes. Journal of Molecular Evolution 53: 144–154. https://doi.org/10.1007/s002390010202

Amadon D (1966) The superspecies concept. Systematic Biology 15(3): 245–249. https://doi.org/10.2307/sysbio/15.3.245

Anderson T (2006) Biology of the Ubiquitous House Sparrow: From Genes to Populations. Oxford University Press, 560 pp. https://doi.org/10.1093/acprof:oso/9780195304114.001.0001

Belkacem AA, Gast O, Stuckas H, Canal D, Lo Valvo M, Giacalone G, Päckert M (2016) North African hybrid sparrows (*Passer domesticus*, *P. hispaniolensis*) back from oblivion – ecological segregation and asymmetric mitochondrial introgression between parental species. Ecology and Evolution 6: 5190–5206. https://doi.org/10.1002/ece3.2274

Bock W (2004) Affinities of *Carpospiza brachydactyla* (Passeriformes; Passeridae). Journal of Ornithology 145(3): 223–226. https://doi.org/10.1007/s10336-004-0034-9

Bock W, Morony Jr JJ (1978) Relationships of the passerine finches. Bonner Zoologische Beiträge 29: 122–147.

Cheng TH (1987) A synopsis of the avifauna of China. Paul Parey, Hamburg Berlin, 1223 pp.

Cicero C, Mason NA, Benedict L, Rising JD (2020) Behavioral, morphological, and ecological trait evolution in two clades of New World Sparrows (*Aimophila* and *Peucaea*, Passerellidae). PeerJ 8: e9249. https://doi.org/10.7717/peerj.9249

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology.. 9:1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Clements JF, Schulenberg TS, Iliff MJ, Roberson D, Fredericks DTA, Sullivan BL, Wood CL (2017) The eBird/Clements checklist of birds of the world: v2017. Downloaded from http://www.birds.cornell.edu/clementschecklist/download (last accessed on 25.07.2018).

Clements JF, Schulenberg TS, Iliff MJ, Billerman SM, Fredericks DTA, Sullivan BL, Wood CL (2019) The eBird/Clements Checklist of Birds of the World: v2019. Downloaded from https://www.birds.cornell.edu/clementschecklist/download (last accessed on 30.12.2020).

Cohen C, Mills M, Francis J (2011) Endemic and special birds of Somaliland. Bulletin of the African Bird Club 18: 86–92.

Cramp S, Perrins CM (1994) Handbook of the birds of Europe the Middle East and North Africa – the birds of the Western Palearctic volume VIII – Crows to Finches. Oxford University Press, Oxford, New York, 956 pp.

Del Hoyo J, Collar NJ (Eds) (2016). Illustrated checklist of the birds of the world. Volume 2 Passerines. Lynx Edicions, Barcelona, 903 pp.

Del Mar Delgado M, Bettega C, Martens J, Päckert M (2019) Ecotypic changes of alpine birds to climate change. Scientific Reports 9: 16082. https://doi.org/10.1038/s41598-019-52483-0

De Silva TN, Peterson AT, Bates JM, Fernando SW, Girard MG (2017) Phylogenetic relationships of weaverbirds (Aves: Ploceidae): A first robust phylogeny based on mitochondrial and nuclear markers. Molecular Phylogenetics and Evolution, 109: 21–32. https://doi.org/10.1016/j.ympev.2016.12.013

De Silva TN, Peterson AT, Perktas U (2019) An extensive molecular phylogeny of weaverbirds (Aves: Ploceidae) unveils broad non-monophyly of traditional genera and new relationships. The Auk 136(3): ukz041. https://doi.org/10.1093/auk/ukz041

Dickinson EC (Ed.) (2003). The Howard and Moore complete checklist of the birds of the world. 3rd edition. Princeton University Press, Princeton and Oxford, 883 pp.

Dickinson EC, Christidis L (Eds) (2014) The Howard and Moore complete checklist of the birds of the world. 4th edition, Volume 2: Passerines. Aves Press, Eastbourne, 804 pp.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Eck S (1996) Die Paläarktischen Vögel – Geospezies und Biospezies. Zoologische Abhandlungen des Museums für Tierkunde Dresden 40 (suppl.): 1–103.

Elgvin TO, Hermansen JS, Fijarczyk A, Bonnet T, Borge T, Stein S, Sæther SA, Voje KL, Sætre GP (2011) Hybrid speciation in sparrows II: a role for sex chromosomes? Molecular Ecology 20: 3823–3837. https://doi.org/10.1111/j.1365-294X.2011.05182.x

Elgvin TO, Trier CN, Tørresen OK, Hagen IJ, Lien S, Nederbragt AJ, Ravinet M, Jensen H, Sætre GP (2017) The genomic mosaicism of hybrid speciation. Science Advances 3: e1602996. https://doi.org/10.1126/sciadv.1602996

Ericson PGP, Johansson US (2003) Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. Molecular Phylogenetics and Evolution 29: 126–138. https://doi.org/10.1016/S1055-7903(03)00067-8

Eroukhmanoff F, Hermansen JS, Bailey RI, Sæther SA, Sætre GP (2013) Local adaptation within a hybrid species. Heredity 111: 286–292. https://doi.org/10.1038/hdy.2013.47

Eroukhmanoff F, Bailey RI, Elgvin TO, Hermansen JS, Runemark A, Trier CN, Sætre GP (2017) Resolution of conflict between parental genomes in a hybrid species. *BioRxiv*. https://doi.org/10.1101/102970

Fjeldså J, Irestedt M, Ericson PGP, Zuccon D (2010) The Cinnamon Ibon *Hypocryptadius cinnamomeus* is a forest canopy sparrow. Ibis 152(4): 747–760. https://doi.org/10.1111/j.1474-919X.2010.01053.x

Gebauer A, Kaiser M (1994) Biologie und Verhalten zentralasiatischer Schneefinken (*Montifringilla*) und Erdsperlinge (*Pyrgilauda*). Journal für Ornithologie 135: 55–71. https://doi.org/10.1007/BF01640275

Gebauer A, Eck S, Kaiser M, Lei FM, Martens J (2006) The Qinghai-Tibet Plateau: center of evolution for snow sparrows (*Montifringilla* s. str.) and mountain-steppe sparrows (*Onychostruthus*, *Pyrgilauda)*. Acta Zoologica Sinica 52 (suppl): 305–309.

Gedeon K, Cauldwell A, Ewnetu M, Regasa F, Schönbrodt R, Töpfer T (2015). House Sparrow *Passer domesticus* and hybrids with Somali Sparrow *Passer castanopterus* in Ethiopia. Bulletin of the African Bird Club 22: 70–73.

Gill F, Donsker D, Rasmussen P (Eds)(2020). IOC World Bird List (v10.1). https://doi.org/10.14344/IOC.ML.10.1 (last accessed on 20.04.2020).

Heath TA, Hedtke SM, Hillis DM (2008) Taxon sampling and the accuracy of phylogenetic analyses. Journal of Systematics and Evolution 46: 239–257. DOI: 10.3724/SP.J.1002.2008.08016

Hermansen JS, Sæther SA, Elgvin TO, Borge T, Hjelle E, Sætre GP (2011) Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture and barriers to gene flow. Molecular Ecology 20: 3812–3822. https://doi.org/10.1111/j.1365-294X.2011.05183.x

Hermansen JS, Haas F, Trier CN, Bailey RI, Nederbragt AJ, Marzal A, Sætre GP (2014) Hybrid speciation through sorting of parental incompatibilities in Italian sparrows. Molecular Ecology 23: 5831–5842. https://doi.org/10.1111/mec.12910

Hering J, Mädlow W, Geiter O, Siegmund A, Eilts HJ, Fuchs E, Müller K, Müller R, Rayaleh H, Vohwinkel R (2020) Untersuchungen zur Avifauna der Mangrovenwälder Dschibutis sowie zur Klärung taxonomischer Fragestellungen bei Buntastrild *Pytilia melba* sowie Haussperling *Passer domesticus* und Somalisperling *Passer castanopterus* – erste Ergebnisse. Vogelwarte 58: 349–362.

Jønsson KA, Fjeldså J (2006) A phylogenetic supertree of oscine passerine birds (Aves: Passeri). Zoologica Scripta 35(2): 149–86. https://doi.org/10.1111/j.1463-6409.2006.00221.x

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Lei F, Qu Y, Song G (2014) Species diversification and phylogeographical patterns of birds in response to the uplift of the Qinghai-Tibet Plateau and Quaternary glaciations. Current Zoology 60: 149–161. https://doi.org/10.1093/czoolo/60.2.149

Lerner HRL, Meyer M, James HF, Hofreiter M, Fleischer RC (2011) Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Current Biology 21: 1–7. https://doi.org/10.1016/j.cub.2011.09.039

Liebl A, Schrey A, Andrew SC, Sheldon EL, Griffith SC (2015) Invasion genetics: Lessons from a ubiquitous bird, the house sparrow *Passer domesticus*. Current Zoology 61: 465–476. https://doi.org/10.1093/czoolo/61.3.465

Martens J, Eck S (1995) Towards an ornithology of the Himalayas. Systematics, ecology and vocalizations of Nepal birds. Bonner Zoologische Monographien 38: 1–454.

Mlíkovský J (2011) Correct name for the Asian Russet Sparrow. Chinese Birds 2: 109–110. https://doi.org/10.5122/cbirds.2011.0016

Moreau RE, Greenway Jr JC (1962) Family Ploceidae, Weaverbirds. In: Mayr E, Greenway Jr JC (eds) Check-List of the Birds of the World. Volume XV. Museum of Comparative Zoology, Cambridge Massachusetts, USA, 327 pp.

Nabhan AG, Sarkar IN (2011) The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. Briefings in Bioinformatics 13: 122–134. https://doi.org/10.1093/bib/bbr014

Päckert M, Martens J, Sun YH, Strutzenberger P (2016) The phylogenetic relationships of Przevalski's Finch *Urocynchramus pylzowi*, the most ancient Tibetan endemic passerine known to date. Ibis, 158: 530–540. https://doi.org/10.1111/ibi.12382

Päckert M, Belkacem AA, Wolfgramm H, Gast O, Canal D, Giacalone G, Lo Valvo M, Vamberger M, Wink M, Martens J, Stuckas H (2019) Genetic admixture despite ecological segregation in a North African sparrow hybrid zone (Aves, Passeriformes, *Passer domesticus* x *Passer hispaniolensis*). Ecology and Evolution 9: 12710–12726. https://doi.org/10.1002/ece3.5744

Päckert M, Favre A, Schnitzler J, Martens J, Sun YH, Tietze DT, Hailer F, Michalak I, Strutzenberger P (2020) Out of Tibet and out of Himalayas: centers of origin and diversification of passerine birds in the Qinghai-Tibet Plateau region. Ecology and Evolution 10: 9283–9300. https://doi.org/10.1002/ece3.6615

Portenko LA, von Vietinghoff-Scheel E (1974) *Montifringilla nivalis* (L.). In: Stresemann E, Portenko LA, Dathe H, Mauersberger G (Eds) Atlas der Verbreitung Palaearktischer Vögel. 4. Lieferung. Akademie Verlag, Berlin.

Praveen J, Jayapal R, Pittie A (2016) A checklist of the birds of India. Indian Birds 11: 113–170.

Qu Y, Ericson PGP, Lei F, Gebauer A, Kaiser M, Helbig A (2006) Molecular phylogenetic relationship of snow finch complex (genera

Montifringilla, *Pyrgilauda*, and *Onychostruthus*) from the Tibetan plateau. Molecular Phylogenetics and Evolution 40: 218–226. https://doi.org/10.1016/j.ympev.2006.02.020

Qu Y, Chen C, Chen X, Hao Y, She H, Wang M, Ericson PGP, Lin H, Cai T, Song G, Jia C, Chen C, Zhang H, Li J, Liang L, Wu T, Zhao J, Gao Q, Zhang G, Zhai W, Zhang C, Zhang YE, Lei F (2021) The evolution of ancestral and species-specific adaptations in snowfinches at the Qinghai–Tibet Plateau. PNAS 118: e2012398118. https://doi.org/10.1073/pnas.2012398118

Rambaut A (2009) FigTree version 1.2.2 - Computer program distributed by the author, website: http://tree.bio.ed.ac.uk (last accessed on 29.11.2010).

Rambaut A, Drummond AJ (2007) TRACER v1.4. http://beast.bio.ed.ac.uk/Tracer

Rasmussen PC, Anderton JC (2005) Birds of South Asia: the Ripley guide. Lynx, Barcelona, 1067 pp.

Ravinet M, Elgvin TO, Trier CN, Aliabadian M, Gavrilov A, Sætre GP (2018) Signatures of human-commensalism in the house sparrow genome. Proceedings of the Royal Society B 285(1884): 20181246. https://doi.org/10.1098/rspb.2018.1246

Resano-Mayor J, Fernández-Martín Á, Hernández-Gómez S, Toranzo I, España A, Gil JA, de Gabriel M, Roa-Álvarez I, Strinella E, Hobson KA, Heckel G, Arlettaz R (2016) Integrating genetic and stable isotope analyses to infer the population structure of the White-winged Snowfinch *Montifringilla nivalis* in Western Europe. Journal of Ornithology 158: 395–405. https://doi.org/10.1007/s10336-016-1413-8

Roselaar CS (1995) Songbirds of Turkey. An atlas of biodiversity of Turkish passerine birds. GMB, Haarlem, 240 pp.

Runemark A, Trier CN, Eroukhmanoff F, Hermansen JS, Matschiner M, Ravinet M, Elgvin TO, Sætre GP (2018) Variation and constraints in hybrid genome formation. Nature Ecology and Evolution 2: 549–556. https://doi.org/10.1038/s41559-017-0437-7

Sætre GP, Riyahi S, Aliabadian M, Hermansen JS, Hogner S, Olsson U, Gonzalez Rojas MF, Sæther SA, Trier CN, Elgvin TO (2012) Single origin of human commensalism in the House Sparrow. Journal of Evolutionary Biology 25: 788–796. https://doi.org/10.1098/rspb.2018.1246

Sætre GP, Cuevas A, Hermansen JS, Elgvin TO, Piñeiro-Fernández L, Sæther SA, Lo Cascio Sætre C, Eroukhmanoff F (2017) Rapid polygenic response to secondary contact in a hybrid species. Proceedings of the Royal Society B, 284(1853): 20170365. https://doi.org/10.1098/rspb.2017.0365

Sangster G (2014) The application of species criteria in avian taxonomy and its implications for the debate over species concepts. Biological Reviews 89: 199–214. https://doi.org/10.1111/brv.12051

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stephan B (2000) Die Arten der Familie Passeridae (Gattungen *Montifringilla*, *Petronia*, *Passer*) und ihre phylogenetischen Beziehungen. Bonner Zoologische Beiträge 49: 39–70.

Summers-Smith JD (2010) The sparrows. T. & A.D. Poyser, Calton, 342 pp.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. https://doi.org/10.1093/molbev/msr121

Tritsch C, Martens J, Sun YH, Heim W, Strutzenberger P, Päckert M (2017) Improved sampling at the subspecies level solves a taxonomic dilemma – A case study of two enigmatic Chinese tit species (Aves, Passeriformes, Paridae, *Poecile*). Molecular Phylogenetics and Evolution 107: 538-550. https://doi.org/10.1016/j.ympev.2016.12.014

Turner DA (2016) Range expansion in the Somali Sparrow *Passer castanopterus* in east and northeast Africa. Scopus 36: 47–49.

Vaurie C (1956) Systematic notes on Palearctic birds. No 24 Ploceidae: the genera *Passer*, *Petronia* and *Montifringilla*. American Museum Novitates 1814: 1–27.

Weir JT, Schluter D (2008) Calibrating the avian molecular clock. Molecular Ecology 17: 2321–2328. https://doi.org/10.1111/j.1365-294X.2008.03742.x

Wiens JJ, Tiu J (2012) Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. PLoS ONE 8: e42925. https://doi.org/10.1371/journal.pone.0042925

Wolters HE (1952) Die Gattungen der westpaläarktischen Sperlingsvögel (Ordn. Passeriformes). Bonner Zoologische Beiträge 3: 231–288.

Wolters HE (1979) Die Vogelarten der Erde. 1.-4. Lieferung. Paul Parey, Hamburg und Berlin, 746 pp.

Zuccon D, Prŷs-Jones R, Rasmussen PC, Ericson, PGP (2012) The phylogenetic relationships and generic limits of finches (Fringillidae). Molecular Phylogenetics and Evolution 62: 581–596. https://doi.org/10.1016/j.ympev.2011.10.002

Zwickl DJ, Hillis DM (2002) Increased taxon sampling greatly reduces phylogenetic error. Systematic Biology 51: 588–598. https://doi.org/10.1080/10635150290102339

Supplementary material

File 1

Authors: Päckert, M, Hering J, Belkacem AA, Sun Y-H, Hille S, Lkhagvasuren D, Islam S, Martens J (2021)
Data type: .docx
Explanation note: Additional samples and sequence data used for analysis of inter- and intraspecific variation of the cytochrome-*b* gene (*Montifringilla*, *Pyrgilauda* and *Petronia*) and the ND2 gene (*Passer*).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/vz.71.e65952.suppl1

File 2

Authors: Päckert, M, Hering J, Belkacem AA, Sun Y-H, Hille S, Lkhagvasuren D, Islam S, Martens J (2021)
Data type: .docx
Explanation note: Gazetteer for collection sites with information on localities and/or coordinates.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/vz.71.e65952.suppl2

Chapter 2: Museomics help resolving the phylogeny of snowfinches (Aves, Passeridae, *Montifringilla* and allies)

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Museomics help resolving the phylogeny of snowfinches (Aves, Passeridae, *Montifringilla* and allies)

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ABSTRACT

Historical specimens from museum collections provide a valuable source of material also from remote areas or regions of conflict that are not easily accessible to scientists today. With this study, we are providing a taxon-complete phylogeny of snowfinches using historical DNA from whole skins of an endemic species from Afghanistan, the Afghan snowfinch, *Pyrgilauda theresae*. To resolve the strong conflict between previous phylogenetic hypotheses, we generated novel mitogenome sequences for selected taxa and genome-wide SNP data using ddRAD sequencing for all extant snowfinch species endemic to the Qinghai-Tibet Plateau (QTP) and for an extended intraspecific sampling of the sole Central and Western Palearctic snowfinch species (*Montifringilla nivalis*).

Our phylogenetic reconstructions unanimously refuted the previously suggested paraphyly of genus *Pyrgilauda*. Misplacement of one species-level taxon (*Onychostruthus tazcanowskii*) in previous snowfinch phylogenies was undoubtedly inferred from chimeric mitogenomes that included heterospecific sequence information. Furthermore, comparison of novel and previously generated sequence data showed that the presumed sister-group relationship between *M. nivalis* and the QTP endemic *M. henrici* was suggested based on flawed taxonomy. Our phylogenetic reconstructions based on genome-wide SNP data and on mitogenomes were largely congruent and supported reciprocal monophyly of genera *Montifringilla* and *Pyrgilauda* with monotypic *Onychostruthus* being sister to the latter. The Afghan endemic *P. theresae* likely originated from a rather ancient Pliocene out-of-Tibet dispersal probably from a common ancestor with *P. ruficollis*. Our extended *trans*-Palearctic sampling for the white-winged snowfinch, *M. nivalis*, confirmed strong lineage divergence between an Asian and

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a European clade dated to 1.5 – 2.7 million years ago (mya). Genome-wide SNP data suggested subtle divergence among European samples from the Alps and from the Cantabrian mountains.

1. Introduction

Natural history collections are biological archives of extant as well of past biodiversity (Meineke et al., 2019; Schindel and Cook, 2018; Hahn et al., 2020; Hilton et al., 2021) and provide basic material for the study of evolutionary changes over time of adaptive phenotypical traits (del Mar Delgado et al., 2019; Mason and Unitt, 2018; Miranda et al., 2021), range expansions in response to climate change (Mende and Hundsdoerfer, 2013) or decline of genetic diversity in response to historical population bottlenecks (Glenn et al., 1999; Godoy et al., 2004; Kuhn et al., 2013; Gauthier et al., 2020). In the field of molecular systematics, genetic analysis of type specimens has become important to trace back their geographic origin (Cong et al., 2021) or for a correct assignment of taxon names to clades of a phylogeny (Tritsch et al., 2017; Kehlmaier

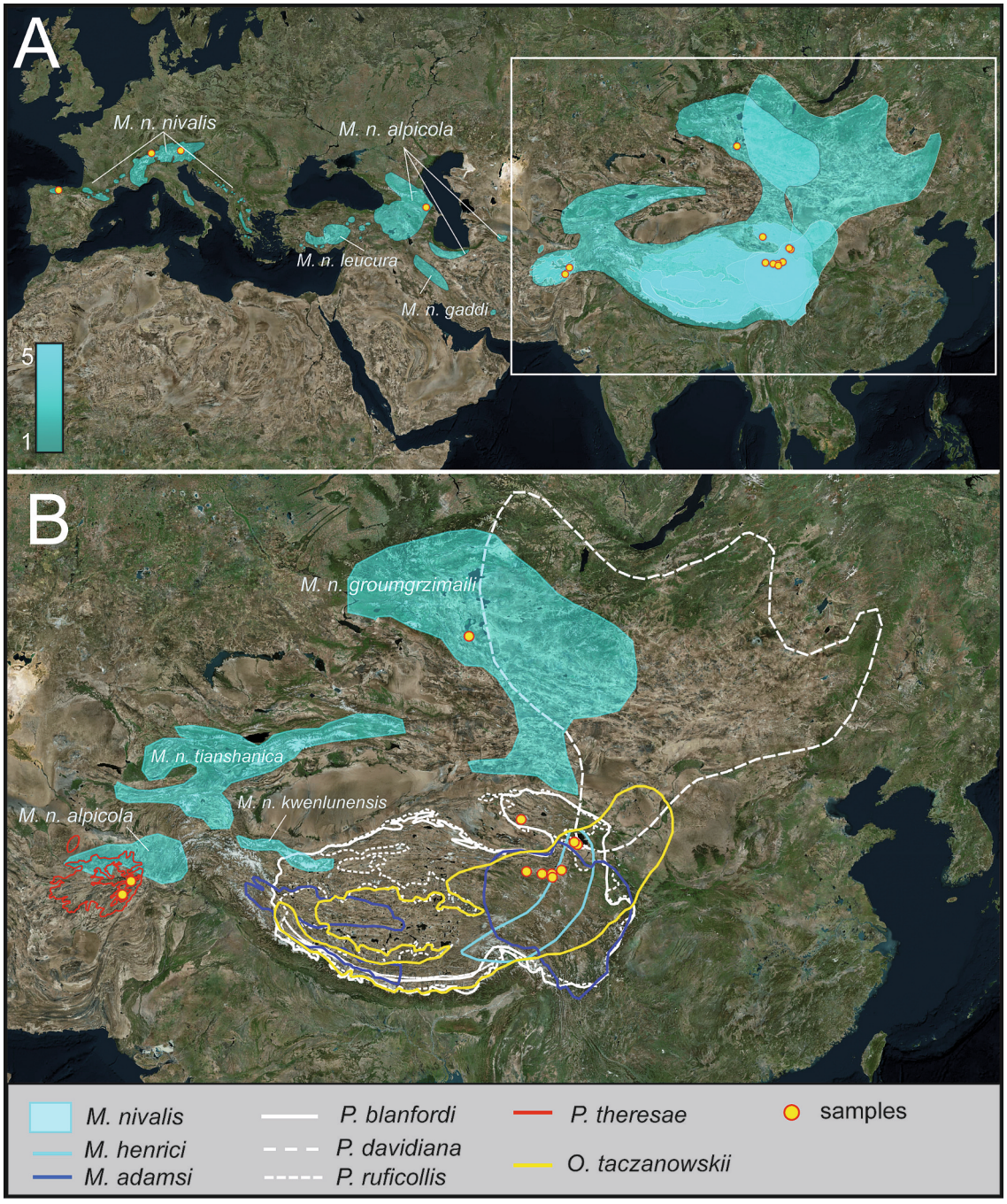

Fig. 1. Distribution of snowfinches (genera *Montifringilla*, *Pyrgilauda*, *Onychostruthus*) A) entire range across Eurasian mountain systems; B) zoom on the Qinghai-Tibet Plateau (QTP) showing distributions and range overlap for seven sympatric or parapatric species. Ranges of *M. nivalis* modified according to Gebauer et al. (2006); collection sites of samples used for this study indicated by yellow dots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 2019; Scherz et al., 2020). Moreover, "historical DNA" (Raxworthy and Tilston Smith, 2021) extracted from museum specimens often provides the only chance to include rare species in phylogenies, e.g. in case of extinct species (Shapiro et al., 2002; Hekkala et al., 2011; Heupink et al., 2014; Podsiadlowski et al., 2017; Kehlmaier et al., 2021; Kearns et al., 2022) or of ultra-rare species represented by a single specimen in collections worldwide (Uva et al., 2018; Jønsson et al., 2018; Schweizer et al., 2020). Collections have therefore become very important for the optimization of phylogenetic data sets based on dense taxon sampling (Fiedler de Abreu Jr et al., 2020; Sampaio et al., 2023). Moreover, along with recent advances in genomic methods, museum collections provide a great potential for a wide variety of studies in the diverse field of so-called "museomics" (Zedane et al., 2016; Gauthier et al., 2020; Card et al., 2021; Raxworthy and Tilston Smith, 2021; Ernst et al., 2022; Lalueza-Fox, 2022). Nevertheless, many phylogenetic studies relied on an incomplete taxon sampling due to a lack of material from inaccessible or remote areas or regions of armed conflict. This is quite problematic, because among the driving forces of biodiversity loss, political conflict is a neglected aspect, though it has manifold negative impacts on natural resources and ecosystems and therefore constitutes a major impediment for biodiversity research (McNeely, 2003; Hanson et al., 2009; Smallwood et al., 2011). As a consequence, the endemic faunas and floras of some conflict regions have remained severely understudied to date. Here, we focus on an example from Central Asia.

Afghanistan is situated at the crossroads of two global biodiversity hotspots: the Himalayas in the East and the Central Asian Mountains in the North (Marchese, 2015; Mittermeier et al., 2004; Hanson et al., 2009; map in Fig. 1). At a narrow spatial scale, Afghanistan harbors several unique ecoregions (some of them shared with neighboring Pakistan), such as the East Afghan Montane Coniferous Forests, the Sulaiman Range Alpine Meadows or the Baluchistan Xeric Woodlands (Wikramanayake et al., 2002). An ornithological survey from 2008 recorded a total of 122 bird species for Bamiyan county (Busuttil & Ayé 2009). Despite its comparatively low regional species richness, Afghanistan harbors refuge areas for major populations of threatened animals, such as the snow leopard, *Uncia uncia*, or the Marco Polo sheep, *Ovis ammon polii* (Saidajan, 2012). However, the local and regional fauna of Afghanistan remains poorly studied. For example, in a recent assessment of the global snow leopard population, occurrence data from Afghanistan was missing (see map in Riordan et al., 2016). Recent genetic studies on material from Afghanistan are scarce, and most of them focused either on domestic animals (Karimi et al., 2016) or on cultivated plant species (Terasawa et al., 2009; Seghal et al., 2015; Gori et al., 2019; Tehseen et al., 2021). Only a few phylogenetic and DNA barcoding studies included recent samples from wild Afghan populations collected in the 21st century (reptiles: Guo et al., 2011; Khan et al., 2021; Kazemi et al., 2021; butterflies: Efetov et al., 2014; Shapoval et al., 2017). In contrast, a recent phylogenetic study on the Afghan pika (*Ochontona rufescens*) relied on material exclusively collected in Iranian populations (Khalilipur et al., 2017). That knowledge gap is typical for the Afghan fauna because over the past decades traveling and field-work have become increasingly risky in this region of continuous conflict and is currently virtually impossible (Böhme and Jablonski, 2022; Maheshwari, 2022).

Among the seven endemic vertebrate species of Afghanistan (Kanderian et al., 2011), the Afghan snowfinch, *Pyrgilauda theresae* is the only bird species (Dementiev, 1963) even though there are occasional winter records from Turkmenistan and doubtful occurrences in neighboring Tajikistan (Busuttil et al., 2010; Tolstoj and Geipel, 1990). This species was first described by Meinertzhagen (1937) from a specimen collected on the 19th of April 1937 at Shibar Pass (Kowtal-e Shibar) at about 2990 m a.s.l. (for reliability of type specimen data, see Rasmussen & Prŷs-Jones, 2003). Though it is currently considered a species of least concern by the IUCN, the global population size of *P. theresae* has not been estimated yet due to data deficiency (BirdLife International, 2021), and today the species is known from nine breeding season localities only, all of them restricted to Afghanistan (map in Vietinghoff-Scheel, 1980).

i) Traditionally, snowfinches were united under the single genus *Montifringilla* (e.g. Vaurie, 1956; Clements et al., 2022), but they are nowadays divided into three genera (*Montifringilla*, *Pyrgilauda,* and *Oynchostruthus*; e.g. Gill et al., 2023; Clements et al., 2023; Table 1). Six species are endemic to the Qinghai-Tibet Plateau (QTP) and its flanking mountain regions (Fig. 1A; Gebauer et al., 2006). Only one species, *M. nivalis*, occurs across a wide *trans*-Palearctic range from the northern QTP margin in the Chinese Altai, central Mongolia (subspecies *M. n. groumgrzimaili*) and west of the QTP region, inhabiting the Caucasus (subspecies *M. n. alpicola*) and European mountain systems (the nominate subspecies *M. n. nivalis*). Despite a large number of genetic studies, the phylogenetic relationships among snowfinch species are still under debate. At the family level, snowfinches represent a monophyletic clade in the Passeridae. Thus they are actually sparrows and not related to the finches, Fringillidae (Päckert et al., 2020b, 2021). So far, their affiliation to Passeridae was not acknowledged by a change of English species names, except a single attempt by Summers-Smith (2009) who listed the small *Pyrgilauda* species (including the larger *P. taczanowskii*; see Table 1) as "ground-sparrows" (but still listed all *Montifringilla* species as "snowfinches"). To date, intraspecific genetic diversification was described only across the *trans*-Palearctic range of the most widespread species, *M. nivalis*. However these findings were based on quite limited sampling (Päckert et al., 2021). Most recent molecular phylogenies of snowfinches were either based on mitochondrial markers alone (Lei et al., 2014; Cobos et al., 2021) or were subject to a strong bias between the signal of highly variable mitochondrial markers versus one or two less variable nuclear introns (Qu et al., 2006; Päckert et al., 2020b, 2021). Tree topologies inferred from these different data sets were conflicting for example concerning the position of the white-rumped snowfinch (*Onychostruthus taczanowskii*) and the relationships between species of the larger genus *Montifringilla* (Fig. 2A, B). Recent studies on large, genome-wide marker sets of snowfinches included only one species per genus (Qu et al., 2021; She et al., 2021). The Afghan snowfinch was missing from all these phylogenies. In such cases, historical material from bird collections becomes important. A historical collection of Afghan vertebrates is hosted at Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany (Jablonski et al., 2019; Böhme and Jablonski, 2022). The ornithological collection of ZFMK hosts 276 bird specimens collected during two expeditions to Afghanistan from 01.09. to 13.09.1965 along an itinerary from Faizabad to Qala-e-Pandja (map in Niethammer, 1973) and seven years later to Darwaz (11.07.1972—31.07.1972) and the Pamir range (03.08. 1972—05.09.1972).

With this paper, we provide a first taxon-complete phylogeny of snowfinches based on two data sets of one mitochondrial marker and genome-wide single nucleotide polymorphisms (SNPs) inferred from a next-generation sequencing (NGS) approach. We aim at.

ii) resolving taxonomic discrepancies among published phylogenies (Fig. 2A, B)
iii) providing a first phylogenetic hypothesis on the position of the Afghan snowfinch, *Pyrgilauda theresae*
iv) exploring the intraspecific diversification of the white-winged snowfinch, *Montifringilla nivalis*.

2. Methods

2.1. Sampling and DNA extraction

We extracted DNA from 40 samples of snowfinches from all eight species of the three genera (*Montifringilla*, *Onychostruthus,* and

Table 1
Classification of snowfinches according to different taxonomic authorities (subspecific taxa, only those listed treated in this study).

	Hartert (1910)	Hartert & Steinbacher (1932)	Vaurie (1956), Mayr & Greenway Jr (1962), Wolters (1979)	Summers-Smith (2009)	Dickinson (2003), Dickinson & Christidis (2014), Summers-Smith & van Balen (2016), Gill et al. (2023)
genera	1	1	1	2	3
species	5	6	7	8	8
White-winged Snowfinch	*Montifringilla nivalis*	*Montifringilla nivalis*	*Montifringilla nivalis*	*Montifringilla nivalis*	*Montifringilla nivalis*
	Montifringilla n. nivalis	*Montifringilla n. nivalis*	*Montifringilla n. nivalis*	*Montifringilla n. nivalis*	*Montifringilla n. nivalis*
	Montifringilla n. alpicola	*Montifringilla n. alpicola*	*Montifringilla n. alpicola*	*Montifringilla n. alpicola*	*Montifringilla n. alpicola*
	Montifringilla n. groumgrzimaili	*Montifringilla n. groumgrzimaili*	*Montifringilla n. groumgrzimaili*	*Montifringilla n. groumgrzimaili*	*Montifringilla n. groumgrzimaili*
Tibetan Snowfinch	*Montifringilla n. henrici*	*Montifringilla n. henrici*	*Montifringilla n. henrici*	*Montifringilla henrici*	*Montifringilla henrici*
Black-winged Snowfinch	*Montifringilla n. adamsi*	*Montifringilla adamsi*	*Montifringilla adamsi*	*Montifringilla adamsi*	*Montifringilla adamsi*
Plain-backed Snowfinch	*Montifringilla blanfordi*	*Montifringilla blanfordi*	*Montifringilla blanfordi*	*Pyrgilauda blanfordi*	*Pyrgilauda blanfordi*
Small snowfinch	*Montifringilla davidiana*	*Montifringilla davidiana*	*Montifringilla davidiana*	*Pyrgilauda davidiana*	*Pyrgilauda davidiana*
Rufous-necked Snowfinch	*Montifringilla ruficollis*	*Montifringilla ruficollis*	*Montifringilla ruficollis*	*Pyrgilauda ruficollis*	*Pyrgilauda ruficollis*
Afghan Snowfinch	–	–	*Montifringilla theresae*	*Pyrgilauda theresae*	*Pyrgilauda theresae*
White-rumped Snowfinch	*Montifringilla mandelli*	*Montifringilla taczanowskii*	*Montifringilla taczanowskii*	*Pyrgilauda taczanowskii*	*Onychostruthus taczanowskii*

Pyrgilauda) and one further sample of the rock sparrow, *Petronia petronia* (Table S1). All samples were either frozen blood or tissue samples preserved in ethanol or preserving buffer except two toe pad samples taken from two historical specimens of the Afghan snowfinch, *P. theresae*. These were collected at Dasht-i-Nawar on 08.06.1965 at 3000 m a.s.l. (MAR1082; field number 495) and at Imai Pass on 29.04.1965 at 2800 m a.s.l. (MAR1083; field number 171). For DNA extraction from frozen blood or tissue samples we used an InnuPREP DNA Mini Kit and an innuPREP Blood DNA Mini Kit (both Analytik Jena AG, Germany) following the manufacturer's protocol except for overnight incubation with proteinase K for cell lysis.

To avoid cross-contamination historical toe pad samples were entirely processed in the clean room of the historical DNA facility of the Museum of Zoology, Senckenberg Dresden. DNA was extracted from toe-pad tissue using the DNeasy Blood & Tissue Kit (Qiagen) and two final elution steps with 2 x 50 µl elution buffer. Before and after each step of the procedure, clean benches were cleaned with DNA away (Molecular Bio Products, Inc.), and benches and the entire room were set under UV light for four hours.

2.2. Mitochondrial DNA – Single marker cytochrome-*b*

A limited series of cytochrome-*b* sequences (cyt*b*) from a subset of our sampling was available from previous studies (Päckert et al., 2020b, 2021). To complete the data set for this mitochondrial marker we amplified a 1079-bp-long cyt*b* fragment for 19 samples using the primer combination of O-L14851/ O-H16065 primers (Weir and Schluter, 2007). The PCR protocol was as follows: i) initial denaturation at 94 °C for 10 min, ii) 35 cycles with denaturation at 92 °C for 60 s, annealing for 60 s at 53 °C and extension at 72 °C for 120 s, iii), final extension at 72 °C for 10 min. We purified PCR products using the ExoSAP-IT enzymatic cleanup (USB Europe GmbH; Staufen, Germany; 1:20 dilution, modified protocol: 30 min at 37 °C, 15 min at 80 °C). Purified PCR products were prepared for sequencing with BigDye^{TM} 3.1 Dye Terminator Cycle Sequencing Kits (Thermo Fisher Scientific, Waltham, MA, USA) and cycle sequencing products were purified by using Sephadex (GE Healthcare, Munich, Germany), and sequenced in both reading directions on an ABI 3730 capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA). We inspected and edited all sequences with Chromas v.2.6.5 (Technelysium Pty Ltd, Brisbane, Australia), and cross-checked chromatograms of forward and reverse sequences of each sample. We used MEGA v. 10.1.8 (Kumar et al., 2018) for editing and alignment of consensus sequences. Newly generated cyt*b* sequences were deposited at GenBank under accession numbers OQ947839 – OQ947857 (compare Table S1).

2.3. Mitogenomes – Library preparation, sequence assembly, and sequence annotation

Because *P. theresae* was the single snowfinch species missing out from mitogenome-based phylogenies so far (Cobos et al., 2021), we generated whole mitochondrial genomes for this species using specific protocols for museum material (Kehlmaier et al., 2019, 2021; Stelbrink et al., 2019). From the two historical toe pads (*P. theresae*) 28 µl of each lysate (11 ng DNA for sample MAR1082, and 305 ng DNA for sample MAR1083) was converted into single-indexed, double-stranded Illumina sequencing libraries (dsLibs) following Meyer and Kircher (2010) with modifications by Fortes and Paijmans (2015). For library preparation DNA concentration was measured with a Qubit 3.0 Fluorometer (Invitrogen, Life Technologies GmbH, Darmstadt, Germany) using the highly sensitive quantitation assay. DNA molecule fragment length was checked on a Tape Station using the D1000 screen tape. Subsequently, DNA extracted from toe pad samples was sheared down to ca. 150 bp using the Covaris ultrasonicator.

To increase the amount of endogenous mitochondrial DNA in the libraries, two rounds of in-solution hybridization capture (Horn, 2012;

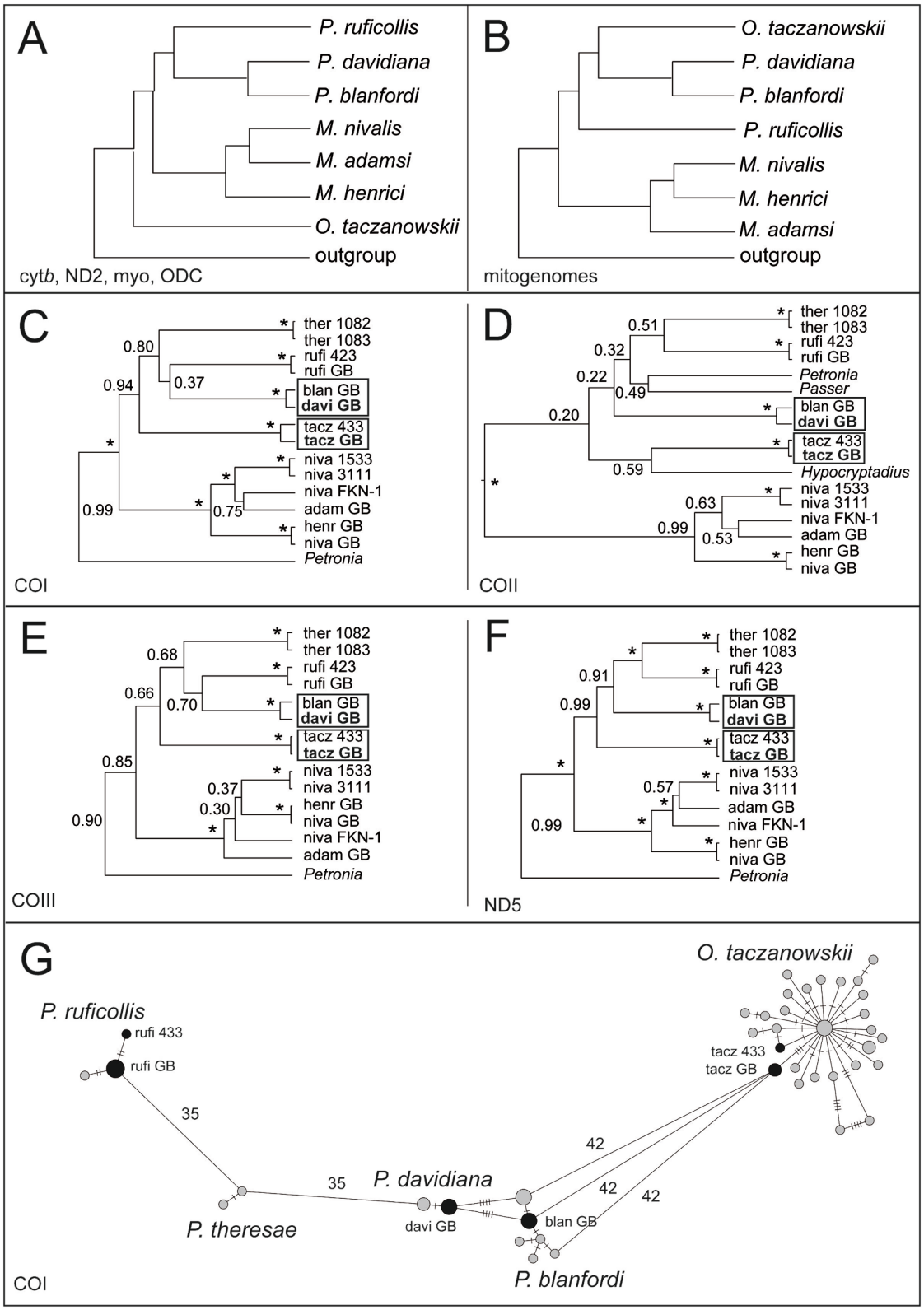

Fig. 2. Competing phylogenetic hypotheses for snowfinches based on A) a multi-locus data set (Päckert et al., 2020b, 2021), B) mitochondrial DNA (Cobos et al., 2021); C-F) Phylogenies of snowfinches inferred from four mitochondrial coding markers (extracted from whole mitogenome sequences; GB = from GenBank) that did not show evidence of heterospecific fragments in the consensus mitogenome sequences (COI, COII, COIII, ND5); the two outgroup taxa (*Passer domesticus* and *Hypocryptadius cinnamomeus*) were pruned from the trees; G) TCS network for an extended data set of the DNA barcoding marker (COI; n = 53; 483 bp), circles denote haplotypes, dashes indicate substitutions between connected haplotypes, black circles indicate haplotype of COI sequences inferred from whole mitogenome data (sequence/sample ID indicated at corresponding haplotypes).

Maricic et al., 2010) were performed in a dedicated capture-only workspace in the main laboratory, using DNA baits generated from long-range PCR products of *Pyrgilauda ruficollis* (sample MAR423). Long-range PCR (LR-PCR) and primer sequences are detailed in the Supplementary Information (Table S2). The PCR protocol was as follows: Denaturation at 93 °C for 3:00 min, 35 cycles of 93 °C for 0:20, 62 °C for 0:30 min, 68 °C for 10:00 min, and final elongation at 68 °C for 20:00 min. Sequencing was performed in-house on an Illumina MiSeq platform, generating 75 bp paired-end reads. To account for intraspecific variation in the mitogenome of *M. nivalis*, we applied the same long-range PCR setup and library preparation method as described above to frozen blood samples from Mongolian, Caucasian, and European samples of this species (plus one sample each of *O. taczanowskii* and *P. ruficollis* for comparison; see Table S1). LR-PCR products were sheared down to ca. 150 bp and mixed at an equimolar ratio before being converted into dsLibs and sequenced.

Mitogenomes were adapter-trimmed with Skewer 0.2.2 (Jiang et al., 2014). BBmap-suite 37.24 (Bushnell et al., 2017) was used for read merging (minimum length 35 bp), quality filtering (minimum Q-score 20), and duplicate removal. The remaining quality-filtered reads (QFR) were screened for contamination using FastQScreen 0.11.4 (Wingett and Andrews, 2018) and a set of predefined mitogenomes (Table S1). Due to no obvious contamination problems, the entire read pool was used for mitogenome assembly with MITObim (Hahn et al., 2013), a two-step baiting and iterative mapping approach, with an allowed mismatch value of 2 and a starting seed KJ148629 (*P. blanfordi*). The resulting scaffolds were visualized and checked for assembly artifacts in Tablet (Milne et al., 2013). Artifacts were manually removed from the assembled contigs and all positions with coverage below 3-fold were masked as ambiguous (N) using the maskfasta subcommand of BEDTools 2.29.2 (Quinlan and Hall, 2010). The sequence length distribution of mapped reads was calculated with a customized awk command and in Microsoft Excel. Newly generated mitogenome sequences were deposited at the European Nucleotide Archive (ENA) under accession numbers OX637969-OX637975.

2.4. Validation and quality check of mitogenome sequences

We compared our seven newly generated mitogenomes to fifteen published mitogenomes of snowfinches available at GenBank (Table S3; e.g. from Ma et al., 2014, 2016). For each snowfinch species, published mitogenome sequences were identical for intraspecific pairwise comparisons, so we used a single published mitogenome sequence per snowfinch species. For hierarchical outgroup rooting we added whole mitogenome sequences of *Passer domesticus* (MN356394) and *Hypocryptadius cinnamomeus* (MN356286) to our dataset. We constructed two alignments for whole mitogenome sequences and for a set of 13 coding mitochondrial genes (n = 17) using MEGA and checked alignments for inconsistencies, such as regions of unusually high numbers of substitutions among conspecific sequences. For comparison of intra- and interspecific divergence levels, we calculated pairwise uncorrected *p*-distances with MEGA for each of the thirteen coding markers. To control for deviations among tree topologies inferred from different markers, we reconstructed mitochondrial phylogenies for all thirteen coding markers in separate runs with BEAST v.2.6.2 (Bouckaert et al., 2014; parameters and settings, see below). Following recommendations for validation of newly generated mitogenomes (and quality check of published mitogenomes) by Botero-Castro et al. (2016), we used larger published sequence data sets of mitochondrial markers (most from phylogeographic studies on snowfinch species by Qu & Lei, 2009; Qu et al., 2006, 2005, 2010) for comparison with homologous fragments of our mitogenome sequences: cytochrome-oxidase subunit 1 [COI]: n = 55, 483 bp; cytochrome-*b* [cyt*b*]: n = 109, 880 bp; d-loop, control region [CR]: n = 22, 288 bp (full documentation see supplementary Table S4). From each of the three data sets, we constructed minimum-spanning networks with PopArt 1.7 (Leigh and Bryant, 2015).

2.5. ddRAD library preparation and sequencing

For inference of a genome-wide SNP data set we used double-digest restriction site associated DNA sequencing (ddRAD seq), which is a frequently applied approach for the study of diverse phylogenomic research questions in birds, such as diversification among island populations (Martin et al., 2021; Cooper and Uy (2017) or resolution of phylogenetic relationships in tits and larks (Stervander et al., 2015, 2016), seabirds (Obiol et al., 2021) and flycatchers (Gwee et al., 2019). DdRAD seq was performed at the Deep Sequencing Facility in the Center for Molecular and Cellular Bioengineering (CMCB) Dresden. We used Qubit (Thermo Fisher Scientific, Waltham, MA, USA), dsDNA High-Sensitivity (HS) and Broad-Range (BR) assays for DNA concentration measurement following the manufacturer's protocol. According to our Qubit measurements, we selected 38 samples with sufficient DNA concentrations for ddRAD seq (compare Table S1). For sample preparation, 50 ng gDNA were double-digested with SbfI and MspI (NEB) for 120 min at 37 °C followed by heat inactivation at 65 °C for 20 min. SbfI specific library barcodes carrying Truseq-i5 Illumina adapters with cohesive ends were ligated to the cohesive ends of the SbfI restriction sites of the digested DNA fragments. The same was done for the MspI site with a MspI-specific truncated universal TruSeq-i7 adapter. Ligation was performed for 120 min at 22 °C followed by heat inactivation at 65 °C for 20 min. Samples with different P5 Barcodes were pooled and purified using XP beads (Beckman Coulter, Krefeld, Germany) at a ratio of 1:1 to remove non-ligated adaptors. Sample-specific library indices were added during PCR (98 °C 30 sec, 15 cycles [98 °C 10 sec, 65 °C 75 sec], 65 °C 5 min) with 1x concentrated NEBNext Q5 Hot Start Hifi PCR Master Mix and standard TruSeq i5/i7 indexing PCR primers. After an XP bead purification (1:1) libraries were quantified using the Fragment Analyzer (Agilent, Waldbronn, Germany). Libraries were equimolarly pooled before sequencing them in single-end mode on an Illumina NextSeq 500 system to a read length of 75 bp and a depth of at least 1 million reads per sample. Individual fast-Q files of ddRAD raw sequencing data were deposited at NCBI SRA (for accession numbers, see Table S1). Sets of SNP alignments and further data files were deposited at Dryad under https://doi.org/10.5061/dryad.m905qfv9k.

2.6. Data assembly and SNP calling

We used the program process_radtags as implemented in Stacks software (Catchen et al., 2013) with default settings applied for demultiplexing of Illumina raw reads and for removal of barcodes, adapters and low-quality reads. We checked average sequence quality per read and GC content using FastQC v0.11.9 (Andrews, 2015). For reference-based assembly of our trimmed reads (Eaton and Ree, 2013) we relied on a multi-reference approach (Bohling, 2020; Valiente-Mullor et al., 2021). First, we mapped our cleaned read data to two reference genomes of snowfinches from Qu et al. (2021): *Pyrgilauda ruficollis* (NCBI acc. no: GCF_017590135.1) and *Onychostruthus tazcanowskii* (NCBI acc. no: GCA_017590055.1) downloaded from NCBI. Genome data for these two snowfinch species was annotated to 15,000 protein-coding genes (Qu et al., 2021) in a first "computational phase" of a two-step process (Ekblom and Wolf, 2014). Since a chromosome-level assembly was not available for the closely related species, and reference genomes from rather distant relatives can generally be used for read mapping and SNP calling (Galla et al., 2019), we also aligned our read data to the house sparrow (*Passer domesticus*) genome which is assembled to chromosome-level (Elgvin et al., 2017; accession no: GCA_001700915.1). We used ipyrad v.0.9.42 (Eaton, 2014; Eaton and Overcast, 2020) for data assembly and read mapping to the three different reference genomes. We applied a clustering threshold of 85 % and a minimum sequencing depth for clustering ≥ 6X. We applied default parameter settings of the reference-based ipyrad pipeline with a maximum of 8 indels, 0.5 heterozygous sites, 20 % SNPs per locus, and a minimum of four samples per locus.

After these first filtering steps, the ipyrad pipeline (Eaton and Overcast, 2020) produced three independent VCF output files from mapping against three different reference genomes. For variant calling we used vcftools 1.1.5 (Danecek et al., 2011) and bcftools v1.8 (Danecek et al., 2021) with a quality value ≥ 30 applied to separate autosomal from Z-chromosomal data sets [which was possible only for the data set inferred from alignment to the house sparrow reference genome that was annotated to the chromosome level]. From each of the VCF files we generated final SNP data sets allowing for 0 %, 10 %, 20 % and 30 % missing data that were used as input data for phylogenetic analysis.

In a final step, we used vcftools for biallelic SNP filtering and thinning of vcf files to retain only one SNP per contig and thus to reduce the effects of physical linkage among markers (O'Leary et al., 2018). We applied the vcftools "thinning" option and tested a range of thinning factors (thin) of which thin = 200 yielded the highest proportion of unlinked SNPs in our thinned data set (i.e. 100 %). Likewise, we generated SNP data sets from each of the three thinned VCF files allowing for 0 %, 10 %, 20 % and 30 % missing data.

2.7. Phylogenetic inference and divergence time-estimates

For Bayesian inference of the species phylogeny, we used BEAST v.2.6.2 (Bouckaert et al., 2014). A-priori we estimated best-fit substitution models with MrModeltest v.2.3 (Nylander, 2004) for all data sets processed in separate BEAST runs (for model settings see Table 2). For test runs with each of the thirteen mitochondrial coding markers, and for genome-wide SNP alignments with varying fractions of missing data, the parameters of BEAST were consistent: an MCMC chain length of 10,000,000 generations (with all model settings estimated and parameters logged and trees sampled every 1,000 generations) under the uncorrelated lognormal clock model, the "auto-optimize" option activated and a Yule prior.

Final optimized runs were performed separately for i) the set of thirteen mitochondrial coding markers (thirteen partitions, with fixed best-fit model settings for each partition; Table 2), ii) the cyt*b* data set for our full sampling and further sequences from GenBank (n = 51; supplementary Tables S1, S4), iii) the three SNP data sets (generated using different reference genomes) with the percentage of missing data that yielded the highest node support in the test runs. We performed three independent runs with BEAST for 30,000,000 generations for the mitogenome data set (with parameters logged and trees sampled every 3,000 generations) and for 50,000,000 generations for the SNP data sets (with parameters logged and trees sampled every 5,000 generations) under the uncorrelated lognormal clock model for all loci, the "auto-optimize" option activated and a Yule prior applied to the tree. We combined log files and tree files from independent BEAST runs using LOGCOMBINER v.1.8.1 (with a burnin of 30 % applied to all tree files) and checked the combined log file in TRACER v. 1.4 (Rambaut & Drummond, 2007) to ensure adequate ESS values for all parameters (all ESS > 200). We constructed consensus trees using TreeAnnotator (burnin of 30 % applied to the combined tree files). We also conducted a tree set analysis as implemented in DensiTree that allows comparing sets of all trees with sets of consensus trees for every possible topology (Bouckaert, 2010).

All obtained phylograms were visualized in FigTree vers. 1.4.2 (Rambaut, 2009). As a control, we repeated phylogenetic reconstructions for all five data sets using a maximum-likelihood approach as implemented in RaxML (Stamatakis, 2006, 2014). The mitogenome data set was partitioned by gene (13 coding markers with the GTR + I + Γ model consistently applied to all partitions), whereas the SNP data sets were left unpartitioned (GTR + I + Γ model applied). For all reconstructions with RaxML, node support was inferred from 1000 thorough bootstrap replicates.

To distinguish between a lack of support due to non-informativeness of the data set and conflicting information between information from different loci we relied on the Quartet Sampling method by Pease et al. (2018), that has previously been applied to that end in phylogenetic studies (Paetzold et al., 2019; Bybee et al., 2021; Kong et al., 2022). Quartet Sampling [QS] quantifies and qualifies discord in a given input tree by calculating quartet topologies for internal branches by sampling subtending terminal taxa to create replicates. The results are given as four scores: Quartet concordance (QC: $-1 <= QC <= 1$) indicating the amount of discordance in the dataset for a given branch; Quartet Differential (QD: $0 <= QD <= 1$) indicates the skew in inferred discordant topologies for that branch (if present); Quartet Informativeness (QI: $0 <= QD <= 1$) indicates informativeness of the data for a given branch; and QF (Quartet Fidelity: QF; $0 <= QF <= 1$) indicates the number of concordant replicates for a given taxon (Pease et al., 2016). Considered together the four values can indicate whether the given dataset has variation of sufficient quality and quantity to inform a given branch (QI), whether this information contradicts the one represented by the tree (QC), and if so, whether this discord is random or skewed (QD). The QD value represents any discordant quartet topology evaluated and the distribution between the two possible discordant relationships across replicates. If the ratio is even (QD = 1), it indicates incomplete lineage sorting as a likely source for the discord, if it is skewed ($QD \ll 1$) this indicates horizontal gene transfer as a source of discord. Finally, the proportion of discordant replicates a specific sample is producing (QF), indicates a possible rogue behavior or low informativeness for the given sample (Pease et al., 2018). Inclusion of "rogue taxa" in a phylogeny might obscure the true evolutionary relationships among clades, often due to long-branch attraction (Westover et al., 2013). To avoid such an effect and to test robustness of resolved relationships, QS analysis can be repeated under exclusion of taxa showing low QF scores.

For QS analysis, we selected the BEAST tree inferred from read mapping to the annotated *P. domesticus* reference genome with 20 % missing (autosomal) data and the corresponding full-length alignment (including invariable sites; alignment length: 517,763 sites). We performed QS with 100 replicates and the default parameters.

We applied two independent approaches to estimate divergence times among extant snowfinch species. First, we calibrated the mitogenome phylogeny by assigning empirical substitution rates by Lerner et al. (2011) to 13 coding markers (with a normal prior distribution, means according to marker-specific estimates by Lerner et al. [2011; see Table 2] and SD set to 10 % of the mean estimate). The same was done for the cyt*b* data set applying the empirical substitution rates by the latter study and by Weir & Schluter (2007) in separate runs. For a secondary calibration of the genome-wide SNP data set, we assigned a fixed node age to the most recent common ancestor (tmcra) of all snowfinches derived from a fossil calibration of the Passeroidea tree by Päckert et al., (2020b,2021). Accordingly, we set the mean tmrca prior for the node uniting all snowfinches to 8.23 Ma and adjusted a normal prior distribution (with sigma = 1.0) according to the 95 % highest posterior density interval (HPDI = [6.45 – 10.24] Ma; Päckert et al., 2020b).

For the reconstruction of unrooted neighbor net networks and TCS networks, we used SplitsTree 4.17.1 (Huson & Bryant, 2006). We examined divergence among species-level taxa and population structure in *M. nivalis* using principal component analysis (PCA) as implemented in the ipyrad.pca tool from the ipyrad pipeline. We performed two separate PCAs for *Montifringilla* one the one hand and for *Pyrgilauda* and *Onychostruthus* on the other hand. For calculation of pairwise F_{ST} values among snowfinch species and among populations of *M. nivalis* we used Stacks 2.60 software (Rochette and Catchen, 2017). Both, PCA and FSt calculations relied on the autosomal SNP data set inferred from read alignment to the annotated *Passer domesticus* genome.

3. Results

3.1. Mitochondrial DNA

We found mismatches in the position of mitogenome sequences in tree topologies for nine out of thirteen mitochondrial coding markers

Table 2
Model settings as inferred from MrModeltest applied to data sets of genome-wide SNP and of mitochondrial markers for inference of phylogeny using BEAST 2; reference genomes (alignment length and model settings for unthinned SNP-data sets with 20 % missing data allowed): ref. 1 = Onychostruthus taczanowskii; ref. 2 = Pyrgilauda blanfordi; Passer = Passer domesticus; Rate(mito): fixed mean rates of mitochondrial markers according to Lerner et al. (2011) assigned to each of the thirteen coding mtDNA markers.

	genome-wide SNPs		SNPs autosomes	SNPS Z-chrom.	Mitogenome (13 coding markers)												
marker	**ref. 1**	**ref. 2**	**ref. *Passer***	**ref. *Passer***	**ND1**	**ND2**	**COI**	**COII**	**ATP8**	**ATP6**	**COIII**	**ND3**	**ND4L**	**ND4**	**ND5**	**Cytb**	**ND6**
bp	39,952	40,695	30,550	899	978	1040	1551	685	168	684	784	351	297	1378	1817	1144	519
model	GTR	GTR	GTR	HKY	GTR + I + Γ	GTR + Γ	GTR + Γ	GTR + Γ	GTR + Γ	GTR + Γ	GTR + Γ	GTR + I + Γ	GTR + Γ	GTR + I + Γ	GTR + I + Γ	GTR + Γ	HKY + I + Γ
πA	0.1856	0.1846	0.3255	0.2353	0.3035	0.3170	0.2842	0.3255	0.3175	0.3366	0.3021	0.3202	0.2828	0.3309	0.3385	0.3044	0.4025
πC	0.3152	0.3166	0.3393	0.2692	0.3543	0.3668	0.2923	0.3393	0.3887	0.4039	0.3830	0.3687	0.3828	0.3895	0.3836	0.3787	0.3832
πT	0.3147	0.3146	0.1302	0.2845	0.1287	0.1064	0.1409	0.1302	0.0662	0.0783	0.1164	0.0979	0.1204	0.0877	0.0990	0.1237	0.0896
πG	0.1846	0.1841	0.2050	0.2110	0.2135	0.2097	0.2826	0.2050	0.2276	0.1812	0.1985	0.2132	0.2140	0.1918	0.1789	0.1932	0.1247
α	–	–	–	–	1.6711	1.1969	0.2795	0.1363	0.2408	0.1374	0.0949	5.1547	0.1310	15.149	2.3496	0.1491	1.0221
I	0	0	0	0	0.5829	0	0	0	0	0	0	0.5879	0	0.5775	0.5785	0	0.5303
Ti/Tv ratio	–	–	–	2.6541	–	–	–	–	–	–	–	–	–	–	–	–	13.8496
R(a) [A-C]	1.1094	0.9889	1.0733	–	2.2496	0.6892	1.6620	0.3604	163478.45	0.08066	0.6243	1289.5616	0.7773	1.1767	1.0733	1408.1095	–
R(b) [A-G]	5.1571	5.0965	5.4676	–	71.8733	30.9663	7.1987	10.0887	5373712.00	57.2492	24.7197	36274.0742	15.1338	31.9667	30.8831	11189.6514	–
R(c) [A-T]	1.2321	1.2493	1.0343	–	1.7034	0.6659	2.0029	1.2930	107937.78	1.9787	0.7366	2030.5664	1.0047	0.8836	2.0316	1531.7542	–
R(d) [C-G]	1.0337	1.0175	1.2287	–	1.2602	0.3265	1.0715	0.0000	0.0000	0.4327	0.4522	583.7142	0.0000	0.0000	0.5698	311.8066	–
R(e) [C-T]	5.1723	5.0834	5.4201	–	36.9278	12.9552	10.0005	11.4824	1093238.88	21.1377	14.7202	16167.6572	10.8711	15.2253	23.3920	15823.3740	–
Rate (mito)					0.025	0.029	0.016	0.019	0.019	0.026	0.019	0.024	0.025	0.022	0.021	0.014	0.024

suggesting the inclusion of heterospecific DNA fragments in the published mitogenomes of *O. tazcanowskii* and *P. davidiana*. In tree topologies inferred from the four remaining coding markers (COI, COII, COIII, ND5) newly generated sequences of the latter two species were resolved within clades of conspecific mitogenome sequences from GenBank at low pairwise distances (<1%; identical sequences for COII and COIII; Table S3, Fig. 2C-F, S1). The topology resulting from COII showed only poor resolution of the deeper splits with the three outgroups nested in *Pyrgilauda*; (Fig. 2D). In the minimum spanning network inferred from a larger data set of the DNA barcoding marker all COI fragments from mitogenomes were nested in the correct cluster (Fig. 2G). In contrast, we found a striking mismatch in the position of the *P. davidiana* mitogenome sequence in six out of thirteen mtDNA markers (Fig. S2): In tree topologies inferred from ATP8, ATP6, ND3, ND4L, ND4, and cyt*b* the fragments representing the *P. davidiana* mitogenome clustered with the two mitogenomes of *O. tazcanowskii* at unexpectedly short branch lengths. For these six markers, the two *O. taczanowskii* mitogenomes and the *P. davidiana* mitogenome were extremely similar (with pairwise comparisons ranging at *p*-distances below 0.01, identical sequences for ATP8, ND3 and ND4L; Table S3, Fig. S1), whereas *p*-distances between *P. davidiana* and *P. blanfordi* were about ten times higher than expected (Fig. S1). That mismatch was even found within a single marker: In the haploytpe network inferred from the first fragment of cyt*b* (403 bp), all sequences inferred from mitogenomes were nested in the correct cluster, whereas in the haplotype network based on the second half of the same marker (477 bp), the mitogenome fragment of *P. davidiana* was identical to the central haplotype of the *O. tazcanowskii* cluster (Fig. S3). The remaining three markers and the non-coding control region showed strong mismatches in the position of two species (Fig. S4): For ND1 and ND2, *O. tazcanowskii* clustered with the sister-species pair *P. davidiana* and *P. blanfordi* (identical ND2 sequences for *O. tazcanowskii* and *P. davidiana;* Table S3, Figs S1, S5). Finally, tree topologies for ND6 and the flanking non-coding control region showed mismatches for mitogenomes of both *O. tazcanowskii* and *P. davidiana* (also confirmed by the minimum-spanning network based on a larger data set for the control region [n = 24]; Fig. S4). Unexpectedly, for the latter three coding markers (ND1, ND2, ND6), *p*-distances between the two *O. taczanowskii* mitogenomes were about twenty times higher (mean p-dist. = 10 %) than for pairwise comparisons with the remaining ten markers (mean p-dist = 0.5 %; Table S1, Fig. S1).

