


# Integrating genetic and stable isotope analyses to infer the population structure of the White-winged Snowfinch *Montifringilla nivalis* in Western Europe

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**Abstract** The population structure and seasonal movements of alpine birds in Europe are still largely unknown. Species living in high mountains now face acute risks of habitat loss, range contractions and local extinction due to current and projected climate change. Therefore, a better understanding of the spatial structuring and exchange among populations of European mountain birds is important from both ecological and conservation points of view. The White-winged Snowfinch *Montifringilla nivalis* is one of the most characteristic passerines of alpine habitats in Europe. Despite the fact that its breeding nuclei are relatively well defined, we still know little about the species' population structure and movements in Western Europe. By analysing two mitochondrial loci (cytochrome *b* and the control region) and stable isotopes of hydrogen ( $\delta^2\text{H}$ ), we assessed to what extent breeding populations of White-winged Snowfinches in the Cantabrian Mountains (CM), the Pyrenees and the Alps, and also a wintering population in the Eastern Pyrenees, function as a metapopulation. We

first show the phylogenetic relationships of the White-winged Snowfinch (*Montifringilla nivalis* subsp. *nivalis*) within the Snowfinch complex. When assessing the population structure in Western Europe, most mitochondrial haplotypes were present in all breeding populations, but one was only found in the