According to this comparison of topologies and pairwise p-distance values across 13 mitochondrial markers we needed to take into account that GenBank mitogenomes of both *P. davidiana* and *O. taczanowskii* included heterospecific DNA sequences. We therefore repeated phylogenetic reconstructions based on the mitochondrial data set after replacing all unreliable fragments of coding markers in the consensus mitogenome sequence of the two latter species by anonymous data („N“; compare Table S1, Fig. S1). The only major difference among consensus trees inferred from the two original mitogenome data sets referred to the position of *Onychostruthus* that was nested in *Pyrgilauda* in the uncorrected consensus tree on the one hand (Fig. 3A), whereas *Montifringilla* and *Pyrgilauda* were reciprocally monophyletic and *Onychostruthus* was sister to the latter in the corrected consensus tree (Fig. 3B). The latter topology was fully congruent with that of the cyt*b* tree inferred from the full set of samples (Fig. S5). Within *Pyrgilauda*, the Afghan snowfinch, *P. theresae*, was resolved as sister to *P. ruficollis* with full support (Fig. 3). In *Montifringilla*, mitogenomes for *M. nivalis* and *M. henrici* downloaded from GenBank formed a monophyletic clade with extremely short within-clade branch lengths (Fig. 3) and *p*-distances ranged between 0.05 and 0.2 % (Table S1). In contrast, our specimens of *M. nivalis* from

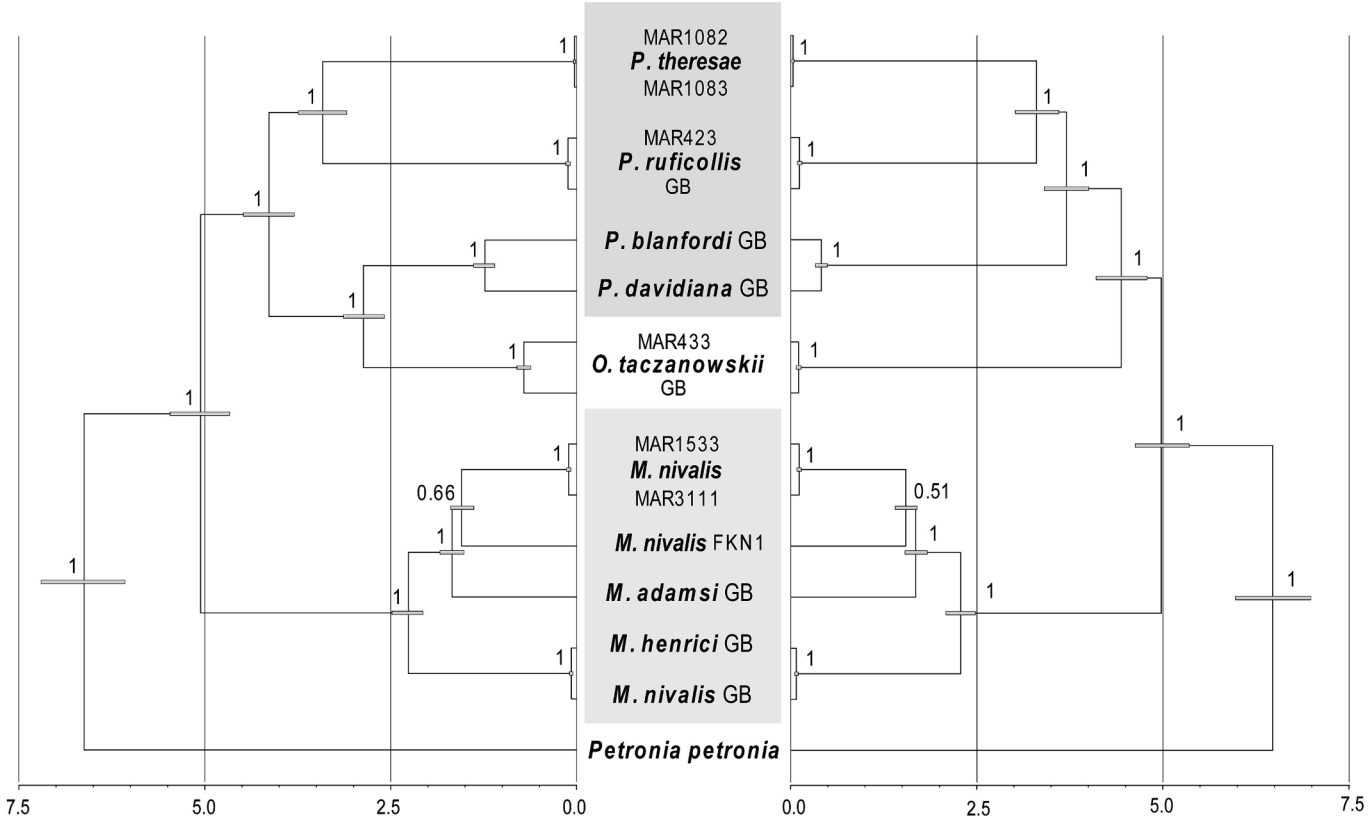

Fig. 3. Phylogenies of snowfinches based on 13 mitochondrial coding regions; the two outgroup taxa (*Passer domesticus* and *Hypocryptadius cinnamomeus*) were pruned from the trees; Bayesian inference of phylogeny with BEAST v.2.6.2, MCMC chain length: 50,000,000 generations, burnin: 30 % of sampled trees; A) uncorrected alignments containing full mitogenome sequences for all species; *Pyrgilauda* is paraphyletic; B) alignments containing missing data for heterospecific markers of the putative chimeric mitogenomes of *P. davidiana* (KJ148632; missing data for ND3, ND4, ND4L, ND6, ATP6, ATP8 and cyt*b* 2nd half) and *O. taczanowskii* (KJ148631; missing data for ND6, ND1 and ND2); *Pyrgilauda* is monophyletic.

Asia and Europe were clearly separated from their sister clade *M. adamsi,* and from a third monophyletic clade including mitogenome sequences from GenBank for *M. henrici* and *M. nivalis* (Fig. 3).

3.2. Genome-wide SNPs

3.2.1. Effects of reference genome choice and missing data

For most samples (n = 35) more than 1 million reads were recovered from the Illumina run. A lower number of reads was recovered only for three frozen tissue samples and one historical toe pad sample of *P. theresae* (compare Table S1). Mapping rates ranged between 70 – 80 %, and were slightly higher for the two ingroup reference genomes with *Pyrgilauda* species and *O. tazcanowskii* scoring best with the respective congeneric reference genome (Table S1; Fig. S6). As the only exception, all *Montifringilla henrici* had lower mapping rates for the two ingroup reference genomes as compared to that of the outgroup (Fig. S6). As could be expected, the historical *P. theresae* sample showed the lowest mapping rate (Fig. S6). After trimming and first filtering steps the sequence matrices inferred from each of the three reference genomes had sizes of 1,664,040 – 2,242,210 sites (with 52.12 – 53.25 % missing sites) and contained 66,729 – 107,218 SNPs (38.41 – 39.28 % missing sites in the SNP matrices; Table S5).

Phylogenies inferred from test runs (MCMC chain length 10 million generations) with unthinned SNP data sets from different reference-mapping yielded congruent tree topologies (Fig. S7). For all three approaches, the highest node support from posterior probabilities was received from data sets with 20 % missing data allowed (Fig. S7). For higher proportions of missing data (30 %) posterior probabilities decreased from strong to poor support at eight nodes for the *O. tazcanowskii* reference genome, at three nodes for the *P. domesticus* reference genome, and at a single node for the *P. ruficollis* reference genome (Fig. S7). Thinning of data yielded a strong increase of posterior probabilities at the node uniting all snowfinches (full support in all reconstructions), and at several nodes of the *Montifringilla* clade for the phylogeny inferred from read mapping to the *O. tazcanowskii* reference genome (Fig. S7). 16 out of 18 tree topologies [thinned and unthinned SNP data] supported i) a sister-group relationship of *Onychostruthus* and *Pyrgilauda* (Fig. S7), and ii) a sister-group relationship between *M. adamsi* and *M. nivalis* (Fig. S7). Three of the four deviating topologies that suggested a sister-group relationship between *M. adamsi* and *M. henrici* (thinned data set, 10 % and 20 % missing data; posterior probabilities 0.97 and 0.79; not shown), were inferred from mapping to the outgroup reference genome (*P. domesticus*; Fig. S7).

For final BEAST runs we selected SNP data sets inferred from

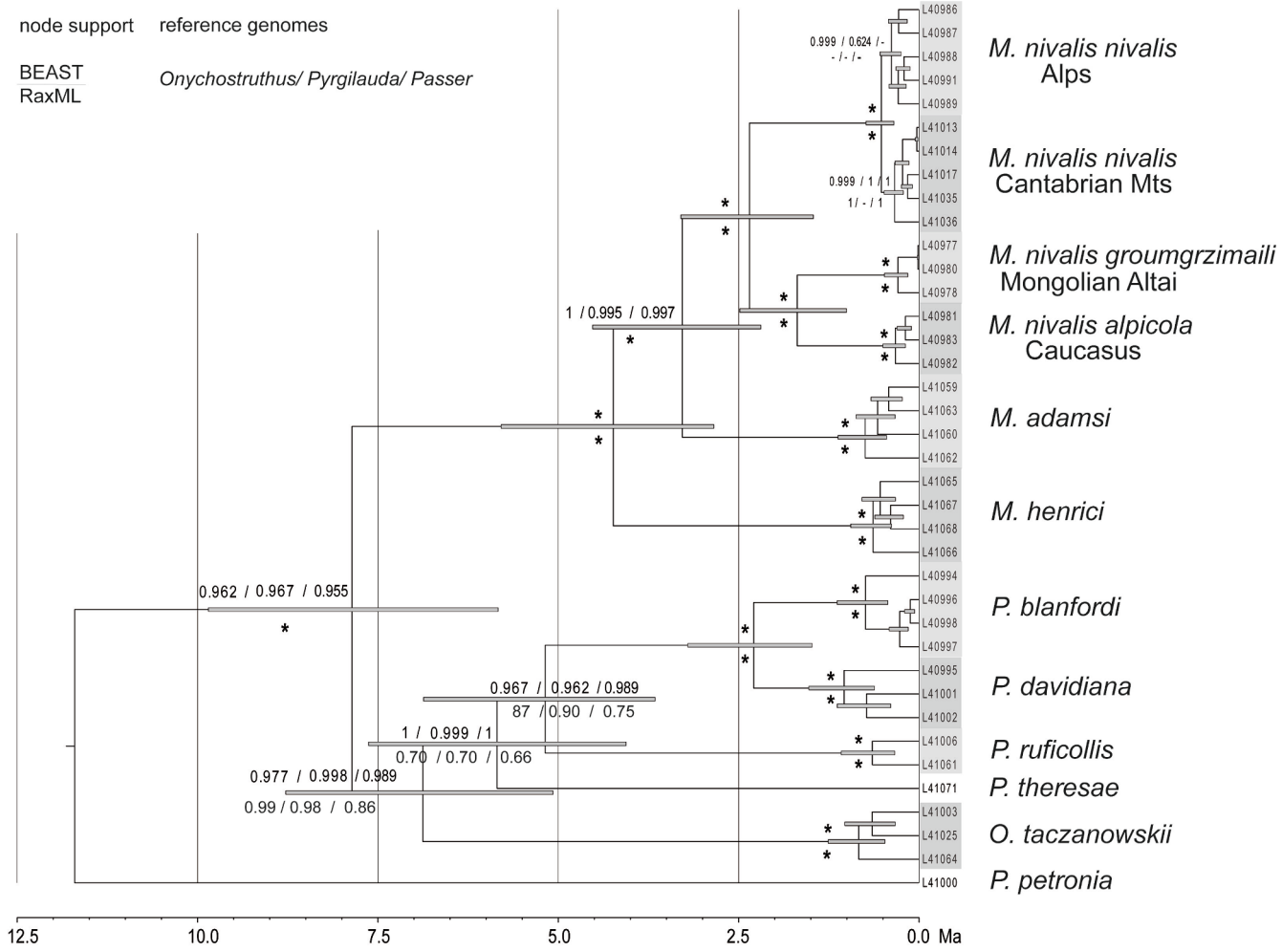

Fig. 4. Phylogeny of snowfinches inferred from three sets of SNPs from cleaned ddRAD seq reads mapped on three different reference genomes: *Passer domesticus*, *Pyrgilauda ruficollis* and *Onychostruthus tazcanowskii*; Bayesian inference of phylogeny relied on three combined runs with BEAST for each data set (MCMC chain length: 50 million generations, trees sampled every 5000 generation, burnin: 30% of sampled trees); the figure shows the time-calibrated topology for the data set inferred from mapping to the *O. taczanowskii* genome (unthinned data); node support values from Bayesian posterior probabilities: above nodes: unthinned data, 20% missing (matrix sizes: 30,550 – 40,695 – 39,952 SNPs), below nodes: thinned data (biallelic unlinked SNPs), 30% missing (matrix sizes: 6,263 – 8,949 – 8,893 SNPs); full node support for all three data sets is indicated by an asterisk.

mapping to all three reference genomes i) allowing 20 % missing data for unthinned SNP data (matrix sizes ranged from 30,550 – 40,695 SNPs), ii) allowing 30 % missing data for the thinned data set (matrix sizes 6,263 – 8,949 biallelic unlinked SNPs; Table S5).

3.2.2. *Inference of phylogeny and divergence times*

Tree topologies were largely congruent i) between BEAST trees (Fig. 4) and RaxML trees (Fig. S8) based on genome-wide SNPs inferred from read mapping to each of the three reference genomes, ii) between all trees based on genome-wide SNPs (Fig. 4, Fig. S8), the corrected mitogenome tree and the cyt*b* tree (Fig. 3, Fig. S5). The three snowfinch genera were reciprocally monophyletic in all phylogenetic reconstructions, with *Onychostruthus* and *Pyrgilauda* being sister taxa and separated from *Montifringilla* by a deep split dated to 7.8 million years ago (mya) in the genome-wide SNP trees and to 5.0 mya in the mitogenome tree (Figs. 3, 4). Tree set analysis of the autosomal data set with DensiTree (Fig. 5A) showed little uncertainty of topology and good concordance of consensus trees with the BEAST trees (Fig. 4). In contrast, the same analysis based on the Z-chromosomal SNP data set showed a much greater uncertainty of topology (decreasing intensity towards the root of both, sets of all trees and of consensus trees; Fig. 5B), which is reflected by the greater reticulation of the Z-chromosomal neighbor net network compared to that inferred from autosomal SNPs (Fig. 5C, D).

The sole incongruence between phylogenies inferred from genome-wide SNPs and from mitochondrial markers concerned the position of the Afghan endemic *P. theresae*. It was resolved as descending from the most ancient offshoot of the *Pyrgilauda* clade in the SNP-based phylogenies (Figs. 4, 5A), instead of being sister to *P. ruficollis* in mitogenome- and cyt*b*-based phylogenies (Fig. 3, Fig. S5). In PCA (inferred from the SNP data set with 20 % missing data allowed), *O. tazcanowski* was strongly separated from all *Pyrgilauda* species along the x-axis (PC1 explaining 49.3 % of the total variation; Fig. 6B). Members of *Pyrgilauda* were mainly separated along PC2 (explaining 20.3 % of the total variation) with clusters of *P. blanfordi* and *P. davidiana* showing some overlap (Fig. 6B). The latter two sister taxa (Figs. 4, 5), appeared as separate clusters only in the scatterplots of PC1 vs PC3 (0 % missing data) and PC1 vs PC4 (20 % missing data; Fig. S9). Inclusion of the historical sample *P. theresae* strongly reduced the number of SNPs (from 8,863 filtered SNPs and 3,233 unlinked SNPs to only 341 filtered and 175 unlinked SNPs with and without *P. theresae*), however, it had no effect on the divisive clustering pattern among *Pyrgilauda* and *Onychostruthus* species (Fig. S9).

For within-clade relationships of *Montifringilla*, all phylogenetic reconstructions suggested a sister-group relationship of *M. nivalis* and *M. adamsi* with *M. henrici* being sister to the latter two (Figs. 4, 5). In

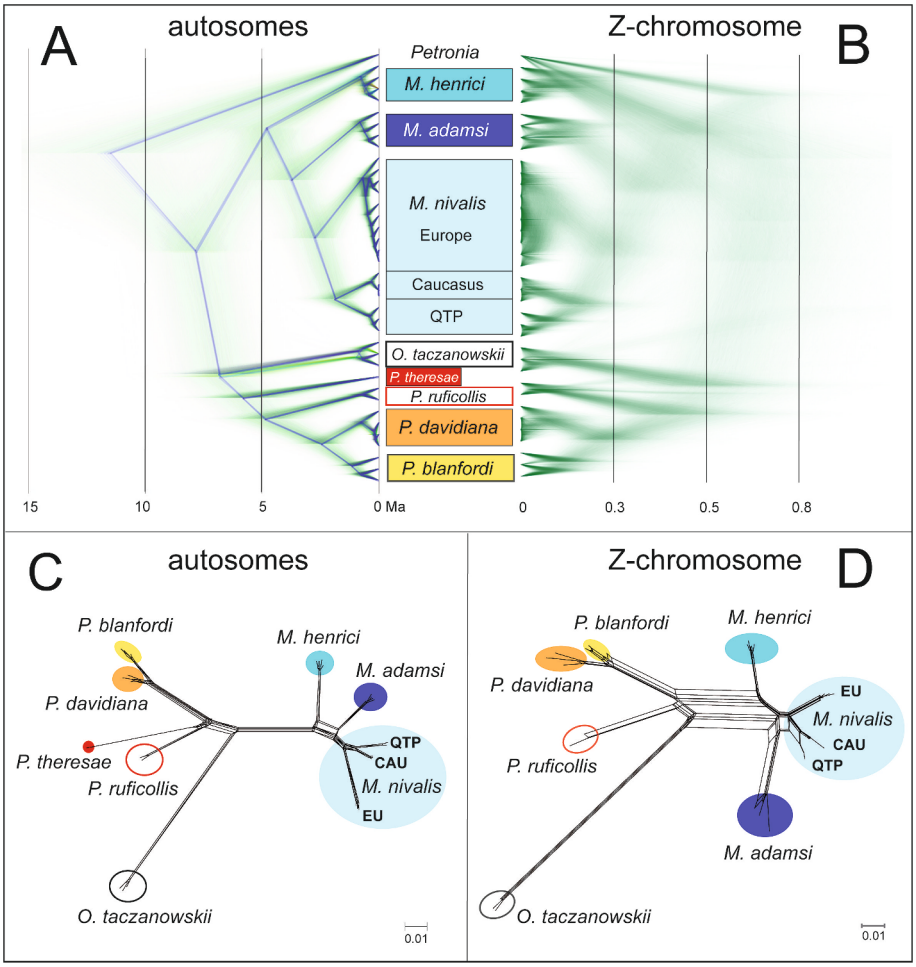

Fig. 5. Phylogenetic relationships of snowfinches inferred from cleaned ddRAD seq reads mapped on the *Passer domesticus* reference genome for two data sets of autosomal SNPs (A, C; unthinned data; n = 30,550 sites; with 20 % missing data allowed) and Z-chromosomal SNPs (B, D; unthinned data; n = 899 SNPs; with 30 % missing data allowed; due to a disproportionately high amount of missing data *P. theresae* was excluded from this analysis); A, B) tree set analysis from three independent runs with BEAST using DensiTree (all trees: green; consensus trees: blue); C, D) unrooted neighbor networks; lineages of *Montifringilla nivalis*: QTP = Qinghai-Tibet Plateau, CAU = Caucasus, EU = Europe. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

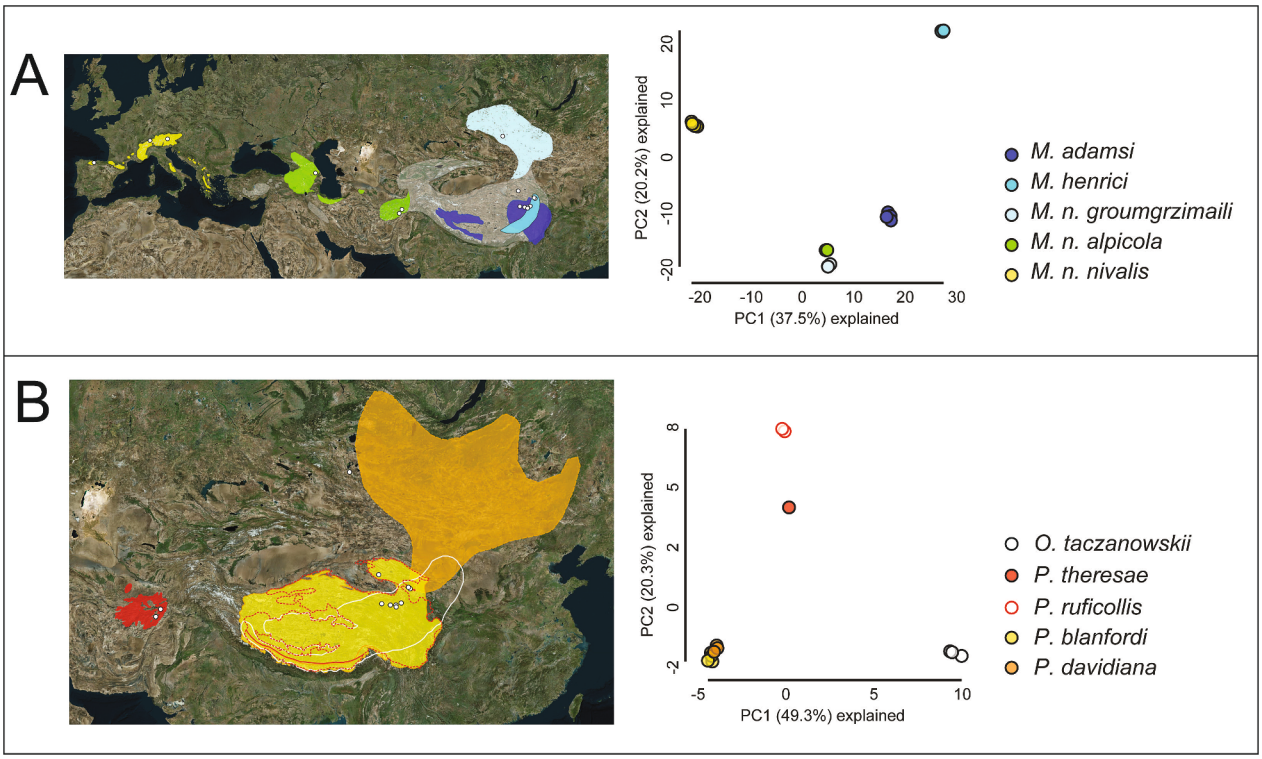

Fig. 6. Genetic divergence among snowfinch taxa as reflected by principal component analysis based on thinned data sets inferred from alignment on the *Passer domesticus* reference genome allowing for 20% missing data; scatterplots of PC1 vs PC2, color codes for taxa in PCA (right) correspond to colors of taxon ranges on the maps (left); A) *Montifringilla*; for *M. nivalis* only the ranges of the three subspecies included in the analysis received a color code, whereas the remaining range (other ssp.) was left transparent; B) *Pyrgilauda* and *Onychostruthus*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PCA, *Montifringilla nivalis* was strongly separated from the other two congenerics along the x-axis (PC1 explaining 37.5 % of the total variation; Fig. 6A) and showed remarkable intraspecific divergence between nominate *M. n. nivalis* from Europe and the two Asian subspecies *M. n. alpicola* and *M. n. groumgrzimaili* (Fig. 6A; PC2 explaining another 20.2 % of the total variation). The split between the Asian and European lineages of *M. nivalis* was dated to 2.3 – 2.7 mya (mitogenomes: 1.53 [1.40 – 1.69] mya). F_{ST} values between the two major lineages (0.39 – 0.48; Table 3) ranged at a similar magnitude like F_{ST} values among *Montifringilla* species (*M. nivalis* vs. *M. adamsi*: 0.34 – 0.41; *M. nivalis* vs. *M. henrici*: 0.39 – 0.47; *M. adamsi* vs. *M. henrici*: 0.34; Table 3). The most recent divergences among terminal sister taxa (Figs. 4, 5) were dated to 1.7 – 1.9 mya for splits among Caucasian *M. n. alpicola* and Mongolian *M. n. groumgrzimaili* (mitogenomes: 0.10 [0.07–0.13] mya) and at 2.3 – 2.4 mya between *P. davidiana* and *P. blanfordi* (mitogenomes: 0.40 [0.32–0.48] mya; smallest F_{ST} value for comparison among species: 0.18; Table 3).

Within European *M. n. nivalis*, samples from the Alps and from the Cantabrian Mountains were reciprocally monophyletic only in Bayesian trees based on SNP data sets inferred from mapping on either of the ingroup reference genomes (Fig. 5). In BEAST trees based on SNPs inferred from mapping on the house sparrow reference genome (and in all three RaxML trees), the Cantabrian snowfinch populations appeared as a monophyletic group that was nested in all other *M. n. nivalis* as a fully supported terminal clade. Accordingly, genetic divergence between the two European populations was limited (F_{ST} value: 0.087; Table 3). In PCA, the two European populations appeared as separate clusters only in the scatterplot of PC1 vs PC4 (Fig. S10).

3.2.3. Quartet sampling

Quartet sampling (QS) scores for the total sampling indicated full support for all terminal clades (species-level or subspecific taxa) and most of the deeper nodes of the snowfinch phylogeny (Fig. 7). Lower QC scores indicated some discord with a skewed distribution of discordant signals in the sister-group relationship of *M. adamsi* and *M. nivalis* and strong discordance again with a skewed ratio in the sister-group relationship of *Onychostruthus* and *Pyrgilauda* (Fig. 7). Quartet informativeness was lowest at nodes i) uniting all *Pyrgilauda* species and ii) uniting the *Pyrgilauda* crown group except *P. theresae* (with 65 % and 48 % of all quartets having passed the likelihood cut-off; Fig. 7). To test for an effect of single branches on nodal support we repeated the QS analysis under exclusion of two individuals that had the lowest quartet fidelity (QF) scores (*P. theresae*: QF = 0.56; one *M. n. nivalis* from the Swiss Alps, L40989 [FKN1]: QF = 0.45; Fig. 7). The second analysis yielded full support for all nodes except for the sister-group relationship between *Onychostruthus* and *Pyrgilauda* (*P. theresae* excluded), which showed a poor QC and QD = 0 despite a moderate QI score (signifying weak support with significant skew towards one alternative relationship; Fig. 7). The Cantabrian *M. nivalis* clade received strong support from QS scores, too (Fig. 7).

4. Discussion

4.1. Quality assessment of mitogenome sequences

Next-generation sequencing (NGS) techniques have recently revolutionized molecular systematics and have been applied to generate high-quality mitogenomes of non-model organisms in the plant and animal kingdoms (Briscoe et al., 2016; Kehlmaier et al., 2019, 2021; Maddock et al., 2016; Yuan et al., 2016). Apart from the finding that a notable percentage of mitogenomes deposited at GenBank was backed by deficient *meta*-data (Strohm et al., 2016), NGS methods have revealed a variety of errors in published mitogenome sequences such as incorrect gene annotation, missing reads or duplications, inclusion of numts or

Table 3
Intra- and interspecific genetic differentiation and distance among snowfinch taxa; F_{ST} values inferred from genome-wide autosomal SNPs (upper diagonal; reference genome *Passer domesticus*; 20 % missing data allowed; n = 30,550 sites); p-distance values inferred from 1144 bp of the mitochondrial cytochrome-*b* (lower diagonal).

	M. n. nivalis Cant.	*M. n. nivalis* Alps	*M. n. alpicola*	*M. n. groumgrzimaili*	*M. adamsi*	*M. henrici*	*P. davidiana*	*P. blanfordi*	*P. ruficollis*	*P. theresae*	*O. tazcanowskii*	*P. petronia*
M. n. nivalis Cantabrian Mts	–	0.0873355	0.421948	0.475532	0.410394	0.468640	0.625886	0.534988	0.617837	0.775211	0.646510	0.816053
M. n. nivalis Alps	0.0021	–	0.386614	0.436230	0.386671	0.443385	0.601708	0.516821	0.590741	0.755804	0.624559	0.797324
M. n. alpicola	0.0442	0.0461	–	0.337359	0.338370	0.394863	0.568617	0.480926	0.559353	0.729820	0.596060	0.781067
M. n. groumgrzimaili	0.0466	0.0485	0.0043	–	0.366867	0.430914	0.606667	0.506102	0.596076	0.722274	0.625105	0.810012
M. adamsi	0.0400	0.0405	0.0411	0.0431	–	0.340002	0.511840	0.448769	0.500629	0.629115	0.546497	0.715263
M. henrici	0.0561	0.0563	0.0597	0.0602	0.0470	–	0.520177	0.451539	0.508726	0.605885	0.552999	0.730608
P. davidiana	0.0776	0.0795	0.0694	0.0713	0.0696	0.0810	–	0.18083	0.458616	0.659060	0.552037	0.753774
P. blanfordi	0.0784	0.0803	0.0704	0.0724	0.0716	0.0818	0.0045	–	0.376991	0.454128	0.485414	0.669589
P. ruficollis	0.0907	0.0914	0.0892	0.0908	0.0812	0.0926	0.0749	0.0761	–	0.559843	0.539417	0.746129
P. theresae	0.0832	0.0849	0.0818	0.0817	0.0710	0.0873	0.0576	0.0586	0.0675	–	0.612398	0.875000
O. tazcanowskii	0.0954	0.0962	0.0978	0.1009	0.0867	0.0977	0.0864	0.0887	0.0978	0.0889	–	0.737817

concatenation of heterospecific DNA fragments (Prada and Boore, 2019; Sangster and Luksenburg, 2020, 2021; Skujina et al., 2017). In a re-evaluation of 1,876 avian mitogenomes, Sangster and Luksenburg (2021) demonstrated that every second phylogenetic study that incorporated mitogenome data from GenBank included at least one problematic sequence. Therefore, a thorough quality assessment of sequence data is recommended prior to phylogenetic analyses. For validation of newly generated mitogenome sequences, Botero-Castro et al. (2016) provided a set of quality control guidelines, such as using larger sets of mtDNA markers (e.g. the DNA-barcoding marker COI) for comparison.

Following those recommendations, we could identify two typical sources of error in published mitogenome sequences of snowfinches. First, mitogenomes of *O. tazcanowskii* and *P. davidiana* available at GenBank contained heterospecific DNA fragments of another snowfinch species (for nine out of thirteen coding markers). Paraphyly of *Pyrgilauda* in our uncorrected mitogenome tree is very likely due to the effect of these two chimeric mitogenomes (compare Sangster and Luksenburg, 2020). For birds, Sangster and Luksenburg (2021) identified 23 published chimeras across the entire class of Aves (1.5 % of their mitogenome data set) and the same team reported an even greater proportion of chimeras in published mitogenomes of fishes (5.7 %, Sangster and Luksenburg, 2020). Inclusion of chimeric mitochondrial DNA sequences in genetic data sets has previously blurred phylogenetic relationships of extinct taxa, such as the Mascarene parrot, *Mascarinus mascarin* (Podsiadlowski et al., 2017). Second, the single published mitogenome assigned to the taxon "*Montifringilla nivalis*" turned out to be nearly identical to that of *M. henrici* and both were deeply diverged from our newly generated mitogenomes for Asian *M. n. groumgrzimaili* and European *M. n. nivalis*. This mismatch is certainly due to flawed taxonomy, because the Tibetan snowfinch was previously included in *M. nivalis* as subspecies *M. n. henrici* (Table 1; compare Cheng, 1987, map on p. 943; Glutz v. Blotzheim and Bauer, 1997). Incorrect assignment of GenBank sequences to species-level taxa due to dated nomenclature or to simple misidentifications was reported as another conspicuous pitfall in phylogenetic reconstructions (Hofstetter et al., 2019; Salvi et al., 2021; Tritsch et al., 2017). Therefore, the use of published mitogenome sequences for *meta*-analyses (such as trait evolution or niche evolution) requires particular care. For example, the phylogenetic backbone used for the reconstruction of niche evolution in snowfinches by Cobos et al., (2021; Fig. 1B) suffered from both sources of error, causing misplacement of *O. taczanowskii* (chimeric mitogenome) and of *M. henrici* (flawed taxonomy; *M. nivalis* is actually missing from their phylogeny; compare Fig. 3).

4.2. Effects of reference genomes and filtering on SNP calling and phylogeny

Generally, independent SNP analyses yielded highly consistent results regardless of the percentage of missing sites or of reference genome choice. For snowfinches, mapping rates (and thus sequence and SNP matrix sized) were slightly higher for the ingroup reference genomes (*P. ruficollis*, *O. taczanowskii*) than for the outgroup reference genome (*P. domesticus*), a general trend that was found in other bird groups (Charadriiformes; Galla et al. 2019) or in ray-finned fishes (Bohling, 2020). However, single outlier taxa might not follow that general pattern, like *M. henrici* in our taxon sampling, that consistently showed lower rates for ingroup-mapping across all samples. Previous studies confirmed possible effects of reference genome choice on divergence patterns reflected by clustering methods or on tree topologies especially in cases of high incomplete lineage sorting (Valiente-Mullor et al., 2021; Rick et al., 2023). However, such effects might have played a minor role for our snowfinch data set, because the outgroup *Passer domesticus* still is a rather close relative to the snowfinches, considering that previous studies had relied on the annotated zebra finch (*Taeniopygia guttata*; Estrildidae) genome for phylogenetic reconstructions of different passerine families, such as larks (Alaudidae; Stervander et al., 2016) or

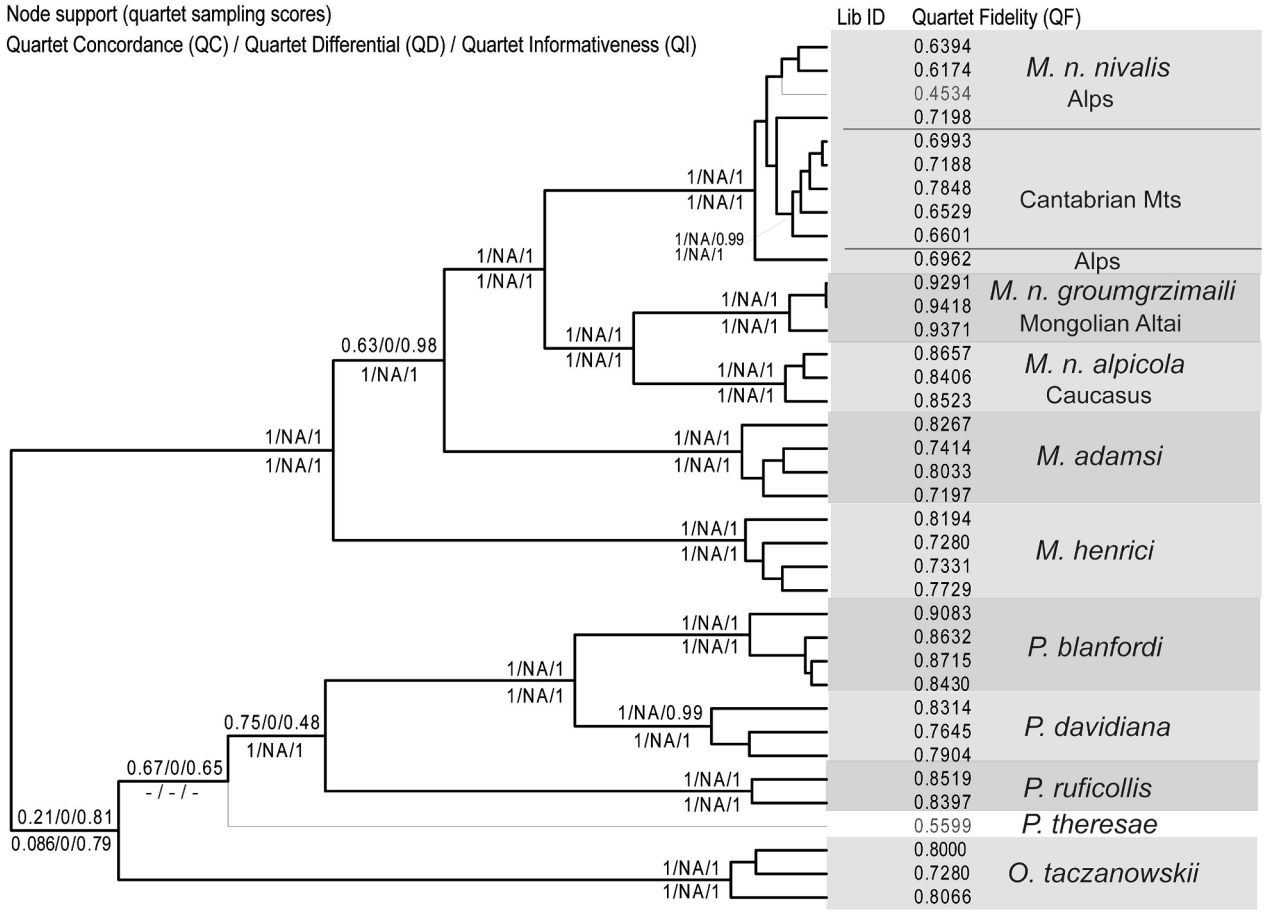

Fig. 7. Phylogeny of snowfinches inferred from cleaned ddRAD seq reads mapped on the *Passer domesticus* reference genome (30,550 SNPs); quartet fidelity (QF) scores shown behind terminal taxa; quartet sampling (QS) results with scores for quartet concordance (QC), quartet differential (QD; NA = not applicable) and quartet informativeness (QI) indicated at nodes (scores for total data set above nodes; scores for reduced data set excluding two individuals with lowest QF score (marked in light grey) below nodes; within-clade scores for terminal taxa not shown).

tits (Paridae; Stervander et al., 2015). Nevertheless, an ingroup reference genome might be advantageous for uncovering shallow divergences reflecting intraspecific diversification, as reciprocal monophyly of the two European mountain clades of *M. n. nivalis* could be resolved only by SNP data sets inferred from ingroup-mapping. For population genomic studies, even a local or regional reference individual can be used to decrease reference bias, as was exemplified for European sticklebacks (*Gasterosteus aculeatus*; Thorburn et al., 2023).

Apart from reference genome choice, filtering thresholds, like proportions of missing data and minor allele count were shown to have major effects on phylogenetic analyses, such as possible removal of true rare or private alleles that are relevant for detection of intraspecific phylogeographic patterns or past demographic events (O'Leary et al., 2018; Rick et al., 2023). The latter effect might be of minor importance for time-calibrated trees covering intergeneric separation across evolutionary time spans of more than 15 myr. However, while we could confirm a postulated effect of missing data proportions on tree topologies or node support (see Eaton et al., 2017), this was not the case for clustering patterns inferred from PCA. Previous studies had inferred fully resolved phylogenies for evolutionary radiations with even greater amounts of missing data (60 % missing data per SNP: Crotti et al., 2019; up to 90 % missing data: Tripp et al., 2017). For our snowfinch data set, data thinning (extracting only biallelic unlinked SNPs) improved node support generally for the most ancient node (uniting the three snowfinch genera), and specifically also for within-clade relationships of *Montifringilla* in the outgroup-reference data set.

4.3. Quartet discordance and mito-nuclear discordance

Generally, QF values for tip clades of the snowfinch phylogeny were similar to those in previous phylogenies that included higher numbers of taxa (e.g. Kong et al., 2022). Neither of the QS analyses supported the phylogenetic hypothesis previously inferred from mitogenome sequences (Cobos et al., 2021) that had placed *Onychostruthus* firmly nested in a paraphyletic *Pyrgilauda* (Fig. 2B). QS scores suggested an effect of poorly informed branches (Pease et al., 2018) only for the *Pyrgilauda* clade, QC and QI scores for this clade improved when the analysis was repeated after removal of two terminal taxa with the lowest QF scores (*P. theresae* and one Swiss sample [FK1] of *M. n. nivalis*). Removal of *P. theresae* from the taxon sampling did not affect the phylogenetic relationships among the remaining snowfinch species, which suggests that *P. theresae* is not a "rogue taxon" per se (Westover et al., 2013), but instead the poor scores for the *Pyrgilauda* clade were caused by the high proportion of missing data in the SNP sequence inferred from the historical *P. theresae* sample. Exclusion of "poor-quality individuals" can improve the robustness of phylogenetic trees (O'Leary et al. 2018), and in fact, in the second QS analysis (without poor-quality individuals) only a single node showed a significant discord, i.e. the sister-group relationship of *Onychostruthus* and *Pyrgilauda*. This might also explain why the position of *P. theresae* is the only major conflict between nuclear and mitochondrial trees in our study (due to the high percentage of missing data inferred from that historical toe-pad sample). The inclusion of poor-quality individuals might also have affected QC and QI scores in the *Montifringilla* clade, because the

sister-group relationship of *M. adamsi* and *M. nivalis* received full support from QS analysis only when the two poor-quality samples were excluded.

The inferred QD value of 0 suggests that discordant quartets exclusively resolved one of the two possible alternative quartet topologies for each of the three most ancient nodes of the *Onychostruthus/Pyrgilauda* clade. This indicates that the discord might be a result of past reticulate evolution, i.e from past horizontal gene transfer between the two sister clades concerned (Pease et al., 2018; Paetzold et al., 2019). Genome-wide sequence data sets provide a useful matrix for tracing past horizontal gene flow even among deeply divergent clades of a phylogeny, as demonstrated for Himalayan and Tibetan pikas (*Ochotona*; Dahal et al., 2023) or for more shallow divergences among species-level taxa during island radiations (Manthey et al., 2020; Martin et al., 2021). Nevertheless, incomplete lineage sorting (ILS) must be considered as a reason for unresolved phylogenetic relationships at shallow levels of divergence (Palacios et al., 2019; Peters et al., 2007; Wang et al., 2018; Liu et al., 2022) or associated with recent range expansions (Mende and Hundsdoerfer, 2013) in snowfinches as well. In snowfinches, poor support for reciprocal monophyly of Alpine and Cantabrian populations of *M. n. nivalis* might be (partly) due to ILS, for example (only the latter group received constant support from different analyses, even from QS analysis). Notably, for such shallow divergences reference genome choice was shown to have strong effects in phylogenetic data sets including high levels of ILS (Rick et al., 2023), and accordingly we found strongest support for two reciprocally monophyletic European snowfinch clades from the two data sets inferred from ingroup-mapping.

In summary, strong node support values and consistency of tree topologies across phylogenetic reconstructions adds further support to previous findings that genome-wide SNP data provide more robust and more reliable phylogenetic hypotheses as compared to a small number of conventional loci combined in multi-locus analyses, for example of mtDNA markers and nuclear introns (Glon et al., 2021). Future studies shall rely on expanded taxon samplings (e.g. subspecific taxa of widespread *M. nivalis*) and data allowing the measurement of gene/locus tree discord to investigate further patterns of diversification and possible reticulate evolution in snowfinches.

4.4. Phylogeny and evolutionary history of snowfinches

Our genome-wide data sets (SNPs and corrected mitogenomes) clearly rejected the postulated paraphyly of the snowfinch genus *Pyrgilauda* (Fig. 2A; Cobos et al., 2021) and confirmed that in accordance with current taxonomy (Gill et al., 2023), *Montifringilla* and *Pyrgilauda* are reciprocally monophyletic (compare Päckert et al., 2021). Furthermore, our phylogenetic reconstructions clearly supported a sister-group relationship of the two genera *Onychostruthus* and *Pyrgilauda*, that were previously united under genus *Pyrgilauda* (Mlíkovský, 1998; Summers-Smith, 2009). Snowfinch vocalizations were shown to facilitate distinction among genera, e.g. according to different flight call types (Gebauer et al., 2006) and to phylogenetic signal of syllable types in songs (Lei et al., 2005). However, a comprehensive taxon-complete bioacoustics analysis for snowfinches is missing so far.

Furthermore, all tree topologies inferred from mitogenomes and genome-wide SNPs clearly confirmed *M. nivalis* as a monophyletic taxon and sister to *M. adamsi*. These findings clearly reject the previously postulated i) paraphyly of *M. nivalis* based on limited sequence information (Päckert et al., 2020b, 2021) and ii) sister-group relationship of *M. nivalis* and *M. henrici* (Cobos et al., 2021), both of which were often treated as conspecifics (Table 1; see also Mayr, 1927; Moreau and Greenway Jr, 1962; Portenko and Vietinghoff-Scheel 1974). Previous authors either argued in favor of a distinctiveness of *M. henrici* against the conspecific *M. nivalis nivalis* and *M. nivalis adamsi* (e.g. Hartert (1921–1922); Cramp and Perrins, 1994), or for a separation of *M. adamsi* against conspecific *M. n. nivalis* and *M. n. henrici* (e.g. Vaurie, 1959). However, due to the long evolutionary time span of their separation (3.5 – 4.5 mya) and reciprocal monophyly they should represent distinct species-level taxa *M. nivalis*, *M. adamsi* and *M. henrici* (in accordance with Gill et al., 2023). Martens and Eck (1995) agreed on a closer relationship of *M. nivalis* and *M. adamsi* based on morphological traits as confirmed by our phylogeny. Vaurie (1959; p. 587) had synonymized *Montifringillia nivalis groumgrzimaili* with *Montifringilla nivalis alpicola*, however, genetic distinctiveness of the latter two warrants recognition of the latter two as separate (at least subspecific) taxa. The to-date unstudied populations of *M. n. kwenluensis* from the Kunlun Shan, Xinjiang Province (Cheng, 1987; Cramp and Perrins, 1994) could shed some light on any as yet undetected cryptic diversification or on the question of whether the phenotypical cline is associated with past or recent gene flow.

Our SNP-based divergence time estimate for the onset of the snowfinch radiation during the mid-Miocene (7.8 mya; see also Päckert et al., 2020b) was in good accordance with the time estimate inferred from whole-genome data (Qu et al., 2021: 8.3 [5.5 – 11. 4] mya). Taking into account, that mitochondrial DNA is inherited only from females and therefore can only elucidate the evolutionary history of female lineages, we consider the more ancient split ages inferred from the genome-wide SNP data set as the more reliable estimates for the timing of the snowfinch radiation. Generally, divergence time estimates can greatly differ between phylogenies inferred from mitochondrial and from nuclear markers even when the same calibration is applied to the data sets (McCormack et al., 2011; Ritchie et al., 2022). Päckert et al. (2021) identified a single Pleistocene out-of-Tibet dispersal event in snowfinches, because molecular sequence data for the Afghan snowfinch, *P. theresae*, was missing at that time. Our taxon-complete phylogeny showed that *P. theresae* likely originated from a more ancient Pliocene out-of-Tibet split between 5.8 mya (SNP data set) and 3.3 mya probably from a common ancestor with *P. ruficollis* (mitogenome data set). The Afghan endemic *P. theresae* would thus represent a comparatively ancient species-level lineage as compared to many other avian endemics from the Western Himalayas and adjacent mountain ranges that represent taxa of Pleistocene origin (deRaad et al., 2022; Wolfgramm et al., 2021).

The second, more recent out-of-Tibet colonization event in snowfinches was associated with westward dispersal of founder populations of extant *M. n. nivalis* into Europe during the early Pleistocene (2.7 – 1.5 mya; see Päckert et al., 2020b). As two major physical barriers, the Pyrenees (and their westward extensions to the Cantabrian Mountains) and the Alps have been identified as centers of recent diversification of several cold-adapted species in interglacial refuges (Hausdorf and Walther, 2021; Stewart et al., 2010; Tribsch and Schönswetter, 2003) and as hotspots of secondary contact zones including hybridization and gene flow across a latitudinal gradient (Ebdon et al., 2021; Hewitt, 2000; Schmitt, 2007). Accordingly, Pleistocene genetic divergence between montane populations from these European mountain systems was detected in European *M. n. nivalis* (this study), conifers (*Abies alba*, Scotti-Saintagne et al., 2021) and butterflies (genus *Erebia*; Schmitt et al., 2016; Vila et al., 2011). Repeated range contractions and expansions due to glacial cycles might have enhanced gene flow within and across mountain systems, particularly in conjunction with range expansion towards lower elevations during glacial periods (Stewart et al., 2010).

5. Conclusions

For current and future biodiversity research in a rapidly changing world, museum collections have become increasingly relevant as archives of occurrence records, species distributions and of phenotypic and genetic diversity (review in Meineke et al., 2019). Research on understudied regional faunal and floral assemblages benefits greatly from inclusion of collection materials to fill in current data and knowledge gaps (Figueira and Lages, 2019). Böhme and Jablonski (2022) emphasized the basic need for multilateral co-operations among

museums and universities from Afghanistan and partner countries for future sustainable research on the country's biodiversity and natural heritage. To date, knowledge on Afghan biodiversity has been almost entirely inferred from collection-based research on material collected prior to the era of molecular genetics (Jablonski et al., 2019, 2021). All endemic vertebrate species of Afghanistan were described from material collected during expeditions in the 1960 s and earlier, such as freshwater fishes (Coad and Bogutskaya, 2012) or racerunner lizards (genus *Eremias*; Anderson and Leviton, 1967; Böhme et al., 1991). Genetic research mainly relied on historical collection material for inclusion of Afghan taxa/populations in molecular phylogenies (lizards: Orlova et al., 2022; birds: Päckert et al., 2020a).

Newer material from wild Afghan populations for genetic analyses could only be collected during a short period of time when international armed forces were present in the country, for example from military camps (Krüger et al., 2011) or from Air Force bases (GenBank sequences included in Khan et al., 2021). Field studies during this short time window enabled the discovery of Afghan populations of rare or narrowly distributed species that had been overlooked or misidentified in ornithological collections like the large-billed reed warbler *Acrocephalus orinus* (Ayé et al., 2011; Timmins et al., 2009,2010). Based on that extended knowledge of Central Asian breeding populations of that species, re-examination and DNA barcoding of Central Asian museum specimens of reed warblers revealed that several historical *A. orinus* specimens had been mistaken for a more widespread congeneric *A. dumentorum* (Koblik et al., 2011). Such novel information and material from modern field research helped refining breeding ranges, but also offered new perspectives for population genetic or genomic research. For example, genetic analyses of rare herbarium specimens from the 20th century (1955 – 1978) documented a decline of genetic diversity over time in an Afghan landrace of wheat (Terasawa et al., 2009). On the one hand, extension of such time-limited series from collections with fresh material from the 21st century will facilitate the detection of up-to-date demographic changes for example in comparison with climate or land-use change.

On the other hand, along with optimization of wet-lab protocols and bioinformatics pipelines for NGS-based data sets, previous (partly incomplete) phylogenetic hypotheses can be tested using high-quality sequence data using freshly collected material. Likewise, typical sources of error in published sequence data can be detected, like in mitogenomes of *O. tazcanowskii* and *P. davidiana*. Dense taxon-sampling and increased robustness of molecular trees will also provide improved phylogenetic backbones for future *meta*-analyses of traits' evolution, niche evolution and many other research questions. As a perspective for snowfinch research, future population genetic studies should rely on a broader, *trans*-European sampling to identify further possible relict lineages on the Italian Peninsula, the Balkan Peninsula or Corsica. Similarly, filling in the sampling gaps in Asia would be necessary to identify a possible isolation-by-distance pattern in the most recent dispersal event of Asian *M. nivalis* to the Caucasus in a stepping-stone process *via* Central Asia (*M. n. tianshanica*), the Hindukush, the Elburz (ssp. *alpicola*) and the Zagros mountains (*M. n. raddei;* see del Hoyo and Collar, 2016). The as yet unstudied populations from Asia Minor (*M. n. leucura* from southern Anatolia) might play a key role in the understanding of geographical limits or potential overlap between Asian and European genetic lineages of the white-winged snowfinch, *M. nivalis*.

CRediT authorship contribution statement

Safiqul Islam: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Claire Peart:** Formal analysis, Methodology, Writing – review & editing. **Christian Kehlmaier:** Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Yue-Hua Sun:** Resources, Writing – review & editing. **Fumin Lei:** Resources, Writing – review & editing. **Andreas Dahl:** Investigation, Methodology, Validation, Writing – review & editing. **Sylvia Klemroth:** Investigation, Methodology, Validation, Writing – review & editing. **Dimitra Alexopoulou:** Formal analysis, Writing – review & editing. **Maria del Mar Delgado:** Funding acquisition, Resources, Writing – review & editing. **Paola Laiolo:** Resources, Writing – review & editing. **Juan Carlos Illera:** Resources, Writing – review & editing. **Sebastian Dirren:** Resources, Writing – review & editing. **Sabine Hille:** Resources, Writing – review & editing. **Davaa Lkhagvasuren:** Resources, Writing – review & editing. **Till Töpfer:** Resources, Writing – review & editing. **Martin Kaiser:** Resources, Writing – review & editing. **Axel Gebauer:** Resources, Writing – review & editing. **Jochen Martens:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Claudia Paetzold:** Formal analysis, Software, Validation, Writing – original draft, Writing – review & editing. **Martin Päckert:** Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of generative AI in scientific writing

No AI-assisted technologies were used in the writing process of this manuscript, except the automatic check for spelling and grammar as implemented in WORD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2024.108135.

References

Anderson, S.C., Leviton, A.E., 1967. A new species of *Eremias* (Reptilia: Lacertidae) from afghanistan. Occasional Papers of the California Academy of Sciences 64, 1–4.

Andrews, S. (2015). *FastQC: A quality control tool for high throughput sequence data* (p. 0.11.4). https://www. bioinformatics.babraham.ac.uk/projects/fastqc/.

Ayé, R., Hertwig, S.T., Schweizer, M., 2011. Discovery of a breeding area of the enigmatic Large-billed Reed Warbler *Acrocephalus orinus*. J. Avian Biol. 41 (5), 452–459. https://doi.org/10.1111/j.1600-048X.2010.05031.x.

BirdLife International. (2021). *Species factsheet: Pyrgilauda theresae. Downloaded from* https://www.birdlife.org on 05/11/2021.

Bohling, J., 2020. Evaluating the effect of reference genome divergence on the analysis of empirical RADseq datasets. Ecol. Evol. 10 (14), 7585–7601. https://doi.org/10.1002/ece3.6483.

Böhme, W., & Ščerbak, N. N. (1991). Ein neuer Wüstenrenner aus dem Hochland Afghanistans, *Eremias* (*Eremias*) *afghanistanica* sp.n. (Reptilia: Sauria: Lacertidae). *Bonner zoologische Beiträge, 42*(2), 137–141.

Böhme, W., Jablonski, D., 2022. Making forgotten information available: An early study on the Afghanistan Mountain Salamander *Paradactylodon (Afghanodon) mustersi* (Smith, 1940) (Caudata: Hynobiidae). Bonn Zoological Bulletin 71 (1), 1–7. https://doi.org/10.20363/BZB-2022.71.1.001.

Botero-Castro, F., Delsuc, F., Douzery, E.J.P., 2016. Thrice better than once: quality control guidelines to validate new mitogenomes. Mitochondrial DNA. Part a, DNA Mapping, Sequencing, and Analysis 27 (1), 449–454. https://doi.org/10.3109/19401736.2014.900666.

Bouckaert, R.R., 2010. DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26 (10), 1372–1373. https://doi.org/10.1093/bioinformatics/btq110.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for bayesian evolutionary analysis. PLoS Comput. Biol. 10 (4), 1–6. https://doi.org/10.1371/journal.pcbi.1003537.

Briscoe, A. G., Hopkins, K. P., & Waeschenbach, A. (2016). High-Throughput Sequencing of Complete Mitochondrial Genomes. *Methods in Molecular Biology (Clifton, N.J.)*, *1452*, 45–64. DOI: 10.1007/978-1-4939-3774-5 3.

Bushnell, B., Rood, J., Singer, E., 2017. BBMerge – Accurate paired shotgun read merging via overlap. PLoS One 12 (10), e0185056.

Busuttil, S., Ayé, R., 2009. Ornithological surveys in Bamiyan province, Islamic republic of Afghanistan. Sandgrouse 31, 146–159.

Busuttil, S., Ayé, R., Shank, C., 2010. Notes on nest and breeding of Afghan snowfinch *Pyrgilauda theresae*, Bamiyan province, Afghanistan. Sandgrouse 32, 13–14.

Bybee, S.M., Kalkman, V.J., Erickson, R.J., Frandsen, P.B., Breinholt, J.W., Suvorov, A., Dijkstra, K.-D.-B., Cordero-Rivera, A., Skevington, J.H., Abbott, J.C., Sanchez Herrera, M., Lemmon, A.R., Lemmon, E.M., Ware, J., 2021. Phylogeny and classification of Odonata using targeted genomics. Mol. Phylogenet. Evol. 160, 107115 https://doi.org/10.1016/j.ympev.2021.107115.

Card, D.C., Shapiro, B., Giribet, G., Moritz, C., Edwards, S.V., 2021. Museum genomics. Annual Review Genetics 55, 633–659. https://doi.org/10.1146/annurev-genet-071719-020506.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., 2013. Stacks : an analysis tool set for population genomics. Mol. Ecol. 3124–3140 https://doi.org/10.1111/mec.12354.

Cheng, T., 1987. A synopsis to the avifauna of China. Paul Parey Scientific Publ, Hamburg, Berlin.

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Fredericks, T. A., Gerbracht, J. A., Lepage, D., Billerman, S. M., Sullivan, B. L. & Wood, C. L. (2022). The eBird/Clements checklist of Birds of the World: v2022. Downloaded from https://www.birds.cornell.edu/clementschecklist/download/ (last accessed on 15.02.2024).

Clements, J. F., Rasmussen, P. C., Schulenberg, T. S., Iliff, M. J., Fredericks, T. A., Gerbracht, J. A., Lepage, D., Spencer, A., Billerman, S. M., Sullivan, B. L., & Wood, C. L. (2023). The eBird/Clements checklist of Birds of the World: v2023. Downloaded from https://www.birds.cornell.edu/clementschecklist/download/ (last accessed on 15.02.2024).

Coad, B.W., Bogutskaya, N.G., 2012. A new species of riffle minnow, *Alburnoides holciki*, from the Hari River basin in Afghanistan and Iran (Actinopterygii: Cyprinidae). Zootaxa 3453, 43–55. https://doi.org/10.11646/zootaxa.3453.1.3.

Cobos, M.E., Cheng, Y., Song, G., Lei, F., Peterson, T.A., 2021. New distributional opportunities with niche innovation in Eurasian snowfinches. J. Avian Biol. 52, e02868.

Cong, Q., Shen, J., Zhang, J., Li, W., Kinch, L.N., Calhoun, J.V., Warren, A.D., Grishin, N. V., 2021. Genomics reveals the origins of historical specimens. Mol. Biol. Evol. 38, 2166–2176. https://doi.org/10.1093/molbev/msab013.

Cooper, E.A., Uy, J.A.C., 2017. Genomic evidence for convergent evolution of a key trait underlying divergence in island birds. Mol. Ecol. 26, 3760–3774. https://doi.org/10.1111/mec.14116.

Cramp, S., Perrins, C.M., 1994. Handbook of the birds of europe the middle east and north africa Volume VIII, Crows to Finches. Oxford University Press, Oxford, New York.

Crotti, M., Barratt, C.D., Loader, S.P., Gower, D.J., Streicher, J.W., 2019. Causes and analytical impacts of missing data in RADseq phylogenetics: Insights from an African frog (*Afrixalus*). Zool. Scr. 48 (2), 157–167. https://doi.org/10.1111/zsc.12335.

Dahal, N., Romine, M.G., Khatiwara, S., Ramakrishnan, U., Lamichhaney, S., 2023. Gene flow drives genomic diversity in Asian Pikas distributed along the core and range-edge habitats in the Himalayas. Ecol. Evol. 13, e10129.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27 (15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., Li, H., 2021. Twelve years of SAMtools and BCFtools. GigaScience 10 (2), giab008. https://doi.org/10.1093/gigascience/giab008.

de Abreu, F., Jr, E., Pavan, S.E., Tschuiya, M.T.N., Wilson, D.E., Percequillo, A.R., Maldonado, J.E., 2020. Museomics of tree squirrels: a dense taxon sampling of mitogenomes reveals hidden diversity, phenotypic convergence, and the need of a taxonomic overhaul. BMC Evol. Biol. 20, 77. https://doi.org/10.1186/s12862-020-01639-y.

de Raad, J., Päckert, M., Irestedt, M., Janke, A., Kryukov, A.P., Martens, J., Red'kin, Y.A., Sun, Y.H., Töpfer, T., Schleuning, M., Neuschulz, E.L., Nilsson, M.A., 2022. Speciation and population divergence in a mutualistic seed dispersing bird. Communications Biology 5 (1), 429. https://doi.org/10.1038/s42003-022-03364-2.

del Hoyo, J., Collar, N.J., 2016. Illustrated checklist of the birds of the world. Passerines, Lynx Edicions, Barcelona.

del Mar Delgado, M., Bettega, C., Martens, J., Päckert, M., 2019. Ecotypic changes of alpine birds to climate change. Sci. Rep. 9 (1), 16082. https://doi.org/10.1038/s41598-019-52483-0.

Dementiev, G., 1963. Theresa's ground finch *montifringilla theresae*. Ibis 105 (1), 107. https://doi.org/10.1111/j.1474-919X.1963.tb02479.x.

Dickinson, E.C., 2003. The howard and moore complete checklist of the birds of the world, Third edition. Princeton University Press, Princeton and Oxford.

Dickinson, E. C., & Christidis, L. (Eds) (2014). The Howard and Moore Complete Checklist of the Birds of the World. Fourth edition, Vol. 2: Passerines. Aves Press, Eastbourne, UK.

Eaton, D.A.R., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30 (13), 1844–1849. https://doi.org/10.1093/bioinformatics/btu121.

Eaton, D.A.R., Overcast, I., 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. Bioinformatics. https://doi.org/10.1093/bioinformatics/btz966.

Eaton, D.A.R., Ree, R.H., 2013. Inferring phylogeny and introgression using RADseq data: An example from flowering plants (*Pedicularis*: Orobanchaceae). Syst. Biol. 62 (5), 689–706. https://doi.org/10.1093/sysbio/syt032.

Eaton, D.A.R., Spriggs, E.L., Park, B., Donoghue, M.J., 2017. Misconceptions on missing data in Rad-seq phylogenetics with a deep-scale example from flowering plants. Syst. Biol. 66 (3), 399–412. https://doi.org/10.1093/sysbio/syw092.

Ebdon, S., Laetsch, D.R., Dapporto, L., Hayward, A., Ritchie, M.G., Dinca, V., Vila, R., Lohse, K., 2021. The Pleistocene species pump past its prime: Evidence from European butterfly sister species. Mol. Ecol. 30 (14), 3575–3589. https://doi.org/10.1111/mec.15981.

Efetov, K.A., Hofmann, A., Tarmann, G.M., 2014. Application of two molecular approaches (use of sex attractants and DNA barcoding) allowed to rediscover *Zygaenoprocris eberti* (Alberti, 1968) (Lepidoptera, Zygaenidae, Procridinae), hitherto known only from the female holotype. Nota Lepidopterologica 37 (2), 151–160. https://doi.org/10.3897/nl.37.7871.

Ekblom, R., Wolf, J.B.W., 2014. A field guide to whole-genome sequencing, assembly and annotation. Evol. Appl. 7 (9), 1026–1042. https://doi.org/10.1111/eva.12178.

Elgvin, T.O., Trier, C.N., Tørresen, O.K., Hagen, I.J., Lien, S., Nederbragt, A.J., Ravinet, M., Jensen, H., Sætre, G.P., 2017. The genomic mosaicism of hybrid speciation. Sci. Adv. 3, e1602996.

Ernst, M., Jonsson, K.A., Ericson, P.G.P., Blom, M.P.K., Irestedt, M., 2022. Utilizing museomics to trace the complex history and species boundaries in an avian-study system of conservation concern. Heredity 128, 159–168. https://doi.org/10.1038/s41437-022-00499-0.

Figueira, R., Lages, F., 2019. Museum and herbarium collections for biodiversity research in angola. In: Huntley, B.J., Russo, V., Lages, F., Ferrand, N. (Eds.), Biodiversity of Angola. Springer, Cham, Switzerland, pp. 513–542.

Fortes, G.G., Paijmans, J.L.A., 2015. Analysis of whole mitogenomes from ancient samples BT - whole genome amplification: methods and protocols. In: Kroneis, T. (Ed.), Whole Genome Amplification. Methods in Molecular Biology. Humana Press, New York, NY, 10.1007/978-1-4939-2990-0_13.

Galla, S.J., Forsdick, N.J., Brown, L., Hoeppner, M.P., Knapp, M., Maloney, R.F., Moraga, R., Santure, A.W., Steeves, T.E., 2019. Reference genomes from distantly related species can be used for discovery of single nucleotide polymorphisms to inform conservation management. Genes 10 (1). https://doi.org/10.3390/genes10010009.

Gauthier, J., Pajkovic, M., Neuenschwader, S., Kaila, L., Schmid, S., Orlando, L., Alvarez, N., 2020. Museomics identifies genetic erosion in two butterfly species across the 20th century in Finland. Mol. Ecol. Resour. 20, 1191–1205. https://doi.org/10.1111/1755-0998.13167.

Gebauer, A., Eck, S., Kaiser, M., Lei, F.M., Martens, J., 2006. The qinghai-tibet plateau : center of evolution for snow sparrows (*Montifringilla* s. str.) and mountain-steppe sparrows (*Onychostruthus*, *Pyrgilauda*). Acta Zool. Sin. 52, 305–309.

Gill, F., Donsker, D., & Rasmussen, P. (Eds)(2023). IOC World Bird List (v13.2). Doi 10.14344/IOC.ML.13.2. http://www.worldbirdnames.org/ (last accessed on 27.02.2024).

Glenn, T.C., Stephan, W., Braun, M.J., 1999. Effects of a population bottleneck on whooping crane mitochondrial DNA variation. Conserv. Biol. 13 (5), 1097–1107. http://www.jstor.org/stable/2641743.

Glon, H., Quattrini, A., Rodríguez, E., Titus, B.M., Daly, M., 2021. Comparison of sequence-capture and ddRAD approaches in resolving species and populations in hexacorallian anthozoans. Mol. Phylogenet. Evol. 163, 107233 https://doi.org/10.1016/j.ympev.2021.107233.

Glutz von Blotzheim, U., & Bauer, K. M. (1997). Handbuch der Vögel Mitteleuropas. Band 14/1. Passeriformes (5. Teil). Aula-Verlag, Wiesbaden, Germany.

Godoy, J.A., Negro, J.J., Hiraldo, F., Donázar, J.A., 2004. Phylogeography, genetic structure and diversity in the endangered bearded vulture (*Gypaetus barbatus*, L) as revealed by mitochondrial DNA. Mol. Ecol. 13 (2), 371–390. https://doi.org/10.1046/j.1365-294x.2003.02075.x.

Gori, M., Pecchioli, S., Giordani, E., Saeedi, M.A., Wafa, F.H., Biricolti, S., 2019. Barcoding assessment of the *Citrus* species cultivated in eastern Afghanistan. Adv. Hortic. Sci. 33 (3), 403–408. https://doi.org/10.13128/ahs-25648.

Guo, X., Dai, X., Chen, D., Papenfuss, T.J., Ananjeva, N.B., Melnikov, D.A., Wang, Y., 2011. Phylogeny and divergence times of some racerunner lizards (Lacertidae: *Eremias*) inferred from mitochondrial 16S rRNA gene segments. Mol. Phylogenet. Evol. 61, 400–412. https://doi.org/10.1016/j.ympev.2011.06.022.

Gwee, C.Y., Eaton, J.A., Garg, K.M., Alström, P., Van Balen, S., Hutchinson, R.O., Prawiradilaga, D.M., Le, M.H., Rheindt, F.E., 2019. Cryptic diversity in *Cyornis* (Aves: Muscicapidae) jungle-flycatchers flagged by simple bioacoustic approaches. Zool. J. Linn. Soc. 186 (3), 725–741. https://doi.org/10.1093/zoolinnean/zlz003.

Hahn, C., Bachmann, L., Chevreux, B., 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads - a baiting and iterative mapping approach. Nucleic Acids Res. 41 (13), e129.

Hahn, E.E., Grealy, A., Alexander, M., Holleley, C.E., 2020. Museum epigenomics: Charting the future by unlocking the past. Trends Ecol. Evol. 35 (4), 295–300. https://doi.org/10.1016/j.tree.2019.12.005.

Hanson, T., Brooks, T.M., Da Fonseca, G.A.B., Hoffmann, M., Lamoreux, J.F., Machlis, G., Mittermeier, C.G., Mittermeier, R.A., Pilgrim, J.D., 2009. Warfare in biodiversity hotspots. Conserv. Biol. 23 (3), 578–587. https://doi.org/10.1111/j.1523-1739.2009.01166.x.

Hartert, E., 1910. Die vögel der paläarktischen fauna. Friedländer & Sohn, Berlin.

Hartert, E., 1921–1922. Die vögel der paläarktischen fauna. Friedländer & Sohn, Berlin.

Hartert, E., Steinbacher, F., 1932. Die vögel der paläarktischen fauna. Friedländer & Sohn, Ergänzungsband, Berlin.

Hausdorf, B., Walther, F., 2021. A forgotten subspecies of the land snail species *Arianta arbustorum* from a Pleistocene refuge in the Western Alps. J. Moll. Stud. 87, 1–7. https://doi.org/10.1093/mollus/eyab017.

Hekkala, E.R., Saumure, R.A., Jaeger, J.R., Herrmann, H.-W., Sredl, M.J., Bradford, D.F., Drabeck, D., Blum, M.J., 2011. Resurrecting an extinct species: archival DNA, taxonomy, and conservation of the Vegas Valley leopard frog. Conserv. Genet. 12, 1379–1385. https://doi.org/10.1007/s10592-011-0229-6.

Heupink, T.H., van Grouw, H., Lambert, D.M., 2014. The mysterious Spotted Green Pigeon and its relation to the Dodo and its kindred. BMC Evol. Biol. 14 (1), 136. https://doi.org/10.1186/1471-2148-14-136.

Hewitt, G., 2000. The genetic legacy of the Ice Ages. Nature 405, 907–913. https://doi.org/10.1038/35016000.

Hilton, E.J., Watkins-Colwell, G.J., Huber, S.K., 2021. The expanding role of natural history collections. Ichthyology and Herpetology 109 (2), 379–391. https://doi.org/10.1643/t2020018.

Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S., Gindro, K., 2019. The unbearable lightness of sequenced-based identification. Fungal Divers. 96 (1), 243–284. https://doi.org/10.1007/s13225-019-00428-3.

Horn, S., 2012. Target enrichment via DNA hybridization capture. Methods Mol. Biol. 840, 177–188. https://doi.org/10.1007/978-1-61779-516-9_21.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23 (2), 254–267. https://doi.org/10.1093/molbev/msj030.

Jablonski, D., Uroševi, A., Andjelković, M., Džukić, G., 2019. An unknown collection of lizards from Afghanistan. ZooKeys 843, 129–147. https://doi.org/10.3897/ZOOKEYS.843.29420.

Jablonski, D., Basit, A., Farooqi, J., Masroor, R., Böhme, W., 2021. Biodiversity research in a changing Afghanistan. Science 6549, 1402. https://doi.org/10.1126/science.abj8118.

Jiang, H., Lei, R., Ding, S.-W., Zhu, S., 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinf. 15 (1), 182. https://doi.org/10.1186/1471-2105-15-182.

Jønsson, K.A., Blom, M.P.K., Päckert, M., Ericson, P.G.P., Irestedt, M., 2018. Relicts of the lost arc: High-throughput sequencing of the *Eutrichomyias rowleyi* (Aves: Passeriformes) holotype uncovers an ancient biogeographic link between the Philippines and Fiji. Mol. Phylogenet. Evol. 120, 28–32. https://doi.org/10.1016/j.ympev.2017.11.021.

Kanderian, N., Lawson, D., Zahler, P., 2011. Current status of wildlife and conservation in Afghanistan. Int. J. Environ. Stud. 68 (93), 281–298. https://doi.org/10.1080/00207233.2011.573960.

Karimi, M.O., Shariati, M.M., Zerehdaran, S., Moradi, M.H., Javadmanesh, A., 2016. Study of genetic diversity of sheep breeds In Afghanistan. Bioscience Biotechnology Research Asia 13 (1), 573–581. https://doi.org/10.13005/bbra/2072.

Kazemi, E., Nazarizadeh, M., Fatemizadeh, F., Khani, A., Kaboli, M., 2021. The phylogeny, phylogeography, and diversification history of the westernmost Asian cobra (Serpentes: Elapidae: *Naja oxiana*) in the Trans-Caspian region. Ecol. Evol. 11, 2024–2039. https://doi.org/10.1002/ece3.7144.

Kearns, A.M., Campana, M.G., Slikas, B., Berry, L., Saitoh, T., Cibois, A., Fleischer, R.C., 2022. Conservation genomics and systematics of a near-extinct island radiation. Mol. Ecol. 31 (7), 1995–2012. https://doi.org/10.1111/mec.16382.

Kehlmaier, C., Zhang, X., Georges, A., Campbell, P., Thomson, S., Fritz, U., 2019. Mitogenomics of historical type specimens of Australasian turtles: clarification of taxonomic confusion and old mitochondrial introgression. Sci. Rep. 9, 5841. https://doi.org/10.1038/s41598-019-42310-x.

Kehlmaier, C., Albury, N.A., Steadman, D.W., Graciá, E., Franz, R., Fritz, U., 2021. Ancient mitogenomics elucidates diversity of extinct West Indian tortoises. Sci. Rep. 11 (1), 1–9. https://doi.org/10.1038/s41598-021-82299-w.

Khalilipur, O., Rezaei, H.R., Shabani, A.A., Kaboli, M., Ashrafi, S., Ashrafzadeh, M.R., 2017. Assessment of the phylogenetic status of afghan pika (*Ochotona rufescens*). Journal of Wildlife and Biodiversity 1 (1), 12–18. https://doi.org/10.22120/jwb.2017.26905.

Khan, M.A., Jablonski, D., Nadeem, M.S., Masroor, R., Kehlmaier, C., Spitzweg, C., Fritz, U., 2021. Molecular phylogeny of *Eremias* spp. from Pakistan contributes to a better understanding of the diversity of racerunners. J. Zool. Syst. Evol. Res. 59, 466–483. https://doi.org/10.1111/jzs.12426.

Koblik, E.A., Red'kin, Y.A., Meer, M.S., Derelle, R., Golenkina, S.A., Kondrashov, F.A., Arkhipov, V.Y., 2011. *Acrocephalus orinus*: A Case of Mistaken Identity. PLoS One 6 (4), e17716.

Kong, H., Condamina, F.L., Yang, L., Harris, A., Feng, C., Wen, F., Kang, M., 2022. Phylogenomic and macroevolutionary evidence for an explosive radiation of a plant genus in the miocene. Syst. Biol. 71 (3), 589–609. https://doi.org/10.1093/sysbio/syab068.

Krüger, A., Strüven, L., Post, R.J., Faulde, M., 2011. The sandflies (Diptera: Psychodidae, Phlebotominae) in military camps in northern Afghanistan (2007–2009), as identified by morphology and DNA 'barcoding'. Ann. Trop. Med. Parasitol. 105 (2), 163–176. https://doi.org/10.1179/136485911X12899838683241.

Kuhn, K., Schwenk, K., Both, C., Canal, D., Johansson, U.S., van der Mije, S., Töpfer, T., Päckert, M., 2013. Differentiation in neutral genes and a candidate gene in the pied flycatcher: using biological archives to track global climate change. Ecol. Evol. 3 (14), 4799–4814. https://doi.org/10.1002/ece3.855.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lalueza-Fox, C., 2022. Museomics. Curr. Biol. 32, 1214–1215. https://doi.org/10.1016/j.cub.2022.09.019.

Lei, F., Qu, Y., Song, G., 2014. Species diversification and phylogeographical patterns of birds in response to the uplift of the Qinghai-Tibet Plateau and Quaternary glaciations. Curr. Zool. 60 (2), 149–161. https://doi.org/10.1093/czoolo/60.2.149.

Lei, F.M., Wang, A.Z., Wang, G., Yin, Z.H., 2005. Vocalizations of red-necked snow finch, *Pyrgilauda ruficollis* on the Tibetan Plateau, China - A syllable taxonomic signal? Folia Zool. 54 (1–2), 135–146.

Leigh, J. W., & Bryant, D. (2015). *POPART : full-feature software for haplotype network construction*. 1110–1116. DOI: 10.1111/2041-210X.12410.

Lerner, H.R.L., Meyer, M., James, H.F., Hofreiter, M., Fleischer, R.C., 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Current Biolology 21, 1838–1844. https://doi.org/10.1016/j.cub.2011.09.039.

Liu, X., Zhang, S., Cai, Z., Li, K., 2022. Genomic insights into zokors' phylogeny and speciation in China. Proc. Natl. Acad. Sci. 119 (19) https://doi.org/10.1073/pnas.2121819119.

Ma, Y., Huang, Y., Lei, F., 2014. Sequencing and phylogenetic analysis of the *Pyrgilauda ruficollis* (Aves, Passeridae) complete mitochondrial genome. Zool. Res. 35 (2), 81–91. https://doi.org/10.11813/j.issn.0254-5853.2014.2.081.

Ma, Y., Wang, X., Hunag, Y., Lei, F., 2016. Complete mitochondrial genome of the *Onychostruthus taczanowskii* (Aves, Passeridae) and its phylogenetic analysis. Mitochondrial DNA 27 (4), 2984–2985. https://doi.org/10.3109/19401736.2015.1060473.

Maddock, S., Briscoe, A., Wilkinson, M., Waeschenbach, A., San Mauro, D., Day, J.J., Littlewood, T.J., Foster, P.G., Nussbaum, R.A., Gower, D.J., 2016. Next-generation mitogenomics: A comparison of approaches applied to caecilian amphibian phylogeny. PLoS One. https://doi.org/10.1371/journal.pone.0156757.

Maheshwari, A., 2022. Biodiversity conservation in Afghanistan under the returned Taliban. Nat. Ecol. Evol. 6 (4), 342–343. https://doi.org/10.1038/s41559-021-01655-1.

Manthey, J.D., Oliveros, C.H., Andersen, M.H., Filardi, C.E., Moyle, R.G., 2020. Gene flow and rapid differentiation characterize a rapid insular radiation in the southwest Pacific (Aves: *Zosterops*). Evolution 74 (8), 1788–1803. https://doi.org/10.1111/evo.14043.

Marchese, C., 2015. Biodiversity hotspots: A shortcut for a more complicated concept. Global Ecol. Conserv. 3, 297–309. https://doi.org/10.1016/j.gecco.2014.12.008.

Maricic, T., Whitten, M., Pääbo, S., 2010. Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. PLoS One 5 (11), 1–5. https://doi.org/10.1371/journal.pone.0014004.

Martens, J., Eck, S., 1995. Towards an ornithology of the Himalayas - Systematics, ecology and vocalizations of Nepal birds. Bonner Zoologische Monographien 38, 1–445.

Martin, C.A., Armstrong, C., Illera, J.C., Emerson, B.C., Richardson, D.S., Spurgin, L.G., 2021. Genomic variation, population history and within-archipelago adaptation between island bird populations. R. Soc. Open Sci. 8, 201146 https://doi.org/10.1098/rsos.201146.

Mason, N.A., Unitt, P., 2018. Rapid phenotypic change in a native bird population following conversion of the Colorado Desert to agriculture. J. Avian Biol. 49 (2), jav-01507. https://doi.org/10.1111/jav.01507.

Mayr, E., 1927. Die schneefinken (Gattungen *Montifringilla* und *Leucosticte*). J. Ornithol. 75, 596–619.

Mayr, E., Greenway, J.r. (Eds.), 1962. Checklist of the Birds of the World. Museum of Comparative Zoology, Cambridge, Massachusetts.

McCormack, J.E., Heled, J., Delaney, K.S., Peterson, A.T., Knowles, L.L., 2011. Calibrating Divergence Times On Species Trees Versus Gene Trees: Implications For Speciation History Of Aphelocoma Jays. Evolution 65 (1), 184–202. https://doi.org/10.1111/j.1558-5646.2010.01097.x.

McNeely, J.A., 2003. Conserving forest biodiversity in times of violent conflict. Oryx 37 (2), 142–152. https://doi.org/10.1017/S0030605303000334.

Meineke, E.K., Davies, T.J., Daru, B.H., Davis, C.C., 2019. Biological collections for understanding biodiversity in the Anthropocene. Philos. Trans. R. Soc., B 374 (1763), 20170386. https://doi.org/10.1098/rstb.2017.0386.

Meinertzhagen, R., 1937. *Montifringilla theresae* sp. nov. Bulletin of the British Ornithological Club 58 (407), 10.
Mende, M.B., Hundsdoerfer, A.K., 2013. Mitochondrial lineage sorting in action - Historical biogeography of the *Hyles euphorbiae* complex (Sphingidae, Lepidoptera) in Italy. BMC Evol. Biol. 13 (1) https://doi.org/10.1186/1471-2148-13-83.
Meyer, M., Kircher, M., 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb Protoc 2010 (6). https://doi.org/10.1101/pdb.prot5448.
Milne, I., Stephen, G., Bayer, M., Cock, P.J.A., Pritchard, L., Cardle, L., Shaw, P.D., Marshall, D., 2013. Using Tablet for visual exploration of second-generation sequencing data. Brief. Bioinform. 14 (2), 193–202. https://doi.org/10.1093/bib/bbs012.
Miranda, I., Giska, I., Farelo, L., Pimenta, J., Zimova, M., Bryk, J., Dalén, L., Mills, L.S., Zub, K., Melo-Ferreira, J., 2021. Museomics dissects the genetic basis for adaptive seasonal coloration in the least weasel. Mol. Biol. Evol. 38 (10), 4388–4402. https://doi.org/10.1093/molbev/msab177.
Mittermeier, R. A., Seligmann, P. A., & Ford, H. (Eds)(2004). Hotspots revisited. CEMEX, Mexico City.
Mlíkovský, J., 1998. Generic Name of Southern Snowfinches. *Forktail* 14, 85.
Moreau, R.E., Greenway Jr., J.C., 1962. Family Ploceidae, Weaverbirds. In: Mayr, E., Greenway Jr, J.C. (Eds.), Check-List of the Birds of the World. Museum of Comparative Zoology, Cambridge Massachusetts, USA.
Niethammer, G., 1973. Zur Vogelwelt des afghanischen Pamir und des Darwaz. Bonner Zoologische Beiträge 24, 270–284.
Nylander, J.A.A., 2004. MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
O'Leary, S.J., Puritz, J.B., Willis, S.C., Hollenbeck, C.M., Portnoy, D.S., 2018. These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. Mol. Ecol. 27, 3193–3206. https://doi.org/10.1111/mec.14792.
Obiol, F., James, J., James, H.F., Chesser, R.T., Bretagnolle, V., González-Solís, J., Rozas, J., Riutort, M., Welch, A.J., 2021. Integrating sequence capture and restriction site-associated DNA sequencing to resolve recent radiations of pelagic seabirds. Syst. Biol. 70 (5), 976–996. https://doi.org/10.1093/sysbio/syaa101.
Orlova, V.F., Solovyeva, E.N., Dunayev, E.A., Ananjeva, N.B., 2022. Integrative taxonomy within *Eremias multiocellata* complex (Sauria, Lacertidae) from the western part of range: Evidence from historical DNA. Genes 13, 941. https://doi.org/10.3390/genes13060941.
Päckert, M., Bader-Blukott, M., Künzelmann, B., Sun, Y.H., Hsu, Y.C., Kehlmaier, C., Albrecht, F., Illera, J.C., Martens, J., 2020a. A revised phylogeny of nuthatches (Aves, Passeriformes, *Sitta*) reveals insight in intra- and interspecific diversification patterns in the Palearctic. Vertebrate Zoology 70 (2), 241–262. https://doi.org/10.26049/VZ70-2-2020-10.
Päckert, M., Favre, A., Schnitzler, J., Martens, J., Sun, Y.H., Tietze, D.T., Hailer, F., Michalak, I., Strutzenberger, P., 2020b. Out of Tibet and out of Himalayas: centers of origin and diversification of passerine birds in the Qinghai-Tibet Plateau region. Ecol. Evol. 10 (17), 9283–9300. https://doi.org/10.1002/ece3.6615.
Päckert, M., Hering, J., Belkacem, A.A., Sun, Y.-H., Hille, S., Lkhagvasuren, D., Islam, S., Martens, J., 2021. A revised multilocus phylogeny of old world sparrows (Aves: Passeridae). Vertebrate Zoology 71. https://doi.org/10.3897/VZ.71.E65952.
Paetzold, C., Wood, K.R., Eaton, D.A.R., Wagner, W.L., Appelhans, M.S., 2019. Phylogeny of hawaiian melicope (Rutaceae): RAD-seq resolves species relationships and reveals ancient introgression. Frontiers of Plant Science 10, 1074. https://doi.org/10.3389/fpls.2019.01074.
Palacios, C., García-R, S., Parra, J.L., Cuervo, A.M., Stiles, F.G., McCormack, J.E., Cadena, C.D., 2019. Shallow genetic divergence and distinct phenotypic differences between two Andean hummingbirds: Speciation with gene flow? Auk 136 (4), 1–21. https://doi.org/10.1093/auk/ukz046.
Pease, J.B., Brown, J.W., Walker, J.F., Hinchliff, C.E., Smith, S.A., 2018. Quartet Sampling distinguishes lack of support from conflicting support in the green plant tree of life. Am. J. Bot. 105 (3), 385–403. https://doi.org/10.1002/ajb2.1016.
Peters, J.L., Zhuravlev, Y., Fefelov, I., Logie, A., Omland, K.E., 2007. Nuclear loci and coalescent methods support ancient hybridization as cause of mitochondrial paraphyly between gadwall and falcated duck (*Anas* spp.). Evolution 61 (8), 1992–2006. https://doi.org/10.1111/j.1558-5646.2007.00149.x.
Podsiadlowski, L., Gamauf, A., Töpfer, T., 2017. Revising the phylogenetic position of the extinct Mascarene Parrot *Mascarinus mascarin* (Linnaeus 1771) (Aves: Psittaciformes: Psittacidae). Mol. Phylogenet. Evol. 107, 499–502. https://doi.org/10.1016/j.ympev.2016.12.022.
Portenko, L.A., von Vietinghoff-Scheel, E., 1974. *Montifringilla nivalis* (L.). In: Stresemann, E., Portenko, L.A., Dathe, H., Mauersberger, G. (Eds.), Atlas Der Verbreitung Palaearktischer Vögel. 4. Lieferung. Akademie Verlag, Berlin.
Prada, C.F., Boore, J.L., 2019. Gene annotation errors are common in the mammalian mitochondrial genomes database. BMC Genomics 20, 73. https://doi.org/10.1186/s12864-019-5447-1.
Qu, Y., Ericson, P.G.P., Lei, F., Li, S.H., 2005. Postglacial colonization of the Tibetan plateau inferred from the matrilineal genetic structure of the endemic red-necked snow finch. Pyrgilauda ruficollis. Molecular Ecology 14 (6), 1767–1781. https://doi.org/10.1111/j.1365-294X.2005.02528.x.
Qu, Y., Lei, F., 2009. Comparative phylogeography of two endemic birds of the Tibetan plateau, the white-rumped snow finch (*Onychostruthus taczanowskii*) and the Hume's ground tit (*Pseudopodoces humilis*). Mol. Phylogenet. Evol. 51 (2), 312–326. https://doi.org/10.1016/j.ympev.2009.01.013.
Qu, Y., Ericson, P.G.P., Lei, F., Gebauer, A., Kaiser, M., Helbig, A.J., 2006. Molecular phylogenetic relationship of snow finch complex (genera *Montifringilla*, *Pyrgilauda*, and *Onychostruthus*) from the Tibetan plateau. Mol. Phylogenet. Evol. 40 (1), 218–226. https://doi.org/10.1016/j.ympev.2006.02.020.
Qu, Y., Lei, F., Zhang, R., Lu, X., 2010. Comparative phylogeography of five avian species: implications for Pleistocene evolutionary history in the Qinghai-Tibetan plateau. Mol. Ecol. 19 (2), 338–351. https://doi.org/10.1111/j.1365-294X.2009.04445.x.
Qu, Y., Chen, C., Chen, X., Hao, Y., She, H., Wang, M., Ericson, P.G.P., Lin, H., Cai, T., Song, G., Jia, C., Chen, C., Zhang, H., Li, J., Liang, L., Wu, T., Zhao, J., Gao, Q., Zhang, G., Lei, F., 2021. The evolution of ancestral and species-specific adaptations in snowfinches at the Qinghai-Tibet Plateau. Proc. Natl. Acad. Sci. 118 (13) https://doi.org/10.1073/pnas.2012398118.
Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics (oxford, England) 26 (6), 841–842. https://doi.org/10.1093/bioinformatics/btq033.
Rambaut, A., & Drummond, A. (2007). *TRACER v1.4.* http://beast.bio.ed.ac.uk/Tracer.
Rambaut A. (2009). *FigTree version 1.2.2 - Computer program distributed by the author, website:* http://tree.bio.ed.ac.uk (last *accessed on 29.11.2010)*.
Rasmussen, P.C., Prŷs-Jones, R.P., 2003. History *vs* mystery: the reliability of museum specimen data. Bulletin of the British Ornithological Club 123A, 66–94.
Raxworthy, C.J., Tilston Smith, B., 2021. Mining museums for historical DNA: advances and challenges in museomics. Trends Ecol. Evol. 36 (11), 1049–1060. https://doi.org/10.1016/j.tree.2021.07.009.
Rick, J.A., Brock, C.D., Lewanski, A.L., Golcher-Benavides, J., Wagner, C.E., 2023. Reference Genome Choice and Filtering Thresholds Jointly Influence Phylogenomic Analyses. Syst. Biol. syad065 https://doi.org/10.1093/sysbio/syad065.
Riordan, P., Cushman, S.A., Mallon, D., Shi, K., Hughes, J., 2016. Predicting global population connectivity and targeting conservation action for snow leopard across its range. Ecography 39, 419–426. https://doi.org/10.1111/ecog.01691.
Ritchie, A.M., Hua, X., Bromham, L., 2022. Investigating the reliability of molecular estimates of evolutionary time when substitution rates and speciation rates vary. BMC Ecology and Evolution 22 (1), 1–19. https://doi.org/10.1186/s12862-022-02015-8.
Rochette, N.C., Catchen, J.M., 2017. Deriving genotypes from RAD-seq short-read data using Stacks. Nat. Protoc. 12 (12) https://doi.org/10.1038/nprot.2017.123.
Saidajan, A., 2012. Effects of war on biodiversity and sustainable agricultural development in Afghanistan. Journal of Developments in Sustainable Agriculture 7 (1), 9–13. https://doi.org/10.11178/jdsa.7.9.
Salvi, D., Berrilli, E., Garzia, M., Mariottini, P., 2021. Yet another mitochondrial genome of the pacific cupped oyster: The Published Mitogenome of *Alectryonella plicatula* (Ostreinae) Is Based on a Misidentified *Magallana gigas* (Crassostreinae). Front. Mar. Sci. 8 (September), 8–13. https://doi.org/10.3389/fmars.2021.741455.
Sampaio, F.L., Day, J.J., Mendis Wickramasinghe, L.J., Cyriac, V.P., Papadopoulou, A., Brace, S., Rajendran, A., Simon-Nutbrown, C., Flouris, T., Kapli, P., Ranga Vidanapathirana, D., Kotharambath, R., Kodandaramaiah, U., Gower, D.J., 2023. A near-complete species-level phylogeny of uropeltid snakes harnessing historical museum collections as a DNA source. Mol. Phylogenet. Evol. 178, 107651 https://doi.org/10.1016/j.ympev.2022.107651.
Sangster, G., Luksenburg, J., 2020. Chimeric mitochondrial genomes: a hazard for phylogenetics and environmental DNA identification of fishes-chimeric-mitochondrial-genomes-a-hazard-for-phylogenetics-and-environmental-dna-identification-of-fishes. Authorea. https://doi.org/10.22541/au.160205226.65255244/v1.
Sangster, G., Luksenburg, J.A., 2021. Scientific data laundering: Chimeric mitogenomes of a sparrowhawk and a nightjar covered-up by forged phylogenies. Biochem. Syst. Ecol. 96, 104263 https://doi.org/10.1016/j.bse.2021.104263.
Scherz, M.D., Rasolonjatovo, S.M., Köhler, J., Rancilhac, L., Rakotoarison, A., Raselimanana, A.P., Ohler, A., Preick, M., Hofreiter, M., Glaw, F., Vences, M., 2020. Barcode fishing' for archival DNA from historical type material overcomes taxonomic hurdles, enabling the description of a new frog species. Sci. Rep. 10 (1), 19109. https://doi.org/10.1038/s41598-020-75431-9.
Schindel, D.E., Cook, J.A., 2018. The next generation of natural history collections. PLoS Biol. 16 (7), 1–8. https://doi.org/10.1371/journal.pbio.2006125.
Schmitt, T., 2007. Molecular biogeography of Europe: pleistocene cycles and postglacial trends. Front. Zool. 4, 11. https://doi.org/10.1186/1742-9994-4-11.
Schmitt, T., Louy, D., Zimmermann, E., Habel, J.C., 2016. Species radiation in the Alps: multiple range shifts caused diversification in ringlet butterflies in the European high mountains. Org. Divers. Evol. 16 (4), 791–808. https://doi.org/10.1007/s13127-016-0282-6.
Schweizer, M., Etzbacher, C., Shirihai, H., Töpfer, T., Kirwan, G., 2020. A molecular analysis of the mysterious Vaurie's Nightjar *Caprimulgus centralasicus* yields fresh insight into its taxonomic status. J. Ornithol. 161, 635–650. https://doi.org/10.1007/s10336-020-01767-8.
Scotti-Saintagne, C., Boivin, T., Suez, M., Musch, B., Scotti, I., Fady, B., 2021. Signature of mid-Pleistocene lineages in the European silver fir (*Abies alba* Mill.) at its geographic distribution margin. Ecol. Evol. 11 (16), 10984–10999. https://doi.org/10.1002/ece3.7886.
Seghal, D., Vikram, P., Sansaloni, C.P., Ortiz, C., Pierre, C.S., Payne, T., Ellis, M., Amri, A., Petroli, C.D., Wenzl, P., Singh, S., 2015. Exploring and mobilizing the gene bank biodiversity for wheat improvement. PLoS One 10 (7), e0132112.
Shapiro, B., Sibthorpe, D., Rambaut, A., Austin, J., Wragg, G.M., Bininda-Emonds, O.R. P., Lee, P.L.M., Cooper, A., 2002. Flight of the dodo. Science (New York, N.Y.) 295, 1683. https://doi.org/10.1126/science.295.5560.1683.
Shapoval, N.A., Yakolev, R.V., Kuftina, G.N., 2017. The taxonomic status of *Cossus cossus* afghanistanus (Lepidoptera, Cossidae) from Afghanistan: insights from molecular and morphological data. Ukrainian Journal of Ecology 7 (3), 134–138.
She, H., Jiang, Z., Song, G., Ericson, P.G.P., Luo, X., Shao, S., Lei, F., Qu, Y., 2021. Quantifying adaptive divergence of the snowfinches in a common landscape. Divers. Distrib. n/a (n/a), 1–14. https://doi.org/10.1111/ddi.13383.

Skujina, I., McMahon, R., Hegarty, M., 2017. Are nuclear/mitochondrial sequence duplications correctly characterised in published sequence databases? Insights in Genetics and Genomics 1 (6), 1.
Smallwood, P., Shank, C., Dehgan, A., Zahler, P., 2011. Wildlife conservation in Afghanistan? Bioscience 61, 506–511. https://doi.org/10.1525/bio.2011.61.7.4.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22 (21), 2688–2690. https://doi.org/10.1093/bioinformatics/btl446.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
Stelbrink, B., Kehlmaier, C., Wilke, T., Albrecht, C., 2019. The near-complete mitogenome of the critically endangered *Pseudocleopatra dartevellei* (Caenogastropoda: Paludomidae) from the Congo River assembled from historical museum material. *Mitochondrial DNA. Part B*. Resources 4 (2), 3229–3231. https://doi.org/10.1080/23802359.2019.1669081.
Stervander, M., Illera, J.C., Kvist, L., Barbosa, P., Keehnen, N.P., Pruisscher, P., Bensch, S., Hansson, B., 2015. Disentangling the complex evolutionary history of the Western Palearctic blue tits (Cyanistes spp.) – phylogenomic analyses suggest radiation by multiple colonization events and subsequent isolation. Mol. Ecol. 24 (10), 2477–2494. https://doi.org/10.1111/mec.13145.
Stervander, M., Alström, P., Olsson, U., Ottosson, U., Hansson, B., Bensch, S., 2016. Multiple instances of paraphyletic species and cryptic taxa revealed by mitochondrial and nuclear RAD data for *Calandrella* larks (Aves: Alaudidae). Mol. Phylogenet. Evol. 102, 233–245. https://doi.org/10.1016/j.ympev.2016.05.032.
Stewart, J.R., Lister, A.M., Barnes, I., Dalén, L., 2010. Refugia revisited: individualistic responses of species in space and time. Proc. R. Soc. B Biol. Sci. 277 (1682), 661–671. https://doi.org/10.1098/rspb.2009.1272.
Strohm, J.H.T., Gwiazdowski, R.A., Hanner, R., 2016. Mitogenome metadata: current trends and proposed standards. Mitochondrial DNA. Part a, DNA Mapping, Sequencing, and Analysis 27 (5), 3263–3269. https://doi.org/10.3109/19401736.2015.1015003.
Summers-Smith, J. D., & van Balen, S. (2016). Family Passeridae (Old-World Sparrows). In: del Hoyo, J., Elliott, A., & Christie, D. (Eds), Illustrated Checklist of the Birds of the World, Vol. 2 – Passerines. Lynx Edicions, Barcelona.
Summers-Smith, J. D. (2009). Family Passeridae (Old-World Sparrows). In: del Hoyo, J., Elliott, A., & Christie, D. (Eds), Handbook of the Birds of the World, Vol. 14 – Bush-shrikes to Old World Sparrows. Lynx Edicions, Barcelona.
Tehseen, M.M., Istipliler, D., Kehel, Z., Sansaloni, C.P., da Silva Lopes, M., Kurtulus, E., Muazzam, S., Nazari, K., 2021. Genetic diversity and population structure analysis of *Triticum aestivum* L. landrace panel from Afghanistan. Genes 12 (3), 340. https://doi.org/10.3390/genes12030340.
Terasawa, Y., Kawahara, T., Sasakuma, T., Sasanuma, T., 2009. Evaluation of the genetic diversity of an Afghan wheat collection based on morphological variation, HMW glutenin subunit polymorphisms, and AFLP. Breed. Sci. 59, 361–371. https://doi.org/10.1270/jsbbs.59.361.
Thorburn, D.-M.-J., Sagonas, K., Binzer-Panchal, M., Chain, M.J.J., Feulner, P.G.D., Bornberg-Bauer, E., Reusch, T.B.H., Samonte-Padilla, I.E., Milinski, M., Lenz, T.L., Eizaguirre, C., 2023. Origin matters: Using a local reference genome improves measures in population genomics. Mol. Ecol. 23 (7), 1706–1723. https://doi.org/10.1111/1755-0998.13838.
Timmins, R.J., Mostafawi, N., Rajabi, A.M., Noori, H., Ostrowski, S., Olsson, U., Svensson, L., Poole, C.M., 2009. The discovery of long-billed reed warblers *acrocephalus orinus* in north-eastern afghanistan. BirdingASIA 12, 42–45.
Timmins, R.J., Ostrowski, S., Mostafawi, N., Noorin, H., Rajabi, A.M., Svensson, L., Olsson, U., Poole, C., 2010. New information on the Large-billed Reed Warbler *Acrocephalus orinus*, including its song and breeding habitat in north-eastern Afghanistan. Forktail 26, 9–23.
Tolstoj, V.A., Geipel, E.F., 1990. First record of Theresa's snow finch (*Pyrgilauda theresae*) in Tadjikistan. Ornitologiya 24, 164.
Tribsch, A., Schönswetter, P., 2003. Patterns of endemism and comparative phylogeography confirm palaeoenvironmental evidence for pleistocene refugia in the eastern alps. Taxon 52 (3), 477–497. https://doi.org/10.2307/3647447.
Tripp, E.A., Tsai, Y.E., Zhuang, Y., Dexter, K.G., 2017. RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. Ecol. Evol. 7 (19), 7920–7936. https://doi.org/10.1002/ece3.3274.
Tritsch, C., Martens, J., Sun, Y.-H., Heim, W., Strutzenberger, P., Päckert, M., 2017. Improved sampling at the subspecies level solves a taxonomic dilemma - A case study of two enigmatic Chinese tit species (Aves, Passeriformes, Paridae, *Poecile*). Mol. Phylogenet. Evol. 107, 538–550. https://doi.org/10.1016/j.ympev.2016.12.014.
Uva, V., Päckert, M., Cibois, A., Fumagalli, L., Roulin, A., 2018. Comprehensive molecular phylogeny of barn owls and relatives (Family: Tytonidae), and their six major Pleistocene radiations. Mol. Phylogenet. Evol. 125, 127–137. https://doi.org/10.1016/j.ympev.2018.03.013.
Valiente-Mullor, C., Beamud, B., Ansari, I., Francés-Cuesta, C., García-González, N., Mejía, L., Ruiz-Hueso, P., González-Candelas, F., 2021. One is not enough: On the effects of reference genome for the mapping and subsequent analyses of short-reads. PLoS Comput. Biol. 17 (1) https://doi.org/10.1371/journal.pcbi.1008678.
Vaurie, C., 1956. Systematic notes on Palearctic birds. No 24. Ploceidae: the genera *Passer*. *Petronia* and *Montifringilla*. *American Museum Novitates* 1814, 1–27. http://hdl.handle.net/2246/5394.
Vaurie, C., 1959. The birds of the palearctic fauna – passeriformes. H. F. & G Witherby Limited, London.
Vietinghoff-Scheel, E., 1980. *Pyrgilauda theresae* Meinertzhagen. In: Dathe, H., Neufeldt, I.A. (Eds.), Atlas Der Verbreitung Palaearktischer Vögel, 8. Akademie Verlag, Berlin, Lieferung.
Vila, M., Marí-Mena, N., Guerrero, A., Schmitt, T., 2011. Some butterflies do not care much about topography: A single genetic lineage of *Erebia euryale* (Nymphalidae) along the northern Iberian mountains. J. Zool. Syst. Evol. Res. 49 (2), 119–132. https://doi.org/10.1111/j.1439-0469.2010.00587.x.
Wang, K., Lenstra, J.A., Liu, L., Hu, Q., Ma, T., Qiu, Q., Liu, J., 2018. Incomplete lineage sorting rather than hybridization explains the inconsistent phylogeny of the wisent. Communications Biology 1, 169. https://doi.org/10.1038/s42003-018-0176-6.
Weir, J.T., Schluter, D., 2007. The latitudinal gradient in recent speciation and extinction rates of birds and mammals. Science. https://doi.org/10.1126/science.1135590.
Westover, K.M., Rusinko, J.P., Hoin, J., Neal, M., 2013. Rogue taxa phenomenon: A biological companion to simulation analysis. Mol. Phylogenet. Evol. 69 (1), 1–3. https://doi.org/10.1016/j.ympev.2013.05.010.
Wikramanayake, E.D., Dinerstein, E., Loucks, C.J., 2002. Terrestrial ecoregions of the Indo-Pacific: A conservation assessment. Island Press, Washington, DC.
Wingett, S.W., Andrews, S., 2018. FastQ Screen: A tool for multi-genome mapping and quality control. F1000Research 7, 1338. https://doi.org/10.12688/f1000research.15931.2.
Wolfgramm, H., Martens, J., Töpfer, T., Vamberger, M., Pathak, A., Stuckas, H., Päckert, M., 2021. Asymmetric allelic introgression across a hybrid zone of the coal tit (*Periparus ater*) in the central Himalayas. Ecol. Evol. 11, 17332–17351. https://doi.org/10.1002/ece3.8369.
Wolters, H.E., 1979. Die vogelarten der erde. Lieferung. Paul Parey, Hamburg und Berlin, p. 4.
Yuan, S., Xia, Y., Zheng, Y., Zeng, X., 2016. Next-generation sequencing of mixed genomic DNA allows efficient assembly of rearranged mitochondrial genomes in *Amolops chunganensis* and *Quasipaa boulengeri*. PeerJ 2016 (12). https://doi.org/10.7717/peerj.2786.
Zedane, L., Hong-Wa, C., Murienne, J., Jeziorski, C., Baldwin, B.G., Besnard, G., 2016. Museomics illuminate the history of an extinct, paleoendemic plant lineage (*Hesperelaea*, Oleaceae) known from an 1875 collection from Guadalupe Island, Mexico. Biol. J. Linn. Soc. 117, 44–57. https://doi.org/10.1111/bij.12509.

Chapter 3: Transpalearctic out-of-Tibet dispersal facilitated divergence of genetic lineages, phenotypes and ecological niche preference in a Eurasian alpine bird (Aves, Passeriformes, *Montifringilla nivalis*)

Transpalearctic out-of-Tibet dispersal facilitated divergence of genetic lineages, phenotypes and ecological niche preference in a Eurasian alpine bird (Aves, Passeriformes, *Montifringilla nivalis*)

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Abstract

The Qinghai-Tibetan Plateau (QTP) is one of the world's key biodiversity hotspots, playing a significance role in shaping ecological and evolutionary processes of many flora and fauna. QTP is considered as the center of the origin of the many species including Snowfinches. Snowfinches diversification began in the late Miocene and has recognized in three genera (*Montifringilla, Pyrgilauda, Onychostruthus*) with eight recognized species. Among them, *Montifringilla nivalis* species shows the out-of-Tibet dispersal, now inhabiting alpine environments across Eurasian Mountain. In this study, we analyzed the intraspecific diversification of *M. nivalis* using genome-wide SNPs, ecological data, and morphological traits. Our findings reveal two major genetic lineages namely Asian and European lineages and diversification began in the Early Pleistocene. Extended Bayesian Skyline Plot (EBSP) analysis suggests long-term population stability of the *M. nivalis* with slight increases during the Holocene. Distinct phenotypic differences between lineages indicate local ecological adaptation. These findings highlight the role of glacial refugia and historical climate events in shaping present snowfinch diversity and distribution.

keywords: alpine environments, climate niche, ddRAD sequencing, Gloger's rule, single nucleotide polymorphisms (SNPs), snowfinches, spectral reflectance

1. Introduction

Among the roughly 30 global biodiversity hotspots (Marchese 2015), mountains play a prominent role. The two mountain systems extending across the largest elevational gradients on Earth, the Sinohimalayas and the Andes, are among the 20 centers of highest vascular plant diversity (Mutke et al. 2011). They stand out (together with the African Arc) as the global areas harboring the highest species richness of vertebrates (Antonelli et al. 2018; Mosbrugger et al. 2018; Rahbeck et al. 2019b).

The Sinohimalayas (including the Himalayas and the Hengduanshan in the East) represent the forested southern margin of the Qinghai-Tibet-Plateau (QTP), the largest topographic feature on Earth (Favre et al. 2015). The two regions are divided by two major diversity gradients. Along a smooth longitudinal gradient bird species richness of the Himalayas declines from the tropical East towards the drier and colder West (Price et al. 2014). Along a rather sharp elevational gradient, forest bird communities of the Sinohimalayas (and its southward extensions to the Indo-Burmese Mountains) are characterized by high numbers of both ancient and young species, whereas alpine bird communities above the timberline typically represent species-poor assemblages (Fjeldså et al. 2012). Moreover, the eastern QTP margin (mainly represented by the Hengduanshan) also harbors the highest proportion of endemic species of all Eurasian mountain systems (Rahbeck et al. 2019b, Deng et al. 2020). Nevertheless, species-poor alpine communities of the core QTP are the home of two ancient (Miocene), endemic, monotypic bird families species; the Przewalski's finch (*Urocynchramus pylzowi*, Urocynchramidae; Päckert et al. 2016) and the ibisbill (*Ibidorhyncha struthersii*, Ibidorhynchidae; Baker et al. 2007). Breeding ranges of a great proportion of Holarctic high-elevation bird specialists are also largely or entirely restricted to the QTP (Scridel et al. 2018), including most snowfinch species (Cobos et al. 2021; Päckert et al. 2020).

Snowfinches are characteristic Tibetan faunal elements, and current taxonomy recognizes eight species in three genera (*Montifringilla*, *Pyrgilauda*, *Onychostruthus*; Gill et al. 2024). They are regarded as a prominent example of out-of-Tibet dispersal (Fig. 1). In mammals, such an out-of-Tibet dispersal has previously been suggested as a major driver of diversification for the cold-adapted megafauna (review in Deng et al. 2020) and for smaller rodents like high alpine pikas (Ochotonidae; subgenus *Pika*) that dispersed out of Tibet into the Nearctic in the late Miocene and further diversified into two extant Nearctic species (Wang et al. 2020, Tang et al. 2022). In fact, most alpine radiations across the Holarctic are characterized by a multidirectional faunal and floral exchange between the QTP and adjacent regions (plants: Ebersbach et al. 2017; vertebrates: Liu et al. 2016; Päckert et al. 2020). Transcontinental colonization events from a Tibetan source area were

often related to dispersing organisms, as it is the case for e.g. some butterflies (genus *Hyles*: Hundsdörfer et al. 2017; genus *Parnassius*: Todisco et al. 2010; Zhao et al. 2022; genus *Oeneis*: Kleckova et al. 2015) and birds (rosy finches, genus *Leucosticte*: Funk et al. 2021; snowfinches: Päckert et al. 2020).

The onset of snowfinch diversification has been dated to the late Miocene at 5.7 – 8.3 mya (Qu et al. 2021) and was mainly characterized by *in-situ* speciation of six endemic and largely sympatric snowfinch species on the QTP and along its margins (Fig. 1B; Päckert et al. 2020, 2021). One small-range endemic from the Hindukush, *Pyrgilauda theresae*, emerged from an ancient late Miocene colonization event from a QTP area of origin (Islam et al. 2024). Only the white-winged snowfinch (*Montifringilla nivalis*) occupies a wide trans-Palearctic distribution range across the major Eurasian mountain systems, where two major genetic lineages of an early Pleistocene divergence have been identified so far: an eastern lineage comprising populations from the Mongolian Altai (*M. n. groumgrzimaili*) and the Caucasus (*M. n. alpicola*), and a western lineage including populations from the European Alps, the Pyrenees, Cantabrian Mountains and the Balkans (*M. n. nivalis*; Resano-Mayor et al. 2017; Islam et al. 2024). The hypothesis of a dramatic niche expansion event in *M. nivalis* inferred from niche evolution modeling (Cobos et al. 2021) was substantially challenged, as it does not seem convincing that a derived thermal niche of European populations of *M. n. nivalis* should be substantially broader than a more narrow ancestral niche of it's Asian relatives (Brambilla et al. 2022). Nevertheless, thermal niches occupied by *M. nivalis* in mountain systems of Europe are undoubtedly divergent from those in the alpine environments of Asian mountain systems, particularly from those at the northern margin of the QTP (Cobos et al. 2021). In other vertebrates, niche expansion or niche shifts were suggested to have accelerated diversification in general (Hu et al. 2015) and can be correlated with diversification of phenotypes, such as in corvids (Garcia-Porta et al. 2022), and in an endemic lazy toad species of the QTP (*Scutiger boulengeri*; Lin et al. 2021).
In this study, we provide a multi-disciplinary analysis of the range-wide intraspecific diversification of *M. nivalis*, including population genomic analyses from five Eurasian mountain systems using ddRAD sequencing, flanked by projections of past and present distributions using ecological niche modeling and a phenotypic analysis of body size and plumage color features. Our initial expectations are

(i) Deep genetic divergence between the European and the Asian clade of *M. nivalis* (Islam et al. 2024) might be reflected by phenotypic trait divergence. According to Bergmann's rule, body size of terrestrial vertebrates should increase with latitude and decrease with temperature (Guo et al. 2024; He et al. 2023). With respect to color, according to

Gloger's rule, climatic effects on melanin pigmentation should result in darker coloration of mammals and birds in warmer and more humid climates (Delhey 2017). However, recent studies have indicated that, owing to complex interactions of antagonistic or additive effects of temperature and precipitation on plumage colour, Gloger's rule does not generally apply to all passerine birds (Delhey et al. 2019; Marcondes et al. 2022).

(ii) Weak evidence of phylogeographic structure across European mountain ranges (Islam et al. 2024) should receive stronger support from population genetic analyses of genome-wide SNP data based on a broader sampling. We include an analysis of demographic histories inferred from population genomic data combined with projections of past distributions to gain further insight into the evolutionary history of the species.

2. Materials and Methods

2.1 Sampling, DNA extraction and Sanger sequencing

We extracted DNA from 68 frozen tissue and blood samples of white-winged snowfinches from the Mongolian Altai, (*M. n. groumgrzimaili*; n= 17), the Caucasus (*M. n. alpicola*; n= 3), the European Alps (*M. n. nivalis*; n= 11) and the Cantabrian Mountains (*M. n. nivalis*; n= 37; for sample origin see Appendix S1; Table S1.1; map in Fig. 2A). For DNA extraction, we used InnuPREP DNA Mini Kit (Analytik Jena AG) for tissue samples and innuPREP Blood DNA Mini Kit (Analytik Jena AG) for blood samples, respectively. In both procedures, we followed manufacturer's protocols except for overnight incubation with proteinase K for cell lysis.

We amplified a 1079-bp fragment of the mitochondrial cytochrome-*b* with the primer combination of O-L14851/O-H16065**.** We followed the PCR and sequencing protocols documented in Islam et al. (2024). Purified PCR products were prepared for sequencing using the BigDyeTM 3.1 Dye Terminator Cycle Sequencing Kits (Applied Biosystems, now at Thermo Fisher Scientific, Waltham, MA, USA), and sequenced in both reading directions on an ABI 3730 capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA). For each sample, we inspected forward and reverse sequences with Chromas v.2.6.5 (Technelysium Pty Ltd, Brisbane, Australia), and we used MEGA v. 10.1.8 (Kumar et al., 2018) for editing and alignment of consensus sequences. From the cyt*b* sequence data set we produced a minimum-spanning haplotype network with PopArt 1.7 (Leigh & Byrant 2015).

2.1 Genome sequencing and de-novo assembly

For our population genetic analysis, we opted for a commonly applied reduced representation

sequencing approach, i.e. double digest restriction-site associated DNA sequencing (ddRAD-seq; Peterson et al. 2012; McCormack et al. 2013). A reference genome for *M. nivalis* was previously not available; we have generated a novel de novo assembly for this species in this study using 10X Genomics linked read technology from an *M. nivalis* blood sample, sample was collected from Spain

Regarding of whole genome reference samples DNA was isolated using the NZY Tissue gDNA Isolation kit, strictly following the manufacturer's instructions. DNA was eluted in a final volume of 50 μL. The integrity of the extracted DNA was checked in a 2% agarose gel stained with GreenSafe (NZYTech) and visualised under UV light. The isolated DNA was quantified with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). A genomic shotgun library was constructed using the TruSeq Nano DNA Library Prep kit (Illumina) following the manufacturer's instructions. The library was quantified with the Qubit dsDNA HS Assay Kit, and its fragment size distribution was checked in the Agilent 2100 Bioanalyzer using the Agilent DNA 1000 Kit (Agilent Technologies). The library was sequenced in the NovaSeq 6000 PE150 platform (Illumina), obtaining 50 gigabases of raw data. Raw sequences were trimmed using trimmomatic v. 0.39 (Bolger et al., 2014) combining adapter trimming (ILLUMINACLIP: adapters: 2: 30: 10: 8: keepBothReads) and quality control (LEADING: 25; TRAILING: 25; SLIDINGWINDOW: 8: 25) removing trimmed reads less than 90 bp in length.

Three different genome assemblers were run to compare results before selecting one assembly to serve as a genomic reference; Abyss (Jackman et al., 2017), Ray (Boisvert et al., 2010) and SOAPdenovo 2 (Luo et al., 2012), all of which had already performed well on bird genomes (Bradnam et al., 2013). To determine the optimal value for the k-mer parameter KmerGenie v. 1.7 (Chikhi and Medvedev, 2014) was used before assembly, which resolved 57 as best-fitting k-mer, and was used for all three assemblers.

For de-novo assembly Abyss v. 2.3.7 was run in Bloom filter and paired end mode with B=50G. Ray v.2.3.1 was run with all parameters except the value for k-mer length left at default. For SOAPdenovo v. 2.4.2 maximum read length was set to 151 bp with length cutoff disabled, average insert size of 350 bp, the mapping distance at 32, and the remaining parameters set to their respective default values. All assemblies were performed on a local workstation with 32 CPUs and 320 GB of RAM. Assembly Statistics were computed using REAPR v1.0.18 (Hunt et al., 2013) and Busco v.5.5.0 (Simão et al., 2015) with the Passeriformes dataset v 10.

2.2 Library preparation and ddRAD sequencing

Double-digested restriction site-associated DNA (ddRAD) sequencing of 68 snowfinch samples was performed at Deep Sequencing Centre at TU Dresden. RADseq libraries were generated from individual samples (50 ng gDNA) in a double digestion with SbfI and MspI (NEB) and specific library barcodes carrying truncated adapters with cohesive ends (Trueq-i5 and TruSeq-i7) ligated to the cohesive ends of restriction sites of digested DNA fragments. Details of library preparation protocols can be inferred from Islam et al. (2024). Equimolarly pooled libraries were sequenced in single end mode on an Illumina NextSeq 500 system to a read length of 75 bp and a depth of at least 1 million reads per sample.

2.3 Reference-based variant calling

Raw Illumina reads were demultiplexed with the process_radtags program with default settings, part of the Stacks software (Catchen et al., 2011, 2013). FastQC v0.11.9 (Andrews, 2015) was used to check sequence quality per read and GC content. For short-read mapping, we used a multi-reference based assembly approach (Bohling, 2020; Valiente-Mullor et al., 2021) with the iPyrad pipeline (Eaton and Ree, 2013). We generated two different SNP data sets from short-read mapping with (i) our own de-novo reference genome for *Montifringilla nivalis*, and (ii) a published house sparrow (*Passer domesticus*) genome that was annotated to chromosome level (Elgvin et al., 2017; accession no: GCA_001700915.1).

We used ipyrad v.0.9.42 (Eaton and Overcast, 2020) for data assembly and read mapping, applying a clustering threshold of 85% and a minimum sequencing depth for clustering ≥6X. We applied default parameter settings of the reference-based ipyrad pipeline with a maximum of 8 indels, 0.5 heterozygous sites, and 20% SNPs per locus, and a minimum of four samples per locus. The ipyrad pipeline (Eaton and Overcast, 2020) produced two separate VCF output files from independent mapping against the two different reference genomes.

We used vcftools 1.1.5 (Danecek et al., 2011) for biallelic SNP filtering, and with a quality value ≥30 applied to separate autosomal from Z-chromosomal data sets (only for the data set inferred from alignment to the house sparrow reference genome annotated to the chromosome level; compare Islam et al. 2024). From each of the two VCF files, we generated final SNP data sets without allowing missing sites as input data for downstream analysis. To reduce effects of physical linkage among markers (O'Leary et al., 2018), we applied the vcftools "thinning" option and set a thinning factor thin=200 to receive an optimal proportion of unlinked SNPs in our thinned data set (compare Islam et al. 2024).

2.4 Clustering analyses

We used the ipyrad.pca tool from the ipyrad pipeline for the principal component analysis. To observe population genetic structure among the Palearctic and European snowfinch populations based on allele frequencies, we used Structure v.2.3.4 (Pritchard et. al 2000) with the ipyrad-analysis toolkit. We have run eight iterations over K ranging from 1 to 8, with each MCMC chain for a single *K* running with a burn in of 5,000 and sampling over 10,000 generations. We ran *CLUMPP* (CLUster Matching and Permutation Program) (Rosenberg et al. 2004) from ipyrad analysis pipeline to visualize the STRUCTURE run results. We identified the optimal K (number of clusters) using the ΔK method (Evanno et al. 2005). To calculate pairwise F_{ST} values among populations of *M. nivalis* we used Stacks 2.60 software (Rochette and Catchen, 2017). We ran all analyses separately for the total Eurasian sampling (n = 67) and for a reduced data set containing samples from European populations only (n = 48).

2.5 Demographic history

We estimated historical population size changes through time using Extended Bayesian Skyline Plots (EBSP; Heled & Drummond 2008) as implemented in BEAST v2.6.2 (Bouckaert et al. 2014). We used gawk the function in the linux system to extract RAD seq loci with more than three SNPs for five sets of populations separately allowing for 50% missing sites. Subsequently, by visual inspection of alignments, we discarded loci with missing data for >5 individuals and/or many missing sites at the beginning/end of the locus. The final input sets contained the mitochondrial cytochrome-*b* plus (i) Europe all 20 (out of 36) RAD seq loci extracted, (ii) Asia all 28 (out of 46) loci, (iii) Mongolia 17 (out of 22) loci; and (iv) Cantabrian Mountains 12 (out of 23) loci (see Appendix S1, Tables S1.2, S1.3; sets of all 81 alignments deposited at DRYAD). In light of the low number of loci extracted (n= 4), we did not perform a separate EBSP analysis for the sampling from the Alps alone.

For each run, we applied the HKY model and a strict clock across all loci and applied an empirical clock rate for the mitochondrial maker (compare Trucchi et al. 2014) cytochrome-b of 0.0105 substitutions per site per lineage per million years (Weir and Schluter 2008). To avoid numeric instability errors (Bouckaert et al. 2019; Finch et al. 2018) that occurred during test runs with BEAST, we modified clock rate priors for RAD seq loci from a default uniform distribution to a normal distribution. We inferred a mean rate across ddRAD seq loci from a phylogenetic study of snowfinches based on the same set of raw data (Islam et al. in prep), and set the prior intervals according to the normal distribution of mean rates checked with TRACER (mean= 0.00995, sigma=

0.0005; for further details see Appendix S2.2). For each data set, we ran the MCMC chain for 500 million generations, and checked ESS values from log files with TRACER. We generated skyline plots following the instructions by Heled (2008; R script provided in the supplementary materials).

2.6 Species distribution models

Locality records for *M. nivalis* and its sub-species were collected from online resources such as the Global Biodiversity Information Facility GBIF (www.gbif.org; n= 13,998 records). We used Geoplaner V3.0 (www.geoplaner.de) to georeference occurrences and verify registered coordinates, and DivaGIS (www.diva-gis.org) to extract elevational information for each record. We discarded vague locality data and occurrences that were certainly based on misidentifications or false taxonomic arrangements, in particular with respect to Asian populations of two related species *M. adamsi* and *M. henrici* (Supplementary Information; Fig. S1). After filtering for duplicate records from the same location, the raw occurrence data set contained 1406 localities.

Species distribution models (SDMs) were computed for the total occurrence data set of Eurasian populations and for two subsets of occurrence data that correspond to the two major genetic lineages (Islam et al. 2024). Separate modelling of these two subspecific units takes into account a large sampling bias between an intensely surveyed area in Europe and less well surveyed regions in Asia (compare Fourcade et al. 2013). The European subset contained all populations of the nominate subspecies *M. n. nivalis*, whereas the eastern subset comprised Near East, Caucasian and Central Asian populations (subspecies *M. n. leucura*, *M. n. alpicola*, *M. n. gaddi*, *M. n. tianshanica*, and *M. n. groumgrzimaili;* see Appendix S1; Table S1.4).

Spatially clustered localities were filtered to a minimum inter-point distance of 5 km using the ‘spatially rarefy occurrence data’ tool implemented into the SDMtoolbox v2.4 (Brown 2014) for ESRI ArcGIS v10.3, retaining 737 unique localities for *M. nivalis:* 492 for the European clade, and 245 for the eastern clade. The impact of uneven sampling effort between the European and eastern subunit were assessed by building models based on randomly selected equal numbers of records. Since the impact of unequal sampling was negligible all records were retained for model building.

A set of bioclimatic predictor variables with a spatial resolution of 2.5’ (about 4.5 km) describing annual trends, seasonality, and limiting factors related to temperature and precipitation, was obtained from WorldClim (www.worldclim.org). To investigate the influence of past climate fluctuations on past distribution of *M. nivalis*, we furthermore derived predictor variables for three projections for

the Last Glacial Maximum (LGM, ~22,000 ya) from global circulation models through the Paleoclimate Modelling Intercomparison Project Phase II (www.worldclim.org). Models included in the analyses were the Community Climate System Model (CCSM3) (Otto-Bliesner et al. 2006), the Max Planck Institute Earth System Model P (MPI-ESM-P), and the Model for Interdisciplinary Research on Climate (MIROC) (Hasumi & Emori 2004). A rectangular bounding box was used as background.

Models were computed using the machine learning algorithm MaxEnt v3.4.1 (Phillips et al. 2017; Phillips et al. 2006), a highly efficient, presence-only modelling algorithm. A subsampling method with 100 replicates, randomly splitting the data set into a training (50%) and a testing subset (50%), was applied. We chose the cloglog suitability output metric. Subsequently, the model was transferred to the three LGM climate scenarios. The area under the curve (AUC), a threshold-independent measure of model performance was used for model evaluation (Ling et al. 2003). An AUC score of 1 corresponds to a perfect fit, whereas a score of 0.5 is performance that matches random expectations (Phillips et al. 2006; Elith 2006). The average projection across all replicate runs was used for further processing, wherein the '10 percentile training presence logistic threshold' was applied as presence-absence threshold.

2.7 Phenotypic traits

For the phenotypic trait analyses, we relied on a published data set of morphological and colouration measurements (Delgado et al. 2019). For our analysis, we included data from 244 specimens from this dataset. Morphological measurements included wing, tail, tarsus, and bill lengths (all in mm) of each bird, as a surrogate of body size. In addition, from each specimen we measured the spectral reflectance of three different body parts representing three different colours (back/brown, head/greyish and wing/blackish), using a 31 Ocean Optics Jaz spectrometer and PX-2 flash lamp (Ocean Optics, Dunedin, FL). We computed six indices that summarise the chromatic variation in the spectral reflectance (Delhey et al. 2015), namely brightness (B2), intensity (B3), contrast (S6), saturation (S7), chroma (S8) and hue (H1; for definitions, see supplementary information). We calculated the above-mentioned 18 colorimetric variables for all recorded spectra (for details of spectrometric measurements, see Delgado et al. 2019).

We furthermore measured seven further parameters referring to the extent of brown, black, and white plumage patches (patch size; Fig. For each sample we took four digital photographs of the body (upper, lower, right and left sides) using a Nikon DF camera with a 50mm lens mounted on a tripod,

thus keeping the bird-camera distance constant. Each sample was placed on a graph paper, in order to have a scale unit as reference. Afterwards, we used the photographs to measure the area and the perimeter of the visible brown patches on the upper side of the body (both the brown of the body and the wings), the brown patch on the tail, the grey of the head and the white of the tail and the wings (Fig. S1.1.A), using the Magic Wand and the Measurement Log tools in Adobe Photoshop CC 2016 (Fig. S1.1. B,C). Prior to each measurement, we set the measurement scale through the specific tool in Photoshop, thus specifying the number of pixels in each image equal to 1 cm scale unit derived from the graph paper. When a certain color was not uniform on the part of the body considered, the Magic Wand tool selected fragmented patches and the Measurement Log tool returned multiple measures (one for each fragment). In these cases, we considered the sum of all the measures for both the area and the perimeter (for a list of variables measured, see supplementary Fig. S3).

We excluded specimens that had missing data for more than two phenotypic variables from the analysis. The phenotypic data were analysed with SPSS 19, in a linear discriminant analysis (LDA) for both original and log-transformed data (Mendez et al. 2002) using i) simultaneous estimation considering all independent variables and ii) stepwise selection of best variables with best univariate discrimination (lowest Wilk's lambda). LDA was previously applied to the challenge of discrimination among sexes of *M. nivalis* (Strinella et al. 2011). Because *M. nivalis* is a sexually dimorphic species, all analyses of phenotypic variables were performed separately for sexes; in addition, only adult individuals were included in the analysis (final n= 169; males: n= 119, females: n= 50). For LDA, we a priori defined three geographic groups that match the three genetic lineages (Islam et al. 2024): i) European populations, ii) Caucasian and Middle Eastern populations, iii) Central Asian populations from Kyrgysztan, Kazakhstan and Afghanistan (supplementary Fig.6). Prior to LDA, we assessed multicollinearity between predictor variables using variance inflation factors (VIFs; Fox & Monette 1992). We ran three separate simple linear models, considering the individual ID as a response variable, assuming that values of VIF higher than 10 indicates collinearity. We estimated the collinearity between (1) body-size features, (2) colorimetric variables and (3) patch size.

We extracted climate variables for all georeferenced collection sites of museum specimens to test for a possible correlation of plumage colour traits with climatic variables according to ecogeographic rules. Due to missing or vague locality information on many labels, reliable georeferencing of collection sites could only be done for a limited number of specimens, which reduced the data set for this analysis (males n= 70; females n= 36).

3. Results

3.1 Whole-genome assembly

We assembled short read sequencing for the whole-genome, sequencing resulted in 174,809,615 paired raw reads with an average GC content of 42%. After trimming, 136,963,515 sequences remained. The three scaffolded assemblies produced by Abyss, Ray and SOAPdenovo2 ranged in total length from 1.06 (Ray) to 1.21 Gbp (Abyss). The longest scaffold overall was produced by SOAPdenovo2 (1.1 Mbp). The Ray scaffolded assembly had the highest number of gaps, whereas the Abyss assembly had the lowest. The Abyss assembly also had the lowest total gap length, at just ~10% of that of the Ray and SOAPdenovo2 assemblies, respectively (Table 1). The N50 value was highest for the Abyss assembly (74,962) with Ray a close second (70,130); SOAPdenovo2 had the lowest (31,662; see Appendix S1; Table S1.6). The BUSCO analysis focused on the 10,844 putatively single-copy genes in the Passeriformes dataset v. 10. The Abyss assembly recovered 85.3% of these completely and in single-copy status; the Ray assembler performed nearly as well (83.2%; Appendix S1; Table S1.6). In contrast, SOAPdeonovo2 recovered only 71.2% of the target genes completely and as single-copy genes. The SOAPdenovo2 assembly missed twice as many target genes as the other two assemblers. The Ray assembler resulted in 150 duplicated BUSCO genes compared to 44 (Abyss) and 34 (Ray; Appendix S1; Table S1.6).

3.2 Raw read mapping

Illumina HiSeq2500 produced an average of 461k ddRAD raw reads per sample. Mean mapping rate was higher, whereas mean coverage depth was slightly lower for read-mapping on the *M. nivalis* genome (mapping rate: 68.4% [54.2 – 73.5%] vs. 58.6% [57.3 – 59.7%]; mean coverage depth: 100.4 [40.1 – 168.1] vs. 104.6 [36.8 – 148.3]; for *M. nivalis* vs. *P. domesticus* reference genomes). From short-read alignment with the *M. nivalis* reference genome, the number of total filtered loci ranged from 7919 – 10,362, the sequence matrix contained 973,812 sites (variable and non-variable; 16.91% missing sites) and the SNP matrix contained 20,687 sites (19.23 % missing sites; see Appendix 1, Table S1.7). In contrast, short-read alignment with the house sparrow reference genome yielded a total number of filtered loci from 6465 – 8477, the sequence matrix contained 720,240 sites (variable and non-variable; 18.86% missing sites) and the SNP matrix contained 13,228 sites (20.08 % missing sites; see Appendix 1, Table S1.8).

For the total data set, allowing for 0% missing data, the SNP matrix contained 7630 sites and 3949 biallelic unlinked SNPs after further filtering. (Read-mapping to the *P. domesticus* reference genome resulted in a matrix size of 4268 sites and 2443 biallelic unlinked SNPs after further filtering.) Separate analyses of European populations were based on a SNP matrix size of 2258 sites and 1690

biallelic unlinked SNPs (read-mapping on the *P. domesticus* reference genome, matrix size: 1562 sites and 1231 biallelic unlinked SNPs).

3.2 Lineage divergence and clustering analyses

Patterns of divergence and admixture based on the two independent SNP data sets inferred from read-mapping to the house sparrow and the white-winged snowfinch genome were fully congruent. In the following, we present the results based on the SNP data set inferred from the conspecific *M. nivalis* reference genome, which, as expected, provided a higher average score for the mean mapping rate.

For the total sampling, PCA and STRUCTURE analysis distinguished three well-separated clusters, corresponding to the Mongolian Altai, Caucasus, and European mountain systems (Fig. 2; k=3, maximal Δk= 12.125; Fig. 2D, Appendix S1, Table S1.9). No signal of a fourth cluster was apparent either in the STRUCTURE plot for k=4 (Fig. 2C) or in the scatterplot of the first two principal components (PC1 explaining 64.9% of the total variation, PC2 explaining 6.4% of the total variation; Fig. 2E). However, European populations from the Alps and the Cantabrian Mountains were separated along PC3, which explained another 1.7% of the total variation (Fig. 2F).

Both the mitochondrial marker and the SNP data set showed a strong separation of Asian and European populations. The two lineages were separated by a minimum of 44 substitutions in the cyt*b* haplotype network (suppl. Fig. S2), which equals a p-distance of roughly 5.0% (F_{ST} values ranged 0.288–0.396; Table 1). The Caucasian population was represented by a single private haplotype that differed by a single substitution from the Mongolian cluster (Fig. S2), whereas the SNP network showed these two populations as strongly separated phylogroups (Fig 2B; p-dist= 0.25%, F_{ST}= 0.198; Table 1).

Considering the separate analyses of European populations, although the optimal number of clusters was estimated at k=4 (Δk= 9.252; Appendix S1, Table S1.9), PCA, STRUCTURE plots, and the SNP network showed only two biologically meaningful clusters: the populations of the Alps and the Cantabrian mountains (Fig. 3; PC1 explained 10.5% and PC2 explained another 4.1% of total variation.). The STRUCTURE plot for k=2 showed the populations from the two European mountain systems as two clusters connected by slightly asymmetric allelic introgression from the Alps into the Cantabrian Mountains (Fig. 3B). Within the latter region, populations from Picos de Europa and from Las Ubiñas could not be separated in the STRUCTURE plot (Fig. 3B), the PCA scatterplot (Fig. 3C), or in the minimum spanning network (Fig. 3D). Uncorrected p-distances among populations from the two European mountain systems ranged 0.16–0.23%, and F_{ST} values ranged 0.051–0.052 (Table 1).

3.3 Demographic history, population size changes

In the pooled Asian data set, the 28 selected RADseq loci (total alignment length 3256 bp) contained 5–10 SNPs each. Seven loci failed to score any read data for 1–5 (out of 20) individuals (Table S1.2a). In the pooled European data set, the 20 selected RADseq loci (total alignment length 2306 bp) contained 5–8 SNPs each. Nine loci failed to score any read data for 1–8 (out of 48) individuals (Table S1.3a). According to EBSPs, both Asian and European snowfinch populations have maintained a rather constant effective population size from the middle Pleistocene (~1 mya) to the end of the LGM (Fig. 4). For the two larger population subsets, EBSPs showed a slight Holocene population size increase starting at ~10,000 ya for Mongolian *M. n. groumgrzimaili* (Fig. 4C, D) and at about 5000 ya for Cantabrian *M. n. nivalis* (after a minor population size decrease during the LGM; Fig. 4G, H).

3.4 SDMs

The discrimination ability of all models was high across replicate runs (all records: AUC = 0.956 ± 0.004 SD; European subset: AUC = 0.984 ± 0.002 SD; eastern subset: AUC = 0.965 ± 0.005 SD). Elevation was consistently important across all three units (all records: 38.9%; European population: 20.0%; eastern subunit: 57.2%). The overall model was affected mainly by elevation (38.9%), followed by precipitation during the coldest quarter (bio 19: 33.0%) and coldest month (bio 14: 16.0%). The distribution of the European population was affected mainly by precipitation of the driest quarter (bio 17: 51.3%) and the driest month (bio 14: 25.8%), followed by elevation (20.0%). Predicted suitability for the eastern subunit was shaped principally by elevation (57.2%) and mean temperature of the wettest quarter (bio 8: 11.2%). For a detailed list on variable contributions see Table 2.

For European populations, the predicted distribution fitted well to the known range of *M. n. nivalis*. Areas of highest suitability appeared confined to high-elevation areas in the Pyrenees and its western extensions (the Cevennes, the Alps, the eastern Dinaric Alps), plus a small area of lower suitability along the eastern Black Sea coast (Fig. 5). For the eastern population, our models identified highly suitable areas in Turkey and western Iran and eastern Afghanistan, with smaller fragmented areas scattered across the Hindu Kush Mountains, all the way to the western Tian Shan Mountains (Fig. 6); a disjunct area of high suitability was also apparent in the northern Altay Mountains in Mongolia. The Caucasus Mountains were accorded only a low predicted suitability. Contrary to current range estimates (BirdLife International 2017) a vast region of the QTP does not harbor any suitable area for

M. nivalis. According to our estimates, the fraction of the IUCN shape file south of the Kunlunshan and its eastern extensions without any occurrence records of *M. nivalis* covered an area of ~1.5 million km^2, which equals >22% of the entire range estimate according to IUCN (BirdLife International 2017, Fig. 7).

Projections of ecological niche models to paleoclimatic conditions for the LGM suggested that *M. nivalis* occupied a range comparable to that of its present-day distribution. The Cantabrian Mountains, the Pyrenees, and the western Alps were likely uninhabitable, suggesting that the remainder of the Alps might have served as a glacial refuge for the species in Europe. For the eastern phylogroup, vast suitable areas existed during the LGM extending from Turkey in the West along the Kopet Dag and the Caucasus Mountains across the Pamir-Alay system and the entire Tian Shan range to the southern margin of the Mongolian Altai in the East (compare Figs 6, 7).

3.5 Phenotypic traits

According to VIF values, there was no collinearity neither among body-size variables nor among metric dimensions of color patches. However, we found a high multicollinearity among colorimetric variables with intensity (B3) and contrast (S6) showing high VIF values for all three color patches (plus brightness [B2] for grey). After exclusion of B3 and S6 from the data set, the problem of collinearity disappeared. Therefore, we deleted B3 and S6 (for all three colors) from the data set and performed LDA with a reduced data set of 22 variables (body-size: n= 4; colorimetric: n= 12; patch size: n= 6).

Irrespective of data transformation and data selection (all simultaneously *vs* stepwise selection) classification accuracy to each of the three *a-priori* groups was high (89.2% - 93.3% of the individuals were assigned to the correct group; Table S1.10). In both sexes, Central Asian populations were separated from their eastern counterparts along DF1 (Fig. 8), that explained 57.5% and 79.7% in males and females and showed significant correlations for color patch dimensions (brown-area, white-LW) and hue (H1: all colors in males; brown and black in females; Table S1.11). In addition, European *M. n. nivalis* were separated from their Near and Middle East counterparts along DF2 (Fig. 8), that explained further 42.5% and 20.3% of the total variance in males and females, and showed significant correlations with color patch dimensions (M2-area) and chroma (S8 for black and brown) in both sexes (Table S1.11). In stepwise discriminant analysis (SDA), three size variables were equally selected for both sexes (bill length; patch sizes: brown-area, M2-area; Table S1.11), for males four further variables were included in the analysis (patch size: Cm-area, white-

LW-area; tail length; S8 black), whereas only chroma (S8) for brown was retained as a fourth variable in females (Table S1.11). Classification accuracy for SDA was a little lower than for DA (all variables simultaneously included) with 89.2% of the males and 88.2% of the females assigned to the correct group (Table S1.10).

To check for possible associations among morphological traits and climate variables, we relied on one temperature variable and two precipitation variables that had the greatest effects on SDMs. Among body size variables, only wing pattern correlated significantly negative with precipitation of the coldest quarter (Bio19) for both sexes and with temperature of the wettest quarter in females ($p < 0.05$, Table 4a; Fig. S3). In addition, male tail and tarsus lenghts increased and wing length also decreased significantly with precipitation of driest quarter (Bio17; $p < 0.01$, Table 4a; Fig. S3).

Among plumage male color variables, 9 (out of 15) variables correlated significantly with precipitation during the driest quarter (Bio17; all variables decreased except saturation [S7] for all three patches; $p < 0.05$; Table 4; Fig. 9). Only four variables correlated significantly with mean temperature of the wettest quarter: brightness (B2), intensity (B3) and contrast (S6) of greyish head decreased ($p < 0.001$), whereas saturation (S7) of greyish head increased ($p < 0.05$; Table 4). Strikingly, only 4 out of 15 colour variables of male plumage correlated significantly with precipitation of the coldest quarter (Bio19): saturation (S7) for black and brightness (B2) and intensity (B3) for grey increased, whereas only chroma (S8) for black decreased with Bio19 (Table 4; Fig. 9). In females, only a single color variable (chroma, S8 grey) decreased significantly with temperature of the wettest quarter (Bio8; $p < 0.05$) and two other variables increased significantly with precipitation of the driest quarter (Bio17; all $p < 0.05$; Table 4).

4. Discussion

4.1 Pleistocene lineage divergence and demographic histories

Currently, *M. nivalis*, is considered the only snowfinch species that shows a broad geographic range and an intraspecific genetic differentiation at the same time, diverging it into three distinct phylogroups (Päckert et al. 2020, 2021; this study). Divergence times between the two major European and Asian lineages were dated to the Early Pleistocene (2.7–1.5 mya; Islam et al. 2024). Likewise, Pleistocene emergence of novel genetic lineages has also been suggested for alpine organisms in other mountain systems of South America and New Zealand (Wallis et al. 2016). Similar phylogeographic disjunctions among Asian and European subspecific lineages have also been documented in other Eurasian mountain specialists like the alpine accentor, *Prunella collaris*, (Päckert et al. 2020), the bearded vulture, *Gypaetus barbatus* (Streicher et al. 2021) and the Eurasian dipper, *Cinclus cinclus* (Hourlay et al. 2018).

While previous studies had suggested strong past population declines for several Palearctic bird species (Song et al. 2021; Miller et al. 2021; Robinson et al. 2021), our EBSPs for both European and Asian phylogroups of *M. nivalis* suggested long-term constancy of effective population sizes over the last 30 – 50 my, followed by a slight Holocene population size increase., i.e. no evidence of a population bottleneck. Similar demographic patterns were reconstructed for other snowfinch species on the QTP (Qu et al. 2010), for some other Holarctic mountain specialists (*Leucosticte*, Miller et al. 2021), but also for other montane and lowland bird species (Ashrafzadeh et al. 2021; Drovetski et al. 2018). This is in accordance with our range projections onto paleoclimatic conditions that suggested large areas of suitable habitat for ancestral *M. nivais* during the LGM across its entire range. In Central Asia a large area of suitable paleohabitat should have existed from the Pamir-Alay system in the South along the entire Tian Shan range to the Mongolian Altai and its eastern extensions (Fig. 7). Those glacial refuges of *M. nivalis* must have been largely separated from those reconstructed for the other snowfinch "platform species" that were supposedly restricted to one rather small glacial refuge at the northeastern QTP margin (Qu et al. 2010, Lei et al. 2014).

Within the European nominate form *M. nivalis nivalis*, shallow genetic divergence among populations from the Alps and those from the Cantabrian mountains likely accumulated in allopatry in separate glacial refuges: one across the entire Alpine Arc and another smaller area in the Pyrenees and their westward extensions (see Fig. 6). Strikingly, our projected distributions did not suggest remarkable range shifts or contractions during the LGM, which is in good accordance with fossil records of *M. nivalis* from the Würmian period that are mostly congruent with the species' extant Pyrenean and Alpine range (with some occurrences farther north of the Alps in southern France and southern Germany; Tyrberg 1991).

There are fossil sites of this species dated between 15,000–28,000 years BP that are closer to the Cantabrian Mountains & Pyrenees (in N Spain) than the remaining Alps (Smith et al. 2013). Like in other European vertebrate species, the signal of admixture in some of the Cantabrian individuals might have resulted from combined effects of incomplete lineage sorting (ILS) and past/ extant gene flow among the Alpine and the Pyrenean-Cantabrian populations (Recuero & García-París 2011; Milá et al. 2013). Phylogeographic patterns involving distinct lineages from different European mountain systems (e.g. Pyrenees, Alps, Carpathians etc.) are manifold and complex across different groups of organisms (review by Schmitt 2009; rock lizards, *Iberolacerta*: Crochet et al. 2004; Apennine-Pyrenees phylogenetic disjunction in beetles: Berilli et al. 2024; mammals, *Rupicapra*:

Rodríguez et al. 2009, Perez et al. 2014, Iacolina et al. 2021). Even within single mountain systems microgeographic patterns of genetic divergence can be observed (review by Schmitt 2009), and accordingly Ceresa et al. (2024) found evidence of small-scale phylogeographic structure among *M. n. nivalis* populations of the central-eastern Alps, suggesting partly restricted gene flow due to isolation-by-distance and high local inbreeding effects. Accordingly, the genetic diversity of European *M. n. nivalis* might still be underestimated, and future phylogeographic and population genomic studies should therefore include so far unstudied snowfinch populations from the Italian Apennines, the Dinaric Alps and the Greek mountains. It might also be worth verifying whether the species still exists in its formerly described breeding grounds on some Mediterranean islands like Corsica and Crete (Portenko & Vietinghoff-Scheel 1974, Hölzinger 2011).

4.2 Trait divergence among phylogroups

Ecological niche modeling by Cobos et al. (2021) suggested that colonization of the Western Palearctic mountain systems from a Tibetan source area was associated with an expansion of the niche of nominate *M. n. nivalis* from colder towards warmer temperature regimes. Even though Cobos et al. (2021) likely overestimated the extent of temperature niche expansion (i.e. methodological issues pointed out by Bambrilla et al. 2022), this is in line with our results. The sister species of the snowfinch clade, the rock sparrow (*Petronia petronia*) shows a similar pattern of divergence and ecological segregation among nominate *P. p. petronia,* from the lowlands on the Iberian Peninsula up to 2000 m in the European Alps (Mingozzi et al. 2021) and genetically divergent Mongolian and Tibetan *P. p. brevirostris* (Päckert et al. 2020) breeding at high alpine elevations up to 4800 m (Summers-Smith 2009). In other passerines, such as corvids, niche expansion has been shown to correlate with diversification of phenotypes (Garcia-Porta et al. 2022). Accordingly, sympatric snowfinch species from the QTP (*Montifringilla adamsi*, *Pyrgilauda ruficollis, Onychostruthus taczanowskii*) show strong divergence of body size traits, whereas only *M. adamsi* showed strong divergence of the climatic niche compared to the other two more closely related species (She et al. 2021). Moreover, even within other sparrow species, like *Passer domesticus,* trait variation seems to follow ecogeographic rules, i.e. changes of body size features along elevational gradients (Bala et al. 2024), and along latitudinal gradients (body size and plumage colour; Cohen & Dor 2018).

In our white-winged snowfinch data set, European populations of nominate *M. n. nivalis* had longer tails and tarsi but shorter wings and beaks than Asian populations (compare measurements in Cramp & Perrins 1994). Significant correlations among plumage color and climate variables were mostly

found for the dorsal patches on the head (grey) and the back (brown), rather than for the lateral patch on the wing (black). Previous studies also suggested stronger effects of precipitation on plumage brightness and 'redness' of the back than of the belly (Marcondes et al. 2021). The marked differences of environmental effects on plumage colors among sexes found in *M. nivalis* (greater effect on male plumage color) might be related to different selective pressures acting on plumage coloration, particularly in sexually dimorphic bird species (Dale et al. 2015). In male *M. nivalis*, brightness (B2), intensity (B3) and contrast (S6) decreased significantly whereas only saturation (S7) increased significantly with both temperature (Bio8) and precipitation (Bio17). This seems to contradict the expectation to find darker plumages in warmer and wetter environments according to Gloger's rule (Delhey 2017) as recently confirmed for some bird species (Flame-colored Tanager, *Piranga bidentata*: Robles-Bello et al. 2022; Barn Owl, *Tyto alba*: Romano et al. 2019). Though the validity of Gloger's rule was already discussed for interspecific comparisons of snowfinch species from different genera (Delgado et al. 2019), recent studies showed that Gloger's rule does not apply generally to all passerine birds (see Lee et al. 2021; example *S. webbiana*) owing to complex interaction of antagonistic or additive effects of temperature and precipitation on plumage color (Marcondes et al. 2021; Koskenpato et al. 2023).

For bird communities of the Iberian Peninsula, melanin-based color traits were shown to correlate with summer ambient temperatures, but not with precipitation (Galvan et al. 2018). However, comparisons among independent studies need to be treated with particular care, because color traits such as "lightness" could have been quantified using quite different methods; e.g. in our study B2 = "Mean relative reflectance over the entire spectral range", whereas Delhey et al (2019) quantified "lightness" according to red-green-blue values obtained from published color plates. Apart from environmental effects, a genetic basis of plumage coloration should also be taken into account, as was shown for rosy finches (*Leucosticte spp.*): the diagnostic phenotypes of three extant Nearctic species (cheek and crown color) started diverging very recently (at ~0.25 mya) and were shown to be associated with candidate regions on several different chromosomes (e.g. melanonesis genes; Funk et al. 2023). However, similar studies of functional genomics are missing for *M. nivalis,* and would be an important further step towards understanding intraspecific diversification of these Palearctic high-alpine birds.

In summary, we conclude that, even though LDA revealed congruence of three morphological clusters with three phylogroups of *M. nivalis*, no diagnostic trait discontinuities exist: the variance of single variables showed large overlaps among the three phylogroups. According to the phenotypic

diagnosis of Vaurie (1959), nominate *M. n. nivalis* is distinguishable from its Asian counterparts by its "ashy grey crown that contrasts with the brown back." The finding that at least some of the spectral variables of plumage color correlated strongly with climate variables might support the idea that an evolutionary niche shift in *M. nivalis* has also promoted phenotypic divergence.

4.3 Range overestimates due to dated taxonomy

Until recently, published distribution maps for *M. nivalis* projected a vast Tibetan range covering the entire QTP from its northernmost margins in the Kunlun Mountains and the Qilian Mountains to the high elevations of the Himalayas in the South and the Hengduanshan in the Southeast (del Hoyo & Collar 2016; BirdLife International 2017, 2024; compare Fig. 7). Such a vast trans-QTP range is only in accordance with a dated taxonomy that recognized a single *Montifringilla* species, i.e. *M. nivalis* sensu lato that includes the subspecific taxa *adamsi* and/or *henrici*, now recognized as full species. Accordingly, previous maps extended the Asian range of *M. nivalis* into NE Qinghai and Xizang (i.e. the range of the subspecific taxon *M. n. henrici*; compare maps in Portenko & Vietinghoff-Scheel, 1974; Cheng, 1987). Most authors who later recognized the Tibetan snowfinch, *M. henrici*, as a species of its own consequently limited the Asian range of *M. nivalis* sensu stricto to the mountain chains north and west of the QTP (Gebauer 2006; Summers-Smith 2009, Summers-Smith and Bonan 2020). Recognition of three species-level taxa *M. nivalis* s. str., *M. henrici*, and *M. adamsi* must necessarily result in range projections that show some overlap only for the latter two species in Qinghai, whereas *M. nivalis* s. str. is nearly entirely allopatric from its congeners except for a marginal overlap of *M. n. kwenlunensis* and nominate *M. a. adamsi* (Fig. 1 in Gebauer et al. 2006). This essential information was likely overlooked by some authors, who apparently did not adjust projected range maps to their proposed taxonomic changes, i.e. they distinguished three *Montifringilla* species but retained an overly broad range map for *M. nivalis* that included an immense taxonomic bias (i.e. covering the entire ranges of previously subspecific *M. n. henrici* and *M. n. adamsi*; del Hoyo & Collar 2016; BirdLife International 2017, 2024). Consequently, these projections show an immense range overestimate of ~1.5M km2 of uninhabited area where *M. nivalis* s. str. does not occur (even a large area on the central QTP that is not occupied by any of the three *Montifringilla* species; compare Fig. 9 and map in Gebauer et al. 2006).

This sort of error needs to be kept in mind when IUCN distribution maps are used for threat assessments in birds and other vertebrates (Santini et al. 2019; Harfoot et al. 2021) or as a basis for population size estimates (Wiedenfeld & Tognelli, 2023). Some authors raised the concern that range

projections for narrow-range endemics may overestimate their true extents rather commonly (Ramesh et al 2017). A recent exploration more generally of the IUCN distribution maps for birds pointed toward rampant and pervasive errors of various sorts (Peterson et al. 2018). Therefore, an account for uncertainty with respect to data quality and biases, published range maps or atlas data is essential for accurate mapping of species' distributions (Rocchini et al. 2011).

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References

Andrews, S. (2015) FastQC: A quality control tool for high throughput sequence data. 0.11.4.

Antonelli A, Kissling D, Flantua SGA, Bermúdez MA, Mulch A, Muellner-Riehl AN, Kreft H, Linder HP, Badgley C, Fjeldså J, Fritz SA, Rahbek C, Herman F, Hooghiemstra H & Hoorn C. 2018. Geological and climatic influences on mountain biodiversity. Nature Geoscience 11, 718–725. https://doi.org/10.1038/s41561-018-0236-z

Ashrafzadeh M R, Khosravi R, Fernandes C, Aguayo C, Bagi Z, Lavadinovic VM, Szendrei L, Beukovic D, Mihalik B, Kusza S (2021) Assessing the origin, genetic structure and demographic history of the common pheasant (*Phasianus colchicus*) in the introduced European range. Sci Rep 11(1), 21721. https://doi.org/10.1038/s41598-021-00567-1

Bala R., Kumar R.S., Gokhale P., Mohan D., Barve S. (2024) House Sparrows *Passer domesticus* increase in body size and dorsal feather insulation along a Himalayan elevational gradient. Journal of Field Ornithology. https://doi.org/10.5751/JFO-00469-950210

Baker, A. J.; S. L. Pereira & T. A. Paton (2007) Phylogenetic relationships and divergence times of Charadriiformes genera: multigene evidence for the Cretaceous origin of at least 14 clades of shorebirds. Biology Letters 3: 205-209.

Berilli E, Biondi M, Garzia M, D'Alessandro P, Salvi D (2024) Apennine–Pyrenees disjunct distribution: an unusual biogeographic pattern revealed in flea beetles of the *Longitarsus candidulus* species-group (Coleoptera, Chrysomelidae). Current Zoology, zoad050. https://doi.org/10.1093/cz/zoad050

BirdLife International (2017) *Montifringilla nivalis* (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2017: e.T103819582A111173412. https://dx.doi.org/10.2305/IUCN.UK.2017-1.RLTS.T103819582A1111734

BirdLife International (2024) Species factsheet: *Montifringilla nivalis*. Downloaded from https://datazone.birdlife.org/species/factsheet/white-winged-snowfinch-montifringilla-nivalis on 16/05/2024.

Boisvert, S., Laviolette, F., and Corbeil, J. (2010). Ray: Simultaneous Assembly of Reads from a Mix of High-Throughput Sequencing Technologies. Journal of Computational Biology. J. Comput. Biol. 17. Available at: https://www.liebertpub.com/doi/full/10.1089/cmb.2009.0238 (Accessed April 8, 2024).

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bohling, J. (2020). Evaluating the effect of reference genome divergence on the analysis of empirical RADseq datasets. Ecology and Evolution, 10(14), 7585–7601. https://doi.org/10.1002/ece3.6483

Bouckaert, R. et al. (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10. https://doi.org/10.1371/journal.pcbi.1003537

Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchêne S., Fourment M., Gavryushkina A., et al. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS computational biology, 15(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bradnam, K. R., Fass, J. N., Alexandrov, A., Baranay, P., Bechner, M., Birol, I., et al. (2013). Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. GigaScience 2, 2047-217X-2–10. https://doi.org/10.1186/2047-217X-2-10

Brambilla, M., Bettega, C., Delgado, M.M., de Gabriel-Hernando, M., Päckert, M., Arlettaz, R., Dirren, S., Fontanilles, P., Gil, J.A., Herrmann, M., Hille, S., Korner-Nievergelt, F., Pedrini, P., Resano-Mayor, J., Schano, C. & Scridel, D. 2022: Insufficient considerations of seasonality, data selection and validation lead to biased species-climate relationships in mountain birds. Journal of Avian Biology, 9, e03015. https://doi.org/10.1111/jav.03015

Brown JL (2014) SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. Methods in Ecology and Evolution, 5(7), 694-700. https://doi.org/10.1111/2041-210X.12200

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J. H. (2011). Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. **1**, 171–182. https://doi.org/10.1534/g3.111.000240

Catchen, J., Hohenlohe, P. A., Bassham, S. & Amores, A. (2013) Stacks: an analysis tool set for population genomics. Mol. Ecol. 3124–3140. https://doi.org/10.1111/mec.12354

Ceresa F, Brambilla M, Kvist L, Vitulano S, Pes M, Tomasi L, Pedrini P, Bettega C, Anderle M, Hilpold A, Kranebitter P (2024) Restricted dispersal and inbreeding in a high-elevation bird across the 'sky islands' of the European Alps. Journal of Biogeography, 51:853–868. https://doi.org/10.1111/jbi.14787

Cheng, T. (1987). A synopsis to the avifauna of China. Paul Parey Scientific Publ., Hamburg, Berlin.

Chikhi, R., and Medvedev, P. (2014). Informed and automated k-mer size selection for genome assembly. Bioinformatics 30, 31–37. https://doi.org/10.1093/bioinformatics/btt310

Cobos, M. E., Cheng, Y., Song, G., Lei, F., & Peterson, T. A. (2021). New distributional opportunities with niche innovation in Eurasian snowfinches. Journal of Avian Biology, 52, e02868. https://doi.org/10.1101/2021.04.06.438738

Cohen SB, Dor R (2018) Phenotypic divergence despite low genetic differentiation in house sparrow populations. Sci Rep 8, 39. https://doi.org/10.1038/s41598-017-18718-8

Cramp, S., Perrins, C. M. (1994). Handbook of the birds of Europe the Middle East and North Africa, Volume VIII, Crows to Finches. Oxford University Press, Oxford,New York.

Crochet PA, Chaline O, Surget-Groba Y, Debai C, Cheylan M. (2004) Speciation in mountains: phylogeography and phylogeny of the rock lizards genus *Iberolacerta* (Reptilia: Lacertidae). Mol Phylogenet Evol 30, 806-866. https://doi.org/10.1016/j.ympev.2003.07.016

Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M (2015) The effects of life history and sexual selection on male and female plumage colouration. Nature 527, 367–370. https://doi.org/10.1038/nature15509

Danecek P., Auton A., Abecasis G., Albers C.A., Banks E., Depristo M.A., Handsaker R.E., Lunter G., Marth G.T., Sherry S.T., Mcvean G, Durbin R. 2011.The variant call format and VCFtools. Bioinformatics, 27 (15): 2156-2158, https://doi.org/10.1093/bioinformatics/btr330

Delgado, M., Bettega, C., Martens, J., & Päckert, M. (2019). Ecotypic changes of alpine birds to climate change. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-52483-0

Delhey, K., Delhey, V., Kempenaers, B. & Peters, A. (2015) A practical framework to analyze variation in animal colors using visual models. Behav. Ecol. 26, 367–375. https://doi.org/10.1093/beheco/aru198

Delhey K (2017) Gloger's rule. Current Biology, 27(14), R689-R691. https://doi.org/10.1016/j.cub.2017.04.031

Delhey K (2019) A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. Biological Reviews, 94(4), 1294-1316. https://doi.org/10.1111/brv.12503

del Hoyo, J., & Collar, N. J. (2016). Illustrated checklist of the birds of the world. Volume 2 Passerines.Lynx Edicions, Barcelona.

Deng, T., Wu, F., Zhous, Z. & Su, T. (2020) Tibetan Plateau: An evolutionary junction for the history of modern biodiversity. Science China – Earth Sciences 63(2), 172–187. https://doi.org/10.1007/s11430-019-9507-5

Drovetski SV, Fadeev IV, Raković M, Lopes RJ, Boano G, Pavia M, Koblik EA, Lohman YV, Red'kin YA, Aghayan SA, Reis S, Drovetskaya SS, Voelker G. (2018). A test of the European Pleistocene refugial paradigm, using a Western Palaearctic endemic bird species. Proc Biol Sci. https://doi.org/10.1098/rspb.2018.1606

Ebersbach J, Muellner-Riehl AN, Michalak I, Tkach N, Hoffmann MH, Röser M, Sun H, Favre A. 2017. In and out of the Qinghai–Tibet Plateau: divergence time estimation and historical

biogeography of the large arctic-alpine genus Saxifraga L. Journal of Biogeography 44: 900–910. https://doi.org/10.1111/nph.19206

Eaton, D. A. R. & Overcast, I. (2020) ipyrad: Interactive assembly and analysis of RADseq datasets, Bioinformatics, Volume 36, Issue 8, April 2020, Pages 2592–2594, https://doi.org/10.1093/bioinformatics/btz966

Eaton, D. A. R. & Ree, R. H. (2013) Inferring Phylogeny and Introgression using RADseq Data: An Example from Flowering Plants (*Pedicularis*: Orobanchaceae). *Systematic Biology*, Volume 62, Issue 5, September 2013, Pages 689–706, https://doi.org/10.1093/sysbio/syt032

Elgvin, T. O., Trier, C. N., Tørresen, O. K., Hagen, I. J., Lien, S., Nederbragt, A. J., Ravinet, M., Jensen, H., & Sætre, G. P. (2017). The genomic mosaicism of hybrid speciation. *Science Advances*, 3. https://www.science.org/doi/abs/10.1126/sciadv.1602996

Elith, J. *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography *(Cop.).* **29**, 129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005 Jul;14(8):2611-20. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Favre A, Päckert M, Pauls SU, Jähnig SC, Uhl D, Michalak I, Muellner-Riehl AN. The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. Biol Rev Camb Philos Soc. 2015 Feb;90(1):236-53. https://doi.org/10.1111/brv.12107

Finch JTD, Power SA, Welbergen JA, Cook JM. 2018. Two's company, three's a crowd: co-occurring pollinators and parasite species in *Breynia oblongifolia* (Phyllanthaceae). BMC Evolutionary Biology 18:193. https://doi.org/10.1186/s12862-018-1314-y

Fjeldså, J., Bowie, R.C. & Rahbek, C. (2012) The role of mountain ranges in the diversification of birds. *Annual Review of Ecology, Evolution, and Systematics* 43, 249–265. https://doi.org/10.1146/annurev-ecolsys-102710-145113

Funk, E. R., Spellman, G. M., Winker, K., Withrow, J. J., Ruegg, K. C., Zavaleta, E., & Taylor, S. A. (2021). Phylogenomic data reveal widespread introgression across the range of an alpine and arctic specialist. Systematic Biology, 70(3), 527-541. https://doi.org/10.1093/sysbio/syaa071

Funk ER, Spellman GM, Winker K, Withrow JJ, Ruegg KC, Taylor SA (2023) The genetic basis of plumage coloration and elevation adaptation in a clade of recently diverged alpine and arctic songbirds. *Evolution* 77(3). https://doi.org/10.1093/evolut/qpac064

Fourcade Y, Engler JO, Besnard AG, Rödder D, Secondi J (2013) Confronting expert-based and modelled distributions for species with uncertain conservation status: A case study from the corncrake (*Crex crex*). Biological Conservation 167, 161-171. https://doi.org/10.1016/j.biocon.2013.08.009

Fox J, Monette G. Generalized collinearity diagnostics. Journal of the American Statistical Association 1992;87:178–83. https://doi.org/10.2307/2290467

Galván I, Rodríguez-Martínez S, Carrascal LM. Dark pigmentation limits thermal niche position in birds. Funct Ecol. 2018; 32: 1531–1540. https://doi.org/10.1111/1365-2435.13094

Garcia-Porta J, Sol D, Pennell M, Sayol F, Kaliontzopolou A, Botero CA (2022) Niche expansion and adaptive divergence in the global radiation of crows and ravens. Nature Communications 13, 2086. https://doi.org/10.1038/s41467-022-29707-5

GBIF.org (26th July 2019). GBIF occurrence download for preserved specimens of *Montifringilla nivalis* (Linnaeus, 1766). doi: https://doi.org/10.15468/dl.vov81x

Gebauer, A., Eck, S., Kaiser, M., Lei, FM, & Martens, J. (2006). The Qinghai-Tibet Plateau : center of evolution for snow sparrows (*Montifringilla* s. str.) and mountain-steppe sparrows (*Onychostruthus*, *Pyrgilauda*). Acta Zoologica Sinica, 52, 305–309.

Gill, F., Donsker, D., & Rasmussen, P. (Eds)(2024). IOC World Bird List (v14.1).

Guo Q, Qian H, Liu P, Zhang J (2024) Bergmann's rule in global terrestrial vertebrates. Biological Journal of the Linnean Society 143(2), blae092. https://doi.org/10.1093/biolinnean/blae092

Harfoot MB, Johnston A, Balmford A, Burgess ND, Butchart SHM, Dias MP, Hazin C, Hilton-Taylor C, Hoffmann M, Isaac NJB, Iversen LL, Outwaithe CL, Visconti P & Geldmann J (2021) Using the IUCN Red List to map threats to terrestrial vertebrates at global scale. Nature Ecology and Evolution 5, 1510-1519. https://doi.org/10.1038/s41559-021-01542-9

He J, Tu J, Yu J, Jiang H (2023) A global assessment of Bergmann's rule in mammals and birds. Global Change Biology 29(18), 5199-5210. https://doi.org/10.1111/gcb.16860

Heled J (2008) Extended Bayesian Skyline Plot tutorial for BEAST 2. Available under: https://www.beast2.org/tutorials/; last accessed on 09.09.2024.

Heled J, Drummond AJ (2008) Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology, 8, 289. https://doi.org/10.1186/1471-2148-8-289

Hasumi H, Emori S (2004) (eds). K-1 coupled GCM (MIROC) description. K-1 Technical Report No. 1. Center for Climate System Research, University of Tokyo, Japan.

Hölzinger J (2011) Schneesperling *Montifringilla nivalis* – Brutvogel auf der Insel Kreta (Griechenland). Monticola 104, 51-61.

Hourlay F, Libois R, D'Amico F, Sarà M, O'Halloran J, Michaux JR. Evidence of a highly complex phylogeographic structure on a specialist river bird species, the dipper (*Cinclus cinclus*). Mol Phylogenet. Evol., 49, 435–444. https://doi.org/10.1016/j.ympev.2008.07.025

Hu J, Jiang Z, Chen J, Qiao H (2015) Niche divergence accelerates evolution in Asian endemic *Procapra* gazelles. Sci Rep 5, 10069. https://www.nature.com/articles/srep10069

Hundsdörfer, A.K., Päckert, M., Kehlmaier, C., Strutzenberger, P. & Kitching, I.J. (2017) Museum archives revisited: Central Asiatic hawkmoths reveal exceptionally high late Pliocene species diversification (Lepidoptera, Sphingidae). Zoologica Scripta 46, 552-570. https://doi.org/10.1111/zsc.12235

Hunt, M., Kikuchi, T., Sanders, M., Newbold, C., Berriman, M., and Otto, T. D. (2013). REAPR: a universal tool for genome assembly evaluation. Genome Biol. 14, R47. https://doi.org/10.1186/gb-2013-14-5-r47

Iacolina L, Buzan E, Safner T, Bašić N, Geric U, Tesija T, Lazar P, Arnal MC, Chen J, Han J, Šprem N. (2021) A Mother's Story, Mitogenome Relationships in the Genus *Rupicapra*. Animals 2021, 11(4), 1065; https://doi.org/10.3390/ani11041065

Islam S., Peart, C., Kehlmaier, C., Sun, Y.H., Lei, F., Dahl, A., Klemroth, S., Alexopoulou, D., Delgado, M., Laiolo, P., Illera, J.C., Dirren, S., Hille, S., Lkhagvasuren, D., Töpfer, T., Kaiser, M., Gebauer, A., Martens, J., Paetzold, C., Päckert, M. (2024) Museomics help resolving the phylogeny of snowfinches (Aves, Passeridae, *Montifringilla* and allies). Molecular Phylogenetics and Evolution, 198, 108135. https://doi.org/10.1016/j.ympev.2024.108135

Jackman, S. D., Vandervalk, B. P., Mohamadi, H., Chu, J., Yeo, S., Hammond, S. A., et al. (2017). ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. *Genome Res.* 27, 768–777. http://www.genome.org/cgi/doi/10.1101/gr.214346.116.

Kleckova I, Cesanek M, Fric Z, Pellisier L (2015) Diversification of the cold-adapted butterfly genus *Oeneis* related to Holarctic biogeography and climatic niche shifts. Mol Phylogenet Ecol 92, 255-265. https://doi.org/10.1016/j.ympev.2015.06.012

Koskenpato, K., Lehikoinen, A., Morosinotto, C., Gunko, R., & Karell, P. (2023). Regional variation in climate change alters the range-wide distribution of colour polymorphism in a wild bird. Ecology and Evolution, 13, e10311. https://doi.org/10.1002/ece3.10311

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lee CC, Fu Y, Yeh CF, Yeung CKL, Hung HY, Yao CJ, Shaner PJL, Li SH (2021) Morphological variations in a widely distributed Eastern Asian passerine cannot be consistently explained by ecogeographic rules. Ecology and Evolution 11(21), 15249-15260. https://doi.org/10.1002/ece3.8208

Lei, F., Qu, Y., & Song, G. (2014). Species diversification and phylogeographical patterns of birds in response to the uplift of the Qinghai-Tibet Plateau and Quaternary glaciations. Current Zoology 60(2), 149–161. https://doi.org/10.1093/czoolo/60.2.149

Leigh JW & Byrant D (2015) popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9), 1110-1116. https://doi.org/10.1111/2041-210X.12410

Lobo, J. M., Jiménez-Valverde, A. & Real, R. (2008) AUC: a misleading measure of the performance of predictive distribution models. Glob. Ecol. Biogeogr. **17**, 145–151. https://doi.org/10.1111/j.1466-8238.2007.00358.x

Ling, C. X., Huang, J. & Zhang, H. (2003) AUC: A Better Measure than Accuracy in Comparing Learning Algorithms BT - Advances in Artificial Intelligence. in (eds. Xiang, Y. & Chaib-draa, B.) 329–341. Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-44886-1_25

Liu, Y., Hu, J., Li, S. H., Duchen, P., Wegmann, D., & Schweizer, M. (2016). Sino-Himalayan mountains act as cradles of diversity and immigration centres in the diversification of parrotbills (Paradoxornithidae). Journal of Biogeography, 43, 1488–1501. https://doi.org/10.1111/jbi.12738

Lin X, Shih C, Hou Y, Shu X, Zhang M, Hu J, Jiang J & Xie F (2021) Climatic-niche evolution with key morphological innovations across clades within *Scutiger boulengeri* (Anura: Megophryidae). Ecology and Evolution, 11:10353–10368. https://doi.org/10.1002/ece3.7838

Luo et al. (2022) Sexual dichromatism and assortative mating by multiple plumage color traits in wild Chestnut Thrush. Avian Research. https://doi.org/10.1016/j.avrs.2022.100033.

Marcondes RS, Nations JA, Seeholzer GF, Brumfield RT. (2021). Rethinking Gloger's Rule: Climate, Light Environments, and Color in a Large Family of Tropical Birds (Furnariidae). The American Naturalist.197(5):592-606. https://pubmed.ncbi.nlm.nih.gov/33908827/

Marchese, C. (2015) Biodiversity hotspots: A shortcut for a more complicated concept. Glob. Ecol. Conserv. 3: 297–309. https://doi.org/10.1016/j.gecco.2014.12.008

McCormack, J.E., Hird, S.M., Zellmer, A.,J., Carstens, B.C. & Brumfield, R.T. (2013) Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. 66,625-638. https://doi.org/10.1016/j.ympev.2011.12.007

Méndez, MA, Christian Hödar, Chris Vulpe, Mauricio González, Verónica Cambiazo (2002). Discriminant analysis to evaluate clustering of gene expression data, FEBS Letters, 522, 24-28. https://doi.org/10.1016/S0014-5793(02)02873-9

Mila B, Surget-Groba Y, Heulin B, Gosá A, Fitze PS (2013) Multilocus phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture zone reveals lowland barriers and high-elevation introgression. BMC Evolutionary Biology, 13:192. https://doi.org/10.1186/1471-2148-13-192

Miller EF, Green RE, Balmford A, Delser PM, Beyer R, Somveille M, Leonardi M, Amos W, Manica A. (2021). Bayesian Skyline Plots disagree with range size changes based on Species Distribution Models for Holarctic birds. Molecular Ecology 30(16), 3993-4004. https://doi.org/10.1111/mec.16032

Mingozzi T, Storino P, Venuto G, Massolo A, Tavecchia G (2022) Climate warming induced a stretch of the breeding season and an increase of second clutches in a passerine breeding at its altitudinal limits. Current Zoology, 68(1), 9–17. https://doi.org/10.1093/cz/zoab029

Mosbrugger, V., Favre, A., Muellner-Riehl, A.N., Päckert, M. & Mulch, A. 2018: Cenozoic Evolution of Geobiodiversity in the Tibeto-Himalayan Region. In: Hoorn, C., Perrigo, A. & Antonelli, A. (eds) Mountains, Climate and Biodiversity: A comprehensive and up-to-date synthesis for students and researchers. Wiley Blackwell, 429-448.

Mutke J, Sommer JH, Kreft H & Barthlott W (2011) Vascular Plant Diversity in a Changing World: Global Centres and Biome-Specific Patterns. In: Habel JC & Zachos F (eds) Biodiversity Hotspots – Evolution and Conservation. Publisher: SpringerEditors. https://doi.org/10.1007/978-3-642-20992-5_5

O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. Molecular Ecology, 27, 3193–3206. https://doi.org/10.1111/mec.14792

Otto-Bliesner, B.L., Brady, E.C., Clauzet, G., Tomas, R., Levis, S., Kothavala, Z. (2006) Last glacial maximum and Holocene climate in CCSM3. J. Clim. 2526–2544. https://doi.org/10.1175/JCLI3748.1

Päckert, M., Martens, J. Sun, Y.-H. & Strutzenberger, P. 2016: The phylogenetic relationships of Przevalski's Finch *Urocynchramus pylzowi*, the most ancient Tibetan endemic passerine known to date. IBIS, 158, 530-540. https://doi.org/10.1111/ibi.12382

Päckert, M., Favre, A., Schnitzler, J., Martens, J., Sun, Y.H., Tietze, D.T., Hailer, F., Michalak, I. & Strutzenberger, P. 2020: "Into and Out of" the Qinghai-Tibet Plateau and the Himalayas: Centers of origin and diversification across five clades of Eurasian montane and alpine passerine birds. Ecology and Evolution, 10(17), 9283–9300. https://doi.org/10.1002/ece3.6615

Päckert, M., Hering, J., Belkacem, A.A., Sun, Y.H., Hille, S., Lkhagvasuren, D., Islam, S. & Martens, J. 2021: A revised multilocus phylogeny of Old World sparrows (Aves: Passeridae). Vertebrate Zoology, 71, 353–366. https://doi.org/10.3897/vz.71.e65952

Perez, T, González I, Essler SE, Fernández M, Domínguez A (2014) The shared mitochondrial genome of *Rupicapra pyrenaica ornata* and *Rupicapra rupicapra cartusiana*: Old remains of a common past. MPE 79, 375-379. https://doi.org/10.1016/j.ympev.2014.07.004

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One. 2012;7(5). https://doi.org/10.1371/journal.pone.0037135

Peterson AT, Navarro-Sigüenza AG, Gordillo A (2018) Assumption-versus data-based approaches to summarizing species' ranges. Conservation Biology 32(3), 568-575. https://doi.org/10.1111/cobi.12801

Phillips, S. J., Anderson, R. P., Dudík, M., Schapire, R. E. & Blair, M. E. (2017) Opening the black box: an open-source release of Maxent. *Ecography (Cop.).* 40, 887–893. https://doi.org/10.1111/ecog.03049

Phillips, S. J., Anderson, R. P. & Schapire, R. E. (2006) Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Portenko LA & Vietinghoff-Scheel (1974) *Montifringilla nivalis* (L.). In: Stresemann E, Portenko LA, Dathe H & Mauersberger G (Eds) Atlas der Verbreitung palaearktischer Vögel. 4. Lieferung. Akademie der Wissenschaften der DDR. Akademie-Verlag, Berlin.

Price, T.D., Hooper, D.M., Buchanan, C.D. et al. (2014) Niche filling slows the diversification of Himalayan songbirds. Nature 509, 222–225. https://doi.org/10.1038/nature13272

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics **155**, 945–959. https://doi.org/10.1093/genetics/155.2.945

Qu Y, Lei F, Zhang R, Lu X. (2010) Comparative phylogeography of five avian species: implications for Pleistocene evolutionary history in the Qinghai-Tibetan plateau. Mol Ecol. 19(2), 338-51. https://doi.org/10.1111/j.1365-294X.2009.04445.x

Qu, Y., Chen, C., Chen, X., Hao, Y., She, H., Wang, M., Ericson, P. G. P., Lin, H., Cai, T., Rosenberg, N. A.Song, G., Jia, C., Chen, C., Zhang, H., Li, J., Liang, L., Wu, T., Zhao, J., Gao, Q., Zhang, G., … Lei, F. (2021). The evolution of ancestral and species-specific adaptations in snowfinches at the Qinghai–Tibet Plateau. Proceedings of the National Academy of Sciences, 118(13). https://doi.org/10.1073/pnas.2012398118

Rahbeck, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N., Nogues-Bravo, D., Whittaker, R.J. & Fjeldså, J. (2019b) Humboldt's enigma: What causes global patterns of mountain biodiversity?. Science 365, 1108–1113 (2019b). https://www.science.org/doi/10.1126/science.aax0149

Ramesh, V., Gopalakrishna, T., Barve, S., & Melnick, D. J. (2017). IUCN greatly underestimates threat levels of endemic birds in the Western Ghats. Biological Conservation, 210, 205–221. https://doi.org/10.1016/j.biocon.2017.03.019

Recuero E, García-París M (2011) Evolutionary history of *Lissotriton helveticus*: Multilocus assessment of ancestral vs. recent colonization of the Iberian Peninsula. Mol Phylogenet Evol 60(1), 170-182). https://doi.org/10.1016/j.ympev.2011.04.006

Resano-Mayor, J. *et al.* (2017) Integrating genetic and stable isotope analyses to infer the population structure of the White-winged Snowfinch *Montifringilla nivalis* in Western Europe. J. Ornithol. ***158***, 395–405. https://doi.org/10.1007/s10336-016-1413-8

Robinson JA, Rauri C.K. Bowie, Olga Dudchenko, Erez Lieberman Aiden, Sher L. Hendrickson, Cynthia C. Steiner, Oliver A. Ryder, David P. Mindell, Jeffrey D. Wall, (2021) Genome-wide diversity in the California condor tracks its prehistoric abundance and decline. Current Biology 31(13), Pages 2939-2946. https://doi.org/10.1016/j.cub.2021.04.035

Robles-Bello SM, Vázquez-López M, Ramírez-Barrera SM, Terrones-Ramírez AK, Hernández-Baños BE (2022) Drivers of phenotypic divergence in a Mesoamerican highland bird. PeerJ 10: e12901. http://doi.org/10.7717/peerj.12901

Rocchini D, Hortal J, Lengyel S, Lobo JM, Jimenéz-Valverde A, Ricotta C, Bacaro G & Chiarucci A (2011) Accounting for uncertainty when mapping species distributions: The need for maps of ignorance. Progress in Physical Geography 35(2): 211-226. https://doi.org/10.1177/03091333113994

Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes from RAD-seq short-read data using Stacks. Nature Protocols, 12(12). https://doi.org/10.1038/nprot.2017.123

Rodríguez F, Hammer S, Pérez T, Suchentrunk F, Lorenzini R, Michallet J, Martinkova N, Albornoz J, Domínguez A. (2009) Cytochrome b Phylogeography of Chamois (*Rupicapra* spp.). Population Contractions, Expansions and Hybridizations Governed the Diversification of the Genus. Journal of Heredity, 100(1), 47–55. https://doi.org/10.1093/jhered/esn074

Romano, A., R. Séchaud, A. H. Hirzel, and A. Roulin. 2019. "Climate-Driven Convergent Evolution of Plumage Colour in a Cosmopolitan Bird." *Global Ecology and Biogeography* **28**, no. 4: 496–507. https://doi.org/10.1111/geb.12870.

Rosenberg, N. A.(2004) distruct: a program for the graphical display of population structure. *Mol. Ecol. Notes* **4**, 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Santini L, Butchart SHM, Rondinini C, Benítez-López A, Hilbers JP, Schipper AM, Cengic M, Tobias JA, Huijbregts MAJ (2019) Applying habitat and population-density models to land-cover time series to inform IUCN Red List assessments. Conservation Biology 33(5), 1084-1093. https://doi.org/10.1111/cobi.13279

Schmitt, T. Biogeographical and evolutionary importance of the European high mountain systems. *Front Zool* **6**, 9 (2009). https://doi.org/10.1186/1742-9994-6-9

Scridel, D., Brambilla, M., Martin, K., Lehikoinen, A., Iemma, A., Matteo, A., Jähnig, S., Caprio, E., Bogliani, G., Pedrini, P., Rolando, A., Arlettaz, R. and Chamberlain, D. (2018), A review and meta-analysis of the effects of climate change on Holarctic mountain and upland bird populations. Ibis, 160: 489-515. https://doi.org/10.1111/ibi.12585

She H, Jiang Z, Song G, Ericson PGP, Luo X, Shao S, Lei F, Qu Y (2021) Quantifying adaptive divergence of the snowfinches in a common landscape. Diversity and Distributions 28(12), 2579-2592. https://doi.org/10.1111/ddi.13383

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351

Smith SE, Gregory RD, Anderson BJ, Thomas CD (2013) The past, present and potential future distributions of cold-adapted bird species. Diversity and Distributions 19(3), 352-362. https://doi.org/10.1111/ddi.12025

Song et al. (2021) Demographic history and divergence of sibling grouse species inferred from whole genome sequencing reveal past effects of climate change. BMC Ecology and Evolution, 21, 194. https://doi.org/10.1186/s12862-021-01921-7

Streicher M, Krüger S, Loercher F & Willows-Munroe S (2021) Evidence of genetic structure in the wide-ranging bearded vulture (*Gypaetus barbatus* (Linnaeus, 1758)). BMC Ecol Evol 21, 42. https://doi.org/10.1186/s12862-021-01760-6

Strinella E, Catoni C, De Faveri A, & Artese C (2011): Biometrics, sexing and moulting of Snow Finch *Montifringilla nivalis* in Central Italy, Ringing & Migration, 26:1, 1-8. https://doi.org/10.1080/00063657.2011.581864

Summers-Smith, D. and A. Bonan (2020). White-winged Snowfinch (*Montifringilla nivalis*), version 1.0. In Birds of the World (J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, and E. de Juana, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.whwsno1.01

Summers-Smith, J. D. (2009). Family Passeridae (Old-World Sparrows). In: del Hoyo, J., Elliott, A., & Christie, D. (Eds), Handbook of the Birds of the World, Vol. 14 – Bush-shrikes to Old World Sparrows. Lynx Edicions, Barcelona.

Tang R, Wang J, Li Y, Zhou C, Meng G, Li F, Lan Y, Price M, Podsiadlowski L, Yu Y, Wang X, Liu Y, Yue B, Liu S, Fan Z, Liu S. (2022). Genomics and morphometrics reveal the adaptive evolution of pikas. Zoological Research, 43(5)813−826. https://doi.org/10.24272/j.issn.2095-8137.2022.072

Todisco T, Gratton P, Cesaroni D, Sbordoni V (2010) Phylogeography of *Parnassius apollo*: hints on taxonomy and conservation of a vulnerable glacial butterfly invader. BJLS 101(1), 169-183. https://doi.org/10.1111/j.1095-8312.2010.01476.x

Trucchi E, Gratton P, Whittington JD, Cristofari R, Le Maho Y, Stenseth NC & Le Bohec C (2014) King penguin demography since the last glaciation inferred from genome-wide data. Proceedings of the Royal Society B 281: 20140528. http://dx.doi.org/10.1098/rspb.2014.0528

Tyrberg, T. (1991) Arctic, montane and steppe birds as glacial relicts in the West Palearctic. Orn. Verh. 25, 29-49.

Valiente-Mullor, C., Beamud, B., Ansari, I., Francés-Cuesta, C., García-González, N., Mejía, L., Ruiz-Hueso, P., & González-Candelas, F. (2021). One is not enough: On the effects of reference genome for the mapping and subsequent analyses of short-reads. PLoS Computational Biology, 17(1). https://doi.org/10.1371/journal.pcbi.1008678

Vaurie C (1956) Systematic notes on Palearctic Birds No. 24 Ploceidae: the Genera *Passer*, *Petronia* and *Montifringilla*. American Museum Novitates 1814, 1-27.

Wallis GP, Waters JM, Upton P & Craw D (2016).Transverse alpine speciation driven by glaciation. Trends Ecol Evol 31(12), 916-926. https://doi.org/10.1016/j.tree.2016.08.009

Wang XY, Liang D, Jin W, Tang MK, Shalayiwu, Liu SY & Zhang P. (2020) Out of Tibet: Genomic Perspectives on the Evolutionary History of Extant Pikas. Mol. Biol. Evol. 37(6):1577–1592. https://doi.org/10.1093/molbev/msaa026

Weir JT, Schluter D (2008) Calibrating the avian molecular clock. Molecular Ecology 17: 2321–2328. https://doi.org/10.1111/j.1365-294X.2008.03742.x

Wiedenfeld DA, Tognelli MF (2023) Relationship between age and method of population estimation and the impact of changes in range-size maps on the IUCN Red List assessments of birds. Bird Conservation International 33:e78. https://doi.org/10.1017/S0959270923000291

Zhao et al. (2022) Phylogeny and Biogeographic History of *Parnassius* Butterflies (Papilionidae: Parnassiinae) Reveal Their Origin and Deep Diversification in West China. Insects, 13(5), 406. https://doi.org/10.3390/insects13050406

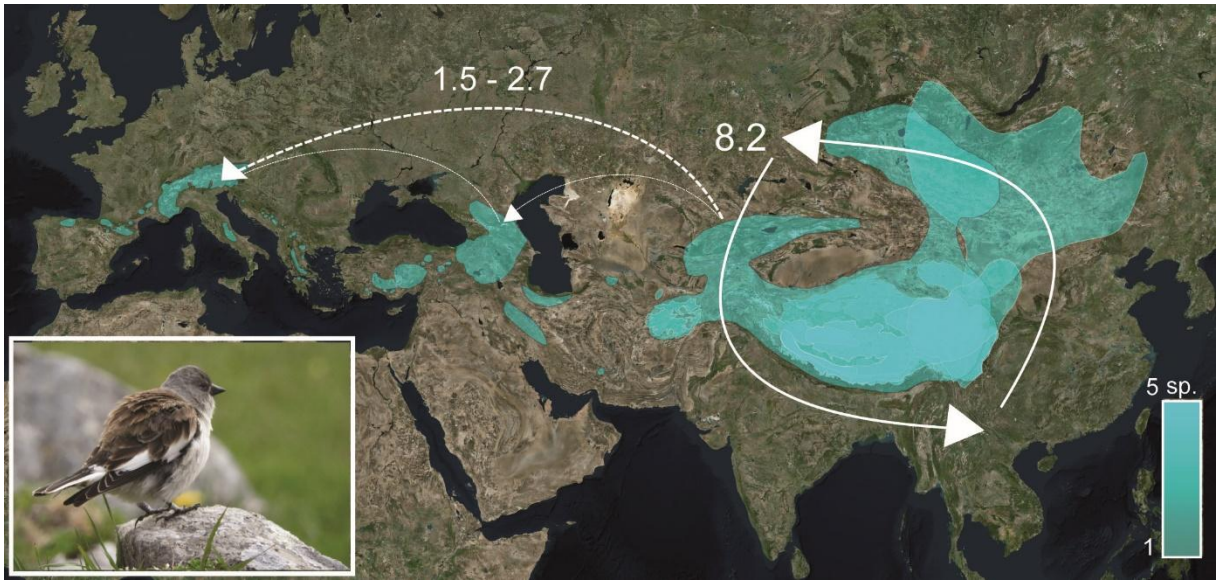

Fig. 1: Late Miocene *in-situ* speciation on the QTP followed by Pleistocene out-of-Tibet colonization events in two clades of alpine passerine birds; numbers indicate age estimates inferred for the onset of in-situ diversification and dispersal events (Päckert et al. 2020); A) mountain finches (*Leucosticte*): transcontinental colonization of the Nearctic from a Tibetan center of origin (three sympatric mountain finch species) to North America, followed by diversification of three extant rosy finch species; B) snowfinches: transpalearctic dispersal of ancestral *Montifringilla nivalis* from a Tibetan area of origin (six sympatric species) to the Caucasus and the Western Palearctic; photo: *M. nivalis* (D.L.); (shape files from BirdLife International 2024).

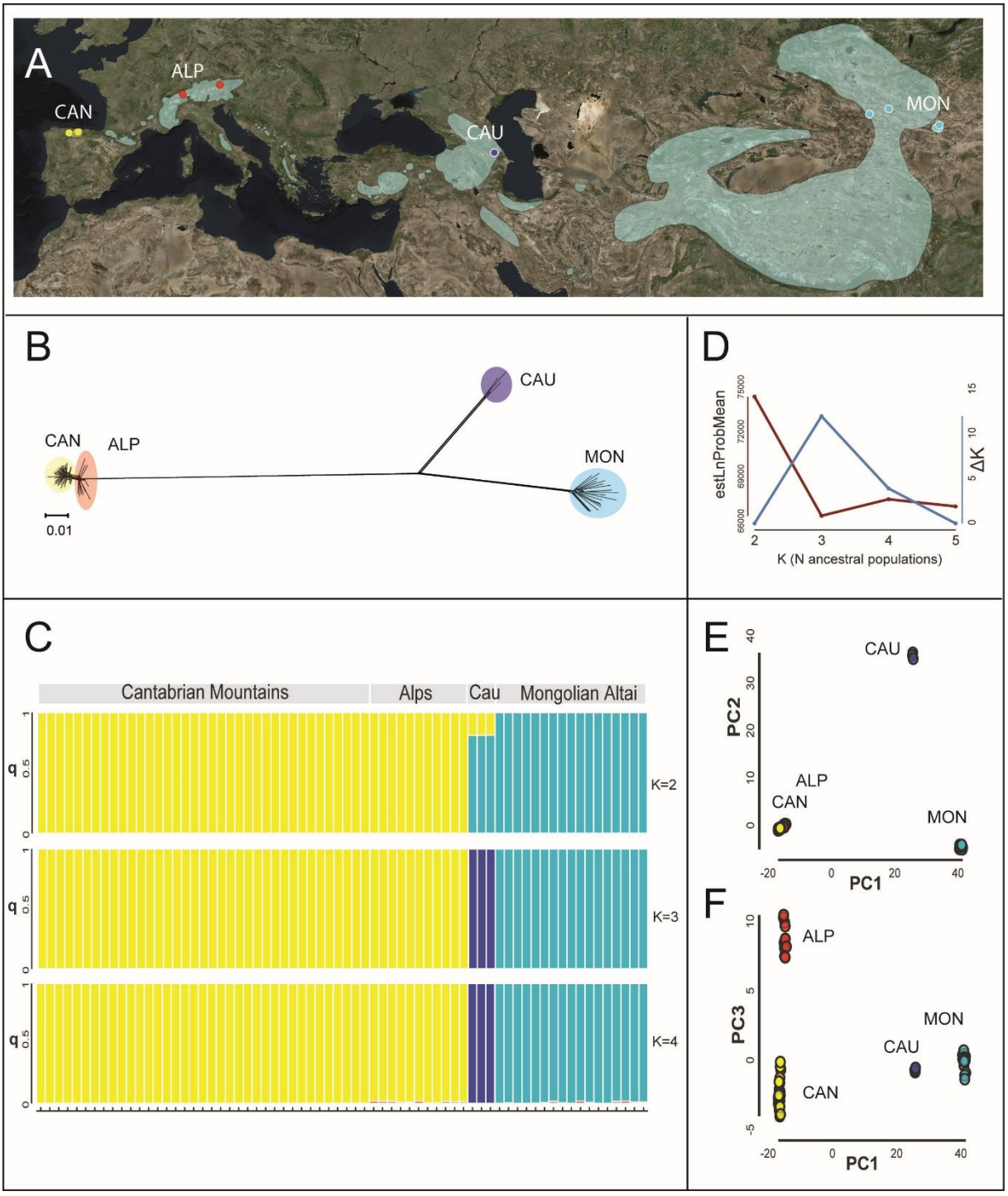

Fig. 2: Intraspecific genetic differentiation among A) European and Asian study populations of *M. nivalis* (collection sites shown as colored dots¸ distribution shape file according to BirdLife International 2014) based on biallelic unlinked genome-wide SNPs (SNP matrix sizes: unfiltered= 7630 bp; after filtering= 3949 bp); B) neighbor-net network reconstructed with SplitsTree; C) Structure plots for K=2, K=3 and K=4; D) identification of the optimal number of genetic clusters according to estLnProbMean and ΔK; E, F) scatterplots of PC1 (explaining 64.9% of the total variation) *vs* PC2 and PC3 (explaining 6.4% and 1.7% of the total variation).

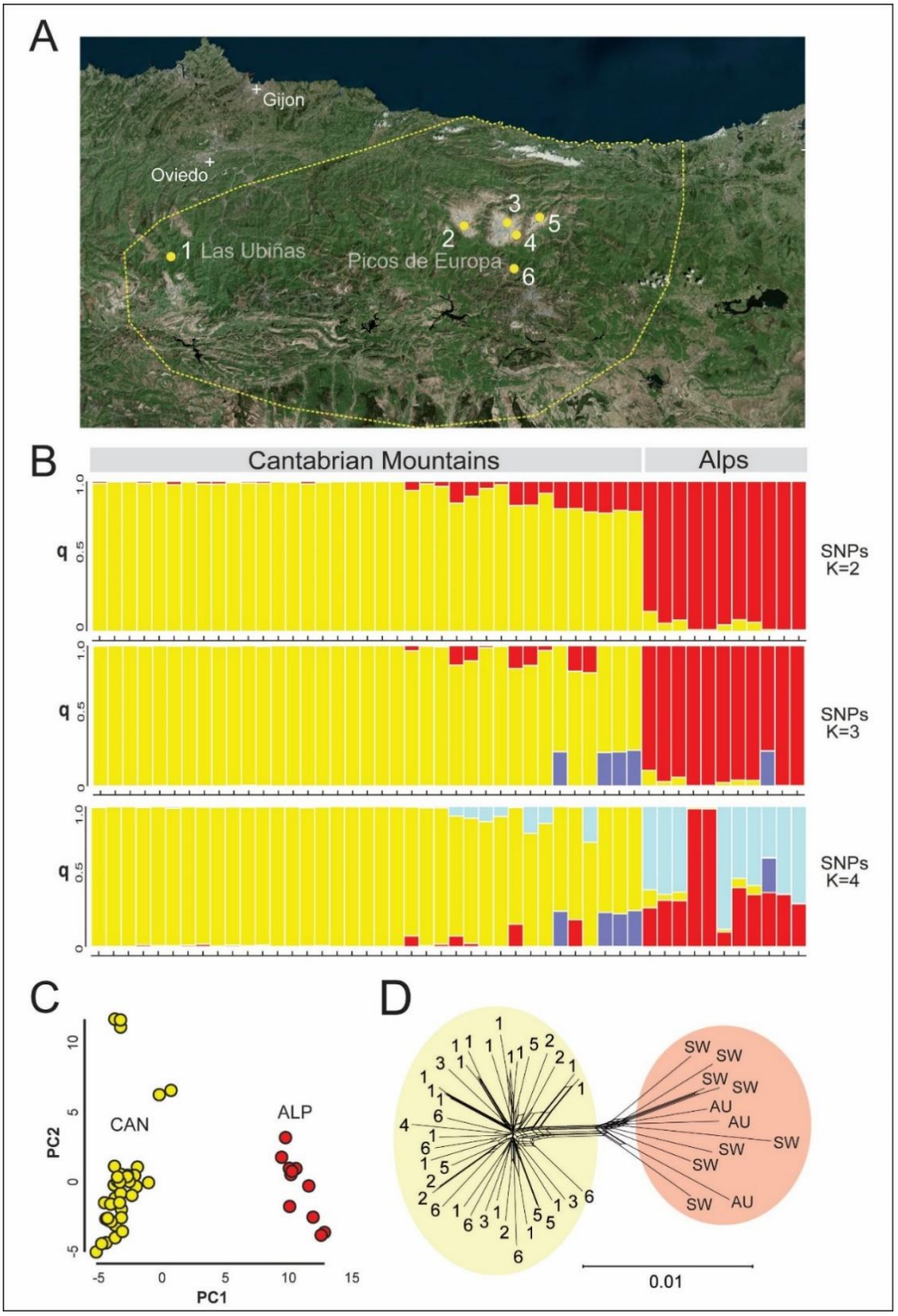

Fig. 3: Genetic differentiation among European populations of *M. nivalis* from the Alps and the Cantabrian Mountains (the latter shown on the map A) based on biallelic unlinked genome-wide SNPs (SNP matrix sizes: unfiltered= 2258 bp; after filtering= 1690 bp); B) Structure plots for K=2, 3 and 4; C) scatterplot of PC1 vs PC3 (explaining 10.5% and 4.1% of the total variation) from principal component analysis (PCA); D) neighbor-net network reconstructed with SplitsTree with populations indicated at tips for the Alps (red cluster): AU= Austria, SW= Switzerland; for the Cantabrian Mountains (yellow cluster) according to numbers shown on map A.

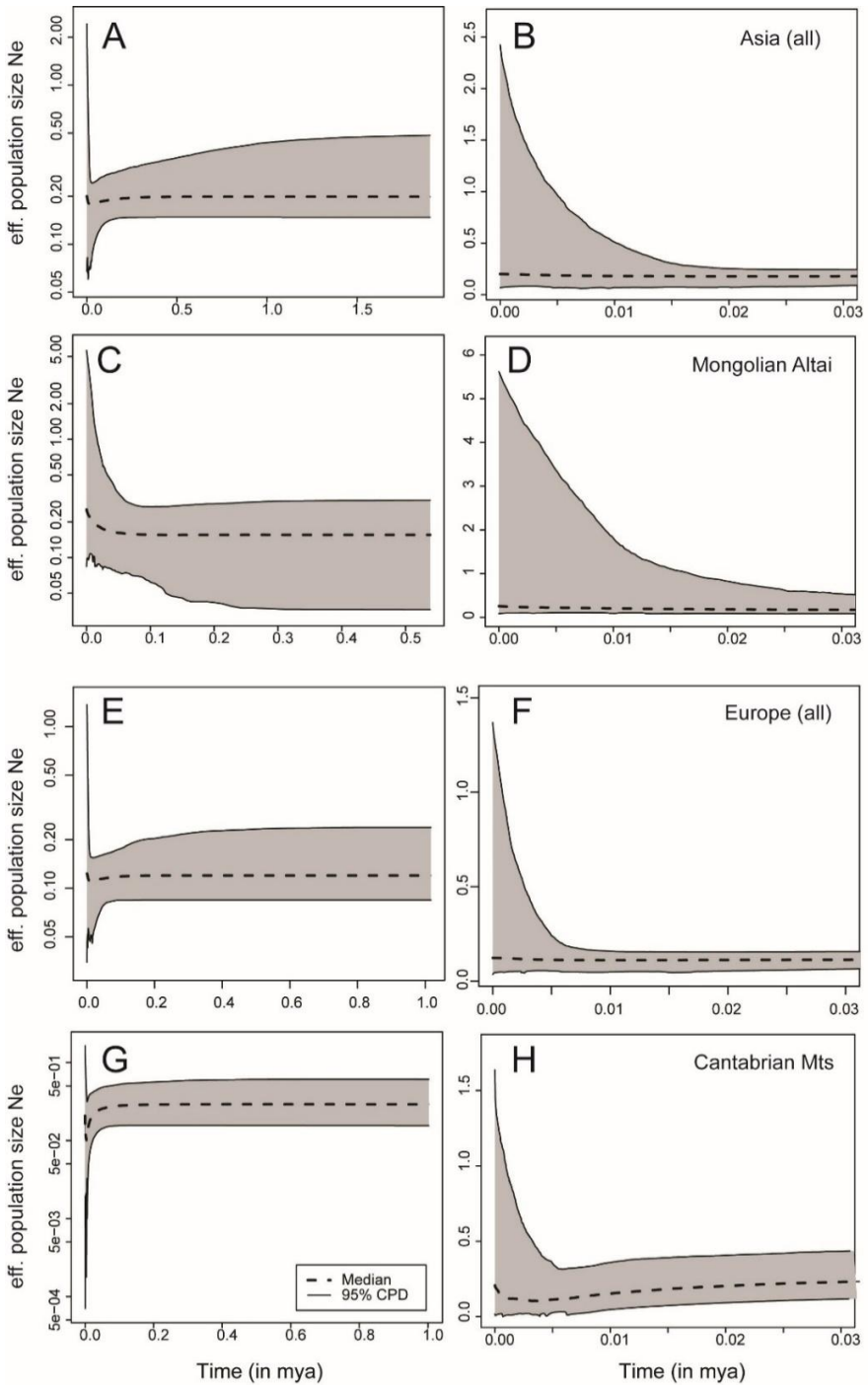

Fig. 4: Extended Bayesian Skyline Plots (EBSP) inferred from multilocus data including the mitochondrial cytochrome-*b* (fixed rate, r= 0.0105 substitutions/site/lineage/myr) and RAD seq loci with n > 3 SNPs (fixed rate, r= 0.00995 substitutions/site/lineage/myr); MCMC chain= 500 million generations; A, B) all Asian populations (*M. n. alpicola* from the Caucasus and *M. n. groumgrzimaili* from the Mongolian Altai), n= 20, 28 RAD seq loci; C, D) only Mongolian populations, n= 17, 17 RAD seq loci; E, F) European populations (*M. n. nivalis* from the Swiss and Austrian Alps and from the Cantabrian Mountains), n= 48, 20 RAD seq loci; G, H) Cantabrian populations alone, n= 37, 12 RAD seq loci.

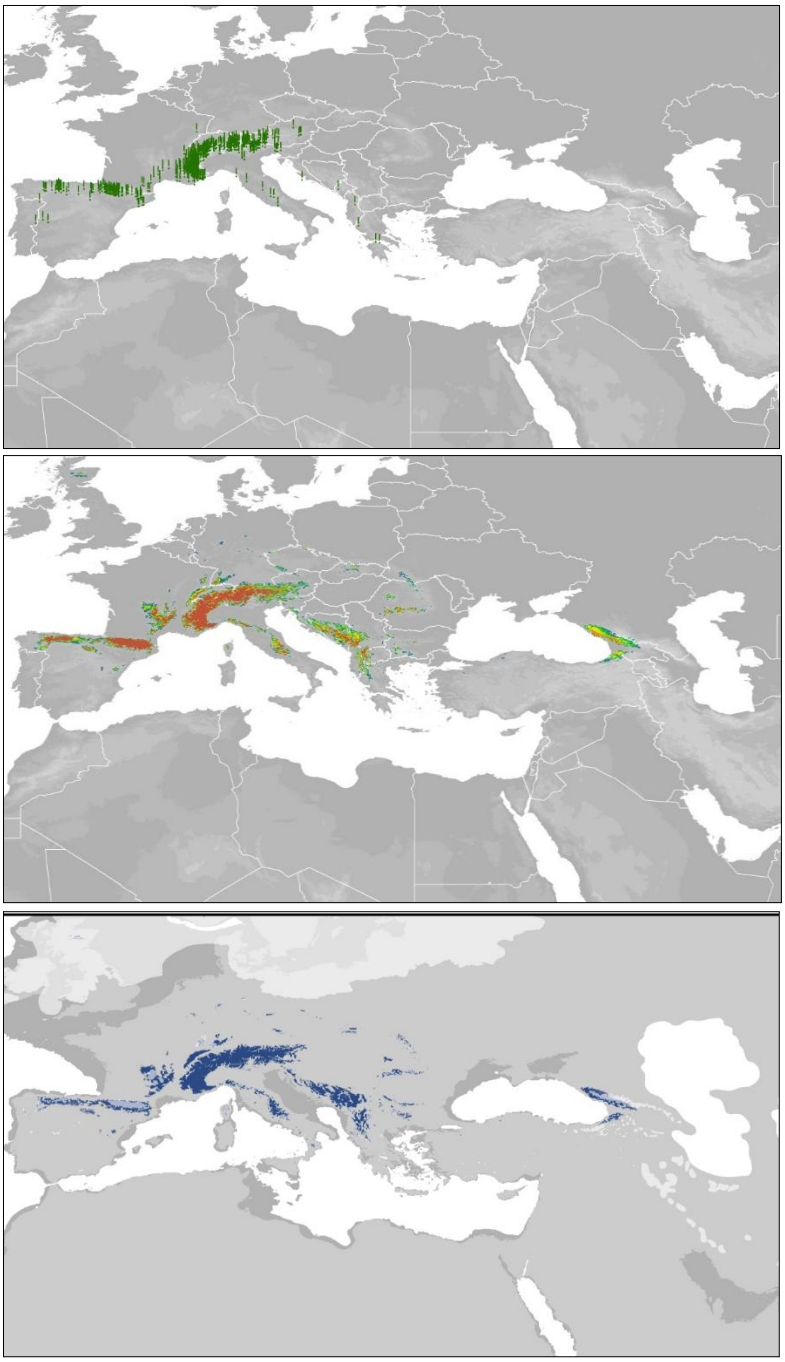

Fig. 5: Species distribution modeling for the European nominate subspecies of the White-winged Snowfinch (*Montifringilla n. nivalis*) (A) occurrence records used to build the model displayed as green dots (B); potential current distribution as derived from MaxEnt, suitability ranges from moderate (dark blue) to high (red); (C) projections onto climatic reconstructions of the Last Glacial Maximum;. Comparison between the three projected LGM models (MPI-ESM-P CCSM3, and MIROC), darker grey shows the LGM land surface while the light grey area represents the present land area, white shaded areas show the extent of ice sheets (https://crc806db.uni-koeln.de/layer/show/6).

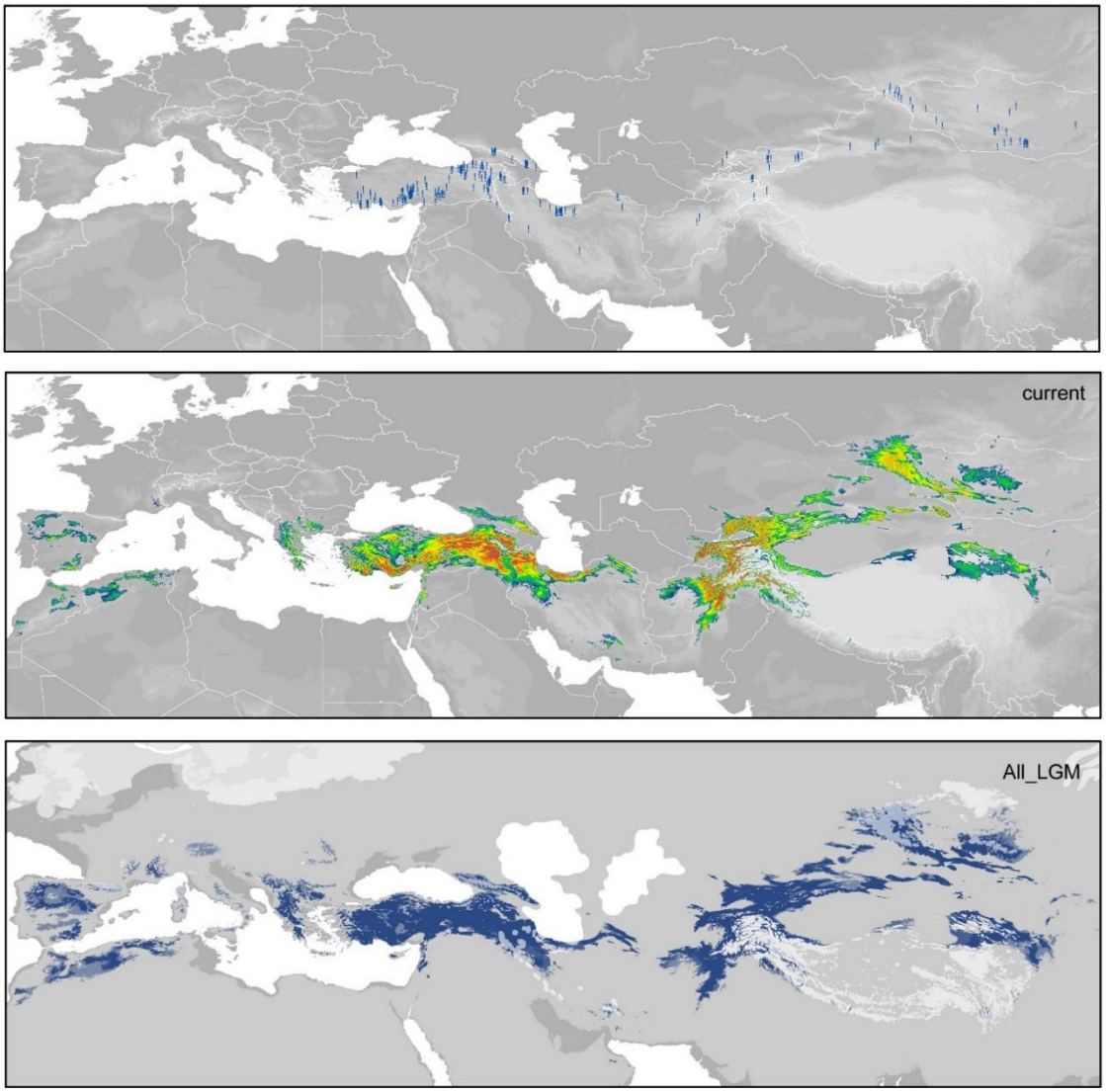

Fig. 6: Species distribution modeling for the Asian subgroup of the White-winged Snowfinch (*Montifringilla nivalis;* incl. ssp. *alpicola*, *gaddi*, *groumgrzimaili*, *leucura*, *tianshanica*) (A) occurrence records used to build the model displayed as blue dots (B) potential current distribution as derived from MaxEnt, suitability ranges from moderate (dark blue) to high (red); (C) projections onto climatic reconstructions of the Last Glacial Maximum;. Comparison between the three projected LGM models (MPI-ESM-P CCSM3, and MIROC), darker grey shows the LGM land surface while the light grey area represents the present land area, white shaded areas show the extent of ice sheets (https://crc806db.uni-koeln.de/layer/show/6).

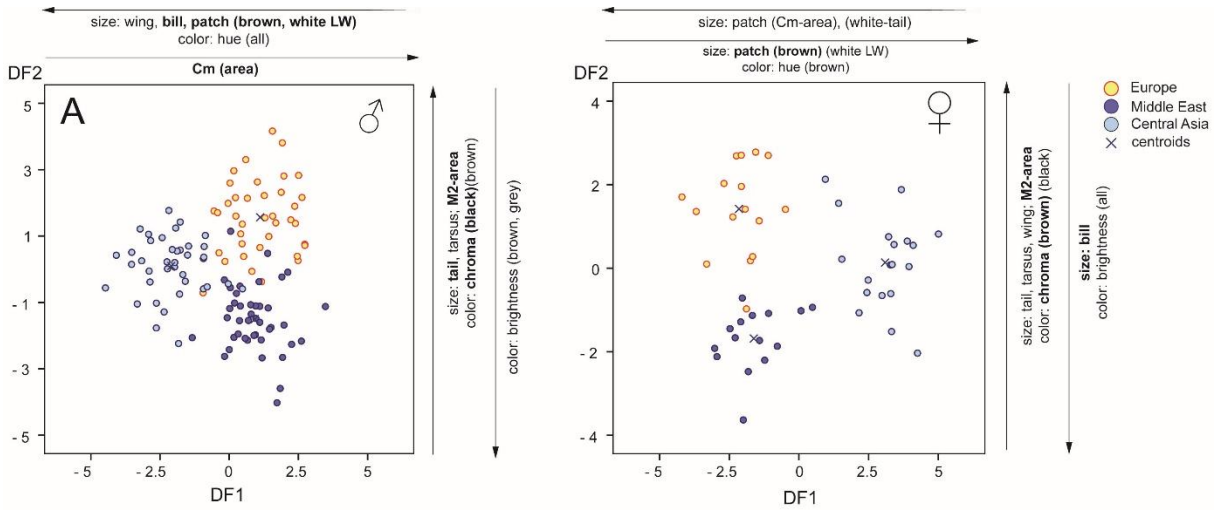

Fig. 7: Intraspecific morphological differentiation of the white-winged snowfinch, *M. nivalis*, linear discriminant analysis (LDA) based on 29 morphological traits; A) males (n= 107) and B) females (n= 59); scatterplot of the first two discriminant functions (DF) 1 and 2.

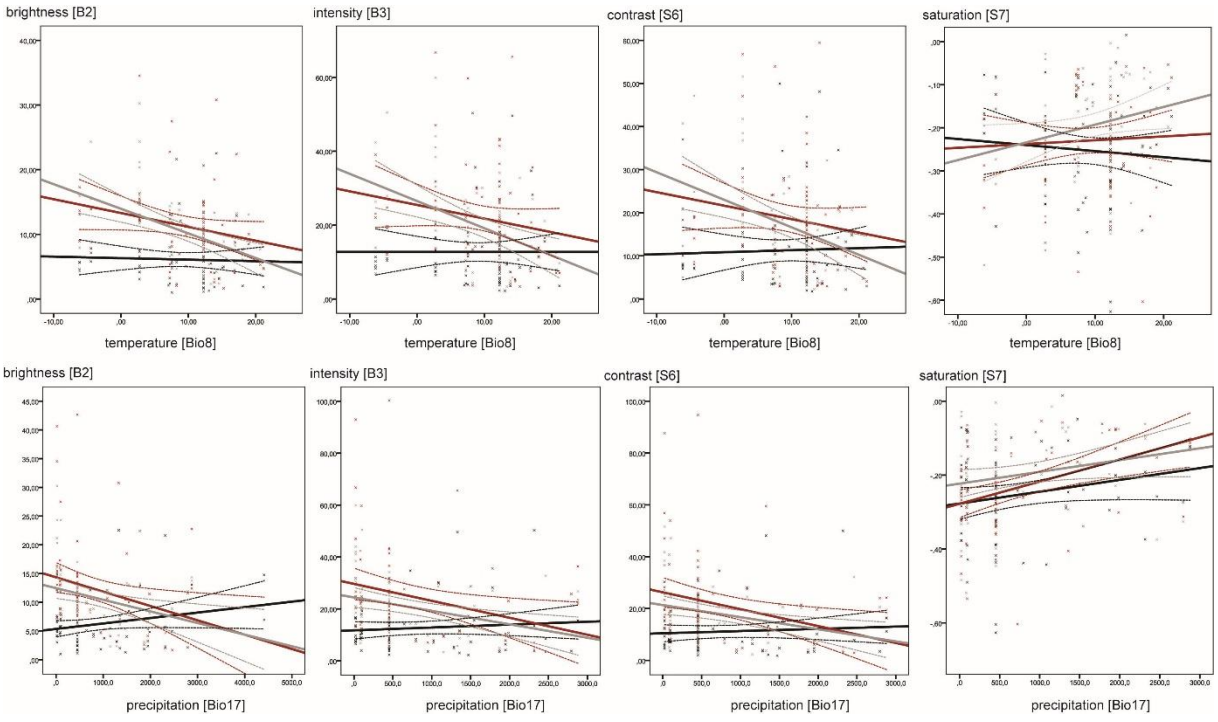

Fig. 8: Correlation between male plumage color variables brightness (B2), intensity (B3), contast (S6) and saturation (S7) for the three color patches with temperature of the wettest quarter [Bio8; above] and precipitation of the driest quarter [Bio17; below]; solid lines are linear regression lines, dotted lines show 95% confidence intervals; line colors refer to patch colors (brown, blackish, greyish).

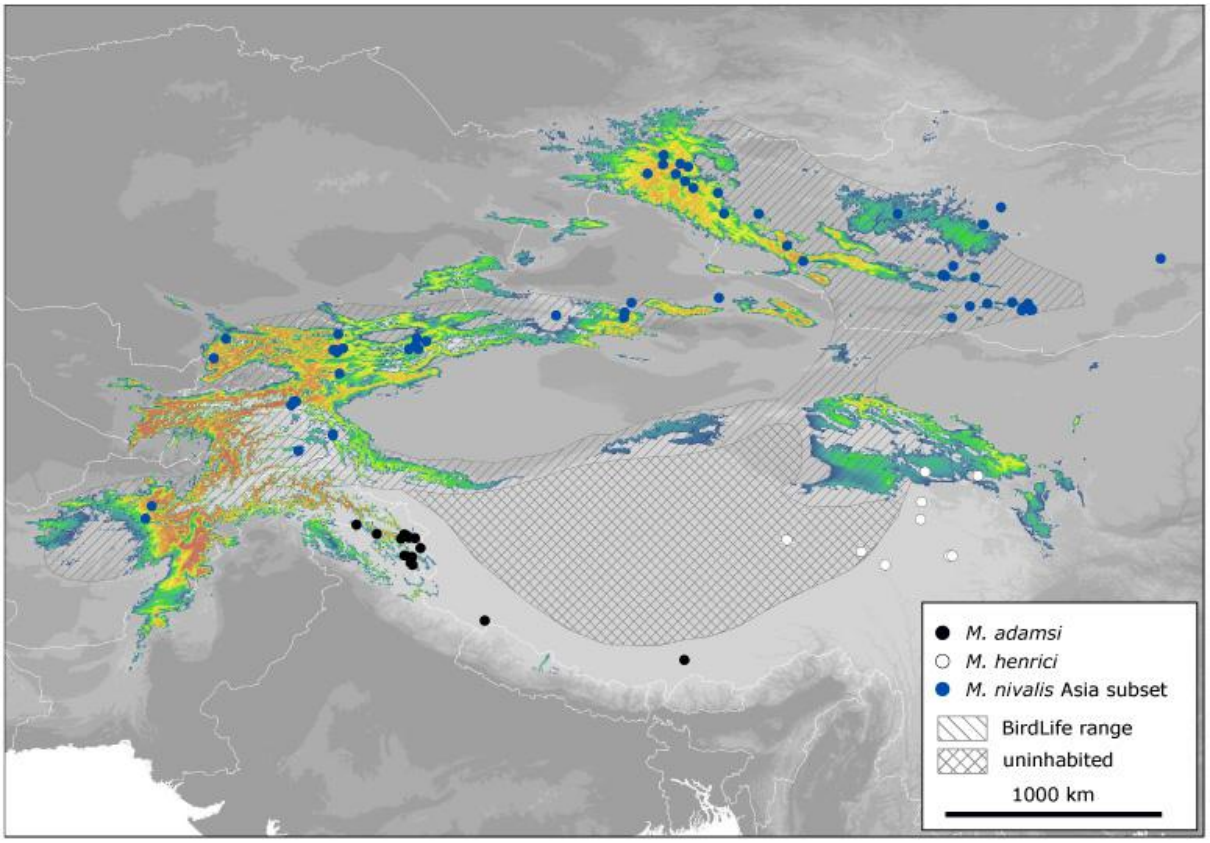

Fig. 9: Asian distribution range of *M. nivalis*; colored areas: SDMs, suitable habitat, current distribution, suitability ranges from moderate (dark blue) to high (red); dots indicate occurrence records (raw data set); blue occurrences were included in the input data set for SDMs, black and white dots were excluded from niche modeling analyses, because they refer to two different Asian snowfinch species (*M. adamsi*= black; *M. henrici* = white; both previously treated as subspecies of *M. nivalis*; taxonomic bias); shaded areas show the Asian distribution of *M. nivalis* according to BirdLife international (2024) including a broad area on the QTP without any reliable occurrence data for this species („uninhabited“).

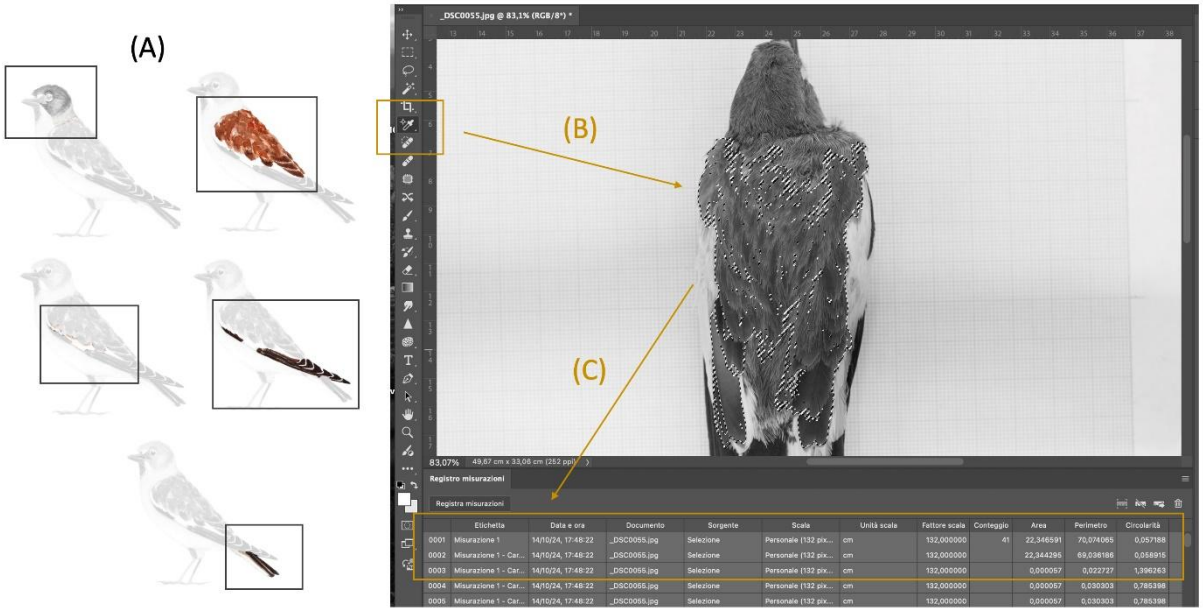

Figure S1.1: Consistent digital photographs of each bird (upper, lower, right, and left sides) were captured, based on which we measured the extent of the visible brown patches on the upper side of the body (including both body and wings), the brown patch on the tail, the grey on the head, and the white on the tail and wings (A). These measurements were executed using the Magic Wand and Measurement Log tools in Adobe Photoshop CC 2016 (B, C).

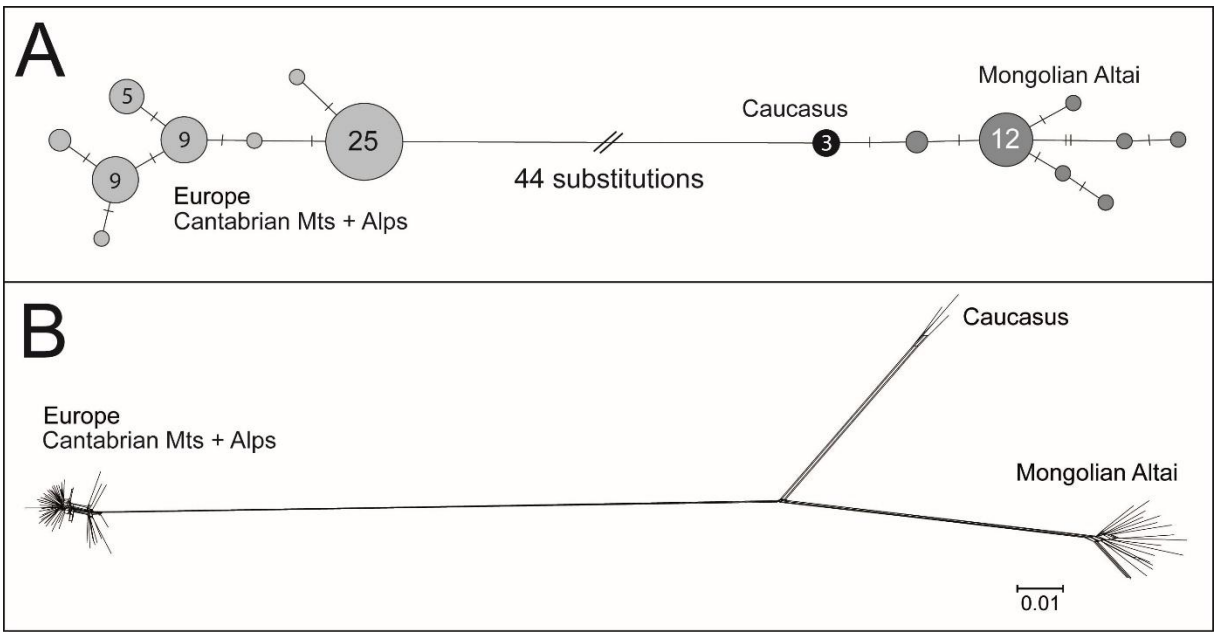

Fig. S2: A)minimum-spanning haplotype network of Eurasian populations of *M. nivalis* (cytochrome-b, 997 base pairs); B) Minimum-spanning networks for autosomal SNP data sets of Eurasian populations of *M. nivalis* generated with SplitsTree

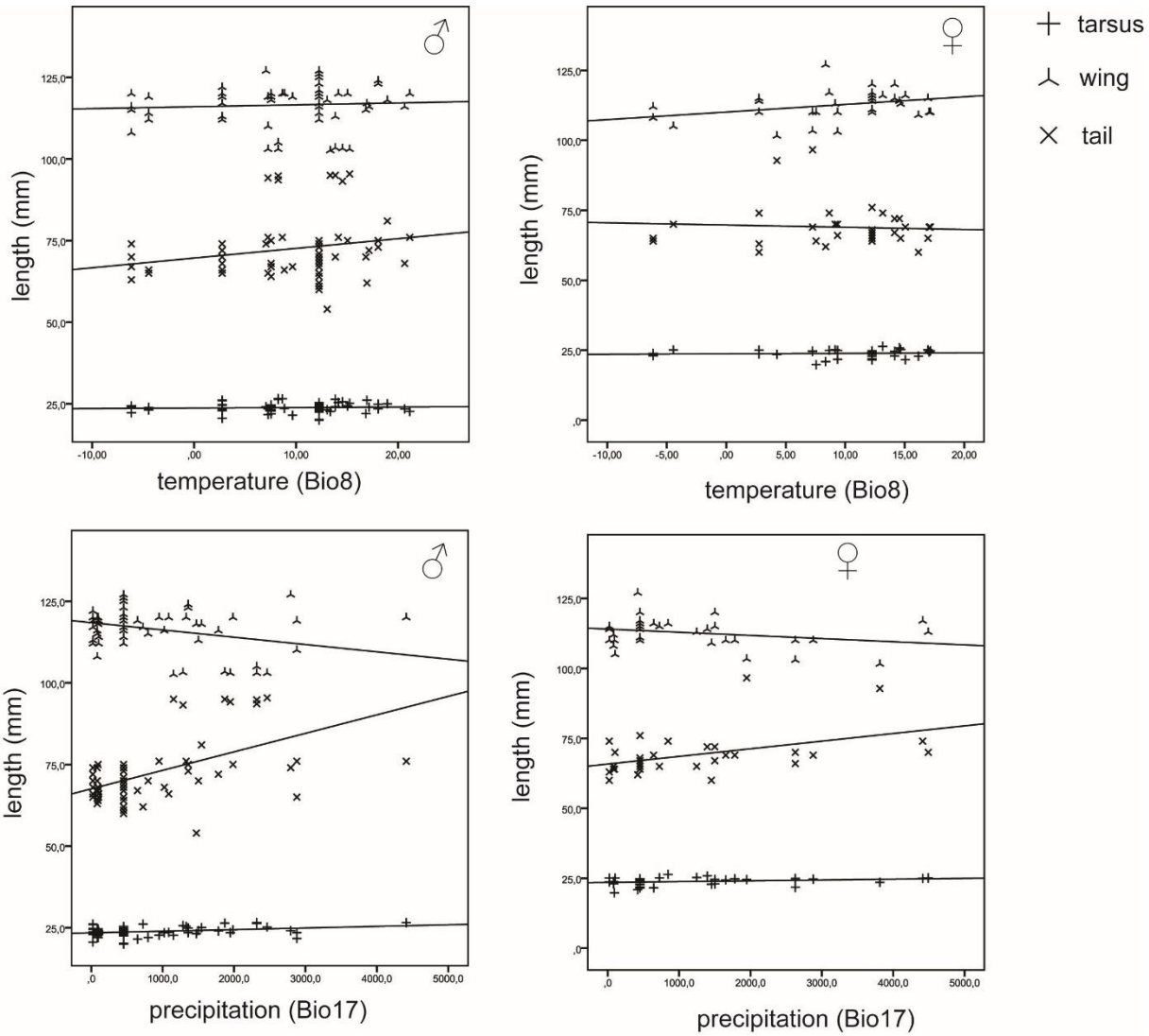

Fig. S3: Correlation of male and female body size parameters (length of tarsus, wing and tail, in mm) with temperature of the wettes quarter (Bio8) and precipitation of the driest quarter (Bio 17).

Discussion

A Tibetan Cradle of Evolution – in-situ speciation and Out-of-Tibet radiations in passerine model species

Discussion

QTP as a Global diversification Hub: Endemism, "Out of Tibet," and Spreading route

Fossil discoveries from the Tibetan Plateau reveal its fundamental role in global biodiversity (Deng 2020); QTP shows the unique physical conditions that dynamically evolved and diversified biodiversity through high rates of speciation and swift diversification, mostly drifted by evolutionary dynamic geological or climatic oscillations (Fjeldså et al., 2012; Favre et al., 2015). The Qinghai-Tibet Plateau shaped by the mountain uplift and glacial cycles, which repetitively fragment and reconnect habitats, speciation (Zhang et al., 2006; Ding et al., 2020). The Qinghai-Tibet Plateau (QTP) is isolated by its unique environmental and physical surroundings, creating a separate high-altitude biodiversity that harbors many endemic species. Additionally, the plateau's uplift history has preserved fossils of many biotas, contribution valuable insights into the evolutionary history of these plants and animals.

The Tibetan Plateau is considered as a key junction in the history of modern biodiversity, showing three major patterns of biotic evolution and dispersal:

a). Local origination of endemism: The fossil records represent that QTP was an ecological island of many endemic species. Fossil records of fish and mammals shows their evolutionary history during the plateau's uplift (Chang et al., 2010, 2016; Deng et al., 2011). For example, QTP endemic fish genera *Gymnocypris*, *Oxygymnocypris*, *Schizopygopsis*, *Platypharodon* and *Chuanchia* of the Schizothoracinae (Cypriniformes: Cyprinidae), which are the dominate QTP fossils of the freshwater fish fauna (Tang et. al. 2019). Fossils show their historical dispersal mirrors the QTP distribution of extant species, reflecting biological responses to the plateau's stepwise uplift (Chang et al., 2010).

b). Local origination and "Out of Tibet" – showed by the evolutionary history of certain mammals and plants those are originated in the QTP and later spread to other parts of the world in a suitable climate. For example, Out-of-Tibet hypothesis considered the Tibetan Plateau as a center of origin for several cold-adapted animals such as woolly rhinoceros, Arctic fox, snow leopards, pikas. The woolly rhinoceros (*Coelodonta*) is good example of Ice Age megafauna which evolved with the large body size including long hair, snow-clearing structures and thick fur to adapted the cold conditions of the Pleistocene (Guérin 1989; Kahlke 2008). Previous fossil evidence could not fully resolve the origin and evolution of the woolly rhinoceros (Deng 2006; Orlando 2003; Qiu 2004). However, the discovery of a new Pliocene (~3.7 Ma) species, *Coelodonta thibetana* sp. nov., from the high-altitude Zanda Basin in Tibet which is suggesting that woolly rhinoceros evolved in the Tibetan Plateau before dispersal to northern Eurasia as the Ice Age began (Deng et al. 2011). The Arctic Fox shows

circumpolar distribution in all Arctic tundra habitats, and the oldest fox fossil evidence from the Early Pliocene of the Himalaya and Kunlun ranges. This evolutionary connection between an ancestral high-elevation species and its modern polar descendant recommends faunal dynamics that linked two distant regions of northern Eurasia (Wang 2014). Fossil evidence suggested two canid lineages in the Tibetan Plateau: one descendent to the Arctic fox lineage, and another to the Tibetan sand fox. The pikas (Lagomorpha: Ochotonidae) is another cold-adapted mammal group presently distributed across alpine and tundra habitats across the Northern Hemisphere (Delibes-Mateos et al. 2011; Wilson & Smith 2015; Smith et al. 2018), and successfully adapted to cold habitat (Wang et al., 2020). Phylogeographic analyses suggested that the last common ancestor of pikas evolved in the mid-Miocene, likely on the Qinghai-Tibetan Plateau and later dispersed across Eurasia and North America (Wang et al., 2020). Snow leopards (*Panthera uncia*) are distributed mountain ranges of Central and South Asia. The earliest known fossil (2.2–2.5 Ma) was discovered near Tibet, and skeletal fossil and phylogenetic analyses suggested that snow leopards dispersed multiple times from the Tibetan Plateau during the Quaternary (Wang et al., 2020). Cold-adapted animals evolved and adapted to high-elevation environments during the uplift of the Tibetan Plateau, which served as a cradle for Ice Age megafauna before their dispersal into colder environments (Deng 2011).

c) Intercontinental dispersal via Tibet:

The Qinghai–Tibet Plateau has played an active role in global biodiversity - sometimes acting as a barrier that isolated species, and other times allowing their movement and interaction. These shifts have influenced the rise and fall of many evolutionary lineages (Vermeij, 1991; Klaus et al., 2016). The Genus *Ailanthus* includes six woody deciduous or evergreen species, those are distributed from China to northern Australia and eastern India to New Guinea, with Southeast Asia as its diversity center (Nooteboom, 1960; Sam et al. 2007; Su et al., 2013; Song & Xu, 2014). Recently discovered fossils from the Deccan Plateau, dated to the latest Cretaceous - earliest Paleocene, represent the earliest record and suggest an Indian origin (Wheeler et al., 2017). *Ailanthus* likely spread to Tibet after the India–Eurasia collision, reaching northern Asia in the Early Eocene and then North America via the Bering land bridge (Corbett & Manchester, 2004; Song et al., 2014). The fish genus Anabas distributed in Asia, while similar species are found in Africa (Norris, 1994). Recently anabantid fossil discovered from central Tibet. Molecular studies suggesting that anabantoid fishes originated in Southeast Asia during the Middle Eocene and later moved into Tibet through interconnected water systems (Wu et al., 2017).

The QTP is an evolutionary center formed by plate collisions, uplift, and climate cooling, and considered an origin and diversification center for many endemic bird groups (families or genera), as well as a key area for understanding colonization events and pathways both out of and into Tibet. For example, avian diversity of the QTP research works using a phylogenetic dataset of the superfamily Passeroidea (660 species; Fig. 4.A) have detected five families that form a hotspot of diversity and endemism on the QTP and its boundaries, including the Himalayas and Hengduanshan. Endemic birds of the QTP have evolved through in-situ speciation and subsequently extended their distribution beyond the QTP's boundaries - a process mentioned to as the "Out-of-Tibet" scenario.

Six of the eight known snowfinch species are endemic to the QTP, representing how they responded to the plateau's massive geological uplift, climate-changes habitat fragmentation, and repetitive cycles of isolation (Päckert et al., 2021). Snowfinches (*Montifringilla* sp *Pyrgilauda* sp, *Onychostruthus* sp.) evolutionary history connects the QTP's dynamic uplift, climatic fluctuations, and habitat diversity have driven rapid diversification and local adaptation in high-altitude species. The onset of alpine radiations has been dated to about 8 million years ago in snowfinches, with terminal dispersal events out of Tibet (via the Himalayas) happening during the Pleistocene (Päckert et al. 2020). These Pleistocene climatic oscillations triggered important range contractions in endemic QTP bird species, followed by Holocene expansions that designed their demographic histories. Similar range shifts and demographic changes were also detected in species across the Western Palearctic (Yang et al. 2009; Lei et al. 2013). Over these short evolutionary timescales, range shifts can be connected with changes in climatic and ecological niches, driving speciation processes (Nyári & Reddy 2013).

Trans-continental and intercontinental colonization pathways have been identified, such as those connecting bridging populations in the Caucasus (*M. nivalis alpicola*) to the European mountain systems (*M. nivalis nivalis*; Fig. 4 "snowfinches"), and extending across the Russian Far East and Kamchatka (*Leucosticte arctoa*) to the Nearctic via the Bering Strait (*L. tephrocotis* and allies; Fig. 4 "mountain finches"). Importantly, the little-studied Afghan snowfinch (*Pyrgilauda theresae*; Fig. 5B) likely arose from an former time, short-distance dispersal event from Tibet to the adjacent Hindukush around 6 million years ago. Genome-wide SNP data have identified late Pleistocene divergence on a smaller scale between European snowfinch populations in the Alps and the Cantabrian Mountains (dating to around 0.6 million years ago; Fig. 4C). Funk et al. (2020) detected similar recent diversification patterns in Nearctic rosy finches, tracing them back to a common ancestor with the Asian rosy finch (*L. arctoa*).

Most of the phylogeographic studies of the QTP's glacial history have been restricted to broad comparisons of unrelated target species, lacking examination of potential shared diversification patterns (Lei et al. 2013). In our present research, we have produced a whole Genome reference genome of *Montifringilla nivalis* and we mapped the ddRAD data with this newly produced reference genome. In our genomic study we could not include all the population samples of the white-winged snowfinch samples. Future studies should include snowfinch samples from additional bridging populations between the QTP and European mountain systems—such as those in the Italian and Balkan Peninsulas (*M. n. nivalis*) and the Near East (*M. n. leucura*). Our projects are focused on unraveling trans-Palearctic dispersal and diversification patterns (Päckert et al. 2015a, Chapter 3; Wang et al. 2015), alongside the demographic histories of snowfinch species.

Current state of knowledge on the biogeography and evolutionary history of snowfinches

The biogeographic analysis presented here offers new insights into the historical dispersal and diversification patterns of snowfinches across the Qinghai-Tibet Plateau and other Palearctic regions. The new phylogenetic reconstructions, supported by genetic data, disclose how past climatic events, such as glaciations, have shaped the distribution and diversification of these species, as supported by previous studies (Hewitt, 2004). These results contribute to a deeper understanding of the evolutionary history of snowfinches, particularly regarding how past ecological changes have influenced their speciation and adaptation processes. In our study, we were intensively focused on snowfinch populations from the Cantabrian and European Alps Mountain ranges. Our phylogenomic research shows the presence of multiple mitochondrial lineages, revel its complex evolutionary history. Our museomics research pointed that *M. nivalis* originated on the Tibetan Plateau and later colonized the Western Palearctic. Specially, *M. nivalis* prove an intraspecific phylogeographic structure across Eurasian Mountain systems - mainly in the Pyrenees, Alps, and Caucasus highlighting the deep influence of Pleistocene-driven diversification on its genetic discrepancy and demographic history. However, the absence of samples from the Balkans restricted to fully explain the population structure of snowfinches across Europe. We also couldn't include samples from Turkey, which would have been important in exploring the population barrier between Europe and Asia. These gaps highlight important areas for future research.

Evolutionary Linkages Between the Qinghai-Tibet Plateau and Himalayan Birds

The uplift of the QTP shaped geological and climatic transformations of the QTP, and formed one of the harshest high-altitude environments on Earth. Besides the development of the high-altitude environments, it is also profoundly influenced the development of adjacent ecosystems, including the Himalayas. Besides the uplifting the Tibet Plateau, it worked as a radiator and a center of evolution of many cold-adapted birds. For example, snowfinches are high-altitude birds group which are mostly radiated within the plateau and its fringes, while ground-tits evolved in low-oxygen alpine environments, that show deep phylogenetic splits that align with main geological and climatic events during the plateau's uplift. In the meanwhile of the uplifting of QTP, Himalayan Forest birds like coal tits (*Periparus ater*), display vicariant patterns, with genetic discrepancy which is designed by topographical barriers such as deep valleys and ridgelines. To observe the secondary contact zones or hybrid zone of Himalayan birds (Päckert et. al. 2023)**,** we explored the legacy of Pleistocene range fragmentation and the subsequent segregation of genetic lineages in glacial refuges. In the Himalayas, the coal tit taxa diversity typically separated during the early or mid-Pleistocene. Previously Wolfgramm et. al. (2021), a population genetic study has been done based on ten microsatellite loci already confirmed large-scale gene flow between western *P. a. melanolophus* and eastern *P. a. aemodius*. However, differences between the phenotypically distinct hybrid populations have not yet been quantified (Wolfgramm et al., 2021). In this study, we generated a dataset of genome-wide single-nucleotide polymorphisms (SNPs) using ddRAD sequencing, which was analyzed using a clustering approach. This analysis clearly depicts, a cross the Dhaulagiri transition zone in Nepal, a narrow hybrid belt exists between two coal tit subspecies: the western *P. a. melanolophus* and the eastern *P. a. aemodius*, with an unusual cinnamon-bellied form presenting locally with these hybrids in the Dhorpatan Valley (Diesselhorst & Martens, 1972; Eck & Martens, 2006; Martens, 1975). We inspected genetic divergence and admixture shapes across Himalayan coal tit populations, from the Hindukush in the west to eastern Nepal, predicting robust genetic mixing in phenotypically intermediate populations of western Nepal and genetic uniqueness in the parental populations at both ends of this cline. Our research relied heavily on natural history collections those were collected in the 1960s and 1970s, and highlights the essential role of natural history collections as biological archives (Kuhn et al., 2013; Mecke et al., 2016; Meineke et al., 2018; Rocha et al., 2014; Winston, 2007). In addition, we studied historical specimens from Hans Löhrl's cross-breeding experiments which were collected in the late 1960s (Fig. 6; Löhrl 1994), including European coal tits (*Periparus ater ater*) and Himalayan spot-winged tits (*P. a. melanolophus*). These recognized F1 and F2 hybrids provided a valuable reference for identifying hybrid populations in the nature, specially within the

natural hybrid zone in the Central Himalayas (Fig. 6). Compared with the known hybrids from Löhrl's experiment, none of the hybrid populations from Nepal show genetic similarities with a hypothetical founding population from an F1 or F2 generation. The local gene pools of the hybrid populations south and southwest of the Dhaulagiri massif resemble backcrosses with *P. a. melanolophus*, while the gene pools of the hybrid populations from the adjacent Kali Gandaki Valley (*P. a. martensi*) and south of the Annapurna massif resemble backcrosses with *P. a. aemodius*.

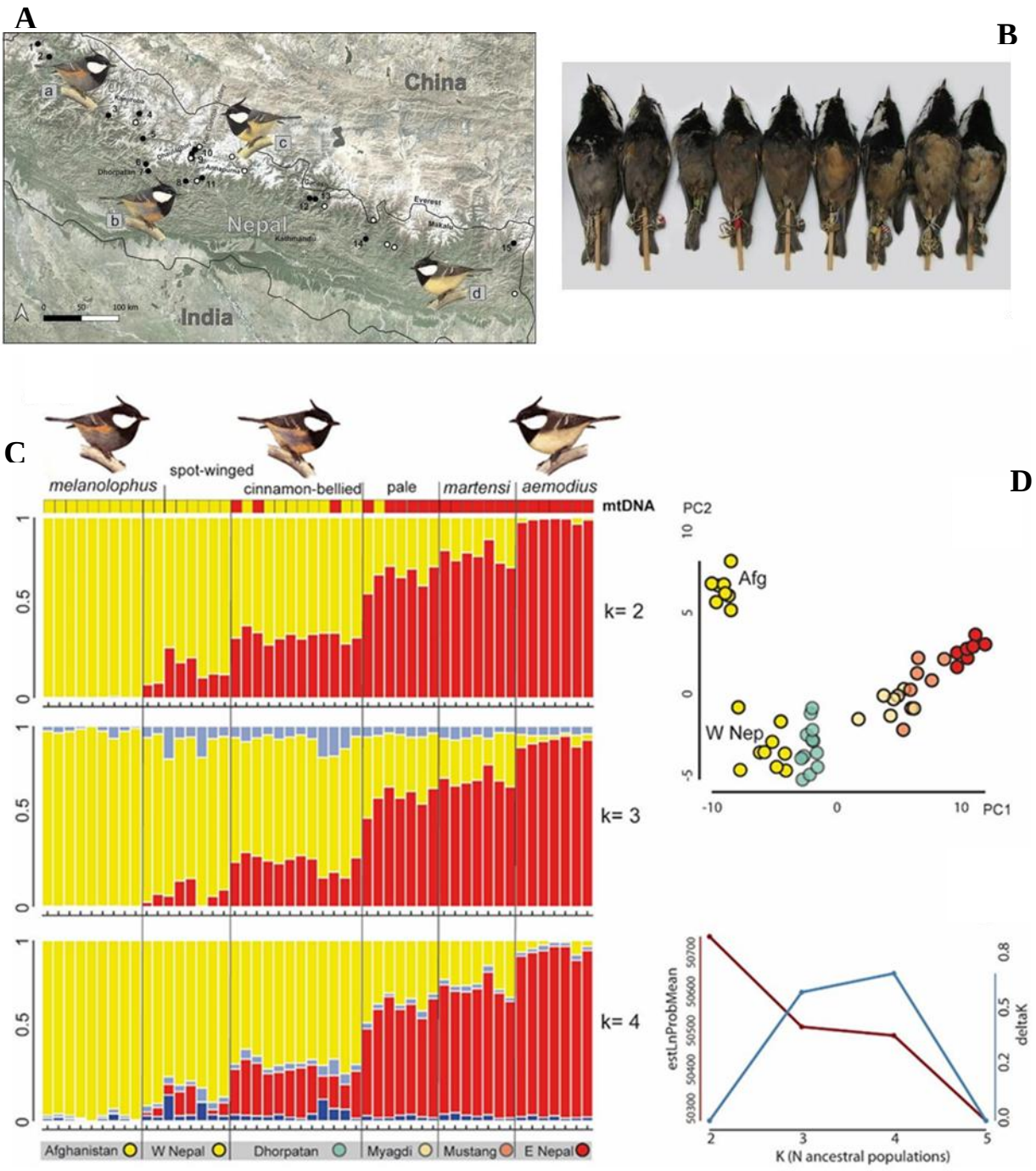

Fig. 5: This study highlights the importance of scientific collections in uncovering historical genetic patterns and evolutionary dynamics. By analyzing DNA from museum specimens of the coal tit (*Periparus ater*) dating back to 1960, illustrate a secondary contact zone in the Western Himalayas. In the Western Himalayas two subspecies *P. a. melanolophus* (population a) and *P. a. aemodius* (population b), where putative hybrid populations with intermediate phenotypes are found in a narrow region of overlap in Central Nepal. A. Map of the Himalayan hybrid zone showing the transition between the western "spot-winged tit," *Periparus s ater melanolophus* (phenotype a; sites: 1 = Chala Valley, 2 = Saipal, 3 = Jagdulah Lekh) and the eastern coal tit subspecies *P. a. aemodius* (phenotype d; sites: 12, 13 = Somdang, west of Syabrubesi, 14 = Dadar Danda, 15 = above Ghunsa). Hybrid populations (from west to east) include: spot-winged hybrids (sensu Harrap & Quinn 1996; sites: 4 = Ringmo at Lake Phoksumdo, 5 = Tarakot, 6 = Thankur); cinnamon-bellied hybrids (phenotype b) occurring in local sympatry with spot-winged hybrids at Dhorpatan (site 7); pale-bellied hybrids (Martens & Eck 1995; site 8 = upper Myagdi Khola); and *P. a. martensi* (sensu Eck 1998; referred to as "coal-tit type hybrids" by Harrap & Quinn 1996; phenotype c; sites: 9 = Thaksang, 10 = Thakkola [Purano Marpha], 11 = between Chitre and Deorali). (Drawings by K. Rehbinder). B. Patterns of divergence and admixture in two overlap zones between western and eastern Himalayan *Periparus* tits, based on STRUCTURE analysis of genome-wide SNP data for k=3. Each vertical column represents one individual, with mitochondrial lineages indicated by the color bars above the STRUCTURE plots. These data highlight zones of admixture in Nepal and China for the coal tit (*Periparus ater*). D. Principal Coordinates Analysis (PCoA) based on genome-wide NGS data, with a triangle diagram illustrating the differences among the various populations.

Methodological advancements in the field

In our research, advanced genomic techniques, such as next-generation sequencing (NGS) and SNP analysis, have been crucial in resolving the complex phylogenetic relationships within Passeridae. These methodological advancements have provided a more comprehensive and precise understanding of avian phylogenetics, overcoming the limitations of previous studies that relied primarily on mitochondrial data (Mardis, 2008). She et al. (2022) explained the adaptive divergence of QTP' sympatric snowfinches (*Montifringilla adamsi*, *Pyrgilauda ruficollis* and *Onychostruthus taczanowskii*) by analysing genomics, morphology, and ecological niche modelling. However, their research did not include the trans-Palearctic white-winged snowfinch (*Montifringilla nivalis*). In our research, we filled this gap by exploring the intraspecific molecular and morphological variation of

the white-winged snowfinch across its Eurasian population. By integrating genomic, morphological, and ecological data, this research has presented a holistic view of snowfinch diversification, setting a pattern for future studies to resolve phylogenetic complexities in other avian groups.

The improvement of Next-Generation Sequencing (NGS) technologies has greatly boosted the facility to study genetic diversity, but collecting fresh samples, especially in regions with harsh weather or ongoing conflicts, remains a significant challenge. In such cases, museum specimens are vital, providing the unique resource of material for phylogeographic studies. However, these samples have some limitations, particularly due to DNA fragmentation over time. Museum specimens classically hold degraded DNA, difficult to extract fragments longer than around 1,000 base pairs, which bounds the use of long-read sequencing technologies. Despite these challenges, museum samples endure critical for research across various disciplines such as phylogenomics, biogeography and evolutionary biology. In our study, we used ddRAD-library preparation, a specific short-read sequencing method, which allowed us to examine around 5000–10000 loci, though these short read NGS libraries do not cover the full genome. Long read whole-genome sequencing could be a future option. We also used an NGS approach for complete mitogenome sequencing, which allowed us to generate complete mitogenomes for key snowfinch taxa, including rare Afghan endemic sample *Pyrgilauda theresae*. This complete mitogenome sequencing revealed that numerous mitogenome sequences on GenBank were chimeric those encompassing fragments from multiple snowfinch species. By recognizing these chimeric sequences, we clarified the discrepancies in previous molecular trees, showing that these errors were responsible for inaccurate placements in snowfinch phylogenies. Looking ahead, future research should aim for continuous sampling across the trans-Palearctic region, exploiting high molecular weight DNA and long-read sequencing technologies. These methods will deliver more comprehensive insights into snowfinch population structure and biogeographical barriers between European and Asian populations, present a deeper understanding of their evolutionary history.

Contribution to evolutionary theory and biogeography

Finally, our work contributes to our collective understanding of evolutionary theory, mainly in the contexts of speciation, adaptive radiation, and the role of harsh environments in shaping biodiversity. By explaining biogeographical history and phylogenetic relationships of the montane and alpine target groups of snowfinches. Our research contributes to the current knowledge on taxon-complete phylogenetic evolution of snowfinches, diversification and climatic challenges of the snowfinches. The insights gained from our research not only advance our understanding of avian evolution but also provide a framework for studying the evolutionary dynamics of other species in extreme environments, bridging the gap between evolutionary biology and ecology.

Challenges and future directions in Passeridae research

Our research has resolved several phylogenetic key questions within Passeridae, but there are still remaining questions. Future research could improve taxon sampling to include more species from underrepresented areas. Moreover, the potential of an integrative taxonomy approach, which could be applied to other avian groups to address similar phylogenetic uncertainties, is particularly promising. The integration of paleontological data with deeper genomic study and findings could also provide a more comprehensive understanding of the evolutionary history of Passeridae, particularly in relation to ecological changes and passerine species diversification.

Importance of scientific collections

Biological archives are essential resources that procedure the foundation for research in systematics, taxonomy, and evolutionary biology, with natural history collections serving as the key reservoirs. Museum collections provide important biological material for classifying and describing new species, tracing past biodiversity (Schindel and Cook, 2018; Hahn et al., 2020; Hilton et al., 2021), and explaining biodiversity patterns across both time and space. By storing specimens alongside their geolocation information, these museum collections allow for increasing comparisons across different topographical regions and historical ages. Today, museum specimens have become important sources of genetic material for molecular analyses, facilitating studies on hybridization, admixture, and phylogeography by bridging the past and present. Natural history collections also act as biological archives, preserve rare or even extinct species, as well as endemics from previously inaccessible or conflicts areas like Afghanistan. We used biological archive samples from museum collections, which provided important genetic material from conflict region of Afghanistan, that are inaccessible to scientists today. In this study, we present the first taxon-complete phylogeny of snowfinches by studying historical genetic materials from whole skins of the Afghan Snowfinch (*Pyrgilauda theresae*) (Fig. 5C). We generated whole mitochondrial genome sequences of the snowfinches taxa and obtained genome-wide SNP data using ddRAD sequencing. This sampling and molecular study approach covered all extant endemic snowfinch species to the Qinghai-Tibet Plateau (QTP) and included extended intraspecific sampling of the only Central and Western Palearctic snowfinch (*Montifringilla nivalis)*. With the advanced next-generation sequencing (NGS) techniques, we successfully created the first comprehensive NGS dataset for *Pyrgilauda theresae* and resolved the molecular phylogeny (Fig. 5. C) of this remarkable group of alpine birds.

As an extended our study to include the phylogeny and phylogeography of the entire genus *Periparus* sp, incorporating data from the Western Palearctic. Comparison of this secondary contact zone or hybrid zone of the Himalayan Birds would not be possible without the valuable historical collection specimens as a comparative reference. Altogether, our findings reveal how scientific collections deepen our understanding of past and present biodiversity while laying the groundwork for future conservation efforts by documenting the dynamic processes shaping life on Earth.

Demographic History, Species Distribution Models (SNMs) of the White-winged Snowfinch (*Montifringilla nivalis*)

In our present projects, we have presented taxon complete phylogeny of the snowfinches. The application of modern NGS technologies in the study of archival samples facilitated us to discover areas with conflicting records or challenging field sampling conditions. Even though we have present taxon complete phylogeny of the snowfinches but we have mainly focused on the white-winged snowfinch (*Montifringilla nivalis*), the only snowfinch species with both a wide trans-palearctic distribution and deep intraspecific genetic structure, split into three main phylogroups (Päckert et al. 2020; Islam et al. 2024). Genetic divergence between European and Asian lineages started in the Early Pleistocene (2.7–1.5 mya), like to patterns observed in other alpine species. Despite population decline in many Palearctic birds (Sanderson 2006), snowfinch populations have stayed remarkably steady since the mid-Pleistocene, with only little Holocene growths in Mongolian and Cantabrian populations.

We used RADseq data from 28 loci in Asia and 20 loci in Europe for the Extended Bayesian Skyline Plot (EBSP) study of Asian and European populations of the white-winged snowfinch (*Montifringilla nivalis*), explaining that both populations continued largely constant effective population sizes from the mid-Pleistocene through the Last Glacial Maximum (LGM), with only minor growth following the LGM. Plumage color and body morphology were connected to climate but displayed no clear diagnostic breaks among genetic groups, suggesting that evolutionary niche shifts have changed subtle phenotypic divergence.

For the species distribution modeling (SDM), we collected over 13,000 distribution records from online resources such as the Global Biodiversity Information Facility GBIF (www.gbif.org; n= 13,998 records) and carefully identified to 1,406 reliable localities. To identify sampling biases, we have done separate species distribution models (SDMs) for European and Asian populations using MaxEnt, with bioclimatic predictors from WorldClim and paleoclimate models (CCSM3, MPI-ESM-P, MIROC).

Model performance was consistently high (AUC > 0.95), with elevation emerging as the most important predictor, especially in Asia, while precipitation was most influential in Europe. Ecological niche models suggest that snowfinch habitat has shifted from colder to warmer conditions during the colonization of the Western Palearctic.

The white-winged snowfinch (*Montifringilla nivalis*) is present across huge areas of the alpine Eurasian mountains habitat, but the range of the Trans-Palaearctic snowfinch has been significantly overestimated due to outdated taxonomy. IUCN maps, for occurrence, incorrectly include large parts of the Qinghai-Tibet Plateau (QTP) - about 1.5 million km² - as appropriate habitat for the snowfinch. This underlines the urgent need for more exact white-winged snowfinch distribution data to avoid biases in conservation and natural planning.

Implications for conservation of Evolutionary Significant Units (ESU)

Understanding the ecological preferences of snowfinches and other Passeridae species, can also have important implications for conservation. Snowfinches distributed across Eurasian mountain systems and some species have very limited ranges, such as the Afghan snowfinch (*Pyrgilauda theresae*).In our study, we are the first to include molecular samples of *P. theresae* from the museum samples. However, additional ecological, behavioural, and population studies on this species are still lacking. This Afghan endemic species need to special conservation efforts due to its limited distribution and specialized adaptations. White-winged snowfinch (*Montifringilla nivalis*) are distributed to the Eurasian mountain and this species has been divided into seven subspecies. In our studies, we have included three subspecies (*M. n. nivalis, M. n. groumgrzimaili* and *M. n. alpicola)* and we could not include four sub species *M. n. leucura, M. n. tianshanica, M. n. kwenlunensis* and *M. n. gaddi*) (Fig. 5A). From our included subspecies samples, we found that these Caucasian populations of the suspecies *M. n. alpicola* genetically and morphologically distinct against the other subspecies. This subspecies qualifies as an Evolutionary Significant Unit (ESU), given its unique and limited distribution and potential ecological importance (Moritz et al. 1994, Casacci et al. 2013). As an ESU, it requires greater focused research and intensive conservation efforts, including detailed studies on its origin, demography, genetic structure, and adaptive traits (Moritz 1994b; Hoelzel 2023). This need is particularly in the context of current habitat fragmentation and climate change. Preserving genetic diversity within species is especially crucial for their long-term adaptability and survival in rapidly changing montane environments. With advances in next-generation sequencing (NGS) techniques, there are now more prospects to uncover cryptic genetic diversity of the snowfinches. For museum samples, utilizing DNA repair kits in the wet lab can improve library preparation by recovering more

loci, improving data quality and depth. Increasing sampling size and applying long read NGS techniques can provide more detailed insights into the genetic landscape of trans-Palearctic snowfinch species, helping to identify Evolutionarily Significant Units (ESUs) with precision. Our findings emphasise the importance of conservation strategies tailored to montane species' unique ecological and genetic needs across trans-Palearctic Mountain ecosystems.

Bibliography

Bibliography:

Aliabadian, M., Roselaar, C. S., Nijman, V., Sluys, R., & Vences, M. 2005: Identifying contact zone hotspots of passerine birds in the Palaearctic region. Biology Letters, 1(1), 21–23. https://doi.org/10.1098/rsbl.2004.0258

Allende, L. M., Rubio, I., Ruíz-del-Valle, V., Guillén, J., Martínez-Laso, J., Lowy, E., Varela, P., Zamora, J., Arnaiz-Villena, A. 2001: The Old World sparrows (Genus *Passer*). Phylogeography and their relative abundance of nuclear mtDNA pseudogenes. Journal of Molecular Evolution. 53: 144–154. https://doi.org/10.1007/s002390010202

Avise, J. C., & Walker, D. 1998: Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London. Series B: Biological Sciences, 265(1395), 457–463. https://doi.org/10.1098/rspb.1998.0317

Casacci, P. L., Barbero F., Balletto E. 2013: The "Evolutionarily Significant Unit" concept and its applicability in biological conservation.Italian Journal of Zoology Volume 81 (pages 182-193) https://doi.org/10.1080/11250003.2013.870240

Chang, M. M., Miao D S, Wang N. 2010: Ascent with modification: Fossil fishes witnessed their own group's adaptation to the uplift of the Tibetan Plateau during the late Cenozoic. In: Long M Y, Gu H Y, Zhou Z H, eds. Darwin's Heritage Today: Proceedings of the Darwin 200 Beijing International Conference. Beijing: Higher Education Press. 60–75

Chang, M. M., Miao D. S. 2016: Review of the Cenozoic fossil fishes from the Tibetan Plateau and their bearings on paleoenvironment (in Chinese). Chin Sci Bull, 61: 981–995

Clayton, J W., Soltis, P S., Soltis, D E. 2009: Recent long-distance dispersal overshadows ancient biogeographical patterns in a pantropical angiosperm family (Simaroubaceae, Sapindales). Systematic Biolgy. Volume 58,(Pages 395-410) doi: https://doi.org/10.1093/sysbio/syp041

Cobos, M. E., Cheng, Y., Song, G., Lei, F., Peterson, A.T. 2021: New distributional opportunities with niche innovation in Eurasian snowfinches. J. Avian Biol. 52. https://doi.org/10.1111/jav.02868

Corbett, S., Manchester R. S. 2004: Phytogeography and Fossil History of Ailanthus (Simaroubaceae). Int J Plant Sci. Volume 165 (4): Pages 671–690. https://doi.org/10.1086/386378

Delibes-Mateos, M., Smith A. T., Slobodchikoff C N, Swenson J E. 2011: The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. Biol Conserv. 144(5):1335–1346.doi. https://doi.org/10.1016/j.biocon.2011.02.012

Deng, T., Wu, F., Zhou, Z. SU T. 2020: Tibetan Plateau: An evolutionary junction for the history of modern biodiversity. Sci. China Earth Sci. 63, 172–187 (2020).

https://doi.org/10.1007/s11430-019-9507-5

Deng, T., Wang, X., Fortelius, M., Li, Q., Wang, Y., Tseng, Z. J., Takeuchi G. T., Saylor J E., Säilä L K., Xie G. 2011: Out of Tibet: Pliocene woolly rhino suggests high-plateau origin of Ice Age megaherbivores. Science. Sep 2;333(6047):1285-8. doi: 10.1126/science.1206594

del Hoyo, J., Collar, N. J. 2016: Illustrated checklist of the birds of the world. Volume 2 Passerines. Lynx Edicions, Barcelona.

Deng T. 2006: Neogene rhinoceroses of the Linxia Basin (Gansu, China). Cour. Forsch.-Inst. Senckenberg.256: 43-56.

Deng, T., Ding, L. 2015: Paleoaltimetry reconstructions of the Tibetan Plateau: progress and contradictions, National Science Review.2 (4) 417–437. https://doi.org/10.1093/nsr/nwv062

Diesselhorst, G., & Martens, J. 1972: Hybriden von *Parus melanolophus* und *Parus ater* im Nepal-Himalaya. Journal für Ornithologie, 113(4), 374–390. https://doi.org/10.1007/BF01647601

Ebersbach, J., Muellner-Riehl, A.N., Michalak, I., Tkach, N., Hoffmann, M.H., Röser, M., Sun, H., Favre, A., (2017). In and out of the Qinghai-Tibet Plateau: divergence time estimation and historical biogeography of the large arctic-alpine genus *Saxifraga* L. J. Biogeogr. 44, 900–910. https://doi.org/10.1111/jbi.12899

Eck, S., & Martens, J. 2006: Systematic notes on Asian birds 49 –A preliminary review of the Aegithalidae, Remizidae and Paridae. Zoologische Mededelingen, 80(5), 1–63.

Ericson P. G P., Irestedt M, Johansson S. U. 2003: Evolution, biogeography, and patterns of diversification in passerine birds. Journal of avian Biology 34:3–15.doi: https://doi.org/10.1034/j.1600-048X.2003.03121.x

Favre, A., Päckert, M., Pauls, S.U., Jähnig, S.C., Uhl, D., Michalak, I., Muellner-Riehl, A.N. 2015: The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. Biol. Rev. 90, 236–253. https://doi.org/10.1111/BRV.12107

Fjeldså, J., Bowie, R.C.K., Rahbek, C. 2012: The role of mountain ranges in the diversification of birds. Annu. Rev. Ecol. Evol. Syst. https://doi.org/10.1146/annurev-ecolsys-102710- 145113

Funk, E.R., Spellman, G.M., Winker, K., Withrow, J.J., Ruegg, K.C., Zavaleta, E., Taylor, S.A. 2021: Phylogenomic Data Reveal Widespread Introgression Across the Range of an Alpine and Arctic Specialist. Syst. Biol. 70, 527–541. https://doi.org/10.1093/sysbio/syaa071

Gebauer, A., Eck, S., Kaiser, M., Lei, F.M., Martens, J. 2006: The qinghai-tibet plateau: center of evolution for snow sparrows (*Montifringilla* s. str.) and mountain-steppe sparrows (*Onychostruthus*, *Pyrgilauda*). Acta Zool. Sin. 52, 305–309.

Gill, F., Donsker, D., Rasmussen, P.,2020 eds:"Family Index". IOC World Bird List Version 10.1. International Ornithologists' Union. Retrieved 26 April 2020.

Gu, Wei, Zhang Ting, Liu Shui-Yin,Tian Qin,Yang Chen-Xuan,Lu Qing,Fu Xiao-Gang,Kates Heather R.,Stull Gregory W.,Soltis Pamela S.,Soltis Douglas E.,Folk Ryan A.,Guralnick Robert P.,Li De-Zhu,Yi Ting-Shuang. 2024: Phylogenomics, reticulation, and biogeographical history of Elaeagnaceae. Plant Diversity, Volume 46, Issue 6. Pages 683-697.doi: https://doi.org/10.1016/j.pld.2024.07.001

Guérin C. 1989: La famille des Rhinocerotidae (Mammalia, Perissodactyla): Systématique, histoire, évolution, paléoécologie. Cranium 2, 3.

Hahn, E.E., Grealy, A., Alexander, M., Holleley, C.E. 2020: Museum epigenomics: Charting the future by unlocking the past. Trends Ecol. Evol. 35 (4), 295–300. https://doi.org/10.1016/j.tree.2019.12.005.

Hewitt, G. 2000: The genetic legacy of the Quaternary ice ages. *Nature* 405, 907–913. https://doi.org/10.1038/35016000

Hilton, E.J., Watkins-Colwell, G.J., Huber, S.K. 2021: The expanding role of natural history collections. Ichthyology and Herpetology 109 (2), 379–391. https://doi.org/10.1643/t2020018

Hoelzel Rus A. 2023: Where to now with the evolutionarily significant unit?. Trends in Ecology & Evolution. Volume 38, Issue 12 (Pages 1134-1142). https://doi.org/10.1016/j.tree.2023.07.005.

Hoorn, C., Perrigo, A., Antonelli, A. 2018: Mountains, climate, and biodiversity. Hoboken, NJ: Wiley.

Islam, S., Peart, C., Kehlmaier, C., Sun, Y.H., Lei, F., Dahl, A., Klemroth, S., Alexopoulou, D., del Mar Delgado, M., Laiolo, P., Carlos Illera, J., Dirren, S., Hille, S., Lkhagvasuren, D., Töpfer, T., Kaiser, M., Gebauer, A., Martens, J., Paetzold, C., Päckert, M. 2024: Museomics help resolving the phylogeny of snowfinches (Aves, Passeridae, *Montifringilla* and allies). Mol. Phylogenet. Evol. 198. https://doi.org/10.1016/j.ympev.2024.108135

Jacques, F. M., Shi, G., Li, H., & Guo, S. 2011: Fossil evidence for the origin of the diverse tropical rainforest regions in Asia during the late Miocene. Quaternary Science Reviews, 30(27-28), 3335-3346.

Janečka, J. E., Jackson. R., Yuquang, Z., Diqiang, L., Munkhtsog, B., Buckley-Beason, V., Murphy, W. J. 2008: Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. Animal Conservation. 11 (5): 401–411. https://doi.org/10.1111/j.1469-1795.2008.00195.x

Jia, D R., Liu T L., Wang L Y., Zhou D W., Liu J Q. 2011: Evolutionary history of an alpine shrub *Hippophae tibetana* (Elaeagnaceae): allopatric divergence and regional expansion. Biological Journal of the Linnean Society 102: 37–50. https://doi.org/10.1111/j.1095-8312.2010.01553.x

Jia, D.R., Abbott, R.J., Liu, T.L., Mao, K.S., Bartish, I. V., Liu, J.Q. 2012: Out of the Qinghai-Tibet Plateau: Evidence for the origin and dispersal of Eurasian temperate plants from a phylogeographic study of *Hippophaë rhamnoides* (Elaeagnaceae). New Phytol. 194, 1123–1133. https://doi.org/10.1111/j.1469-8137.2012.04115.x

Jones, K.E., Korotkova, N., Petersen, J., Henning, T., Borsch, T. and Kilian, N. 2017: Dynamic diversification history with rate upshifts in Holarctic bell-flowers (*Campanula* and allies). Cladistics, 33: 637-666. https://doi.org/10.1111/cla.12187

Jønsson, K.A., Fjeldså, J. 2006: A phylogenetic supertree of oscine passerine birds (Aves: Passeri). Zool. Scr. 35, 149–186. https://doi.org/10.1111/j.1463-6409.2006.00221.x

Kahlke R.-D., Lacombat F. 2008: The earliest immigration of woolly rhinoceros (Coelodonta tologoijensis, Rhinocerotidae, Mammalia) into Europe and its adaptive evolution in Palaearctic cold stage mammal faunas. Quat. Sci. Rev. 27, 1951.Doi: 10.1016/j.quascirev.2008.07.013

Klaus S, Morley RJ, Plath M, Zhang YP, Li JT. 2016: Biotic interchange between the Indian subcontinent and mainland Asia through time. Nat Commun. Jul 4;7:12132. https://doi.org/10.1038/ncomms12132

Kuhn, K., Schwenk, K., Both, C., Canal, D., Johansson, U. S., Mije, S., Töpfer, T., Päckert, M. 2013: Differentiation in neutral genes and a candidate gene in the pied flycatcher: Using biological archives to track global climate change. Ecology and Evolution, 3(14), 4799–4814. https://doi.org/10.1002/ece3.855

Lei, F., Qu, Y., Song, G. 2014: Species diversification and phylogeographical patterns of birds in response to the uplift of the Qinghai-Tibet Plateau and Quaternary glaciations 60, 149–161.

Löhrl, H. 1994: Beitrag zum noch offenen Fragenkomplex der Hybridisation von Tannen-und Schopfmeise (*Parus ater*, *P. melanolophus*) (Aves: Passeriformes: Paridae). Zoologische Abhandlungen des Museums für Tierkunde in Dresden, 48, 149–157.

Martens, J. 1975: Akustische Differenzierung verwandtschaftlicher Beziehungen in der *Parus* (*Periparus*)-Gruppe nach Untersuchungen im Nepal -Himalaya. Journal für Ornithologie, 116(4), 369–433.

Martens, J., Eck, S. 1995: Towards an ornithology of the himalayas: systematics, ecology and vocalizations of nepal birds (p. 445). Bonner Zoologische Monografien 38, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

Marchese, C. 2015: Biodiversity hotspots: A shortcut for a more complicated concept. Global Ecology and Conservation. Conserv. 3: 297–309. https://doi.org/10.1016/j.gecco.2014.12.008

Mardis, E.R. 2008: Next-generation DNA sequencing methods. Annu. Rev. Genomics Hum.Genet. https://doi.org/10.1146/annurev.genom.9.081307.164359

Martens, J., Eck, S.1995: Towards an ornithology of the Himalayas: systematics, ecology and vocalizations of Nepal birds. Bonner Zoologische Monografien 38, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

Mecke, S., Mader, F., Kieckbusch, M., Kaiser, H., Böhme, W., Ernst, R. 2016: Tracking a syntype of the Australian skink Anomalopus leuckartii (Weinland, 1862): 'Lost' treasures in the Senckenberg Natural History Collections Dresden highlight the importance of reassessing and safeguarding natural history collections. Vertebrate Zoology, 66(2), 169–177.

Meineke, E. K., Davies, T. J., Daru, B. H., Davis, C. C. 2018: Biological collections for understanding biodiversity in the Anthropocene. Philosophical Transactions of the Royal Society B, 374(1763), 20170386. https://doi.org/10.1098/rstb.2017.0386

Moritz, C. 1994.a:Defining 'Evolutionarily Significant Units' for conservation. Trends in Ecology & Evolution. Volume 9 (Issue 10). Pages 373-375 doi: https://doi.org/10.1016/0169-5347(94)90057-4

Moritz, C 1994.b: Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology. Volume 3 (Issue 4). https://doi.org/10.1111/j.1365-294X.1994.tb00080.x

Mosbrugger, V., Favre, A., Muellner-Riehl, A., Päckert, M., Mulch, A. 2018: Cenozoic Evolution of Geobiodiversity in the Tibeto-Himalayan Region. In: Hoorn, C., Perrigo, A. & Antonelli, A. (eds) Mountains, Climate and Biodiversity: A comprehensive and up-to- date synthesis for students and researchers, in: Wiley Blackwell. pp. 429–448.

Muellner-Riehl, A. N. 2019: Mountains as evolutionary arenas: Patterns, emerging approaches, paradigm shifts and their implications for plant phylogeographic research in the Tibeto-Himalayan Region. Frontiers in Plant Science, 10, 195. https://doi.org/10.3389/fpls.2019.00195

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., Kent, J. 2000: Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853-858. DOI: 10.1038/35002501

Nooteboom, H. 1960: Simaroubaceae. Flora Malesiana-Series 1, Spermatophyta, 6: 193-226

Norris, S M. 1994: The osteology and phylogenetics of the Anabantidae (Osteichthyes, Perciformes). Dissertation for Doctoral Degree. Tempe: Arizona State University

Nyári, A.S. & Reddy, S. 2013: Comparative Phyloclimatic Analysis and Evolution of Ecological Niches in the Scimitar Babblers (Aves: Timaliidae: Pomatorhinus). PLOSone. https://doi.org/10.1371/journal.pone.0055629

Li, J., Fang, X.1999: Uplift of the Tibetan Plateau and environmental changes. Chin. Sci. Bull. 44, 2117–2124 (1999). https://doi.org/10.1007/BF03182692

Löhrl, H. 1994: Beitrag zum noch offenen Fragenkomplex der Hybridisation von Tannen- und Schopfmeise (Parus ater,P. melanolophus) (Aves: Passeriformes: Paridae). Zool. Ab-handl. Mus. Tierk. Dresden 48: 149–157.

Orlando, L., Leonard J A, Thenot, A., Laudet, V., Guerin, C., Hänni, C. 2003: Ancient DNA analysis reveals woolly rhino evolutionary relationships. Mol Phylogenet Evol. doi: https://doi.org/10.1016/S1055-7903(03)00023-X

Päckert, M., Martens, J., Sun, Y.H., Strutzenberger, P. 2016: The phylogenetic relationships of Przevalski's Finch Urocynchramus pylzowi, the most ancient Tibetan endemic passerine known to date. Ibis (Lond. 1859). 158, 530–540. https://doi.org/10.1111/ibi.12382

Päckert, M., Islam, S., Töpfer, T., Vamberger, M., Martens J. 2023: Genomische Analyse von Tannenmeisenhybriden aus historischen Kreuzungsexperimenten. Vogelwarte - Zeitschrift für Vogelkunde. conference contributions 61:2023 (page. 300-301).

Päckert, M., Favre, A., Schnitzler, J., Martens, J., Sun, Y.H., Tietze, D.T., Hailer, F., Michalak, I. Strutzenberger, P. 2020: "Into and Out of" the Qinghai-Tibet Plateau and the Himalayas: Centers of origin and diversification across five clades of Eurasian montane and alpine passerine birds. Ecology and Evolution, 10(17), 9283–9300. https://doi.org/10.1002/ece3.6615

Päckert, M., Hering, J., Belkacem, A.A., Sun, Y.-H., Hille, S., Lkhagvasuren, D., Islam, S., Martens, J. 2021: A revised multilocus phylogeny of Old World sparrows (Aves: Passeridae). Vertebr. Zool. 71, 353–366. https://doi.org/10.3897/vz.71.e65952

Päckert, M., Sun, Y.-H., Strutzenberger, P., Valchuk, O., Tietze, D.T., Martens, J. 2015b: Phylogenetic relationships of endemic bunting species (Aves, Passeriformes, Emberizidae, Emberiza koslowi) from the eastern Qinghai-Tibet Plateau. Vert. Zool. 65, 135-150. https://doi.org/10.3897/vz.65.e31516

Päckert, M., Favre, A., Schnitzler, J., Martens, J., Sun, Y.-H., Tietze, D. T., Hailer, F., Michalak, I., & Strutzenberger, P. 2020: 'Into and Out of the Qinghai-Tibet Plateau and the Himalayas: Centers of origin and diversification across five clades of Eurasian montane and alpine passerine birds. Ecology and Evolution, 10(17), 9283–9300. https://doi.org/10.1002/ece3.6615

Price, T.D., Hooper, D.M., Buchanan, C.D., Johansson, U.S., Tietze, D.T., Alström, P., Olsson, U., Ghosh-Harihar, M., Ishtiaq, F., Gupta, S.K., Martens, J., Harr, B., Singh, P., Mohan, D. 2014: Niche filling slows the diversification of Himalayan songbirds. Nature 509, 222–225. https://doi.org/10.1038/nature13272

Qi, D., Guo, S., Chao, Y., Kong, Q., Li, C., Xia, M., Xie, B., Zhao, K. 2015: The biogeography and phylogeny of schizothoracine fishes (Schizopygopsis) in the Qinghai-Tibetan Plateau. Zoologica Scripta, 44, 523–533.doi: https://doi.org/10.1111/zsc.12116

Qiu, Z.-x., Deng, T., Wang, B. Y. 2004: Early Pleistocene mammalian fauna from Longdan, Dongxiang, Gansu, China. Palaeontol. Sinica New Series C Number 27.

Qu, Y., Ericson P. G. P., Lei F., Gebauer A., Kaiser M., Helbig J A. 2006: Molecular phylogenetic relationship of snow finch complex (genera Montifringilla, Pyrgilauda, and Onychostruthus) from the Tibetan plateau. Molecular Phylogenetics and Evolution Volume

40, Issue 1.Pages 218-226. doi: https://doi.org/10.1016/j.ympev.2006.02.020

Rahbek, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N., Nogues-Bravo, D., Whittaker, R.J., Fjeldså, J. 2019: Humboldt's enigma: What causes global patterns of mountain biodiversity?. Science (80). 365, 1108–1113. https://doi.org/10.1126/science.aax0149

Rocha, L. A., Aleixo, A., Allen, G., Almeda, F., Baldwin, C. C., Barclay, M. V. L., Bates, J. M., Bauer, A. M., Benzoni, F., Berns, C. M., Berumen, M. L., Blackburn, D. C., Blum, S., Bolaños, F., Bowie, R. C. K., Britz, R., Brown, R. M., Cadena, C. D., Carpenter, K.,Witt, C. C. 2014: Specimen collection: An essential tool. Science, 344(6186), 814–815. DOI:10.1126/science.344.6186.814

Sam, V. H., Nooteboom H P. 2007: Ailanthus Vietnamensis (Simaroubaceae): A new species from Vietnam. Blumea, 52: 555–558. doi:10.3767/000651907X608918

Sanderson, Fiona & Donald, Paul & Pain, Deborah & Burfield, Ian & Bommel, Frans. 2006: Long-term declines in Afro-Palearctic migrant birds. Biological Conservation. 131. 93-105.. https://doi.org/10.1016/j.biocon.2006.02.008

Schmitt, T. 2007: Molecular biogeography of Europe: pleistocene cycles and postglacial trends. Front. Zool. 4, 11. https://doi.org/10.1186/1742-9994-4-11

Schindel, D.E., Cook, J.A. 2018: The next generation of natural history collections. PLoS Biol. 16 (7), 1–8. https://doi.org/10.1371/journal.pbio.2006125.

Selvatti A. P., Gonzaga P L., Russo A. C., 2015: A Paleogene origin for crown passerines and the diversification of the Oscines in the New World. Molecular Phylogenetics and Evolution. Volume 88. Pages 1-15. doi: https://doi.org/10.1016/j.ympev.2015.03.018

Shukla, A, Mehrotra, R C., Spicer, R A., Spicer, T E V. 2016: Aporosa Blume from the paleoequatorial rainforest of Bikaner, India: Its evolution and diversification in deep time. Rev Palaeobot Palynol, 232: 14–21. doi:https://doi.org/10.1016/j.revpalbo.2016.05.006

She, H., Jiang, Z., Song, G., Ericson P. G., Luo, X., Shao, S., Lei, F., Qu, Y. 2021: Quantifying adaptive divergence of the snowfinches in a common landscape. Diversity and Distributions. 28.2579-2592. DOI:10.1111/ddi.13383

Smith, A T., Johnston, C H., Alves, P C., Hackl, K. 2018: Lagomorphs: pikas, rabbits, and hares of the world. Washington: Johns Hopkins University Press.

Song, Z., Xu, D. 2014: The identity of *Ailanthus guangxiensis* (Simaroubaceae) and lectotypification of A. integrifolia Lamarck. Phytotaxa, 173 (2): 177–180. doi: http://dx.doi.org/10.11646/phytotaxa.173.2.10

Stewart, J.R., Lister, A.M., Barnes, I., Dal´en, L. 2010: Refugia revisited: individualistic responses of species in space and time. Proc. R. Soc. B Biol. Sci. 277 (1682), 661–671. https://doi.org/10.1098/rspb.2009.1272

Su, T., Jacques, F M B., Spicer, R A., Liu, Y S., Huang, Y J., Xing, Y W., Zhou, Z K. 2013: Post-liocene establishment of the present monsoonal climate in SW China: Evidence from the late Pliocene Longmen megaflora. Climate of the Past.Volume 9 (pages: 1911–1920). doi: https://doi.org/10.5194/cp-9-1911-2013

Sun, H., Zhang, J., Deng, T., Boufford, DE. 2017: Origins and evolution of plant diversity in the Hengduan Mountains, China. Plant Diversity 39: 161–166.

Tang, Z., Wang, Z., Zheng, C., & Fang, J. 2006: Biodiversity in China's mountains. Frontiers in Ecology and the Environment, 4(7), 347-352.

Tang, Y., Li, C., Wanghe, K., Feng, C., Tong, C., Tian, F., Zhao, K. 2019: Convergent evolution misled taxonomy in schizothoracine fishes (Cypriniformes: Cyprinidae),Molecular Phylogenetics and Evolution.Vol 134.Page. 323-337. https://doi.org/10.1016/j.ympev.2019.01.008

Tietze, D. T., Päckert, M., Martens, J. Lehmann, H. 2013: Complete phylogeny and historical biogeography of true rosefinches (Aves: *Carpodacus*). Zool.J.Linn.Soc. 169, 215-234. doi: https://doi.org/10.1111/zoj.12057

Vermeij, G J. 1991: When biotas meet: Understanding biotic interchange. Science, 253: 1099–1104. DOI:10.1126/science.253.5024.1099

Wang, X., Tseng, Z J., Li, Q., Takeuchi, G T., Xie, G. 2014: From 'third pole' to north pole: a Himalayan origin for the arctic fox. Proc Biol Science. Doi: https://doi.org/10.1098/rspb.2014.0893

Wang, N. 2015: Cenozoic vertebrate evolution and paleoenvironment in Tibetan Plateau: Progress and prospects. Gondwana Res. https://doi.org/10.1016/j.gr.2014.10.014

Wang, X., Liang, D., Jin W, Tang M, Shalayiwu, Liu S., Zhang, P. 2020: Out of Tibet: Genomic Perspectives on the Evolutionary History of Extant Pikas. Mol. Biol. Evol. 37(6):1577–1592 doi: https://doi.org/10.1093/molbev/msaa026

Wen, J., Zhang, J Q., Nie, Z L., Zhong, Y., Sun, H. 2014: Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. Front Genet. DOI: 10.3389/fgene.2014.00004

Wheeler, E A., Srivastava, R., Manchester, S R., Baas, P., Wiemann, M. 2017: Surprisingly modern latest Cretaceous-earliest Paleocene woods of India. IAWA Journal. Volume: 38 (pages: 456–542). doi: 10.1163/22941932-20170174

Wilson, M.C., Smith, A.T. 2015: The pika and the watershed: The impact of small mammal poisoning on the ecohydrology of the Qinghai-Tibetan Plateau. AMBIO 44, 16–22. https://doi.org/10.1007/s13280-014-0568-x

Winston, J. E. 2007: Archives of a small planet: The significance of museum collections and museum-based research in invertebrate taxonomy. Zootaxa, 1668(1), 47–54. https://doi.org/10.11646/zootaxa.1668.1.6

Wolfgramm, H., Martens, J., Töpfer, T., Vamberger, M., Pathak, A., Stuckas, H., Päckert, M. 2021: Asymmetric allelic introgression across a hybrid zone of the coal tit (Periparus ater) in the central Himalayas. Ecology and Evolution, 11, 17332–17351. https://doi.org/10.1002/ece3.8369

Wu, F X, Miao D S, Chang, M M, Shi, G L, Wang, N. 2017: Fossil climbing perch and associated plant megafossils indicate a warm and wet central Tibet during the late Oligocene. *Sci Rep* 7, 878. https://doi.org/10.1038/s41598-017-00928-9

Zhou, C., Xiao, S., Liu, Y., Mou, Z., Zhou, J., Pan, Y., Zhang, C., Wang, J., Deng, X., Zou, M., Liu, H. 2020: Comprehensive transcriptome data for endemic Schizothoracinae fish in the Tibetan Plateau. Scientific data. Article number 28 (2020). https://doi.org/10.1038/s41597-020-0361-6

Chapter VI: Range-wide and regional distribution of the Western Tragopan *Tragopan melanocephalus* and effects of disturbance on local abundances

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Range-wide and regional distribution of the Western Tragopan *Tragopan melanocephalus* and effects of disturbance on local abundances

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Summary

The Western Tragopan *Tragopan melanocephalus* is endemic to the Western Himalayas and currently listed as 'Vulnerable' on the IUCN Red List which also emphasizes a data deficiency regarding its distribution and population size. With this study we provide new data from the Palas Valley, northern Pakistan and deliver a range wide estimate of the species current, past, and future potential distribution as derived from environmental niche models. In the Palas Valley, Western Tragopans occupied different summer habitats on north-facing slopes and winter habitats on south-facing slopes. A quantitative estimate of local populations in six side valleys was inferred from individual call-count surveys during two breeding seasons (April and May 2017, 2018) and disturbance factors were evaluated from information of local people provided in questionnaires. Generalized-linear models (GLMs) showed a significant effect of disturbance factors on Western Tragopans, i.e. local abundances decreased with increasing disturbance from livestock, collectors and hunters visiting the area. This effect was visible across survey years and at both, south- as well as north-facing slopes. While the known distributional range of the Western Tragopan is small and fragmented, our niche models inferred climatically suitable space between Himachal Pradesh and northwestern Pakistan to be more continuous. Given the species sensitivity to disturbance, these findings indicate that the observed fragmentation of the current range might also be attributed to habitat transformation or anthropogenic disturbance rather than climatic suitability. During the Last Glacial Maximum (LGM) *T. melanocephalus* was probably restricted to small forest refugia, whereas projections onto eleven future climate simulations were inconclusive with the majority suggesting that climatically suitable space for *T. melanocephalus* will likely expand in response to anthropogenic climate change. In conclusion, we recommend that future conservation measures should be planned with regard to the species' sensitivity to anthropogenic disturbances.

Introduction

The Himalayas are one of the most diverse regions of the Northern Hemisphere (Fjeldså *et al.* 2012, Favre *et al.* 2015, Martens 2015) and recognized as one of the 36 global biodiversity hotspots (Marchese 2015). The World Wildlife Fund (WWF) ranked the Western Himalayas as an ecoregion with several sub-regions, including the Western Himalayan Sub-alpine Conifer Forests (Wikramanayake *et al.* 2002). The latter extends along an elevational belt ranging from 3,000 to 3,500 m asl from the Kali Gandaki River Valley in central Nepal across north-west India to eastern Pakistan and it harbours 285 bird species, nine of which are considered "endemic or near-endemic" (Wikramanayake *et al.* 2002). The Western Himalayan Sub-alpine Conifer Forest is home to the Koklass Pheasant *Pucrasia macrolopha*, Himalayan Monal *Lophophorus impejanus*, and the near-endemic Western Tragopan *Tragopan melanocephalus*. With an estimated population of about 3,300 mature individuals and a decreasing population trend, the Western Tragopan is presently classified as 'Vulnerable' on the IUCN Red List (BirdLife International 2017) and is included in CITES Appendix 1.

The distribution range of *T. melanocephalus* extends from the Swat Valley in northern Pakistan toward Indus Kohistan, the Kaghan Valley, Kashmir, Himachal Pradesh Gharwal, and possibly into Uttarakhand (Fig. 1; BirdLife International 2001, 2020, Del Hoyo and Collar 2014). Within this range, the species inhabits extremely steep terrain covered with undisturbed

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moist deciduous forests and temperate coniferous forests with a dense shrub understorey (Ali and Ripley 1983, Islam and Crawford 1987). However, IUCN emphasized a need for further surveys to "increase the knowledge of its current distribution and abundance".

Generally, expert-prepared range maps (e.g. BirdLife International 2020), are approximations of a species' distribution and differences from occurrence point maps or modeled range estimates are expected. A recent study demonstrated that the IUCN range maps overestimated distributional ranges of 294 galliform species when compared to occurrence point maps (Ramesh *et al.* 2017, Rotenberry and Balasubramaniam 2020). For the Western Tragopan, IUCN range maps extend onto the high alpine area of the Qinghai-Tibet Plateau in Ladakh where the species has never been recorded, while numerous recent records confirm occurrences from well outside of the estimated range (Fig. 1, areas 1-3) including the Palas Valley in northern Pakistan (Fig. 1, area 1).

The Palas Valley provides a large extent of pristine forests and represents one of the most important refugia for the Western Tragopan (Raja *et al.* 1999, Saqib *et al.* 2013). While the species has been intensively surveyed in other parts of its range (Fig. 1A; Miller 2010, Saqib *et al.* 2013, Shabbir *et al.* 2018) the Palas Valley has received little attention. Himalayan pheasant species are generally sensitive to disturbance (Wikramanayake *et al.* 2002) and the presence of herders, livestock, and collectors of medicinal plants and fungi have been discussed as major threats for the Western Tragopan (BirdLife International 2001, Saberwal *et al.* 2001, Mahabal and Tak 2002, Miller 2010). Local case studies (e.g. Miller 2010, Jolli and Pandit 2011a,b), and interviews with local people (e.g. Awan and Buner 2014) identified negative effects of disturbance on *T. melanocephalus* but did not quantify pressure levels.

Here we provide new occurrences and abundance data from the Palas Valley collected between 2017 and 2019 and assess major anthropogenic disturbance factors. In addition, we present range estimates derived from environmental niche models (ENMs) to determine the present and infer the past and potential future distribution of the Western Tragopan. Niche modelling approaches have previously been applied to identify suitable habitat for *T. melanocephalus* in north-east Pakistan (Ahmad *et al.* 2017, Ali *et al.* 2015), and the Indian Western Himalaya (Singh *et al.* 2020). However, due to a very regional focus of these studies results may not be transferrable across the entire distributional range of the species. Thus, ENMs based on range-wide occurrence data might provide an improved estimate of the extent of climatically suitable space for the Western Tragopan and the potential impacts of anthropogenic climate change.

Methods

Study area

The Palas Valley (Kohistan district, Pakistan) situated on the eastern bank of the Indus River, covers an area of ~1,400 km^2 and an elevation of ~1,000 to ~5,200 m. It extends across two drainage basins (upper and lower Palas) isolated by a ridge. The area is home to at least 157 bird species (Raja *et al.* 1999). Our surveys were conducted in six side-river valleys of the upper Palas (Figs. 1B, 2) in 2017, 2018, and 2019 respectively. Three of these valleys (Takhto, Singara, and Karoser) are located on south-facing slopes north of the Musha'ga river, while the remaining three (Moru, Kabkot, and Diwan) are situated on the north-facing slopes south of Musha'ga.

Call count surveys

Dawn call-counts (Duke 1991) were used to quantify breeding populations. A total of 23 census points situated within suitable

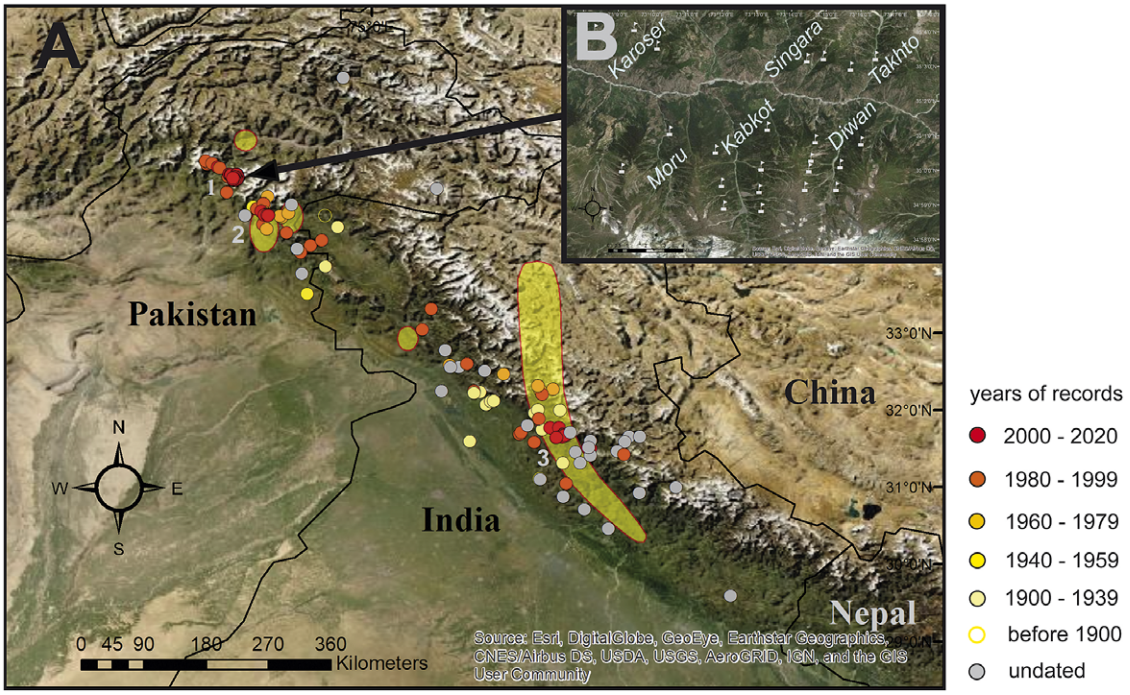

Figure 1. A) Distribution of the Western Tragopan *Tragopan melanocephalus.* The yellow shaded area represents the distribution range according to BirdLife International; dots mark occurrences before spatial filtering, colors refer to collection dates; numbers show hotspots of regional surveys and monitoring programs in the 21st century: 1 = Palas Valley, 2 = Machiara Valley, 3 = Great Himalayan National Park; B) study site Palas Valley, Pakistan, with 23 census points (flagged sites) in six side valleys.

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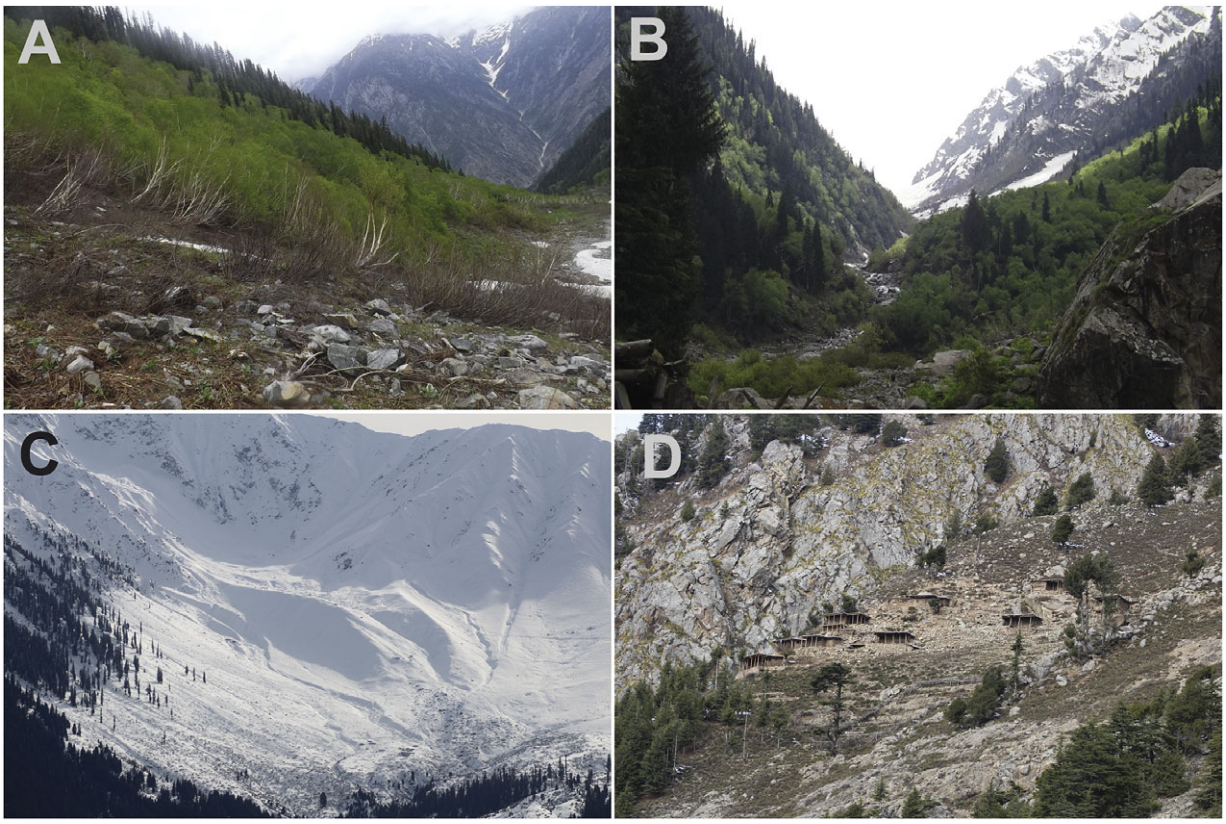

Figure 2. Study sites in the Palas Valley during summer surveys (A, B: June 2018) and winter surveys (C, D: November 2019); A) Diwan Valley, dense stands of *Betula utilis* in the foreground, view towards less densely forested Takhto Valley in the North; B) Diwan Valley, dense mixed conifer forest; C) Moru Valley, snow-covered northern aspects provide suboptimal habitat; D) Karoser Valley, southern aspects with less snow-cover provide suitable winter habitats.

habitat were selected (Fig. 3) and surveyed for 30 minutes in the early morning as recommended by Gaston *et al.* (1981) and Duke (1991).

Because the vocal activity of Asian pheasants is highest at the beginning of the breeding period (Duke 1991, Miller 2010) and strongly decreases in response to unfavourable weather conditions (e.g. heavy rainfall), call count surveys were restricted to dry and warm weather in April–May 2017 and 2018. At each census point the number of calling individuals was recorded along with altitude, GPS coordinates, and habitat characteristics such as predominant tree and shrub species. We surveyed one census point per morning. We arrived at a census point before dawn and started our observation period with the first call of a Western Tragopan, recording the number of calls during a time period of 30 min. To distinguish between calling individuals we noted the direction and the approximate distance of calling individuals within a listening radius of 300 m (Gaston *et al.* 1981, Miller 2010, Awan and Buner 2014).

Surveys of seasonal distribution

Comparison quantified local abundances in summer and winter habitats via call count surveys is problematic because vocal activity of Western Tragopans strongly decreases outside the breeding season. Therefore, seasonal distributions of pheasants across the six side-valleys were previously determined and compared using 'opportunistic sampling' (Soldatini *et al.* 2010, Elsen *et al.* 2017, Ramesh *et al.* 2017) that included all records of sightings, calls, and other evidence such as lost feathers in an area. To account for seasonal changes in local distribution, we surveyed all six side valleys towards the end of the breeding season (June 2018; Fig. 2A, B) and winter (November 2019; Fig. 2C, D). For each occurrence, the date, GPS coordinates, and elevation were recorded. Local summer and winter occurrences were visualized in a map using ArcGIS. Because no local abundances were evaluated in these surveys these data were not subject to any further statistical analysis.

Anthropogenic disturbance analyses

We estimated potential sources of anthropogenic disturbance on the basis of 106 questionnaires handed out to local villagers. We inferred sizes of herds (goats and sheep), numbers of herding dogs, hunters, and collectors of mushrooms and medicinal plants who regularly visit the area during highly sensitive periods of the breeding season (incubation and parental care). To quantify local disturbance, we calculated a total of six categorical disturbance indices for livestock (sheep, goats) and herding dogs and human disturbance (hunters and collectors) each based on the number of individuals that invaded the breeding areas. Based on these counts, we assigned low, moderate, or high disturbance levels/categories to each census point (Table 1, and Tables S1, S2 in the online supplementary material). To account for temporal effects, disturbance indices for livestock (goats, sheep, and dogs) were corrected and set to 0 for those sites where the major parts of herds arrived at a very late stage of the breeding season. Since human disturbance (plant and in particular mushroom collection) starts in early March (Sher *et al.* 2015, Laala *et al.* 2020) and therefore overlaps call counts, no correction was required.

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Table 1. Four categories of disturbance levels based on livestock numbers and people visiting the study area per season as inferred from questionnaires of 106 local persons; three livestock disturbance levels were classified according to numbers of goats, sheep and dogs accompanying herds; three anthropogenic disturbance levels were classified according to numbers of mushroom collectors, medicinal plant collectors and hunters.

Livestock disturbance		Goats/sheep	Dogs	Anthropogenic disturbance		Collectors (plants/ mushrooms)	Hunters
Very low	0	0	0	Very low	0	0	0
Low	1	1–250	1–15	Low	1	1–20	1–10
Moderate	2	251–1000	16–35	Moderate	2	21–99	11–20
High	3	>1000	36–150	High	3	>100	>20

To test for possible effects of environmental variables on local abundance we applied Generalized Linear Models (GLMs) with Poisson-error structure and local count numbers (from 23 census sites) as response variable, using R version 3.6.1 (R Core Team 2019). In the first pilot runs with GLMs that included all environmental predictor variables, models did not converge due to very strong collinearity between predictors (e.g. the variables "goats", "sheep" and "dogs" contained almost the same information; correlation coefficient close to 1). We therefore decided to condense the six environmental variables using principal component analysis (PCA). We relied on the first two principal component axes (PC1 and PC2) to model the effects of livestock and anthropogenic disturbance factors on local *T. melanocephalus* abundance. To account for an effect of multiple counts from the same census site (from successive annual surveys) we decided to run separate models for the different survey years (2017 and 2018). For each survey year, we ran two GLMs: a) including PC1 and PC2 as fixed effects and b) including PC1, slope (north-facing or south-facing) and the interaction between PC1 and slope as fixed effects. The intention of (b) was to allow testing for the impact of livestock and anthropogenic disturbance independently for north- and south-facing slopes. To test whether slopes predicted by the models differed from 0, we used the *emmeans* package (Lenth 2020). All tests were performed on the log-link scale of the original model coefficients. We provide the R script in Appendix S1.

Environmental niche models

We applied ENMs to estimate the current and infer the past and future potential distribution of the Western Tragopan. Occurrences from our own field research were supplemented with records from online databases (GBIF: http://data.gbif.org, VertNet: http://vertnet.org [metadatabase of mainly US American museum collections], Xeno-canto: https://www.xeno-canto.org [global database of sound recordings], and the Oriental Bird Club Image database: http://orientalbirdimages.org [image database of Asian bird species]), records obtained from BirdLife International (2001), and scientific publications (Table S3). All records were examined for vague or imprecise locality information to ascertain their reliability (Awan *et al.* 2016). Records lacking coordinates were georeferenced using Google Earth. The final set of 176 records (Fig. 1A; Table S3) was filtered to a Euclidian distance of 5 km to remove spatially clustered occurrences, retaining 83 unique records for model building. As predictors we obtained a global terrain model (GEBCO Compilation Group, 2020) and a set of 19 bioclimatic variables with a spatial resolution of 2.5 arc-minutes representing annual trends, seasonality, and limiting environmental factors from WorldClim (http://worldclim.org, Hijmans *et al.* 2005). To prevent multicollinearity among predictors during projection (Braunisch *et al.* 2013, Dormann *et al.* 2013) we selected nine uncorrelated (Pearson correlation coefficient $R^2 \leq 0.70$) predictors (bio 2, bio 3, bio 7, bio 10, bio 13, bio 15, bio 17, bio 19, and elevation) based on jackknifing and variable importance of an initial run. To reconstruct the potential distribution during the Last Glacial Maximum (LGM; ~22,000 years before present) we obtained matching predictors from climate reconstructions based on three general circulation models (GCMs); namely the Community Climate System Model (CCSM4) (Gent *et al.* 2011), the Max-Planck-Institute Earth System Model P (MPI-ESM-P), and the Model for Interdisciplinary Research on Climate (MIROC) (Hasumi and Emori 2004) from WorldClim. To predict the potential impacts of future climate change we obtained 11 future simulations for 2070 (representing an average for 2061–2080) derived from downscaled climatologies of the Coupled Modelling Intercomparison Project 5 (CMIP5). For each simulation four representative concentration pathways (RCPs), describing possible future greenhouse gas concentration trajectories including moderate (RCP 2.6), intermediate (RCP 4.5, RCP 6.0) and extreme conditions (RCP 8.5) were obtained from WorldClim.

We employed the machine learning algorithm MaxEnt 3.4.1 (Phillips *et al.* 2006, 2019, Phillips and Dudík 2008) with a circular buffer of 200 km surrounding each record used as background for model training and a rectangular bounding box as projection area. The feature classes linear, quadratic, and product were selected. A bootstrapping method with 100 replicates, randomly splitting the data set into a training (80%) and a testing subset (20%), was applied. Extrapolation was not allowed to reduce uncertainties due to projections onto non-analogous climates (Fitzpatrick and Hargrove 2009, Rocchini *et al.* 2011). Subsequently the resulting model was projected onto paleoclimatic conditions of the LGM and future climate change scenarios respectively. For all projections we applied multivariate environmental similarity surface (MESS) analyses (Elith *et al.* 2010) to identify areas where one or more predictor variables experience conditions beyond the respective calibration range.

For current as well as LGM and future projections, the area under the curve (AUC) was used as a threshold-independent measure of discrimination ability (Swets 1988, Ling *et al.* 2003). An AUC score of 1 refers to a perfect fit of the data while a score of 0.5 is considered no better than random (Elith *et al.* 2006, Phillips *et al.* 2006). The average projection across all replicate runs was used for further processing, wherein the '10 percentile training presence cloglog threshold' was applied as presence-absence threshold.

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Results

Field surveys

During the breeding season, calling individuals could be recorded at 15 of the 23 census points located in three valleys: Kabkot and Diwan on the north-facing slopes, and Singara at the south-facing slopes (Figs 3, 4). In Kabkot and Diwan, Western Tragopans were recorded in dense mixed coniferous-deciduous forests (Fig. 2A, B) dominated by *Abies pindrow* and *Pinus wallichiana* with a higher proportion of birch *Betula utilis* in Diwan (Fig. 2A). Forest vegetation in the Singara Valley was characterized by the presence of oak *Quercus* spp. During the winter survey in November 2019, Western Tragopan were exclusively recorded from two side valleys on the sun-exposed south-facing slopes north of the main river (Figs. 2D, 4), whereas the species was not recorded in breeding habitats on the now snow-covered north-facing slopes (Figs 2C, 4).

Local numbers of birds per census point inferred from call counts during the breeding season ranged between two and seven in 2017 and 5 and 10 in 2018, with highest individual numbers recorded in Kabkot Valley (Table 2; Fig. 3).

Anthropogenic and livestock pressure differed greatly among the six side valleys. Highest disturbance indices were estimated for the Moru Valley, where highest numbers of herds were registered (120–150 herds totalling 2,000–2,500 individuals; Tables S1, S2). With no regular visits of herds during the study period, Singara had the lowest livestock disturbance.

In both PCAs (disturbance variables uncorrected and corrected for timing of arrival) the first principal component (PC1) explained 63% and 67% of the total variation, respectively, and showed strongest positive correlations with livestock pressure variables (numbers of sheep, goats, and dogs; factor loadings ranged between 0.53 and 0.56; for details see Table S4). PC2 explained 14% and 24% of the total variance, respectively, but, unlike PC1, was positively correlated with livestock pressure variables but negatively correlated with anthropogenic pressure variables (Table S4). For both study seasons (2017, 2018) GLMs revealed a significant effect of disturbance levels on local abundance of Western Tragopans (Tables 3a, 3b) with numbers of calling individuals decreasing with increasing local disturbance from livestock (Fig. 5; Table 3a; only the effect of PC1 in 2017 was marginally not significant [P = 0.051]). This effect was generally confirmed when testing separately for data subsets from opposite slopes in the Pallas Valley (shown for PC1 in Fig. 6; model coefficients under "PC1" in Table 3b refer to the south-facing slope; for the north-facing slope, on the log-link scale [2017/2018]: estimate = –0.213/–0.278, SE = 0.091/0.076, z = –2.343/–3.683, P = 0.019/ <0.001).

Species distribution models

The discrimination ability of the model for current climatic conditions was high across replicate runs ($AUC_{training}$ = 0.95 ± 0.01 *SD*; AUC_{test} = 0.94 ± 0.01 *SD*). The model predicted climatically suitable space to stretch along the Pakistan and India portions of the Western Himalayas (Fig. 7). The extent of potentially suitable space measures approximately 750 km x 50 km and extends from Mingora in northern Pakistan to Uttarkashi in India with an area of highest predicted suitability for Western Tragopan between Palampur and Rohru in India (Fig. 7).

Model projections onto paleoclimatic reconstructions received high AUC scores as well (AUC_{test} CCSM4 = 0.94 ± 0.01 SD;

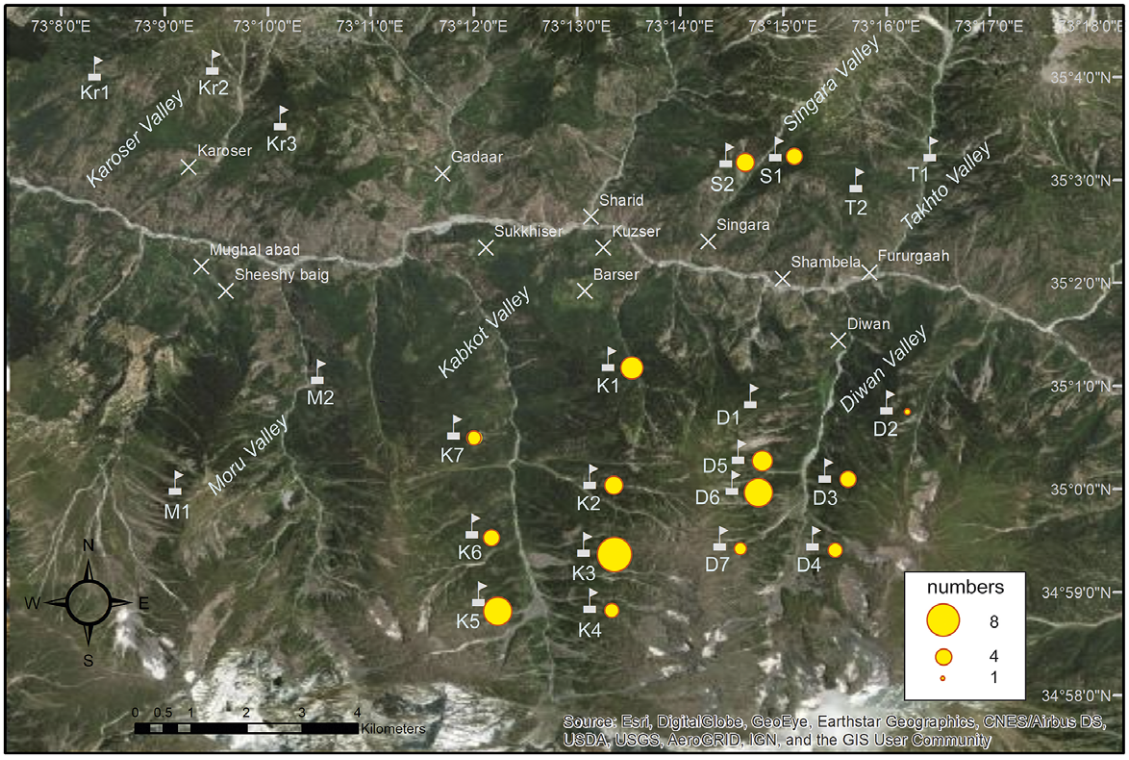

Figure 3. Local abundances of Western Tragopan *Tragopan melanocephalus* in the Palas Valley, Pakistan, inferred from call count surveys across 23 census sites (flagged sites: (Kr1-Kr3, S1-S2, T1-T2, M1-M2, K1-K7, D1-D7), during the breeding season (surveys in April and May 2017 and 2018); circle size equivalent to mean numbers of counted individuals across two years of survey.

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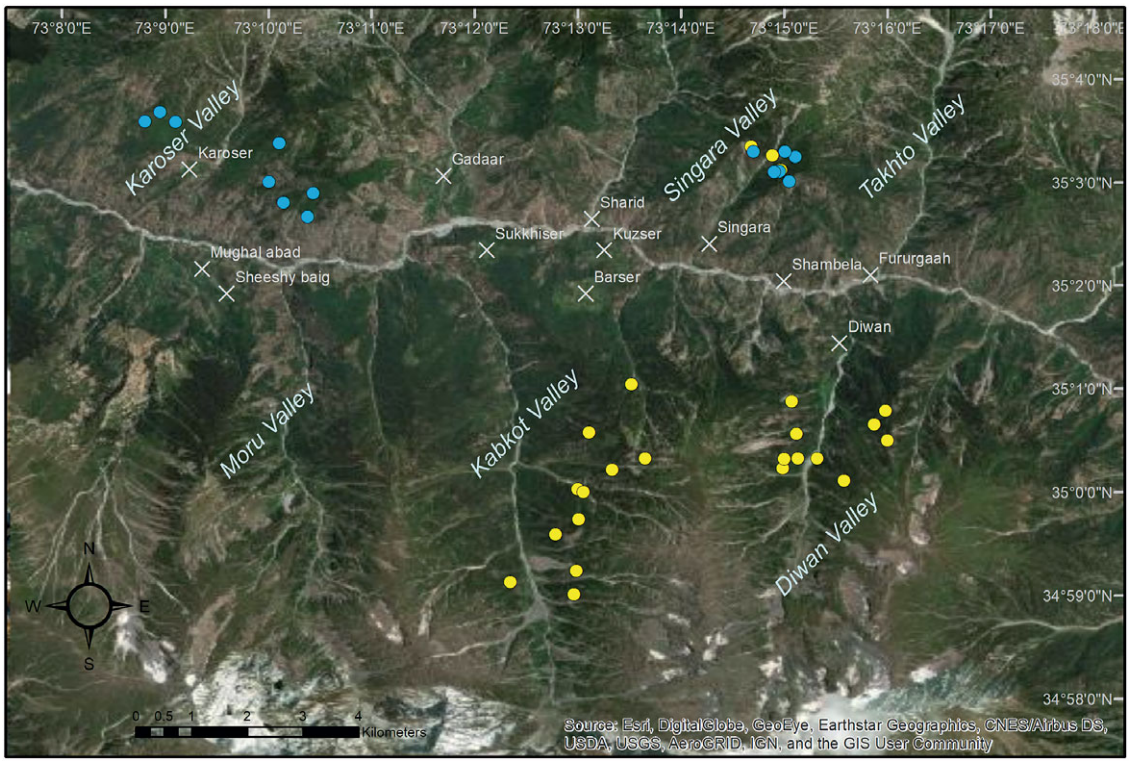

Figure 4. Local distribution of Western Tragopan (*Tragopan melanocephalus*) occurrences in the Palas Valley, Pakistan, during the breeding season (June 2018; yellow dots) and winter season (November 2019; blue dots); each dot refers to one sighting.

Table 2. Individual numbers of Western Tragopan *Tragopan melanocephalus* observed at 23 census points (Kr1-Kr3, S1-S2, T1-T2, M1-M2, K1-K7, D1-D7) during call count surveys in 2017 and 2018 (including means from both years); population density estimated for an assumed survey area of 0.28 km square per census point.

Valley	Number of individuals (*n*)/ calling sites		
	2017	2018	Mean
Karoser	**0**	**0**	**0**
Kr1	0	0	0
Kr2	0	0	0
Kr3	0	0	0
Singara	**5**	**12**	**4.3**
S1	3	5	4
S2	2	7	4.5
Takhto	**0**	**0**	**0**
T1	0	0	0
T2	0	0	0
Moru	**0**	**0**	**0**
M1	0	0	0
M2	0	0	0
Kabkot	**32**	**42**	**5.2**
K1	5	4	4.5
K2	3	6	4.5
K3	7	10	8.5
K4	2	5	3.5
K5	7	9	8
K6	4	4	4
K7	2	5	3.5
Diwan	**16**	**32**	**3.4**
D1	0	0	0
D2	0	1	0.5
D3	3	5	4
D4	2	7	4.5
D5	4	6	5
D6	5	9	7
D7	2	4	3

AUC$_{test}$ MPI-ESM-P = 0.94 ± 0.01 SD; AUC$_{test}$ MIROC = 0.94 ± 0.01 SD). While all three models consistently suggest that the extent of suitable space for Western Tragopan was smaller during the LGM, they significantly differ in predicted extents of suitable space. The CCSM4 projection predicted the largest extent (72% of the area presently predicted as suitable) and suggests that suitable space has only slightly shifted north since the LGM (Fig. 7). However, the MPI-ESM-P and MIROC projections concordantly suggest that suitable space will be restricted to small glacial refugia (measuring

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Table 3a. Model coefficients (including standard error= SE and z-test statistics when tested vs. 0) of GLMs for field data from all 23 census points for the first two principal components (based on six environmental variables of livestock and anthropogenic disturbance) for each of the two survey seasons (2017 and 2018); coefficients and SE are presented on the log-link scale; z value= estimate / SE; significance codes: n.s. = not significant; p< 0.05 = *; p < 0.01 = **; p < 0.001 = ***.

	Year	Estimate	SE	z	p-value	Code
Intercept	2017	0.587	0.167	3.509	< 0.001	***
	2018	1.113	0.130	8.546	< 0.001	***
PC1	2017	−0.180	0.092	−1.955	0.051	n.s.
	2018	−0.291	0.077	−3.773	< 0.001	***
PC2	2017	−0.515	0.134	−3.836	< 0.001	***
	2018	−0.396	0.099	−3.985	< 0.001	***

Table 3b. Model coefficients (including standard error = SE and z-test statistics when tested vs. 0) of GLMs for field data from all 23 census points for slope (south- or north-facing), the first principal component (based on six environmental variables of livestock and anthropogenic disturbance) and their interaction, for each of the two survey seasons (2017 and 2018); coefficients and SE are presented on the log-link scale; z value= estimate / SE; significance codes: n.s. = not significant; *P* < 0.05 = *; *P* < 0.01 = **; *P* < 0.001 = ***.

	year	estimate	SE	z	p-value	Code
Intercept	2017	−3.160	1.912	−1.653	0.098	n.s.
	2018	−3.228	1.639	−1.970	0.049	*
PC1	2017	−1.861	0.911	−2.043	0.041	*
	2018	−2.313	0.759	−3.048	0.002	**
slope	2017	4.197	1.918	2.189	0.029	*
	2018	4.723	1.643	2.874	0.004	**
PC1 : slope	2017	1.648	0.915	1.800	0.072	n.s.
	2018	2.035	0.763	2.669	0.008	**

only 24% and 39% of the area of the current climate model respectively) situated in the eastern parts of the present distributional range (Fig. 7) including areas, where the species is not present today, e.g. western Nepal (Fig. 7).

Projections onto future climate change scenarios derived from 11 GCMs were inconclusive. Seven simulations predicted suitable space to increase across all four RCPs while two GCMs were conclusive across all RCPs in predicting suitable space to shrink in response to climate change (Fig. 8, Figs. S1-S5; Table 4, Table S1). The remaining two GCMs yielded mixed results, with a slight decrease predicted for the moderate RCP 2.6 and increases predicted for RCPs 4.5, 6.0, and 8.5 respectively.

Both GCMs previously used for paleoclimatic reconstructions (CCSM4 and MIROC-ESM) consistently suggest climatically suitable space to expand across all RCPs by 2070. While the CCSM4 projections predict an increase between 50 and 101% (given as percentage of the current extent of suitable space) the MIROC-ESM projections suggest an increase between 37% and 79% (Fig. 8, Table 4). Variable contribution for current, past, and future climatic conditions was similar with mean temperature annual range (bio 7) having the highest contribution (permutation importance: 24.8-28.4%) to the model, followed by precipitation seasonality (Bio 15, permutation importance: 21.1-24.2%), precipitation of the driest quarter (Bio 17, permutation importance: 16.1-18.3%), mean temperature of the warmest quarter (bio 10, permutation importance: 10.4-15.2%), elevation (permutation importance: 7.0-12.1%), and isothermality (bio 3, permutation importance: 5.2-5.8%). Contribution of the remaining predictors did not exceed 5%. For details on variable contributions across all models see Table 4 and Table S1.

Discussion

Population survey and potential disturbance factors

We confirmed that 15 of the 23 census points located in the Palas Valley currently hold breeding populations of Western Tragopan and recorded a mean of 0.5–8.5 calling individuals per site. These numbers correspond to those inferred from previous monitoring results from the late 1990s and emphasize the importance of the Palas Valley for the conservation of the species (BirdLife International 2001). GLMs revealed significantly lower abundances at disturbed sites supporting the postulated sensitivity of the species to disturbance (Miller 2010, Jolli and Pandit 2011a,b, Awan and Buner 2014). We could no longer confirm the species at two sites located on north-facing slopes within the Moru sub-valley although it was previously reported to occur in this area (BirdLife International 2001). There, disturbance is associated with highest numbers of both livestock and of mushroom and plant collectors (Table S2) whose activities overlap with the early breeding season (Sher *et al.* 2015). Generally, we found a greater negative effect of livestock disturbance on the Western Tragopan in the Palas Valley as compared to anthropogenic disturbance, such as collectors visiting the area. In that context, we might consider that even regular presence of livestock during the late breeding season could lead to generally less suitable or less attractive habitat for pheasant or grouse species due to a long-term effect of overgrazing (BirdLife International 2001, Dettenmaier *et al.* 2017). Accordingly, densities of other Himalayan pheasants were also found to decrease with increasing livestock pressure within the course of one year (Bhattacharya *et al.* 2009). In contrast, a comparatively lower sensitivity to regular presence of collectors or tourists might be related to the flexible foraging behaviour of some pheasant species like the Himalayan Monal *Lophophorus impejanus*, that was shown to shift its peak of daily activity to avoid temporal overlap with human activities (Sharief *et al.* 2022).

Apart from anthropogenic disturbance, the exposure of a slope might help to explain the distribution and local abundance of Western Tragopans in the study area. According to BirdLife International (2001) sun-exposed, south-facing slopes with reduced snow cover are preferred during winter but knowledge on seasonal movements is scarce and compromised by uncertainties and discrepancies between studies and winter occurrences on scarcely vegetated, sun-exposed north-facing slopes were also reported (Mahabal and Tak 2002, Sing and Tu 2008). Nevertheless, seasonal habitat preferences might explain why we did not observe Western Tragopans at undisturbed sites in Karoser Valley on the south-facing slopes during the breeding season in three successive years, whereas birds were present in that area in winter (Fig. 4).

Past, present, and future distribution of the Western Tragopan

Compared to the species' range depicted in the BirdLife International shape file (Fig. 1A), our model suggested a broader and

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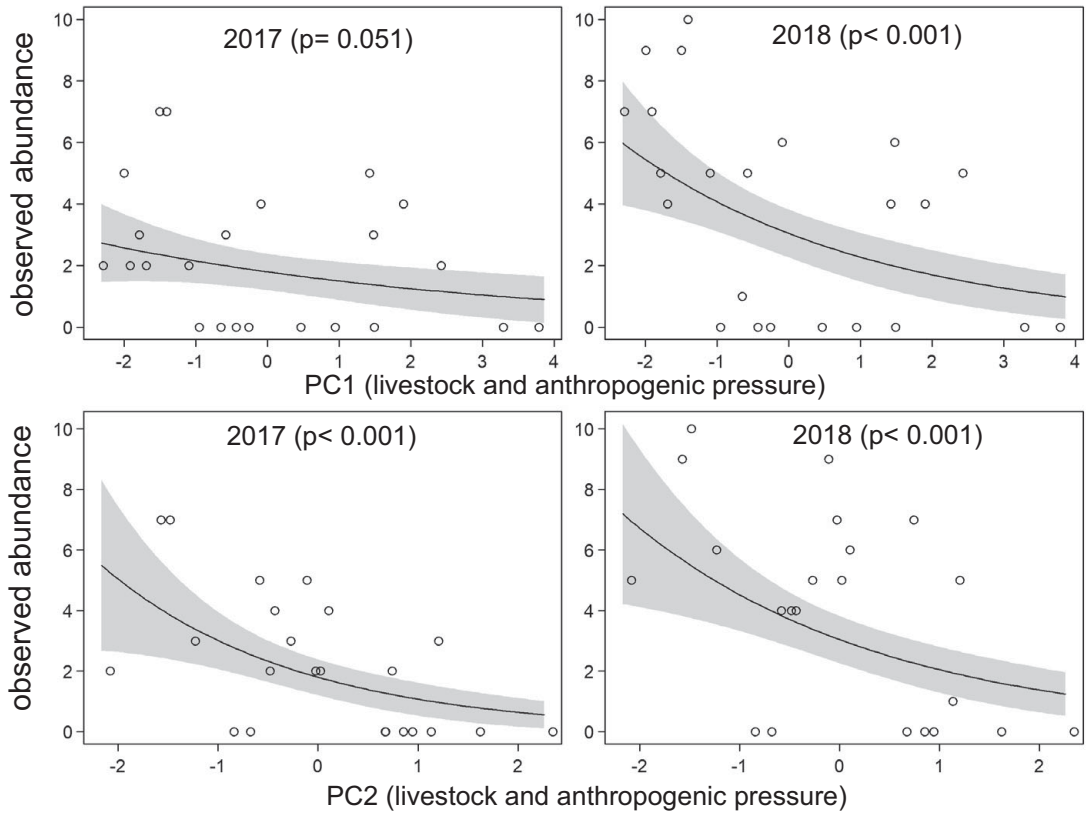

Figure 5. Local abundances of calling Western Tragopans across all 23 survey sites in response to PCA-based disturbance variables (PC1 and PC2 for six predictor variables [numbers of goats, sheep, dogs, plant collectors, mushroom collectors and hunters]) during two field seasons; solid lines = GLM smoothing curves, grey shades = 95% confidence bands.

less fragmented area to be climatically suitable (Fig. 7). Considering the species' sensitivity to disturbance, these discrepancies could indicate that the observed distribution is already a result of habitat transformation and anthropogenic disturbance. Records since 2000 represented only a very limited area of the projected range, i.e. a few major national parks or nature reserves (Fig. 1: sites 1-3). From these records it is difficult to judge whether the lack of recent observations is due to a greater focus on local monitoring projects during the past two decades or whether populations where the species occurred in the 20th century have indeed gone extinct.

Because analogous data for biologically meaningful predictor variables such as vegetation or land-use are regrettably not available for the selected past and future scenarios, our niche models solely rely on terrain and climatic predictor variables. The use of predictors derived from a digital elevation model and remote sensing data in recent SDM approaches, restricted to current climatic conditions, resulted in a significantly more scattered and patchier estimate of potentially suitable space for Western Tragopans in Pakistan (Ali *et al.* 2015). However, due to the very localized focus of that study which was restricted to only 32 sightings, all from the species' extreme north-western range limits, it is possible that only a fraction of the species' climatic niche was captured.

Paleoclimatic projections suggested that the Western Tragopan has likely survived the LGM in glacial refugia located in the Western Himalayas. According to the palynological record, paleoforest vegetation in the Western Himalayas underwent regular shifts between conifer forests dominated by *Pinus* and *Abies* species during warm cycles and evergreen oak *Quercus semecarpifolia* and alder *Alnus* forests during cold cycles (Manish and Pandit 2018). While these paleoforests might have harboured suitable glacial refugia for the species, fossil pollen records indicate that the higher elevations of the Western Himalayas were presumably covered by a community of *Artemisia* spp., chenopods, and grass, lacking suitable forest habitat (Behrensmeyer *et al.* 1992). According to the CCSM4 and the MIROC-ESM projections of the model climatically suitable space for the Western Tragopan was indeed restricted to small, isolated patches and had probably completely vanished from the western part of its current range. Accordingly, the mountain forests of Pakistan would have been (re-)colonized during post-glacial range expansion along forest corridors in the Kashmir Valley region.

Projections onto 11 global circulation models were inconclusive but the majority of models (including the previously used GCMs CCSM4 and MIROC-ESM) suggest that climatically suitable space for the Western Tragopan will expand rather than shrink. On that point, our results strongly disagree with Singh *et al.* (2020) who projected their SDM onto three RCPs (4.5, 6.0, and 8.5) of a single GCM (Miroc5) and postulated that suitable habitat in the Indian Western Himalaya will strongly decrease by 2070. In contrast the projection of our model onto the same GCM suggests climatically suitable space to increase significantly across all four RCPs (Table S1). These differences may be attributed in parts to the limited regional focus of the former study capturing only a fraction of the species' environmental niche. However, such conflicting results demonstrate the importance of evaluating several preferably unrelated GCMs before drawing conclusions.

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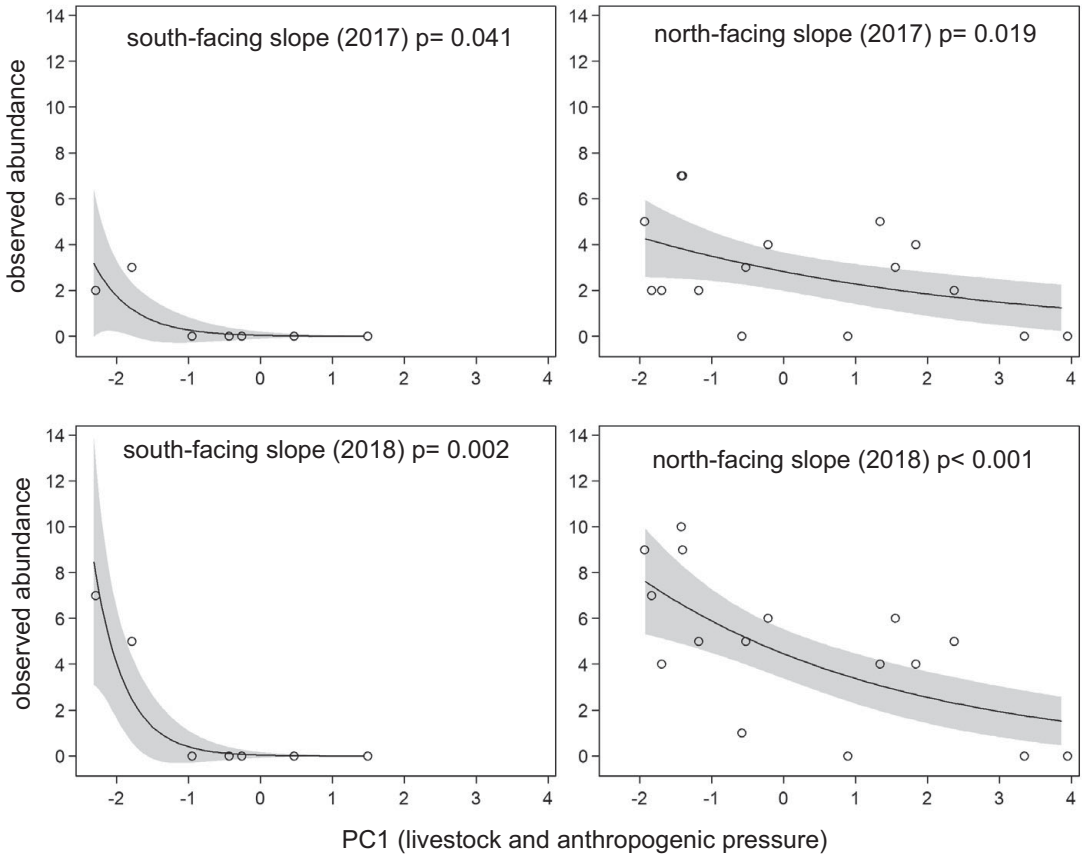

Figure 6. Local abundance of calling Western Tragopans in response to PCA-based disturbance variables (PC1 for six predictor variables [numbers of goats, sheep, dogs, plant collectors, mushroom collectors and hunters]) at different slopes (south-facing: $n = 7$ sites; north-facing, $n = 16$ sites) during two field seasons; solid lines = GLM smoothing curves, grey shades = 95% confidence bands.

While environmental niche models are a suitable tool to aid conservation management decisions it is of utmost importance to be aware of their limitations. For instance, environmental niche models inferred from climate data alone cannot reflect dispersal limitations, biotic interactions, or effects of metapopulation processes. In addition, other drivers of population decline and local extinction, such as anthropogenic pressure or land use change, are not captured by climatic models but will also heavily affect the species' future distribution. These impacts might even counteract any positive effect of the predicted increase of suitable space.

Implications for conservation management

In the 1990s, the Western Tragopan became the flagship species of the Himalayan Jungle Project. After initial opposition from villagers in remote areas, such as the Palas Valley, a continuous dialogue on sustainable forest management and hunting policies was established (Fuller and Garson 2000, Knudsen 2009). Since 2001 the Palas Conservation and Development Project (PCDP) as the successor initiative, conducted several surveys and monitoring programmes for investigation and protection of the natural and cultural heritage of Pakistan. While in many parts of the Western Himalayas Western Tragopans suffered from habitat loss due to increased commercial exploitation of forest ecosystems (BirdLife International 2001), the Palas Valley still harbours large areas of undisturbed 'pristine landcover' (Saqib *et al.* 2013). Yet also in the Palas Valley, logging and illegal hunting have been identified as major threats for the Western Tragopan and other pheasant species (Sutherland 2000). Future conservation measures should be planned in close dialogue with local people, because for example morel *Morchella* spp. trade represents an important economic factor in the Palas Valley (Hamayun *et al.* 2006, Sher *et al.* 2015, Laala *et al.* 2020). At present only 2% of the species' potential habitat in Pakistan is covered by protected areas (Awan *et al.* 2021). For other areas, including the Great Himalayan National Park, GIS modelling of species-habitat associations suggested that only 10% of the protected area provides highly suitable habitat, whereas about 44% was unsuitable for Western Tragopans (Naithani *et al.* 2018). Future research should further determine impacts of disturbance on the local abundance of Western Tragopan. In addition, intensive surveys beyond the known major populations (Fig. 1A) are required e.g., in Northwest India (Himachal Pradhesh, Jammu and Kashmir) to assess whether extant populations of the Western Tragopan still exist west of the Great Himalayan National Park.

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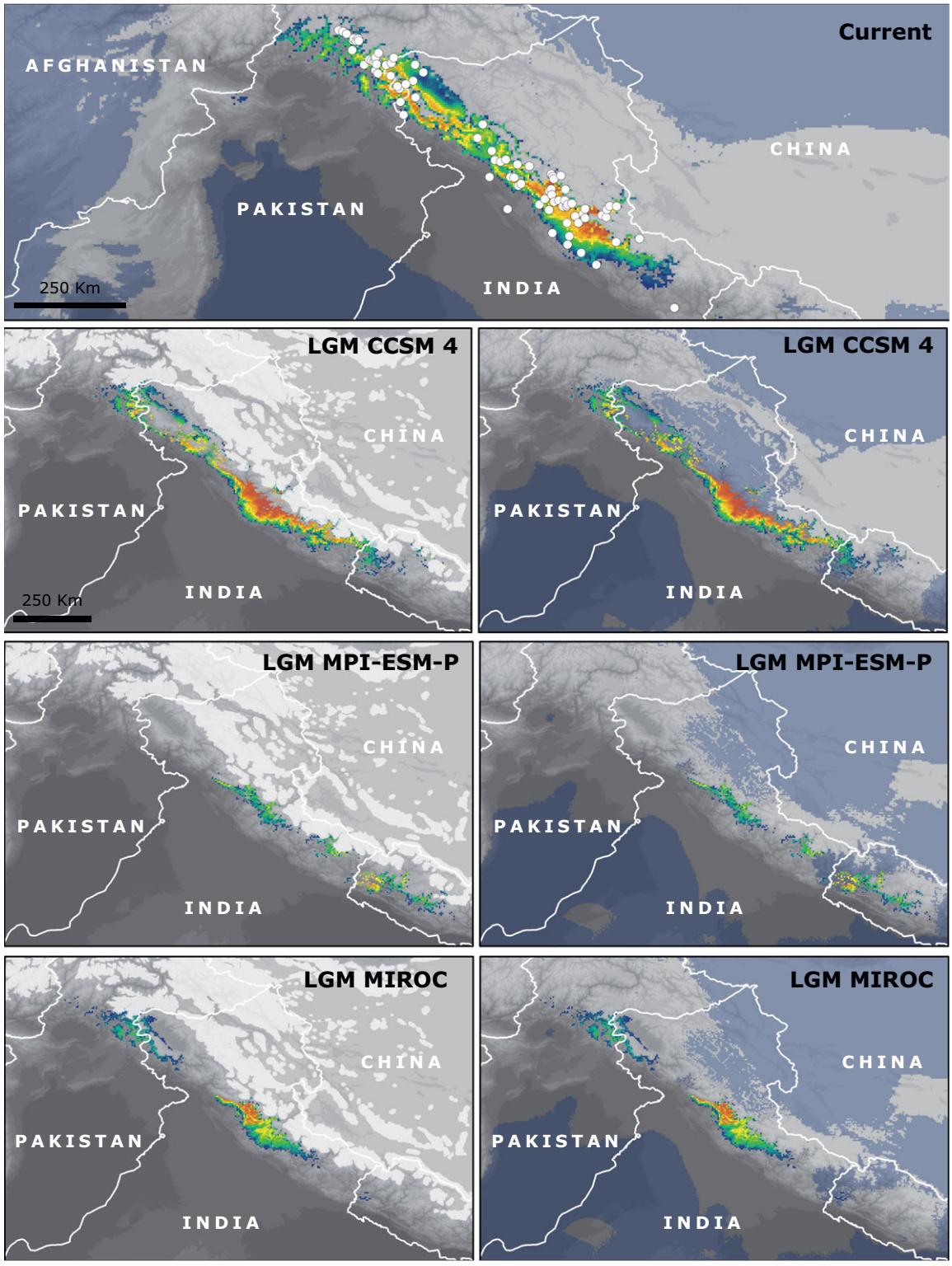

Figure 7. Top: Current potential distribution of the Western Tragopan as derived from MaxEnt with records used to build the model displayed as white dots. Below: Projections onto climatic conditions of the Last Glacial Maximum (LGM) according to the global circulation models CCSM4, MPI-ESM-P, and MIROC. Suitability ranges from moderate (dark blue) to high (red). On the left, the extent of ice sheets during the LGM is displayed as light grey area (https://crc806db.uni-koeln.de/layer/show/6) while on the right MESS areas where climatic conditions exceed those of the calibration range are displayed in blue.

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Table 4. Contribution of environmental predictor variables, AUC values, and extent of environmentally suitable space for current, past, and future climatic conditions.

time period		Percent contribution [%] / Permutation importance [%]											
						future projections							
model			LGM projections			CCSM4				MIROC-ESM			
Variable		current	CCSM4	MPI-ESM-P	MIROC	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5
Bio 2	Mean diurnal T range	8.7/1.0	7.6/2.0	6.6/1.5	7.1/1.3	6.9/2.2	6.8/0.7	7.8/1.2	7.4/1.8	6.9/1.9	7.5/2.5	6.7/1.5	7.4/1.5
Bio 3	Isothermality	4.2/5.7	4.2/5.8	4.3/5.5	4.1/5.2	4.2/6.6	4.4/4.8	4.4/5.3	4.4/6.2	4.5/3.7	4.7/6.3	4.6/6.6	4.3/7.0
Bio 7	Mean T annual range	15.4/27.2	15.3/28.4	14.4/28.1	13.4/24.8	14.3/30.0	13.8/26.3	15.2/27.7	12.7/28.5	15.3/28.9	15.6/21.5	14.6/28.7	13.8/30.6
Bio 10	Mean T of the warmest quarter	2.9/15.2	2.3/13.1	1.9/10.4	2.6/13.9	2.0/11.9	2.5/13.2	2.9/10.4	2.7/12.1	2.6/14.0	2.6/10.6	2.4/11.4	2.3/7.8
Bio 13	Precipitation of the wettest month	1.5/2.5	1.6/2.9	1.9/4.8	1.7/4.8	1.4/2.1	1.8/4.0	2.0/4.7	1.5/2.3	1.8/3.3	1.5/3.6	1.3/3.3	1.5/3.2
Bio 15	Precipitation seasonality	8.2/21.1	8.4/21.8	8.0/21.4	8.1/24.2	7.5/19.6	7.9/ 26.1	8.1/25.1	7.2/23.0	8.4/18.1	8.0/26.7	8.7/19.9	7.8/25.3
Bio 17	Precipitation of the driest quarter	43.3/18.1	49.2/16.1	51.2/18.3	51.1/16.8	52.1/18.5	51.9/14.2	45.9/13.1	52.7/17.8	46.8/20.4	45.2/19.2	50.4/19.7	51.4/14.9
Bio 19	Precipitation of the coldest quarter	9.7/2.2	5.3/2.8	5.8/3.6	5.5/2.3	5.3/2.9	5.4/3.4	7.7/2.8	5.3/2.4	8.2/3.4	9.5/3.0	5.1/3.0	5.2/3.2
Elevation	Elevation	6.1/7.0	6.1/7.2	5.7/6.3	6.4/6.7	6.2/6.2	5.5/7.2	6.1/9.7	6.1/5.9	5.5/6.4	5.3/6.6	6.2/5.9	6.3/6.5
Area [%]		100	72	24	39	150	162	197	201	179	137	153	159
Training AUC ± *SD*		0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.94 ± 0.01
Test AUC ± *SD*		0.94 ± 0.02	0.94 ± 0.01	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.01	0.94 ± 0.02	0.94 ± 0.02

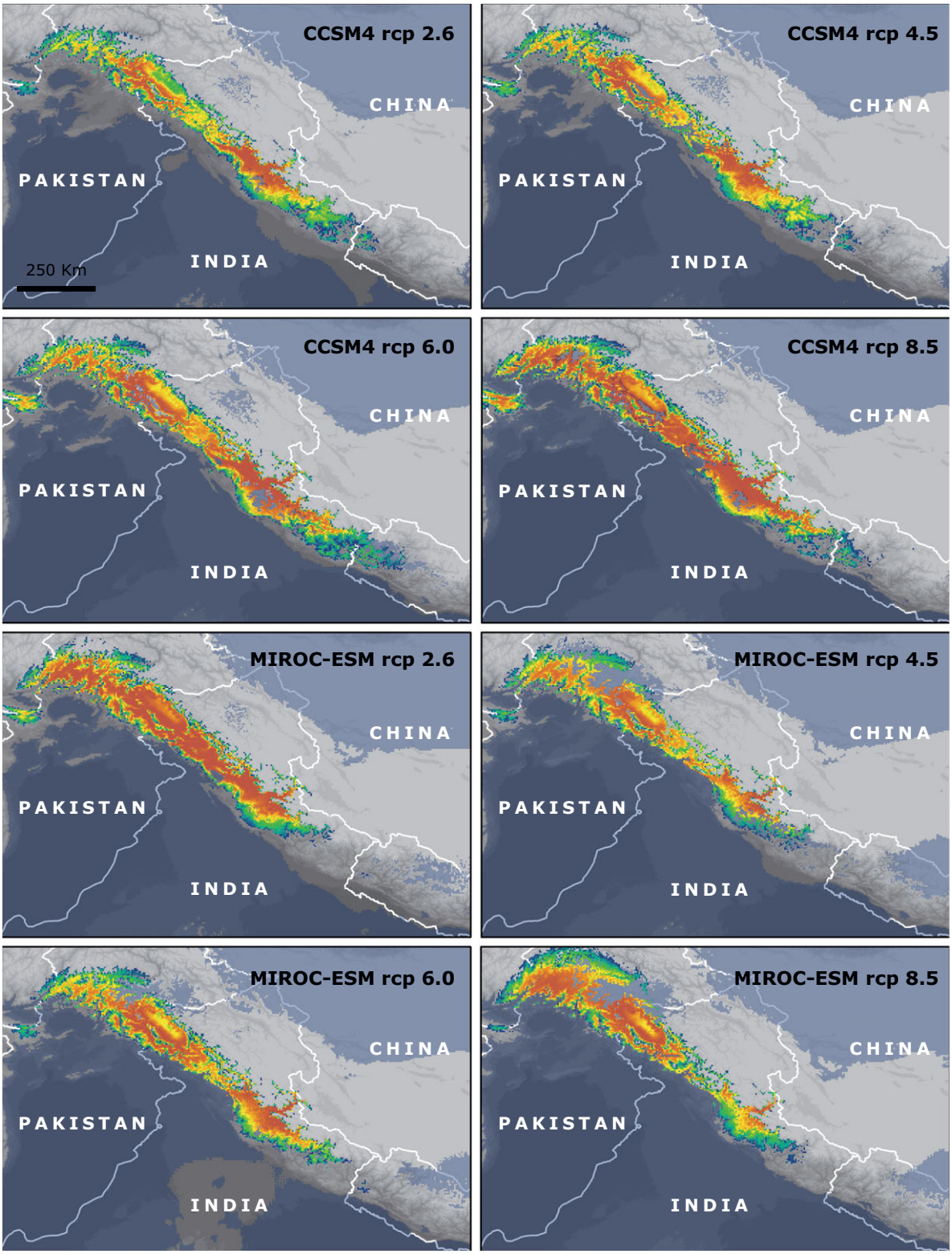

Figure 8. Potential future distribution of the Western Tragopan *Tragopan melanocephalus* according to MaxEnt models projected onto climatic conditions for 2070 as derived from the global circulation models; CCSM4 and MIROC. Suitability ranges from moderate (dark blue) to high (red). Regions where climatic conditions exceed those of the calibration range (MESS) are displayed in blue.

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Supplementary Materials. To view supplementary material for this article, please visit http://doi.org/10.1017/S0959270922000120.

References

Ahmad, R., Sharma, N., Pacchnanda, U., Suhai, I., Deb, K., Bhatnagar, Y. V. and Kaul, R. (2017) Distribution and conservation status of the western tragopan *Tragopan melanocephalus* in Jammu and Kashmir, India. *Curr. Sci.* **112**: 1948–1953.

Ali, H., Akram, U., Abbas, S., Ahmed, M. S., Qamer, F. M., Khan, B., Awan, M. N., Ali, Z., Chaudry, A. A., Saghir, A. and Nagai, M. (2015) Predicting the potential habitat and distribution of Western Tragopan (*Tragopan melanocephalus*) in selected areas of AJ&K, Pakistan: a Maxent Modelling approach. *J. Anim. Plant. Sci.* **25**: 318–323.

Ali, S. and Ripley, S. D. (1983) *Handbook of the birds of India and Pakistan*. New Delhi, Oxford, New York: Oxford University Press.

Awan, M. N. and Buner, F. (2014) Conservation of the Western Tragopan (*Tragopan melanocephalus*) around Salkhala Game Reserve, Azad Kashmir, Pakistan. *Birding ASIA* **21**: 107–111.

Awan, M. N., Buner, F. and Kingdon, N. (2016) A review of published and unpublished surveys of a red-listed flagship species, the Western Tragopan *Tragopan melanocephalus* in Azad Jammu and Kashmir, Pakistan. *Bird Conserv. Internatn.* **26**: 380–395.

Awan, M. N., Geldmann, J., Buner, F., Saqib, Z., Pervez, A., Mahmood, Q., Hashem, A., Al-Arjani, A. F., Alqarawi, A. A., Adh Allah, E. F. and Akbar, T. A. (2021) The effectiveness of protected areas in conserving globally threatened Western Tragopan *Tragopan melanocephalus*. *Animals* **11**: 680.

Behrensmeyer, A. K., Damuth, J. D., diMichele, W. A., Potts, R., Sues, H. D. and Wing, S. L. (eds) (1992) *Terrestrial ecosystems through time – evolutionary paleoecology of terrestrial plants and animals*. Chicago and London: University of Chicago Press.

Bhattacharya, T., Sathyakumar, S. and Rawat G. S. (2009) Distribution and abundance of Galliformes in response to anthropogenic pressures in the buffer zone of Nanda Devi Biosphere Reserve. *Int. J. Galliformes Conserv.* **1**: 78–84.

BirdLife International (2001) *Threatened birds of Asia: the BirdLife International Red Data Book*. Cambridge, UK: Birdlife International.

BirdLife International (2017) *Tragopan melanocephalus* (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2017: e.T22679147A112467383. https://doi.org/10.2305/IUCN.UK.2017-1.RLTS.T22679147A112467383.en. Downloaded on 19 May 2021.

BirdLife International (2020) Species factsheet: *Tragopan melanocephalus*. http://www.birdlife.org. Accessed on 15 April 2020

Braunisch, V., Coppes, J., Arlettaz, R., Suchant, R., Schmid, H. and Bollmann, K. (2013) Selecting from correlated climate variables: A major source of uncertainty for predicting species distributions under climate change. *Ecography* **36**: 971–983.

DelHoyo, J. and Collar, N. J. (2014) *HBW and BirdLife International illustrated checklist of the Birds of the World. Volume* **1**: *Non-passerines*. Barcelona, Spain and Cambridge, UK: Lynx Edicions and BirdLife International.

Dettenmaier, S. J., Messmer, T. A., Hovick, T. J. and Dahlgren, D. K. (2017) Effects of livestock grazing on rangeland biodiversity: A meta-analysis of grouse populations. *Ecol. Evol.* **7**: 7620–7627.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., García Marquéz, J. R., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüler, T., McClean, C., Osborne, P. E., Reineking, B., Schrörder, B., Skidmore, A. K., Zurell, D. and Lautenbach, S. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* **36**: 27–46.

Duke, G. (1991) Using call counts to compare western tragopan populations in Pakistan's Himalaya. Pp. 116–122 in D. A. Hill, P. J. Garson and D. Jenkins, eds. *Pheasants in Asia 1989*. Readings, UK: World Pheasant Association.

Elith, J., Kearney, M., and Phillips, S. (2010) The art of modelling range-shifting species. *Methods Ecol. Evol.* **1**: 330–342.

Elith, J., Graham, C. H., Anderson, R. P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R. J., Huettmann, F., Leathwick, J. R., Lehmann, A., Le, J., Lohmann, L. G., Loiselle, B. A., Manion, G., Moritz, C., Nakamura, C., Nakazawa, Y., Overton, J. McC., Peterson, A. T., Phillips, S. J., Richardson, K., Scachetti-Pereira, R., Schapire, R. E., Soberón, J., Williams, S., Wisz, M. S. and Zimmermann, N. E. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**: 129–151.

Elsen, P. R., Kalyanaraman, R., Ramesh, K. and Wilcove, D. (2017) The importance of agricultural lands for Himalayan birds in winter. *Conserv. Biol.* **31**: 416–426.

Favre, A., Päckert, M., Pauls, S. U., Jähnig, S. C., Uhl, D., Michalak, I. and Muellner-Riehl, A. N. (2015) The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biol. Rev.* **90**: 236–253.

Fitzpatrick, M. C. and Hargrove, W. W. (2009) The projection of species distribution models and the problem of nonanalog climate. *Biodivers. Conserv.* **18**: 2251–2261.

Fjeldså, J., Bowie, R. C. and Rahbek, C. (2012) The role of mountain ranges in the diversification of birds. *Annu. Rev. Ecol. Evol. Syst.* **43**: 249–265.

Fuller, R. A. and Garson, P. J. eds. (2000) *Pheasants. Status survey and conservation action plan 2000–2004*. IUCN, Gland. Switzerland and Cambridge, UK: WPA/ BirdLife/SSC Pheasant Specialist Group and Reading, UK: World Pheasant Associatiion.

Gaston, A. J., Hunter, M. L. and Garson, P. J. (1981) Present distribution and status of pheasants in Himachalpradesh, western Himalayas. *J. World Pheas. Assoc.* **6**: 10–30.

GEBCO Compilation Group (2020) *GEBCO 2020 Grid*. doi:10.5285/a29c5465-b138-234d-e053-6c86abc040b9.

Gent, P. R., Danabasoglu, G., Donner, L. J., Holland, M. M., Hunke, E. C., Jayne, S. R., Lawrence, D. M., Neale, R. B., Rasch, J., Vertenstein, M., Worley, P. H., Yang, Z.-L. and Zhang, M. (2011) The community climate system model version 4. *J. Clim.* **24**: 4973–4991.

Hamayun, M., Khan, S. A., Ahmad, H. Shin, D.-H. and Lee, I.-L. (2006) Morel collection and marketing: A case study from the Hindu-Kush mountain region of Swat, Pakistan. *Lyonia* **11**: 7–13.

Hasumi, H. and Emori, S., eds. (2004) *K-1 coupled GCM (MIROC) description. K-1 Technical Report No. 1*. Tokyo, Japan: Center for Climate System Research, University of Tokyo.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *Internatn. J. Climatol.* **25**: 1965–1978.

Islam, K. and Crawford, J. A. (1987) Habitat use by Western Tragopans *Tragopan melanocephalus* (Gray) in Northeastern Pakistan. *Biol. Conserv.* **40**: 101–115.

Jolli, V. and Pandit, M. K. (2011a) Influence on human disturbance on the abundance of Himalayan pheasant (Aves, Galliformes) in the temperate forest of Western Himalaya, India. *Vestnik Zoologii* **45**: e-40–e-47.

Jolli, V. and Pandit, M. K. (2011b) Monitoring pheasants (Phasianidae) in the Western Himalayas to measure the impact of hydro-electric projects. *The Ring* **33**: 37–46.

Knudsen, A. (2009) *Violence and belonging – Land, love and lethal conflict in the North-West Frontier Province of Pakistan*. Copenhagen: Nias Press.

Laala, G. E., Raja, M. U., Irshad, G., Gardezi, S. R. H. and Akram, A. (2020) Four species of true morels (*Morchella*) recorded from Poonch District (Azad Jammu and Kashmir). *J. Hortic. Sci. Technol.* **3**: 52–55.

Lenth, R. V. (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.6. https://CRAN.R-project.org/package=emmeans.

Ling, C. X., Huang, J. and Zhang, H. (2003) AUC: a better measure than accuracy in comparing learning algorithms. Pp. 329–341 in Y. Xiang and C. D. Brahim, eds. *Advances in artificial intelligence*. Berlin, Heidelberg: Springer-Verlag.

Mahabal, A. and Tak, P. C. (2002) *Status and review of the Western Tragopan*, Tragopan melanocephalus *(J. E. GRAY)*. Kolkata: Zoological Survey of India.

Manish, K. and Pandit, M. K. (2018) Geophysical upheavals and evolutionary diversification of plant species in the Himalaya. *PeerJ* **6**: e5919.

Marchese, C. (2015) Biodiversity hotspots: A shortcut for a more complicated concept. *Glob. Ecol. Conserv.* **3**: 297–309.

Martens, J. (2015) Fauna–Himalayan patterns of diversity. Pp 211–249 in G. Miehe and C. Pendry, eds. *Nepal: an introduction to the natural history,*

ecology and human environment of the Himalayas. Edinburgh, UK: Royal Botanic Garden.

Miller, J. R. B. (2010) Survey of Western Tragopan, Koklass Pheasant, and Himalayan Monal populations in the Great Himalayan National Park, Himachal Pradesh, India. *Indian Birds* **6**: 60–65.

Naithani, S., Mathur, V. B. and Jeganathan, C. (2018) Habitat characterization of Western Tragopan (*Tragopan melanocephalus*), Great Himalayan National Park, India. Internatn. *J. Zool. Stud.* **3**: 46–53.

Phillips, S. J. and Dudík, M. (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* **31**: 161–175.

Phillips, S. J., Anderson, R. P. and Schapire, R. E. (2006) Maximum entropy modeling of species geographic distributions. *Ecol. Modell.* **190**: 231–259.

Phillips, S. J., Dudík, M., Robert, E. and Schapire, R. E. (2019) Maxent software for modeling species niches and distributions (Version 3.4.1). Available from url: http://biodiversityinformatics.amnh.org/open_source/maxent/. Accessed on 27 April 2017.

R Core Team (2019) *R: a language and environment for statistical computing, R version 3.6.1*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Raja, N. A., Davidson, P., Bean, N., Drijvers, R., Showler, D. A. and Barker, C. (1999) The birds of Palas, North-West Frontier Province, Pakistan. *Forktail* **15**: 77–85.

Ramesh, V., Gopalakrishna, T., Barve, S. and Melnick, D. J. (2017) IUCN greatly underestimates threat levels of endemic birds in the Western Ghats. *Biol. Conserv.* **210**: 205–221.

Rocchini, D., Hortal, J., Lengyel, S., Lengyel, S., Jiménez-Valverde, A., Ricotta, C., Bacaro, G. and Chiarucci, A. (2011) Accounting for uncertainty when mapping species distributions: the need for maps of ignorance. *Prog. Phys. Geog.* **35**: 211–226.

Rotenberry, J. T. and Balasubramaniam, P. (2020) Connecting species' geographical distributions to environmental variables: range maps versus points of occurrence. *Ecography* **43**: 1–17.

Saberwal, V. K. and Chhatre, A. (2001) The Parvati and the Tragopan: Conservation and development in the Great Himalayan National Park. *Himalayan Res. Bull.* **21**: 79–88. Available at: https://digitalcommons.macalester.edu/himalaya/vol21/iss2/13

Saqib, Z., Malik, R. N. and von Wehrden, H. (2013) Landcover dynamics in relation to Western Tragopan occurrence in Pakistan: a regional assessment. *Pak. J. Bot.* **45**: 551–559.

Shabbir, A., Anwar, M., Mahmood, T. and Beg, M. A. (2018) Population status of the Western Tragopan (*Tragopan melanocephalus*) in Machiara National Park, Azad Jammu and Kashmir, Pakistan. *J. Anim. Plant. Sci.* **28**: 1542–1546.

Sharief, A., Singh, H., Joshi B. D., Singh, I., Mukherjee, T., Chandra, K., Thakur, M. and Sharma, L. K. (2022) Understanding distribution and occupancy of Himalayan monal in Uttarkashi district, Uttarakhand for conservation and management planning. *Wildlife Biol.* **2022**: e01013.

Sher, H., Aldosari, A. and Bussmann, R. W. (2015) Morels of Palas Valley, Pakistan: A potential source for generating income and improving livelihoods of mountain communities. *Econ. Bot.* **69**: 345–359.

Singh, H., Kumar, N., Kumar, M. and Singh, R. (2020) Modelling habitat suitability of western tragopan (*Tragopan melanocephalus*) a range-restricted vulnerable bird species of the Himalayan region, in response to climate change. *Clim. Risk Manag.* **29**: 100241.

Sing, S. and Tu, F. (2008) A preliminary survey for Western Tragopan *Tragopan melanocephalus* in the Daranghati Wildlife Sanctuary, Himachal Pradesh. *Indian Birds* **4**: 42–55.

Soldatini, C., Albores-Barajas, Y.V. and Pellizi, B. (2010) Habitat preferences of high-altitude Galliformes in Sagarmatha National Park, Nepal. *Ital. J. Zool.* **77**: 347–353.

Sutherland, W. J. (2000) *The conservation handbook: Research, management and policy*. Oxford: Blackwell Science Ltd.

Swets, J. A. (1988) Measuring the accuracy of diagnostic systems. *Science* **240**: 1285–1293.

Wikramanayake, E. D., Dinerstein, E. and Loucks, C. J. (2002) *Terrestrial ecoregions of the Indo-Pacific: A conservation assessment*. Washington, DC: Island Press.

https://doi.org/10.1017/S0959270922000120 Published online by Cambridge University Press

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Appendix 1: A revised multilocus phylogeny of Old World sparrows (Aves: Passeridae)

Table S1: Additional samples and sequence data used for analysis of inter- and intraspecific variation of the cytochrome-*b* gene (*Montifringilla*, *Pyrgilauda* and *Petronia*) and the ND2 gene (*Passer*).

sample ID	Species	GB acc no	Country	State/Region	Locality	reference
MAR3110	*Montifringilla nivalis*	MZ005599	Mongolia		Bondoch Gol, Altai	this study
MAR3112	*Montifringilla nivalis*	MZ005600	Mongolia		Bondoch Gol, Altai	this study
MAR1533	*Montifringilla nivalis*	MZ005594	Russia		Dagestan, N Caucasus, Kurush	this study
MAR1534	*Montifringilla nivalis*	MZ005595	Russia		Dagestan, N Caucasus, Kurush	this study
MAR2426	*Montifringilla nivalis*	MZ005598	Switzerland			this study
MAR4842	*Montifringilla nivalis*	MZ005601	Austria			this study
MAR10185	*Montifringilla nivalis*	MZ005608	Austria		near Mühlberg at Hochkönig	this study
MAR10186	*Montifringilla nivalis*	MZ005609	Austria		near Mühlberg at Hochkönig	this study
MAR11140	*Montifringilla nivalis*	MZ005593	Austria			this study
GB	*Montifringilla nivalis*	JX236394	Mongolia			Fregin et al. (2012)
CM1-CM26	*Montifringilla nivalis*	KX369047-KX369051 KX369059-KX369079	Spain		Cantabrian Mountains	Resano-Mayor et al. (2016)
CP1-CP3 EP1-EP38	*Montifringilla nivalis*	KX369044-KX369046 KX369080-KX369117	Spain		Pyrenees	Resano-Mayor et al. (2016)
SA1-SA6	*Montifringilla nivalis*	KX369053-KX369058	Switzerland		Southwestern Swiss Alps	Resano-Mayor et al. (2016)
MAR989	*Pyrgilauda blanfordi*	MZ005602	China	Qinghai	Madoi, south of Qinghai Lake	this study
MAR990	*Pyrgilauda blanfordi*	MZ005603	China	Qinghai	Madoi, south of Qinghai Lake	this study
MAR1776	*Pyrgilauda blanfordi*	MZ005596	China	Qinghai	steppe highlands near Madoi	this study
MAR2217	*Pyrgilauda blanfordi*	MZ005612	China	Qinghai	NE shores of Tsaring Nor	this study
MAR2218	*Pyrgilauda blanfordi*	MZ005611	China	Qinghai	NE shores of Tsaring Nor	this study
MAR2222	*Pyrgilauda blanfordi*	MZ005610	China	Qinghai	NE shores of Tsaring Nor	this study
voucher 15	*Pyrgilauda blanfordi*	FJ624113	China	Qinghai	-	Qu et al. 2010
voucher 19	*Pyrgilauda blanfordi*	FJ624114	China	Qinghai	-	Qu et al. 2010
voucher 20	*Pyrgilauda blanfordi*	FJ624115	China	Qinghai	-	Qu et al. 2010
zhao12	*Pyrgilauda blanfordi*	FJ624116	China	Qinghai	-	Qu et al. 2010
QZ1106	*Pyrgilauda blanfordi*	FJ624117	China	Qinghai	-	Qu et al. 2010
QZ1135	*Pyrgilauda blanfordi*	FJ624118	China	Qinghai	-	Qu et al. 2010
QZ1136	*Pyrgilauda blanfordi*	FJ624119	China	Qinghai	-	Qu et al. 2010

QZ1149	*Pyrgilauda blanfordi*	FJ624120	China	Qinghai	-	Qu et al. 2010
QPB	*Pyrgilauda blanfordi*	DQ244063	China	Tibet	Tanggula Mountains	Qu et al. 2006
mitogenome	*Pyrgilauda blanfordi*	NC_025912	China	-	-	unpublished
mitogenome	*Pyrgilauda blanfordi*	KJ148629	China	-	-	unpublished
MAR994	*Pyrgilauda davidiana*	MZ005604	China	Qinghai	Qinghai Lake, Heimahe	this study
MAR997	*Pyrgilauda davidiana*	MZ005605	China	Qinghai	Qinghai Lake, Heimahe	this study
MAR998	*Pyrgilauda davidiana*	MZ005606	China	Qinghai	Qinghai Lake, Heimahe	this study
MAR2208	*Pyrgilauda davidiana*	MZ005597	China	Qinghai	Qinghai Lake, Heimahe	this study
QPD	*Pyrgilauda davidiana*	DQ244064	China	Qinghai	Tijanjun	Qu et al. 2006
UWBM 57838	*Pyrgilauda davidiana*	EF530034	Mongolia	Töv Aymag		Klicka et al. 2007
mitogenome	*Pyrgilauda davidiana*	NC_025915	China	-		unpublished
mitogenome	*Pyrgilauda davidiana*	KJ148632	China	-		unpublished
L383 Va6	*Petronia petronia*	KY378769	Spain	Canary Islands	Tenerife	Valente et al. 2017
Petpet241	*Petronia petronia*	AF230914	Spain	-	Madrid	Allende et al. 2001
QPP	*Petronia petronia*	DQ244065	China	Qinghai	Tijanjun	Qu et al. 2006
IZAS uncat.	*Petronia petronia*	AY228074	China	-		Ericson and Johansson 2003
mitogenome	*Petronia petronia*	MF071218	China	-		unpublished
PIAG1	*Passer iagoensis*	MZ005613	Cape Verde	Raso		this study
PIAG4	*Passer iagoensis*	MZ005620	Cape Verde	Raso		this study
PIAG5	*Passer iagoensis*	MZ005622	Cape Verde	Raso		this study
PIAG13	*Passer iagoensis*	MZ005614	Cape Verde	Raso		this study
PIAG14	*Passer iagoensis*	MZ005632	Cape Verde	Raso		this study
PIAG16	*Passer iagoensis*	MZ005631	Cape Verde	Raso		this study
PIAG18	*Passer iagoensis*	MZ005615	Cape Verde	Raso		this study
PIAG20	*Passer iagoensis*	MZ005630	Cape Verde	Raso		this study
PIAG23	*Passer iagoensis*	MZ005616	Cape Verde	Raso		this study
PIAG27	*Passer iagoensis*	MZ005617	Cape Verde	Raso		this study
PIAG29	*Passer iagoensis*	MZ005618	Cape Verde	Raso		this study
PIAG39	*Passer iagoensis*	MZ005619	Cape Verde	Raso		this study
PIAG42	*Passer iagoensis*	MZ005621	Cape Verde	Raso		this study
PIAG53	*Passer iagoensis*	MZ005624	Cape Verde	São Nicolau		this study

PIAG56	*Passer iagoensis*	MZ005628	Cape Verde	São Nicolau		this study
PIAG57	*Passer iagoensis*	MZ005625	Cape Verde	São Nicolau		this study
PIAG-C	*Passer iagoensis*	MZ005626	Cape Verde	Maio		this study
PIAG-D	*Passer iagoensis*	MZ005627	Cape Verde	Maio		this study
PIAG-F	*Passer iagoensis*	MZ005623	Cape Verde	Maio		this study
MTD_2008-80	*Passer domesticus*	KX370736	Germany	Saxony	Kurort Hartha	Päckert et al. 2019
MTD_2008-206	*Passer domesticus*	KX370737	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_2010-71	*Passer domesticus*	KX370742	Germany	Saxony	Auerbach, Rempesgrün	Päckert et al. 2019
MTD_2010-161	*Passer domesticus*	KX370738	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_2010-162	*Passer domesticus*	KX370739	Germany	Saxony	Kurort Hartha	Päckert et al. 2019
MTD_2012-60	*Passer domesticus*	KX370740	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_2012-70	*Passer domesticus*	KX370741	Germany	Saxony	Colmnitz	Päckert et al. 2019
MTD_2012-131	*Passer domesticus*	KX370743	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_2012-132	*Passer domesticus*	KX370744	Germany	Saxony	Neustadt/Sachsen	Päckert et al. 2019
MTD_2012-133	*Passer domesticus*	KX370745	Germany	Saxony	-	Päckert et al. 2019
MTD_2012-231	*Passer domesticus*	KX370746	Germany	Saxony	Klingenberg	Päckert et al. 2019
MTD_65/3	*Passer domesticus*	KX370747	Germany	Saxony	Annaberg	Päckert et al. 2019
MTD_398/2	*Passer domesticus*	KX370750	Germany	Saxony	Wiesa	Päckert et al. 2019
MTD_421/2	*Passer domesticus*	KX370748	Germany	Saxony	Geyer	Päckert et al. 2019
MTD_429/2	*Passer domesticus*	KX370749	Germany	Saxony	Annaberg	Päckert et al. 2019
MTD_TC372	*Passer domesticus*	KX370755	Germany	Sachsen-Anhalt	Bösenburg b. Köthen	Päckert et al. 2019
MTD_TC503	*Passer domesticus*	KX370751	Germany	Saxony	Radebeul	Päckert et al. 2019
MTD_TC536	*Passer domesticus*	KX370752	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_TC555	*Passer domesticus*	KX370753	Germany	Saxony	Dresden	Päckert et al. 2019
MTD_TC367	*Passer domesticus*	KX370754	Germany	Saxony	Crottendorf	Päckert et al. 2019

Additional references

Ericson PGP, Johanson US (2003) Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. Molecular Phylogenetics and Evolution 29: 126–138.

Fregin S, Haase M, Olsson U, Alström P (2012) New insights into family relationships within the avian superfamily Sylvioidea (Passeriformes) based on seven molecular markers. BMC Evolutionary Biology 12, Article number 157.

Klicka J, Burns K, Spellman G (2007) Defining a monophyletic Cardinalini: A molecular perspective. Molecular Phylogenetics and Evolution 45: 1014-1032.

Qu Y, Lei F, Zhang R, Lu X (2010) Comparative phylogeography of five avian species: implications for Pleistocene evolutionary history in the Qinghai–Tibetan plateau. Molecular Ecology 19: 338–351.

Valente L, Illera JC, Havenstein K, Pallien T, Etienne RS, Tiedemann R (2017) Equilibrium Bird Species Diversity in Atlantic Islands. Current Biology 27, 1660–1666.

Appendix 2: Museomics help resolving the phylogeny of snowfinches (Aves, Passeridae, *Montifringilla* and allies)

Supplementary Table S1: Origin of samples used for analysis and accession numbers of sequence data ar

library id	sample id	species	country	region
L40977	MAR3110	*Montifringilla nivalis groumgrzimaili*	Mongolia	Mongolian Altai
L40978	MAR3111	*Montifringilla nivalis groumgrzimaili*	Mongolia	Mongolian Altai
L40980	MAR3113	*Montifringilla nivalis groumgrzimaili*	Mongolia	Mongolian Altai
L40981	MAR1532	*Montifringilla nivalis alpicola*	Russia	Dagestan, N Caucasus
L40982	MAR1533	*Montifringilla nivalis alpicola*	Russia	Dagestan, N Caucasus
L40983	MAR1534	*Montifringilla nivalis alpicola*	Russia	Dagestan, N Caucasus
L40986	MAR11139	*Montifringilla nivalis nivalis*	Austria	Salzburg
L40987	MAR11140	*Montifringilla nivalis nivalis*	Austria	Salzburg
L40988	MAR2426	*Montifringilla nivalis nivalis*	Switzerland	
L40989	FKN1	*Montifringilla nivalis nivalis*	Switzerland	
L40991	FKN3	*Montifringilla nivalis nivalis*	Switzerland	
L41013	UMIB2A290998	*Montifringilla nivalis nivalis*	Spain	Cantabrian Mountains
L41014	UMIB2A290103	*Montifringilla nivalis nivalis*	Spain	Cantabrian Mountains
L41017	UMIB2A290144	*Montifringilla nivalis nivalis*	Spain	Cantabrian Mountains
L41035	UMIB1KA52723	*Montifringilla nivalis nivalis*	Spain	Cantabrian Mountains
L41036	UMIB1KA52724	*Montifringilla nivalis nivalis*	Spain	Cantabrian Mountains
L41059	MAR991	*Montifringilla adamsi*	China	Qinghai
L41060	MAR995	*Montifringilla adamsi*	China	Qinghai
L41062	MAR2213	*Montifringilla adamsi*	China	Qinghai
L41063	MAR2226	*Montifringilla adamsi*	China	Qinghai
L41065	MAR992	*Montifringilla henrici*	China	Qinghai
L41066	MAR993	*Montifringilla henrici*	China	Qinghai
L41067	MAR1000	*Montifringilla henrici*	China	Qinghai
L41068	MAR2204	*Montifringilla henrici*	China	Qinghai
L40994	MAR989	*Pyrgilauda blanfordi*	China	Qinghai
L40996	MAR2217	*Pyrgilauda blanfordi*	China	Qinghai
L40997	MAR2220	*Pyrgilauda blanfordi*	China	Qinghai
L40998	MAR2221	*Pyrgilauda blanfordi*	China	Qinghai
L40995	MAR1776	*Pyrgilauda davidiana*	China	Qinghai
L41001	MAR998	*Pyrgilauda davidiana*	China	Qinghai
L41002	MAR2208	*Pyrgilauda davidiana*	China	Qinghai
L41006	MAR2205	*Pyrgilauda ruficollis*	China	Qinghai
L41061	MAR2206	*Pyrgilauda ruficollis*	China	Qinghai
-	MAR423	*Pyrgilauda ruficollis*	China	Qinghai
-	MAR1082	*Pyrgilauda theresae*	Afghanistan	Ghazni
L41071	MAR1083	*Pyrgilauda theresae*	Afghanistan	Wardak
L41003	MAR2209	*Onychostruthus taczanowskii*	China	Qinghai
L41025	MAR426	*Onychostruthus taczanowskii*	China	Qinghai
-	MAR433	*Onychostruthus taczanowskii*	China	Qinghai
L41064	MAR7544	*Onychostruthus taczanowskii*	China	Gansu
L41000	MAR407	*Petronia petronia*	China	Qinghai

ıd raw read data (GenBank: GB; European Nucleotide Archive: ENA), plus information on ddRAD sequencing s

locality	acc no cytb GB	acc no mitogenome ENA	acc no FASTQ ENA	raw reads	reads passed filter
Bondoch Gol	MZ005599	-	SRR27997285	3819067	3816989
Bondoch Gol	MN337353	OX637970	SRR27997284	3092492	3090776
Bondoch Gol	OQ947839	-	SRR27997273	3169438	3167707
Kurush	MN337352	-	SRR27997262	1775869	1775086
Kurush	MZ005594	OX637971	SRR27997253	2963375	2961979
Kurush	MZ005595	-	SRR27997252	3012391	3011016
Mühlberg, Hochkönig	OQ947840	-	SRR27997251	1710153	1709191
Mühlberg, Hochkönig	MZ005593	-	SRR27997250	1951325	1950353
	MZ005598	-	SRR27997249	1320248	1319538
Furkapass	-	OX637969	SRR27997248	1944816	1943717
Furkapass	OQ947841	-	SRR27997283	4962159	4959590
Picos de Europa	OQ947842	-	SRR27997282	2787594	2786284
Picos de Europa	OQ947843	-	SRR27997281	3542407	3540622
Picos de Europa	OQ947844	-	SRR27997280	3931068	3929089
Las Ubiñas	OQ947845	-	SRR27997279	2981553	2980083
Las Ubiñas	OQ947846	-	SRR27997278	2904328	2902791
Maduo	OQ947847	-	SRR27997277	2693423	2692233
Heimahe	OQ947848	-	SRR27997276	3416137	3414484
Huashixia	OQ947849	-	SRR27997275	2707778	2706441
Huashixia	OQ947850	-	SRR27997274	2416736	2415633
Huashixia	OQ947851	-	SRR27997272	5352346	5349779
Huashixia	OQ947852	-	SRR27997271	4092284	4090417
Heimahe	OQ947853	-	SRR27997270	3256376	3254868
Nanshan	OQ947854	-	SRR27997269	3191419	3189955
Maduo	MZ005602	-	SRR27997268	4914765	4912099
NE shores of Oring Nor	MZ005612	-	SRR27997267	4932053	4929546
NE shores of Oring Nor	MZ005611	-	SRR27997266	156843	156737
NE shores of Oring Nor	MZ005611	-	SRR27997265	370663	370457
near Maduo	MZ005596	-	SRR27997264	5020885	5018192
Heimahe	MZ005606	-	SRR27997263	2941064	2939560
Heimahe	MZ005597	-	SRR27997261	2126451	2125262
Heimahe	OQ947857	-	SRR27997260	2210442	2209275
Heimahe	MN337354	-	SRR27997259	2243353	2242148
Heimahe	-	OX637975	-	-	-
Dasht-i-Nawar	-	OX637972	-	-	-
Imai Pass (= Unai Pass)	-	OX637973	SRR27997258	64311	64265
Huashixia	OQ947855	-	SRR27997257	2703260	2701673
Heimahe	MN337355	-	SRR27997256	3098499	3096684
Heimahe	-	OX637974	-	-	-
W Qilian Mts	OQ947856	-	SRR27997255	463943	463683
Heimahe	-	-	SRR27997254	1083778	1083262

succes (numbers of raw reads, reading depth, mapping rates etc.)

av. read depth	mapped reads *P. domesticus*	mapping rate (%)	mapped reads *P. ruficollis*	mapping rate (%)	mapped reads O. *tazcanowskii*	mapping rate (%)
183.79	2728805	71,5	2913837	76,3	2923411	76,5
143.54	2221653	71,9	2382956	77,09	2379404	76,9
157.58	2264421	71,5	2401025	75,79	2415355	76,2
104.57	1175865	66,2	1156254	65,1	1224306	68,9
145.63	2054896	69,4	2142910	72,3	2183644	73,7
150.05	2116783	70,3	2187565	72,6	2201385	73,1
98.37	1228831	71,9	1270964	74,3	1264892	74
106.53	1383220	70,9	1434676	73,5	1431205	73,3
86.21	935482	70,9	941030	71,3	951746	72,1
98.17	1387629	71,4	1445451	74,3	1435120	73,8
117.75	3528395	71,1	3638429	73,3	3649481	73,5
133.84	1994688	71,6	2015242	72,3	2068225	74,2
164.59	2560780	72,3	2618433	73,9	2672698	75,4
176.48	2845908	72,4	2885076	73,4	2959872	75,3
145.60	2172432	72,9	2257583	75,7	2262773	75,9
141.54	2093500	72,1	2140801	73,7	2195214	75,6
136.88	1989531	73,9	1812604	67,3	1915920	71,1
160.88	2447823	71,7	2464750	72,1	2526951	74
132.23	1989999	73,5	1968862	72,7	1972324	72,8
127.08	1839574	76,2	1620400	67	1815383	75,1
250.28	3767268	70,4	3254057	60,8	2991475	55,9
224.40	3111683	76,1	2250905	55	2560027	62,5
172.44	2323039	71,4	2051991	63	1906886	58,5
176.84	2246059	70,4	1924694	60,3	1776203	55,6
205.55	3553316	72,3	3818436	77,7	3774896	76,8
189.44	3197854	64,9	3416026	69,2	3383486	68,6
15.22	110712	70,6	119755	76,4	118338	75,5
25.90	265095	71,6	287449	77,5	282626	76,2
223.64	3570518	71,2	3789194	75,5	3673979	73,2
140.77	2107430	71,7	2227560	75,7	2139004	72,7
105.28	1478035	69,5	1596349	75,1	1535019	72,2
113.77	1527799	69,2	1699677	76,9	1590592	71,9
114.96	1608124	71,7	1791459	79,8	1694777	75,5
-	-	-	-	-	-	-
-	-	-	-	-	-	-
16.50	23858	37,1	24676	38,3	24108	37,5
125.83	1897937	70,3	1994939	73,8	2065324	76,4
145.56	2268309	73,2	2359710	76,2	2439955	78,7
-	-	-	-	-	-	-
34.48	335561	72,4	341459	73,6	338561	73
60.92	746619	68,9	660341	60,9	657465	60,6

Appendix 3: Transpalearctic out-of-Tibet dispersal facilitated divergence of genetic lineages, phenotypes and ecological niche preference in a Eurasian alpine bird (Aves, Passeriformes, *Montifringilla nivalis*)

Supplementary Material, Appendix S1, supplementary Tables

TableS1.1: Origin of samples used for genetic analysis

Taxon	Country	Locality	**Library ID**	**Lab_ID**	**Sex**	Cyt b	ddRAD
M. n. nivalis	Austria	unknown	L40976	MAR_4842	**H**	xxx	xxx
M. n. nivalis	Austria	Arthurhaus,Salzburg	L40984	MAR_10185	**M**	xxx	xxx
M. n. nivalis	Austria	Arthurhaus,Salzburg	L40985	MAR_10186	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40986	MAR_11139	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40987	MAR_11140	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40988	MAR_2426	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40989	FKN_1	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40990	FKN_2	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40991	FKN_3	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40992	FKN_4	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40993	FKN_5	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41004	UMIB_2A290159	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41005	UMIB_2A290175	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41007	UMIB_2A290162	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41008	UMIB_2A290177	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41009	UMIB_2A290161	**?**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41010	UMIB_2A290150	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41011	UMIB_2A290146	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41012	UMIB_2A290141	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41013	UMIB_2A290998	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41014	UMIB_2A290103	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41015	UMIB_2A290104	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Puertos de Aliva	L41016	UMIB_2L05515	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41017	UMIB_2A290144	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41018	UMIB_2A290109	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41019	UMIB_2A290193	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41020	UMIB_2A290195	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41021	UMIB_2A290187	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41022	UMIB_2A290142	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41023	UMIB_2A290145	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41024	UMIB_1KA52702	**M**	xxx	xxx

M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41026	UMIB_1KA52706	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41027	UMIB_1KA52707	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41028	UMIB_1KA52709	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41029	UMIB_1KA52710	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41030	UMIB_1KA52711	**?**		
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41031	UMIB_1KA52712	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41032	UMIB_1KA52714	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41033	UMIB_1KA52715	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41034	UMIB_1KA52719	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41035	UMIB_1KA52723	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41036	UMIB_1KA52724	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41037	UMIB_1KA52726	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41038	UMIB_1KA52728	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41039	UMIB_1KA52729	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41040	UMIB_1KA52730	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41041	UMIB_1KA52731	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41042	UMIB_1KA52732	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41043	KU_20377	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41044	KU_20378	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41045	KU_20410	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41046	KU_20460	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41047	KU_20466	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41048	KU_20473	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41049	KU_20476	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41050	KU_20653	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41051	KU_20658	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41052	KU_20739	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41053	KU_204759	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41054	KU_204760	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41055	KU_204768	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41057	KU_204770	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41058	KU_204774	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Bondoch Gol	L40977	MAR_3110	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Bondoch Gol	L40978	MAR_3111	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40981	MAR_1532	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40982	MAR_1533	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40983	MAR_1534	**-**	xxx	xxx

Table S1.2a: ddRAD seq loci included in EBSP analysis of the pooled Asian data set (*M. n. alpicola* and *M. n. groumgrzimaili*; n= 20); length of sequence given per cell; 0= failed to score (no read data extracted for locus x of individual y).

		350	**1055**	**1502**	**1572**	**1660**	**1861**	**2087**	**2405**	**2907**	**3233**	**3805**	**4183**	**4492**	**4979**	**5025**	**5149**	**5416**	**6000**	**6047**	**6670**	**6899**	**7842**	**8238**	**8546**	**8713**	**9012**	**9055**	**9061**
Lib ID	**n SNPs**	5	5	5	5	6	7	5	5	5	7	5	5	5	6	6	5	5	5	6	7	6	4	5	5	4	14	6	10
	sample ID																												
L40977	MAR3110	136	70	136	127	135	136	135	136	71	136	136	136	70	139	135	70	0	70	124	136	72	66	136	1st half	71	136	68	137
L40978	MAR3111	136	70	136	127	135	136	135	136	71	136	136	136	71	139	135	70	70	70	124	136	136	66	136	1st half	71	136	68	137
L40981	MAR1532	136	70	70	127	135	136	135	136	71	136	70	136	70	70	135	70	70	70	64	136	136	66	136	1st half	71	136	68	137
L40982	MAR1533	136	70	136	127	135	136	135	136	71	136	71	136	71	139	135	70	70	70	124	136	73	66	136	1st half	71	0	68	137
L40983	MAR1534	136	70	136	127	135	136	135	136	71	136	70	136	70	70	135	70	70	70	124	136	136	66	136	1st half	71	136	68	137
L41043	KU20377	136	70	136	127	135	136	135	136	71	136	136	136	136	76	135	70	70	70	124	136	136	66	136	136	71	136	0	137
L41044	KU20378	136	70	136	127	135	136	135	126	71	136	136	136	136	139	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41045	KU20410	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	70	70	124	107	136	66	136	136	71	136	68	137
L41046	KU20460	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41047	KU20466	136	70	136	56	135	136	135	136	71	136	136	136	136	139	135	70	0	70	124	136	136	66	136	136	71	136	68	137
L41048	KU20473	136	70	136	0	135	136	135	136	71	136	136	136	70	0	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41049	KU20476	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	70	70	124	136	136	66	136	136	0	136	68	137
L41050	KU20653	136	70	136	62	135	136	135	136	71	136	136	136	136	71	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41051	KU20658	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41052	KU20739	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	0	70	124	136	136	66	136	136	0	0	68	137
L41053	KU204759	136	70	136	56	135	136	135	136	71	136	136	136	136	70	135	70	70	70	124	136	136	66	136	136	0	0	68	137
L41054	KU204760	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	70	70	124	136	136	66	136	136	71	136	68	137
L41055	KU204768	136	70	136	56	135	136	135	136	71	136	136	136	136	70	135	70	70	70	124	136	72	66	136	136	0	136	0	137
L41057	KU204770	136	70	136	127	135	136	135	136	71	136	136	136	136	139	135	70	0	70	124	136	136	66	136	136	71	136	68	137
L41058	KU204774	136	70	136	127	135	136	135	136	71	136	136	136	136	71	135	70	0	70	124	136	136	66	136	136	71	136	68	137

Table S1.3a: ddRAD seq loci included in EBSP analysis of the pooled Asian data set (*M. n. nivalis*; n= 48); length of sequence given per cell; 0= failed to score (no read data extracted for locus x of individual y).

	Loc ID	**825**	**1172**	**1528**	**2112**	**2501**	**2586**	**3404**	**3730**	**3976**	**5389**	**5561**	**5652**	**5924**	**6119**	**6599**	**6679**	**7233**	**7533**	**8255**	**8398**
Lib ID	**n SNPs**	8	5	5	???	4		3	6	5	4	4	5	4	5	5	4	7	5	5	4
L40976	MAR4842	0	71	0	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L40984	MAR10185	0	0	76	188	136	136	66	131	71	70	126	0	131	136	137	94	131	134	135	69
L40985	MAR10186	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L40986	MAR11139	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	69
L40987	MAR11140	135	71	76	188	136	136	66	131	71	76	126	133	131	136	137	94	131	134	135	69
L40988	MAR2426	135	0	76	188	136	0	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L40989	FKN1	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L40990	FKN2	135	71	76	188	136	136	66	131	71	63	126	133	131	136	137	94	131	134	135	69
L40991	FKN3	135	71	0	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L40992	FKN4	135	71	76	188	136	136	66	131	71	0	126	133	131	136	137	94	131	134	135	69
L40993	FKN5	135	71	76	188	136	136	66	131	71	76	126	133	131	136	137	94	131	134	135	69
L41004	UMIB2A290159	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41005	UMIB2A290175	135	71	76	188	136	0	66	131	71	70	126	133	131	136	137	94	131	134	135	0
L41007	UMIB2A290162	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41008	UMIB2A290177	135	0	76	188	136	136	66	0	71	70	126	133	131	136	137	94	131	134	135	69
L41009	UMIB2A290161	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41010	UMIB2A290150	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	0
L41011	UMIB2A290146	135	71	76	188	136	0	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41012	UMIB2A290141	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41013	UMIB2A290998	135	0	76	188	136	0	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41014	UMIB2A290103	135	0	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	69
L41015	UMIB2A290104	135	71	76	188	136	136	66	131	71	71	126	133	131	136	137	94	131	134	135	69
L41016	UMIB2L05515	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41017	UMIB2A290144	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	0	134	135	69

L41018	UMIB2A290109	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	0
L41019	UMIB2A290193	135	71	76	188	136	136	66	131	71	76	126	133	131	136	137	94	0	134	135	69
L41020	UMIB2A290195	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41021	UMIB2A290187	135	0	76	188	136	136	66	131	71	71	126	133	131	136	137	94	131	134	135	69
L41022	UMIB2A290142	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	0
L41023	UMIB2A290145	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41024	UMIB1KA52702	135	71	76	188	136	136	66	0	71	70	126	133	131	136	137	94	131	134	135	69
L41026	UMIB1KA52706	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	69
L41027	UMIB1KA52707	135	71	76	188	136	136	66	131	71	74	126	133	131	136	137	94	131	134	135	0
L41028	UMIB1KA52709	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	0	134	135	69
L41029	UMIB1KA52710	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41030	UMIB1KA52711	135	71	76	188	136	136	66	131	71	69	126	133	131	136	137	94	131	134	135	69
L41031	UMIB1KA52712	135	71	76	188	136	136	66	0	71	70	126	133	131	136	137	94	131	134	135	69
L41032	UMIB1KA52714	135	0	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41033	UMIB1KA52715	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41034	UMIB1KA52719	135	71	76	188	136	136	66	0	71	69	126	133	131	136	137	94	131	134	135	69
L41035	UMIB1KA52723	135	71	76	188	136	136	66	131	71	73	126	133	131	136	137	94	131	134	135	0
L41036	UMIB1KA52724	135	71	0	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	0
L41037	UMIB1KA52726	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41038	UMIB1KA52728	135	71	76	188	136	0	66	131	71	69	126	133	131	136	137	94	131	134	135	0
L41039	UMIB1KA52729	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41040	UMIB1KA52730	135	71	76	188	136	136	66	131	71	70	126	133	131	136	137	94	131	134	135	69
L41041	UMIB1KA52731	135	71	76	188	136	136	66	0	71	70	126	133	131	136	137	94	131	134	135	69
L41042	UMIB1KA52732	135	71	0	188	136	0	66	0	71	69	126	133	131	136	137	94	131	134	135	69

Table S1.4: Morphological matrix
(Data available on request. Email. safiq713@gmail.com)

Table S1.5: Occurrence records used for SDMs
(Data available on request. Email. safiq713@gmail.com)

Table S1.6. Statistics for the *Montifringilla nivalis* denovo genome assembly statistics for the scaffolded data resulting from assembly with Abyss, the Ray assembler, and SOAPdenovo as inferred by Reaps and BUSCO analysis.

	Abyss	Ray	SOAPdenovo2
Total length:	1,210,351,634	1,057,029,690	1,151,388,652
Number of sequences:	162,0470	71,353	978,078
Mean sequence length:	746.91	14,814.09	1,177.2
Length of longest sequence:	715,952	528,067	1,126,520
N50	74,962, n = 3953	70,130, n = 4320	31,662, n = 7973
Number of gaps:	57,270	32,397	418,167
Total gap length:	1,106,482	10,595,966	10,445,091
Total BUSCO groups searched	10844		
Complete BUSCOs (C)	9292 (85.7%)	9175 (84.6%)	7760 (71.5%)
Complete and single-copy BUSCOs (S)	9248 (85.3%)	9025 (83.2%)	7726 (71.2%)
Complete and duplicated BUSCOs (D)	44 (0.4%)	150 (1.4%)	34 (0.3%)
Fragmented BUSCOs (F)	453 (4.2%)	491 (4.5%)	720 (6.6%)
Missing BUSCOs (M)	1099 (10.1%)	1178 (10.9%)	2364 (21.9%)

Table S1.7: Quantity of raw reads, Filtered reads, PYARD clusters total, PYRAD Clusters hidepth, Hetero est, error estimation, Reads consensus and Loci in assembly (reference genome *M. nivalis*, own *de-novo* assembly)

Subspecies	Library ID	reads raw	reads passed filter	mapped reads	avg depth total	Mapping rate (%)	clusters total	clusters hidepth	avg depth	error est	reads consens	Loci in assembly
nivalis	L40976	1431209	1430515	966534	2762	67.56	35000	13365	69.27	0.003770	12964	9804
nivalis	L40984	3819067	3816989	2593558	6797	67.94	38160	15683	162.80	0.002783	15298	10205
nivalis	L40985	3092492	3090776	2112646	5249	68.35	40246	16361	126.38	0.003304	15895	10127
nivalis	L40986	1775869	1775086	962673	4553	54.2	21142	11877	79.47	0.003638	11606	9231
nivalis	L40987	2963375	2961979	1858862	6766	62.75	27473	14910	122.96	0.003444	14553	10361
groumgrzimaili	L40977	3012391	3011016	1906950	5084	63.33	37510	14684	127.10	0.002904	14304	10362
groumgrzimaili	L40978	932896	932433	607628	2595	65.16	23419	11630	50.24	0.003770	11332	9201
groumgrzimaili	L41043	3006834	3005279	1984218	6733	66.02	29470	14359	136.17	0.003353	13999	10143
groumgrzimaili	L41044	1710153	1709191	1147355	3726	67.12	30791	13283	83.95	0.003799	12935	9907
groumgrzimaili	L41045	1951325	1950353	1302122	4210	66.76	30930	13812	91.96	0.004129	13462	10082
groumgrzimaili	L41046	1320248	1319538	843583	3499	63.93	24106	11663	70.31	0.003452	11397	9159
groumgrzimaili	L41047	1944816	1943717	1315125	2831	67.66	46454	14889	84.21	0.003711	13985	10086
groumgrzimaili	L41048	1850548	1849571	1260476	3246	68,14	38829	13842	87.68	0.003736	13295	9902
groumgrzimaili	L41049	4962159	4959590	3299448	2785	66.52	118462	32606	96.39	0.003008	26110	10287
groumgrzimaili	L41050	1227469	1226780	838219	2109	68.32	39748	13601	58.08	0.003717	13133	9402
groumgrzimaili	L41051	1845368	1844457	1263912	1605	68.52	78761	30208	38.31	0.003280	23417	9806
groumgrzimaili	L41052	2552925	2551547	1752374	6600	68.67	26550	14105	122.65	0.003522	13789	10041
groumgrzimaili	L41053	2486238	2485005	1747267	6376	70.31	27405	14776	116.61	0.003711	14470	10190
groumgrzimaili	L41054	2351645	2350430	1589466	5780	67.62	27498	13863	112.92	0.003522	13543	10003
groumgrzimaili	L41055	1002344	1001882	684355	2755	68.30	24837	12890	51.27	0.003796	12595	9150
groumgrzimaili	L41057	793440	793075	552527	2670	69.66	20697	10932	48.86	0.003454	10783	7919
groumgrzimaili	L41058	1985677	1984616	1349809	4647	68.01	29048	14467	91.39	0.003995	14104	9800
alpicola	L40981	1861720	1860869	1295937	5711	69.64	22690	13566	94.24	0.004129	13274	9507
alpicola	L40982	3498456	3496669	2374366	8019	67.9	29610	15622	150.42	0.003471	15272	10152
alpicola	L40983	2787594	2786284	1839253	5755	66.01	31961	16002	113.01	0.003711	15651	10162
nivalis	L41004	3542407	3540622	2391767	7071	67.55	33824	16828	140.21	0.003522	16458	10253
nivalis	L41005	2864860	2863514	2042565	5299	71.33	38543	16066	124.73	0.003501	15666	10223
nivalis	L41007	624025	623706	442199	1957	70.89	22594	11425	36.78	0.003796	11152	8878
nivalis	L41008	3931068	3929089	2617508	7376	66.61	35487	17415	148.26	0.003017	17030	10305
nivalis	L41009	1835875	1834882	1314593	4418	71.64	29758	14262	90.04	0.003736	13889	9771
nivalis	L41010	3350385	3348765	2349144	5431	70.14	43255	16514	139.27	0.003855	15958	10261
nivalis	L41011	3290495	3288836	2192650	6373	66.66	34408	16565	130.29	0.003353	16138	10232
nivalis	L41012	2943642	2942038	2015491	7981	68.50	25255	14850	134.40	0.003591	14540	10149
nivalis	L41013	2977642	2976159	1982304	6263	66.60	31650	15728	124.04	0.003471	15358	10127
nivalis	L41014	2158102	2157034	1442415	4608	66.87	31303	14612	96.55	0.003344	14235	9926
nivalis	L41015	1153183	1152631	799613	3354	69.37	23841	12628	61.55	0.003901	12358	9521
nivalis	L41016	1823910	1822895	1249307	4416	68.53	28290	13868	88.02	0.003832	13511	9777
nivalis	L41017	2220630	2219522	1494989	4932	67.35	30315	14813	98.85	0.004129	14424	10031
nivalis	L41018	2660738	2659348	1870598	6027	70.34	31036	15164	121.30	0.003471	14826	10111
nivalis	L41019	2703715	2702278	1810018	5914	66.98	30605	15409	115.48	0.003655	15046	10131

nivalis	L41020	716838	716432	508376	2521	70.95	20168	10825	45.27	0.003911	10617	8640
nivalis	L41021	3129677	3127974	2167582	6297	69.29	34420	15721	135.74	0.003446	15370	10288
nivalis	L41022	1012307	1011815	697967	2961	68.98	23573	12467	54.23	0.003835	12175	9359
nivalis	L41023	3138735	3137057	2164381	6621	68.99	32692	16004	133.28	0.003344	15612	10252
nivalis	L41024	2142852	2141658	1545230	5090	72.15	30357	14960	101.31	0.003832	14600	10089
nivalis	L41026	2981553	2980083	2093730	6646	70.25	31505	16003	128.97	0.004194	15660	10258
nivalis	L41027	2904328	2902791	1970487	5909	67.88	33345	15953	121.42	0.003471	15589	10214
nivalis	L41028	2191943	2190877	1535083	5307	70.067	28923	14855	101.50	0.003832	14507	10143
nivalis	L41029	2347389	2346109	1667303	5599	71.06	29778	15003	109.27	0.003711	14633	10168
nivalis	L41030	1104736	1104169	794615	2963	71.96	26816	12565	61.11	0.003676	12288	9389
nivalis	L41031	989318	988865	701752	2918	70.96	24052	12976	52.38	0.003796	12668	9142
nivalis	L41032	3005279	3003758	2064433	5634	68.72	36640	17219	117.69	0.003446	16800	10131
nivalis	L41033	2236094	2234988	1597211	4854	71.46	32908	15767	99.19	0.003832	15372	9951
nivalis	L41034	2186877	2185776	1514384	3581	69.28	42294	16072	91.20	0.004144	15638	9822
nivalis	L41035	2210134	2208950	1581185	4586	71.58	34478	14837	104.21	0.003444	14468	9637
nivalis	L41036	2709424	2708003	1913928	5524	70.67	34646	16324	115.10	0.003731	15905	10038
nivalis	L41037	3456259	3454525	2539125	4891	73.50	51911	18247	135.57	0.003444	17387	10133
nivalis	L41038	2337121	2335816	1676068	4090	71.75	40983	15906	102.58	0.003444	15430	9952
nivalis	L41039	798707	798356	570550	1914	71.46	29810	12036	44.85	0.003716	11705	8675
nivalis	L41040	3168879	3167288	2236301	5117	70.60	43700	16750	130.75	0.003189	16305	10130
nivalis	L41041	2311020	2309726	1646225	3888	71,27	42340	15581	102.66	0.003674	15092	9824
nivalis	L41042	1954787	1953808	1429052	4168	73.14	34289	15077	92.47	0.003949	14674	9771
nivalis	L40988	2168487	2167168	1515070	4432	69.91	34181	14552	101.77	0.003304	14162	9696
nivalis	L40989	2118348	2117259	1502822	4444	70.97	33814	15036	97.68	0.003731	14673	9855
nivalis	L40990	2692024	2690605	1830304	4428	68.02	41332	15963	111.84	0.003564	15510	9983
nivalis	L40991	1808274	1807368	1298155	3762	71.82	34506	14209	88.92	0.003423	13868	9647
nivalis	L40992	2515360	2513928	1765906	4357	70.24	40533	15725	109.53	0.003304	15230	9941
nivalis	L40993	2353040	2351772	1675006	3560	71.22	47057	15987	101.38	0.003754	15482	9939
	Minumum	4962159	4959590	3299448	8019	66.52	118462	32606	-	-	26110	10362
	Average	2307893	2306704.26	1583324.3	4710.95	68.64	34441.35	15172,985		-	14605.51	9849.29
	Maximum	624025	623706	442199	1605	70.89	20168	10825	-	-	10617	7919

Table S1.8: Quantity of raw reads, filtered reads, PYARD clusters total, PYRAD Clusters hidepth, Hetero est, error estimation, Reads consensus, and Loci in assembly (reference genome *Passer domesticus;* GCA_001700915.1)

Subspecies	Sample Name	raw raw	reads passed filter	mapped reads	Avg Depth total	mapping rate (%)	Clusters total	Clusters hidepth	hetero_est	avg_depth	error_est	Reads consens	Loci in assembly
nivalis	L40976	1431209	1430515	812098	2787	56.76	29137	10772	0.001681	72.22	0.002724	10487	8007
groumgrzimailii	L40977	3819067	3816989	2209087	6924	57.87	31904	12941	0.002125	168.12	0.002084	12633	8325
groumgrzimailii	L40978	3092492	3090776	1821022	5368	58.91	33923	13479	0.002307	132.29	0.002508	13134	8280
alpicola	L40981	1775869	1775086	905978	5156	51.03	17572	9887	0.003222	90.05	0.002569	9678	7536
alpicola	L40982	2963375	2961979	1656529	7299	55.92	22696	12425	0.002821	131.65	0.002531	12158	8475
alpicola	L40983	3012391	3011016	1681580	5338	55.84	31505	12228	0.002971	134.74	0.002100	11941	8477
nivalis	L40984	932896	932433	526071	2754	56.41	19105	9421	0.002016	53.82	0.002666	9224	7501
nivalis	L40985	3006834	3005279	1696284	7090	56.44	23925	11652	0.001722	143.60	0.002320	11413	8297
nivalis	L40986	1710153	1709191	980448	3879	57.36	25275	10851	0.001798	87.94	0.002671	10597	8112
nivalis	L40987	1951325	1950353	1093655	4319	56.07	25320	11225	0.002789	95.11	0.003008	10971	8237
nivalis	L40988	1320248	1319538	734515	3746	55.66	19607	9501	0.001946	75.31	0.002466	9320	7505
nivalis	L40989	1944816	1943717	1113751	2756	57.30	40405	12025	0.001609	88.18	0.002708	11447	8219
nivalis	L40990	1850548	1849571	1065308	3227	57.59	33012	11300	0.001750	90.76	0.002700	10934	8137
nivalis	L40991	4962159	4959590	2843489	2723	57.33	104410	25756	0.001762	104.62	0.002070	21502	8412
nivalis	L40992	1227469	1226780	714535	2103	58.24	33973	11107	0.001676	60.59	0.002589	10756	7659
nivalis	L40993	1845368	1844457	1086254	1455	58.89	74661	24342	0.001216	40.05	0.002280	19970	8002
nivalis	L41004	2552925	2551547	1491139	6945	58.44	21471	11445	0.001484	128.74	0.002498	11178	8185
nivalis	L41005	2486238	2485005	1466383	6604	59.00	22206	12044	0.001262	120.15	0.002664	11804	8337
nivalis	L41007	2351645	2350430	1358097	6214	57.78	21855	11256	0.001281	118.98	0.002513	11017	8154
nivalis	L41008	1002344	1001882	587045	2886	58.59	20340	10525	0.001630	53.94	0.002654	10291	7454
nivalis	L41009	793440	793075	465014	2718	58.63	17111	8949	0.001511	50.26	0.002476	8813	6465
nivalis	L41010	1985677	1984616	1168670	4927	58.88	23720	11785	0.001739	97.26	0.002835	11516	8006
nivalis	L41011	1861720	1860869	1090109	5881	58.58	18536	11083	0.001676	97.07	0.002971	10868	7748
nivalis	L41012	3498456	3496669	2031860	8473	58.10	23979	12751	0.001514	157.82	0.002438	12474	8266
nivalis	L41013	2787594	2786284	1583581	6075	56.83	26069	13080	0.001418	119.16	0.002738	12811	8302
nivalis	L41014	3542407	3540622	2056942	7437	58.09	27659	13637	0.001697	148.89	0.002596	13381	8368
nivalis	L41015	2864860	2863514	1721893	5459	60.13	31540	13183	0.001406	128.24	0.002491	12893	8361
nivalis	L41016	624025	623706	372059	2019	59.65	18429	9254	0.001736	38.25	0.002682	9066	7212
nivalis	L41017	3931068	3929089	2262739	7810	57.58	28973	14207	0.001574	157.23	0.002145	13902	8426
nivalis	L41018	1835875	1834882	1113343	4561	60.67	24410	11679	0.001532	93.19	0.002617	11407	8004
nivalis	L41019	3350385	3348765	1975077	5353	58.97	36894	13425	0.001703	144.00	0.002827	13039	8380
nivalis	L41020	3290495	3288836	1880712	6695	57.18	28090	13412	0.001453	138.14	0.002313	13131	8349
nivalis	L41021	2943642	2942038	1709535	8309	58.10	20574	12053	0.001565	140.52	0.002538	11808	8276
nivalis	L41022	2977642	2976159	1734664	6685	58.28	25948	12840	0.001441	133.10	0.002492	12569	8291
nivalis	L41023	2158102	2157034	1251142	4956	58.00	25247	11914	0.001467	102.88	0.002403	11641	8135
nivalis	L41024	1153183	1152631	683325	3521	59.28	19405	10258	0.001503	64.84	0.002714	10048	7771
nivalis	L41026	1823910	1822895	1082187	4700	59.36	23026	11305	0.001266	93.67	0.002745	11061	7982
nivalis	L41027	2220630	2219522	1285560	5207	57.92	24689	12008	0.001922	104.98	0.003067	11731	8195
nivalis	L41028	2660738	2659348	1589865	6259	59.78	25401	12441	0.001514	125.76	0.002414	12202	8254

nivalis	L41029	2703715	2702278	1554098	6221	57.51	24983	12554	0.001575	121.81	0.002659	12293	8305
nivalis	L41030	716838	716432	434374	2631	60.63	16507	8815	0.001408	47.57	0.002677	8665	7045
nivalis	L41031	3129677	3127974	1848195	6716	59.08	27520	12746	0.001334	142.91	0.002432	12457	8387
nivalis	L41032	1012307	1011815	594267	3100	58.73	19167	10109	0.001553	57.03	0.002739	9888	7599
nivalis	L41033	3138735	3137057	1851750	6938	59.02	26689	13024	0.001444	140.25	0.002397	12737	8384
nivalis	L41034	2142852	2141658	1302354	5253	60.81	24792	12208	0.001266	104.71	0.002745	11947	8243
nivalis	L41035	2981553	2980083	1757335	6823	58.96	25755	13037	0.001672	132.93	0.002992	12773	8385
nivalis	L41036	2904328	2902791	1686004	6221	58.08	27100	12978	0.001643	127.84	0.002496	12678	8334
nivalis	L41037	2191943	2190877	1292426	5452	58.99	23706	12080	0.001266	105.14	0.002745	11831	8288
nivalis	L41038	2347389	2346109	1407930	5781	60.01	24356	12188	0.001766	113.65	0.002745	11907	8297
nivalis	L41039	1104736	1104169	669011	3073	60.58	21771	10289	0.001312	62.91	0.002556	10096	7697
nivalis	L41040	989318	988865	595621	3018	60.23	19734	10650	0.001491	54.21	0.002708	10400	7424
nivalis	L41041	3005279	3003758	1768520	5857	58.87	30195	14101	0.001514	123.21	0.002412	13779	8289
nivalis	L41042	2236094	2234988	1350691	4981	60.43	27117	12966	0.001766	102.07	0.002745	12672	8149
groumgrzimaili	L41043	2186877	2185776	1277230	3552	58.43	35955	13463	0.001943	91.80	0.003085	13133	8006
groumgrzimaili	L41044	2210134	2208950	1329827	4541	60.20	29288	12392	0.002169	104.91	0.002509	12109	7869
groumgrzimaili	L41045	2709424	2708003	1618726	5502	59.77	29422	13673	0.001923	116.22	0.002729	13349	8187
groumgrzimaili	L41046	3456259	3454525	2155874	4727	62.40	45612	15219	0.002244	137.88	0.002526	14643	8284
groumgrzimaili	L41047	2337121	2335816	1415963	4087	60.61	34649	13290	0.002064	103.73	0.002508	12941	8091
groumgrzimaili	L41048	798707	798356	482842	1897	60.47	25456	9959	0.002714	45.81	0.002842	9695	7049
groumgrzimaili	L41049	3168879	3167288	1892458	5033	59.75	37603	13947	0.002047	132.81	0.002338	13615	8262
groumgrzimaili	L41050	2311020	2309726	1398560	3853	60.55	36298	12983	0.002194	104.65	0.002749	12618	8002
groumgrzimaili	L41051	1954787	1953808	1188218	4077	60.81	29141	12568	0.002789	92.17	0.003008	12265	7960
groumgrzimaili	L41052	2168487	2167168	1285736	4474	59.32	28741	12079	0.002313	104.05	0.002458	11777	7890
groumgrzimaili	L41053	2118348	2117259	1267283	4436	59.85	28570	12602	0.001729	98.28	0.002667	12317	8043
groumgrzimaili	L41054	2692024	2690605	1560947	4457	58.01	35020	13355	0.001888	114.04	0.002625	13023	8156
groumgrzimaili	L41055	1808274	1807368	1087241	3748	60.15	29007	11860	0.001988	89.22	0.002496	11591	7882
groumgrzimaili	L41057	2515360	2513928	1503847	4316	59.82	34841	13117	0.002009	111.79	0.002441	12745	8122
groumgrzimaili	L41058	2353040	2351772	1411484	3521	60.01	40086	13328	0.002172	102.43	0.002809	12986	8109
	MAX	4962159	4959590	2843489	8473	57.33	104410	25756				21502	8477
	Average	2307893.01	2306704.26	1351328.77	4866.23	58.58	28839.45	12426.85				12025.67	8041.76
	MIN	624025	623706	372059	1455	59.65	16507	8815				8665	6465

Table S1.9: Evanno

Optimal k – Evanno, total set, Monti ref

etable = struct.get_evanno_table([2, 3, 4, 5])
etable

	Nreps	lnPK	lnPPK	deltaK	estLnProbMean	estLnProbStdev
2	3	0.000	0.0	0.000	-74514.433	618.916
3	3	8002.333	9112.1	12.125	-66512.100	751.510
4	3	-1109.767	1592.2	3.952	-67621.867	402.887
5	3	482.433	0.0	0.000	-67139.433	802.263

get canvas object and set size

Optimal k – Evanno, Europe only, Monti ref
etable = struct.get_evanno_table([2, 3, 4, 5])
etable

	Nreps	lnPK	lnPPK	deltaK	estLnProbMean	estLnProbStdev
2	3	0.000	0.000	0.000	-50965.833	236.630
3	3	443.533	840.800	7.219	-50522.300	116.464
4	3	-397.267	4011.067	9.252	-50919.567	433.516
5	3	-4408.333	0.000	0.000	-55327.900	3825.243

import toyplot.pdf

Table S1.10: Classification accuracy of linear discriminant analysis (predicted group memberships, % of individuals assigned to the correct group) with original data (both all variables considered simultaneously and stepwise selection) and log-transformed data (log-trans).

	males				females			
	Total (n=119)	Europe (n= 38)	Caucasus (n= 42)	Central Asia (n= 39)	Total (n= 50)	Europe (n= 16)	Caucasus (n= 15)	Central Asia (n=19)
original	93,3	89,5	95,2	94,9	98	93,8	100	100
log-trans	92,4	89,5	92,9	94,9	98	93,8	100	100
stepwise	89,2	84,6	92,9	89,7	88,2	81,3	87,5	94,7

Table S1.11: Results of discriminant analysis (DA; log-transformed data, all variables simultaneously included) and stepwise discriminant analysis (SDA)= stepwise analyis, uncorrected data) for 19 morphological variables; separate analyses for males and females; Eigenvalues, percentages of the total variance explained and correlations between predictor variables and each of the two discriminant functions (DF) 1 and 2;*= greatest absolute correlation between predictor variable and either of the two DF.

males (n=119)						females (n=50)				
	DA		SDA				DA		SDA	
DF	1	2	1	2		DF	1	2	1	2
Eigenvalue	2,194	1,621	1,995	0,994		Eigenvalue	6,269	1,594	3,36	0,962
% of variance	57,5	42,5	66,7	33,3		% of variance	79,7	20,3	77,8	22,2
brown-area	-0,516*	0,107	-0,504*	0,384		brown-area	0,336*	0,02	0,487*	0,149
Cm-area	0,267*	0,016	0,300*	-0,149		S8grey	0,173*	-0,123	-	-
white-LW-area	-0,209*	0,045	-0,051	0,521*		white-tail-area	-0,129*	0,028	-	-
H1grey	-0,204*	-0,166	-	-		Cm-area	-0,096*	-0,01	-	-
S8grey	-0,184*	-0,01	-	-		whiteLW-area	0,067*	-0,063	-	-
bill	-0,179*	-0,155	-0,229*	-0,091		H1brown	0,020*	-0,01	-	-
H1black	-0,162*	0,031	-	-		H1black	-0,017*	-0,015	-	-
wing	-0,123*	-0,004	-	-		M2-area	-0,175	0,540*	-0,353	0,799*
H1brown	-0,081*	0,065	-	-		tail	-0,046	0,317*	-	-
M2-area	0,27	0,385*	0,406	0,410*		bill	0,189	-0,289*	0,284	-0,286*
white-RW-area	-0,185	0,366*	-	-		S8brown	0,237	0,285*	0,28	0,340*
tail	0,125	0,361*	0,237	0,368*		S8black	0,1	0,271*	-	-
B2brown	-0,086	-0,303*	-	-		whiteRW-area	0,112	0,216*	-	-
B2grey	0,11	-0,265*	-	-		B2brown	0,016	-0,203*	-	-
S8black	-0,203	0,236*	-0,114	0,312*		wing	0,142	0,200*	-	-
tarsus	0,091	0,232*	-	-		B2black	0,07	-0,187*	-	-
S8brown	-0,168	0,181*	-	-		B2grey	-0,095	-0,134*	-	-
white-tails-area	0,084	-0,106*	-	-		tarsus	0,005	0,083*	-	-
B2black	0,011	0,033*	-	-		H1grey	0,062	-0,065*	-	-

S1.12: R Script to separate Loci More than three SNPs from the Ipyrad loci output file:

```
gawk -v RS="\\\\|\n" -v FS="\n" -v OFS="\n" '{if(gsub(/*/,"*", $NF)>3)print$0"|"}' snow-ref-snow.loci > snow-ref-snow_filt.loci
gawk -v RS="\\\\|\n" -v FS="\n" -v OFS=">" '{match($0, /\|(.*)/, x); for(i=1; i<NF; i++) {gsub("        ", "\n", $i); printf(">%s\n", $i) >>
"locus_"x[1]".fa"}}' snow-ref-snow_filt.loci
tar -czvf fastas.tar.gz ./*fa
```

Supplementary Material, Appendix S1, supplementary Tables

TableS1.1: Origin of samples used for genetic analysis

Taxon	Country	Locality	**Library ID**	**Lab_ID**	**Sex**	Cyt b	ddRAD
M. n. nivalis	Austria	unknown	L40976	MAR_4842	**H**	xxx	xxx
M. n. nivalis	Austria	Arthurhaus,Salzburg	L40984	MAR_10185	**M**	xxx	xxx
M. n. nivalis	Austria	Arthurhaus,Salzburg	L40985	MAR_10186	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40986	MAR_11139	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40987	MAR_11140	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40988	MAR_2426	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40989	FKN_1	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40990	FKN_2	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40991	FKN_3	**M**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40992	FKN_4	**H**	xxx	xxx
M. n. nivalis	Switzerland	Furkapass	L40993	FKN_5	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41004	UMIB_2A290159	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41005	UMIB_2A290175	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41007	UMIB_2A290162	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41008	UMIB_2A290177	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Vega Huerta	L41009	UMIB_2A290161	**?**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41010	UMIB_2A290150	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41011	UMIB_2A290146	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41012	UMIB_2A290141	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41013	UMIB_2A290998	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41014	UMIB_2A290103	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41015	UMIB_2A290104	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Puertos de Aliva	L41016	UMIB_2L05515	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41017	UMIB_2A290144	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Andara	L41018	UMIB_2A290109	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41019	UMIB_2A290193	**H**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41020	UMIB_2A290195	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Urriellu	L41021	UMIB_2A290187	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41022	UMIB_2A290142	**M**	xxx	xxx
M. n. nivalis	Spain	Picos de Europa, Coriscao	L41023	UMIB_2A290145	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41024	UMIB_1KA52702	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41026	UMIB_1KA52706	**M**	xxx	xxx

M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41027	UMIB_1KA52707	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41028	UMIB_1KA52709	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41029	UMIB_1KA52710	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41030	UMIB_1KA52711	**-**		
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41031	UMIB_1KA52712	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41032	UMIB_1KA52714	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41033	UMIB_1KA52715	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41034	UMIB_1KA52719	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41035	UMIB_1KA52723	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41036	UMIB_1KA52724	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41037	UMIB_1KA52726	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41038	UMIB_1KA52728	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41039	UMIB_1KA52729	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41040	UMIB_1KA52730	**H**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41041	UMIB_1KA52731	**M**	xxx	xxx
M. n. nivalis	Spain	La Mesa, Las Ubiñas	L41042	UMIB_1KA52732	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41043	KU_20377	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41044	KU_20378	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41045	KU_20410	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41046	KU_20460	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41047	KU_20466	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41048	KU_20473	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Omnogobi	L41049	KU_20476	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41050	KU_20653	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41051	KU_20658	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41052	KU_20739	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41053	KU_204759	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41054	KU_204760	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41055	KU_204768	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41057	KU_204770	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Gobi Altai	L41058	KU_204774	**H**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Bondoch Gol	L40977	MAR_3110	**M**	xxx	xxx
M. n. groumgrzimaili	Mongolia	Bondoch Gol	L40978	MAR_3111	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40981	MAR_1532	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40982	MAR_1533	**M**	xxx	xxx
M. n. alpicola	Russia	Kurush,N-Caucasus	L40983	MAR_1534	**-**	xxx	xxx

Appendix IV: Range-wide and regional distribution of the Western Tragopan *Tragopan melanocephalus* and effects of disturbance on local abundances

Supplementary Material

Range-wide and regional distribution of the Western Tragopan *Tragopan melanocephalus* and effects of disturbance on local abundances

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Table S1. Livestock disturbance at census points as inferred from 106 questionnaires filled out by local people; arrival and departure peak: period when herds are arriving at and leaving an area; herds: estimated total number of herds visiting the area (both goats and sheep); goats/sheep/dogs: estimated total numbers of goats, sheep and accompanying dogs in an area; dist. goats/sheep: disturbance level classes for both goats and sheep: 0 (very low) = no goats and sheep in the area, 1 (low) = 1-250 individuals, 2 (moderate) = 251 – 1000 individuals, 3 (high) = more than 1000 individuals; dist, dogs: disturbance level classes for accompanying dogs: 0 (very low) = no dogs in the area, 1 (low) = 1-15 individuals, 2 (moderate) = 16 – 35 individuals, 3 (high) = 36 - 150 individuals; at six sites disturbance level of herds and dogs was set to zero ("/0") for GLM analysis due to late arrival peak of herds at the end of the breeding season.

census point	closest settlement	arrival	departure	herds	goats	dist. goats	sheep	dist. sheep	dogs	dist. dogs
Damzai1	Karoser, Mughal Abad	end of May	September	15	500-550	2	0	0	10 to 15	1
Damzai 2	Karoser	start of June	September	3	150-160	1	40-45	1	4 to 5	1
Damzai 3	Karoser, Gadar	start of June	end of September	10	700-750	2	600	2	10 to 15	1
Singara 1	Lower Singara	not regular	not regular	0	0	0	0	0	0	0
Singara 2	Lower Singara	not regular	not regular	0	0	0	0	0	0	0
Takhto 1	Farwurgah	start of June	August	10	200-250	1	50-60	1	10 to 15	1
Takhto 2	Farwurgah	start of June	September	15-18	600-650	2	500-550	2	15-16	2
Moru	Bar paro	June	September	120-150	2000-2500	3	200-2500	3	100-150	3
Moru Nala	Sheeshy Baig	start of June	end of September	80-100	1500-1800	3	1500-1600	3	100-110	3
Kabkot 1	Barser	end of May	end of August	20	300-350	2	200-250	1	20-25	2
Kabkot 2	Barser	end of May	August	15	200-250	1	300-350	2	15 to 20	2
Kabkot 3	Barser	end of July	end of August	14	400-450	2/0	250-300	2/0	14-16	1/0
Kabkot 4	Barser	August	September	25	700-750	2/0	600-650	2/0	25-30	2/0
Kabkot 5	Barser	August	end of September	30	1000-1200	3/0	800-1000	2/0	30-35	2/0
Kabkot 6	Barser	June	August	20	900-950	2	700-750	2	20-25	2
Kabkot 7	Barser	May	September	25	1200-1250	3	600-650	2	25-30	2
Diwan 1	Diwan	end of May	August	10	250-280	2	150-200	1	10 to 15	1
Diwan 2	Diwan	end of May	August	5 to 6	150-200	1	80-100	1	5 to 7	1
Diwan 3	Diwan	June	August	3	100-150	1	100-120	1	3 to 4	1
Diwan 4	Diwan	July	August	4 to 6	150-200	1/0	100-120	1/0	5 to 10	1/0
Diwan 5	Diwan	June	August	5	100-110	1	100-110	1	5 to 8	1
Diwan 6	Diwan	July	end of August	7	300-350	2/0	200-210	1/0	7 to 8	1/0

Diwan 7	Diwan	end of July	end of August	9	400-450	2/0		200-250	1/0	9 to 12	1/0

Table S2. Anthropogenic disturbance from hunters and collectors of mushrooms and medical plants at census points as inferred from 106 questionnaires filled out by local people; collectors (mushrooms and med. plants) and hunters: estimated total number of visitors in the area; dist. coll1 (mushrooms) and coll2 (medical plants): disturbance level classes for collectors: 0 (very low) = no collectors in the area, 1 (low) = 1-20 individuals, 2 (moderate) = 21 – 99 individuals, 3 (high) = more than 100 individuals; dist, hunting: disturbance level classes for hunters: 0 (very low) = no hunters in the area, 1 (low) = 1-10 individuals, 2 (moderate) = 11– 20 individuals, 3 (high) = more than 20 individuals.

census point	closest settlement	mushrooms	dist. coll1	med. plants	dist. coll2	hunters	dist. hunting	anth. disturbance
Damzai1	Karoser, Mughal Abad	15-16	1	20-25	2	5 to 10	1	1,33
Damzai 2	Karoser	3 to 4	1	0	0	0	0	0,33
Damzai 3	Karoser, Gadar	10 to 20	1	5 to 6	1	7 to 8	1	1
Singara 1	Lower Singara	50-55	2	20-25	2	8 to 10	1	1,67
Singara 2	Lower Singara	20-25	2	4 to 5	1	0	0	1
Takhto 1	Farwurgah	20-25	2	8 to 10	1	5 to 10	1	1,33
Takhto 2	Farwurgah	20-30	2	15-20	1	10 to15	2	1,67
Moru	Bar paro	300-350	3	100-120	3	15-20	3	3
Moru Nala	Sheeshy Baig	50-60	2	30-35	2	10 to 15	2	2
Kabkot 1	Barser	50-60	2	20-25	2	20-25	3	2,33
Kabkot 2	Barser	100-110	3	50-60	2	20-25	3	2,67
Kabkot 3	Barser	100-120	3	40-50	2	15-20	2	2,33
Kabkot 4	Barser	150-160	3	50-60	2	20-25	3	2,67
Kabkot 5	Barser	100-110	3	40-50	2	10 to 15	2	2,33
Kabkot 6	Barser	50-60	2	30-40	2	20-25	3	2,33
Kabkot 7	Barser	100-110	3	40-50	2	12 to 15	2	2,33
Diwan 1	Diwan	50-60	2	20-30	2	15-20	3	2,33
Diwan 2	Diwan	10 to 15	1	8 to 10	1	5 to 6	1	1
Diwan 3	Diwan	10 to 15	1	5 to 6	1	5 to 7	1	1
Diwan 4	Diwan	5 to 6	1	1,2	1	10 to 12	2	1,33
Diwan 5	Diwan	20-30	2	15-20	1	10 to 12	2	1,67
Diwan 6	Diwan	20-25	1	15-20	1	10 to 12	2	1,33
Diwan 7	Diwan	50-60	2	10 to 15	1	10 to 15	2	1,67

Table S3. Occurrence records of the Western Tragopan *Tragopan melanocephalus* used for species distribution modeling; occurrences from online databases such as GBIF (specimens, observations), Oriental Bird Club (OBC) image data base, sound archives (xenocanto; XC) and published literature (for references, see below) and from own field data in Pakistan; collection acronyms: CLO= Cornell Laboratory of Ornithology; FMNH= Field Museum of Natural History, Chicago, USA; MCZ= Museum of Comparative Zoology, Cambridge, USA; NHMUK= Natural History Museum London, UK; UMMZ= University of Michigan Museum of Zoology, USA; WFVZ= Western Foundation of Vertebrate Zoology, Camarillo, Canada; ROM= Royal Ontario Museum, Canada; SMF= Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Germany; YPM= Yale Peabody Museum, USA; months from i (= January) to xii (= December).

No	Location	Province	Country	Elevation (m asl)	Latitude	Longitude	Month	Source
1	Kulu	Punjab	India	1207-1232	31.958	77.111	i, ii, iii, iv, v, xii	Specimen, (MCZ, UMMZ, NHMUK, WFVZ, FMNH), Image (OBC)
2	Oot	Himachal Pradesh	India	1000	31.743	77.209	i	Specimen, (UMMZ)
3	Manali, 2 mi E	Himachal Pradesh	India	1900	32.245	77.187	ii	Specimen, (UMMZ)
4	Kashmir	Jammu and Kashmir	India	4253	34.867	75.842		Specimen, (ROM)
5	top of Sulah and Raipur	Himachal Pradesh	India	1008	32.067	76.483	iii	Specimen, (YPM)
6	Shogran	Himachal Pradesh	India	2335	34.630	73.460	xii	Specimen,(FMNH)
7	Kotgarh	Himachal Pradesh	India	1933	31.311	77.474	i, iii, iv, x	Specimen, (MCZ, NHMUK, ROM, SMF)
8	Mussoorie	Uttarakhand	India	1958	30.460	78.064		Specimen (UMZC)
9	Sainj - Siund Road, Sainj	Himachal Pradesh	India	1276	31.767	77.307	iv	Observation (CLO)
10	Rupi Bhaba Wildlife Sanctuary	Himachal Pradesh	India	2225	31.597	77.836	iv	Observation (CLO)
11	Khorli Poi	Himachal Pradesh	India	3013	31.664	77.511	vi	Observation (CLO)
12	Lapah	Himachal Pradesh	India	2168	31.771	77.427	vi	Observation (CLO)
13	Khrungcha to Chota Vasu	Himachal Pradesh	India	2255	31.664	77.471	ii	Observation (CLO)
14	Great Himalayan NP, Shilte	Himachal Pradesh	India	2828	31.683	77.480	vi, vii	Observation (CLO)
15	Great Himalayan NP, Choidwari	Himachal Pradesh	India	3087	31.689	77.484	iii, v, vi	Observation (CLO)
16	Rolla-Bandru-Duranga-Rolla	Himachal Pradesh	India	2365	31.675	77.487	ii, v	Observation (CLO)
17	Choidwr To Rakhundi	Himachal Pradesh	India	2872	31.690	77.488	ii	Observation (CLO)
18	Choiduar To Shilat	Himachal Pradesh	India	2882	31.692	77.490	i	Observation (CLO)
19	Grani	Himachal Pradesh	India	2882	31.689	77.490	iv	Observation (CLO)

20	Choidwar	Himachal Pradesh	India	2882	31.690	77.488	xii	Observation (CLO)
21	Talai	Himachal Pradesh	India	2791	31.647	77.465	xii	Observation (CLO)
22	Grehni to Rolla	Himachal Pradesh	India	2882	31.687	77.488	xii	Observation (CLO)
23	Bundru to Choiduar	Himachal Pradesh	India	2414	31.679	77.488	i	Observation (CLO)
24	Sarkhan Thatch	Himachal Pradesh	India	3607	31.498	77.833	v	Observation (CLO)
25	Great Himalayan NP	Himachal Pradesh	India	1489	31.641	77.387	I, ii	Observation (CLO)
26	Great Himalayan NP--Rolla camp	Himachal Pradesh	India	2365	31.675	77.487	ix	Observation (CLO)
27	Qadir Gali	North-West Frontier	Pakistan	2991	34.637	73.586	v	Observation (CLO)
28	Giga Nulla - Bandalo	North-West Frontier	Pakistan	2694	35.186	72.861	v	Observation (CLO)
29	Maidan Nulla	North-West Frontier	Pakistan	2098	35.206	72.884	v	Observation (CLO)
30	Upper Maidan Nulla	North-West Frontier	Pakistan	2708	35.226	72.845	v	Observation (CLO)
31	Kholi Poyi	Himachal Pradesh	India	2950	31.674	77.498	iv	Observation (CLO)
32	8,400 ft on the hills w. of Wular	Kashmir	Pakistan	1580	34.370	74.558	ii, iii, vi	Specimen (NHMUK)
33	Simla	Himachal Pradesh	India	1977	31.105	77.173	i, ii, iii, vii	Specimen (NHMUK)
34	the hills behind Dharmsala	Punjab	India	1369	32.219	76.323	ii	Specimen (NHMUK)
35	Chamba Chambra	Himachal Pradesh	India	878	32.553	76.126		Specimen (NHMUK)
36	Almorah, Himalayas	Himachal Pradesh	India	1662	29.589	79.647		Specimen (NHMUK)
37	Baghnala Palumpur, Kangra	Punjab	India	548	31.593	76.267	xii	Specimen (NHMUK)
38	Neelum Valley; Machyara River	Azad Jammu and Kashmir	Pakistan	1506	34.500	73.833	v	Observation (CLO)
39	Palampur, Bugh Nala	Himachal Pradesh	India	1008	32.067	76.483	iii	Specimen (YPM)
40	near Dharmsala	Himachal Pradesh	India	2199	32.233	76.400	i	Specimen (YPM)
41	Great Himalayan NP	Himachal Pradesh	India	1488	31.641	77.387	iv	Observation (CLO)
42	Sarahan Pheasantry	Himachal Pradesh	India	2275	31.507	77.796	iv, v, vi	Call (XC), Image (OBC)
43	Besri, Kaghan	Khyber Pukhtunkhwa	Pakistan	2981	34.661	73.607		Newlands, 1974
44	Behari, Kaghan	Khyber Pukhtunkhwa	Pakistan	2828	34.765	73.651		Newlands, 1974
45	Malakandi	Khyber Pukhtunkhwa	Pakistan	2736	34.610	73.509		Chaudhry, 1992
46	Manur	Khyber Pukhtunkhwa	Pakistan	2474	34.674	73.596		Chaudhry, 1992
47	Malakandi	Khyber Pukhtunkhwa	Pakistan	2736	34.610	73.509		Chaudhry, 1992
48	Manur	Khyber Pukhtunkhwa	Pakistan	2474	34.674	73.596		Chaudhry, 1992

49	Machiara	Azad Jammu and Kashmir	Pakistan	3126	34.563	73.602	Mirza, et al. 1978
50	Jagran	Azad Jammu and Kashmir	Pakistan	2615	34.515	73.806	Mirza, et al. 1978
51	Salkhala	Azad Jammu and Kashmir	Pakistan	2398	34.555	73.915	Mirza, et al. 1978
52	Pir Chinasi	Azad Jammu and Kashmir	Pakistan	2535	34.406	73.585	Islam, 1982
53	Dubair, Indus Kohistan	Khyber Pukhtunkhwa	Pakistan	3129	35.199	72.931	Grimmett and Robson, 1986
54	Kabkot, Palas indus kohistan	Khyber Pukhtunkhwa	Pakistan	2679	35.011	73.213	Duke, 1989
55	Muro, Palas Indus Kohistan	Khyber Pukhtunkhwa	Pakistan	2652	35.022	73.138	Duke, 1989
56	Diwan, Palas Indus Kohistan	Khyber Pukhtunkhwa	Pakistan	3021	35.008	73.263	Duke, 1989
57	Khowari, Palas Indus kohistan	Khyber Pukhtunkhwa	Pakistan	2570	35.020	73.185	Duke, 1989
58	Patan, Indus Kohistan	Khyber Pukhtunkhwa	Pakistan	2823	35.152	73.004	Buner and Cornell, 2008
59	Palas, Indus Kohistan	Khyber Pukhtunkhwa	Pakistan	2444	35.003	73.206	Buner and Cornell, 2008
60	Limber Valley	Kashmir	India	1972	34.130	74.203	Javed, 1992; Ahmad et al. 2017
61	Kishtwar	Kashmir	India	1630	33.312	75.766	Gaston et, al. 1983
62	Doda, bhadarwah	Kashmir	India	1356	33.040	75.654	Gaston et, al. 1983
63	Hunza	Gilgit Baltistan	Pakistan	2110	36.307	74.631	Birdlife International 2001
64	Kayal	Khyber Pukhtunkhwa	Pakistan	1771	35.139	73.030	Birdlife International 2001
65	Kanshian nalla balakot	Khyber Pukhtunkhwa	Pakistan	1041	34.524	73.359	Birdlife International 2001
66	Allai	Khyber Pukhtunkhwa	Pakistan	1627	34.818	73.121	Birdlife International 2001
67	Pir Hasimar	Azad Jammu and Kashmir	Pakistan	2915	34.345	73.636	Birdlife International 2001
68	Qazinag	Azad Jammu and Kashmir	Pakistan	1635	34.197	74.350	Birdlife International 2001
69	Leepa valley	Azad Jammu and Kashmir	Pakistan	2051	34.298	73.895	Birdlife International 2001
70	Haji Pir	Azad Jammu and Kashmir	Pakistan	1674	34.038	74.073	Birdlife International 2001
71	Gangotri	Uttar Pradesh	India	3066	30.993	78.940	Birdlife International 2001
72	Keran	Jammu and Kashmir	India	1833	34.665	73.961	Birdlife International 2001
73	Lolab valley	Jammu and Kashmir	India	1751	34.524	74.392	Birdlife International 2001
74	Uri	Jammu and Kashmir	India	1309	34.087	74.033	Birdlife International 2001
75	Poonch	Jammu and Kashmir	India	908	33.766	74.094	Birdlife International 2001
76	Sandran	Jammu and Kashmir	India	1398	33.506	74.161	Birdlife International 2001
77	Pir Panjal	Jammu and Kashmir	India	4035	33.857	74.399	Birdlife International 2001
78	Gamgul Siyabehi Wildlife Sanctuary	Himachal Pradesh	India	1664	32.776	75.949	Birdlife International 2001

79	Bhataal	Himachal Pradesh	India	1124	31.685	76.912	Birdlife International 2001
80	Sahu	Himachal Pradesh	India	1397	32.600	76.233	Birdlife International 2001
81	Ravi valley	Himachal Pradesh	India	1556	32.587	76.009	Birdlife International 2001
82	Tundah	Himachal Pradesh	India	2343	32.503	76.466	Birdlife International 2001
83	Kugti	Himachal Pradesh	India	2776	32.464	76.704	Birdlife International 2001
84	Tali	Himachal Pradesh	India	2219	31.046	77.516	Birdlife International 2001
85	Kalatop	Himachal Pradesh	India	2348	32.552	76.018	Birdlife International 2001
86	Hamta	Himachal Pradesh	India	4337	32.270	77.347	Birdlife International 2001
87	Macleod Ganj	Himachal Pradesh	India	1699	32.243	76.321	Birdlife International 2001
88	Manalsu	Himachal Pradesh	India	1965	32.254	77.184	Birdlife International 2001
89	Dharamsala	Himachal Pradesh	India	1321	32.219	76.323	Birdlife International 2001
90	Palampur	Himachal Pradesh	India	1271	32.107	76.541	Birdlife International 2001
91	Kandbari	Himachal Pradesh	India	1488	32.116	76.581	Birdlife International 2001
92	Jagatsukh	Himachal Pradesh	India	2014	32.202	77.205	Birdlife International 2001
93	solang nala	Himachal Pradesh	India	2573	32.308	77.151	Birdlife International 2001
94	Kais	Himachal Pradesh	India	2174	32.000	77.154	Birdlife International 2001
95	Pulga	Himachal Pradesh	India	2350	31.992	77.438	Birdlife International 2001
96	Kanawar	Himachal Pradesh	India	1287	31.882	77.164	Birdlife International 2001
97	Nargu	Himachal Pradesh	India	770	31.708	76.932	Birdlife International 2001
98	Khokan sanctuary	Himachal Pradesh	India	1184	31.798	77.018	Birdlife International 2001
99	Kashapat	Himachal Pradesh	India	2474	31.407	77.836	Birdlife International 2001
100	Rampur	Uttarakhand	India	1175	31.451	77.639	Birdlife International 2001
101	Rupi Bhaba sanctuary	Himachal Pradesh	India	2538	31.597	77.836	Birdlife International 2001
102	Lippa sanctuary	Himachal Pradesh	India	3394	31.654	78.331	Birdlife International 2001
103	Pangi	Himachal Pradesh	India	2655	31.589	78.278	Birdlife International 2001
104	Kinnaur	Himachal Pradesh	India	3568	31.651	78.476	Birdlife International 2001
105	Tirthan	Himachal Pradesh	India	3107	31.705	77.569	Birdlife International 2001
106	Barua	Himachal Pradesh	India	2216	31.465	78.178	Birdlife International 2001
107	Sangla	Himachal Pradesh	India	2590	31.424	78.265	Birdlife International 2001
108	Daranghati	Himachal Pradesh	India	2480	31.516	77.807	Birdlife International 2001

109	Tharjot	Himachal Pradesh	India	1841	31.578	77.105		Birdlife International 2001
110	Sungri	Himachal Pradesh	India	2606	31.310	77.702		Birdlife International 2001
111	Jakhu	Himachal Pradesh	India	2445	31.101	77.184		Birdlife International 2001
112	Talra	Himachal Pradesh	India	549	32.241	75.903		Birdlife International 2001
113	Tons river	Himachal Pradesh	India	694	30,708	77,755		Birdlife International 2001
114	Chur Mountain	Himachal Pradesh	India	3594	30,871	77,480		Birdlife International 2001
115	Uttarakashi district	Uttar Pradesh	India	3245	30,924	78,465		Birdlife International 2001
116	Raveri, Machiara	Azad Jammu and Kashmir	Pakistan	2845	34,578	73,561		Birdlife International 2001
117	Mali, Machiara	Azad Jammu and Kashmir	Pakistan	3131	34,534	73,641		Birdlife International 2001
118	Kuthiali, Machiara	Azad Jammu and Kashmir	Pakistan	3039	34,535	73,617		Birdlife International 2001
119	Moryan, Machiara	Azad Jammu and Kashmir	Pakistan	2568	34,531	73,608		Birdlife International 2001
120	Charyal, Machiara	Azad Jammu and Kashmir	Pakistan	2800	34,528	73,648		Birdlife International 2001
121	Chita Kushkar, Serli Sacha	Azad Jammu and Kashmir	Pakistan	3397	34,527	73,661		Birdlife International 2001
122	Sehr, Serli Sacha	Azad Jammu and Kashmir	Pakistan	2787	34,530	73,644		Birdlife International 2001
123	Daper, Serli Sacha	Azad Jammu and Kashmir	Pakistan	3175	34,522	73,656		Birdlife International 2001
124	Chain baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3150	354	73,222	v	Own data, this study
125	Chain baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	2859	350	73,217	v	Own data, this study
126	Eel baig, Kabkot palas	Khyber Pukhtunkhwa	Pakistan	3085	35,010	73,218	v	Own data, this study
127	Eel baig, Kabkot palas	Khyber Pukhtunkhwa	Pakistan	2785	35,017	73,225	v	Own data, this study
128	Chain baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3313	355	73,228	v	Own data, this study
129	Chain baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	2890	350	73,218	v	Own data, this study
130	Derh, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3215	34,983	73,216	v	Own data, this study
131	Hubaig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3005	34,993	73,213	v	Own data, this study
132	Konari, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3061	34,985	73,206	v	Own data, this study
133	Chain baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3147	34,987	73,216	v	Own data, this study
134	Eel baig, Kabkot Palas	Khyber Pukhtunkhwa	Pakistan	3257	34,996	73,217	v	Own data, this study
135	Guraj baig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	2849	355	73,255	v	Own data, this study
136	Shamir baig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3125	35,013	73,266	v	Own data, this study
137	Hubaig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3035	354	73,250	v	Own data, this study
138	Shamir baig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3280	358	73,267	v	Own data, this study

139	Shamir baig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3018	35,011	73,265	v	Own data, this study
140	Guraj baig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3281	352	73,260	v	Own data, this study
141	Hubaig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	3014	355	73,250	v	Own data, this study
142	Dadar baig, Diwan palas	Khyber Pukhtunkhwa	Pakistan	2881	359	73,252	v	Own data, this study
143	Dadar baig, Diwan palas	Khyber Pukhtunkhwa	Pakistan	2805	35,015	73,251	v	Own data, this study
144	Hubaig, Diwan Palas	Khyber Pukhtunkhwa	Pakistan	2864	355	73,252	v	Own data, this study
145	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3015	35,054	73,248	v	Own data, this study
146	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3062	35,052	73,249	v	Own data, this study
147	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3109	35,056	73,245	v	Own data, this study
148	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2687	35,061	73,149	xi	Own data, this study
149	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2555	35,060	73,152	xi	Own data, this study
150	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2854	35,060	73,147	xi	Own data, this study
151	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2236	35,050	73,167	xi	Own data, this study
152	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2822	35,056	73,168	xi	Own data, this study
153	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2486	35,048	73,174	xi	Own data, this study
154	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2163	35,047	73,169	xi	Own data, this study
155	Karoser, Palas	Khyber Pukhtunkhwa	Pakistan	2193	35,044	73,173	xi	Own data, this study
156	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3021	35,050	73,251	xi	Own data, this study
157	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	2901	35,052	73,249	xi	Own data, this study
158	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3244	35,054	73,252	xi	Own data, this study
159	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3157	35,055	73,250	xi	Own data, this study
160	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	3025	35,055	73,245	xi	Own data, this study
161	Singara, Palas	Khyber Pukhtunkhwa	Pakistan	2970	35,052	73,248	xi	Own data, this study
162	Singara upper	Khyber Pukhtunkhwa	Pakistan	3225	35,054	73,241	iv	Own data, this study
163	Singara lower	Khyber Pukhtunkhwa	Pakistan	2962	35,055	73,249	iv	Own data, this study
164	Shamir baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3104	35,014	73,267	iv	Own data, this study
165	Guraj baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3251	353	73,257	iv	Own data, this study
166	Sheeshy baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3325	34,992	73,255	iv	Own data, this study
167	Hu baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3039	356	73,243	iv	Own data, this study
168	Kachal baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3274	351	73,242	iv	Own data, this study

169	Barid baig, Diwan	Khyber Pukhtunkhwa	Pakistan	3413	34,992	73,240	iv	Own data, this study
170	Eel baig, Kabkot	Khyber Pukhtunkhwa	Pakistan	2921	35,021	73,222	iv	Own data, this study
171	Chain baig, Kabkot	Khyber Pukhtunkhwa	Pakistan	3012	352	73,219	iv	Own data, this study
172	Hu baig, Kabkot	Khyber Pukhtunkhwa	Pakistan	3336	34,991	73,218	iv	Own data, this study
173	Derh, Kabkot	Khyber Pukhtunkhwa	Pakistan	3154	34,982	73,219	iv	Own data, this study
174	Konari, Kabkot	Khyber Pukhtunkhwa	Pakistan	3621	34,983	73,201	iv	Own data, this study
175	Chupper baig, Kabkot	Khyber Pukhtunkhwa	Pakistan	3208	34,994	73,200	iv	Own data, this study
176	Chui baig, Kabkot	Khyber Pukhtunkhwa	Pakistan	2835	35,010	73,197	iv	Own data, this study

References listed under "source"

Ahmad, R., Sharma, N., Pacchnanda, U., Suhail, I., Deb, K., Bhatnagar, Y.V., Kaul, R. (2017) Distribution and conservation status of the western tragopan *Tragopan melanocephalus* in Jammu and Kashmir, India. Current Science, 112: 1948-1953.

BirdLife International (2001) Threatened Birds of Asia: the BirdLife International Red Data Book. Cambridge, Birdlife International; species factsheet for the Western Tragopan on p. 870-887.

Buner , F., Connell , J . (2008) Galliform monitoring and conservation project in Pakistan's Palas Valley . Progress report to the World Pheasant Association and the British Ornithological Society. Unpublished report.

Chaudhry , A. A . (1992) Distribution and status of pheasants in Pakistan. In: Jenkins , D. (Ed.) Pheasants in Asia 1992. World Pheasant Association, Reading, UK (1993), p. 7-14.

Duke, G. (1989) Survey of the Western Tragopan Tragopan melanocephalus and its montane forest habitat in the Palas and Kandia valleys, Indus Kohistan, Pakistan. May–June 1989. Final report, ICBP/WPA project; (cited in: BirdLife International 2001)

Gaston , A. J. , Islam , K. and Crawford , J. A . (1983) The current Status of the Western Tragopan (*Tragopan melanocephalus*). World Pheasant Association Journal 8:40 – 49

GBIF.org (15 January 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.fx3wsr

Grimmett, R., Robson, C. (1984) A preliminary survey of the Western Tragopan in Indus Kohistan and the Kaghan Valley in Pakistan. Unpublished Appendix to report submitted to National Council for Wildlife Preservation, Government of Pakistan; (cited in: BirdLife International 2001)

Islam , K . (1982) Status and distribution of the Western Tragopan in Northeastern Pakistan . Proc. Int. Symp. Pheasants in Asia 2:44 – 51

Javed, S. (1992) Birds of Limber valley forest (Jammu and Kashmir). Newsletter for Birdwatchers 3:13 – 15

Mirza, Z. B., Aleem, A., Asghar, M. (1978) Pheasant surveys in Pakistan. J. Bombay Nat. Hist. Soc. 75:292 – 296

Newlands, W. A. (1974) Kaghan Valley: Home of the Western Tragopan. Wildlife 16:436 – 441

Table S4. Results of principal component analysis based on six disturbance indices for numbers of goats, sheep, dogs, mushroom collectors (mush. coll.), medicinal plant collectors (plant coll.); eigenvalues, proportion of variation explained (prop. var= absolute, prop. cum = cumulative) shown for six principal components (PC1-PC6) explaining 100% of the total variation, including factor loadings for the six predictor variables.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Eigenvalue	3.0	1.2	< 0.5	< 0.5	< 0.5	< 0.5
prop. var.	0.6254	0.2416	0.0624	0.0458	0.0150	0.0099
prop. cum.	0.6254	0.8670	0.9293	0.9751	0.9901	1.0000
	factor loadings					
goats	0.559	0.256		0.529	0.557	0.169
sheep	0.525	0.200	-0.139	-0.518	-0.283	0.563
dogs	0.532	0.146		-0.122	-0.246	-0.787
mush.coll	0.137	-0.541	-0.709	-0.18	0.383	
plant.coll	0.199	-0.404	-0.16	0.603	-0.618	0.162
hunters	0.265	-0.646	0.67	-0.205	0.143	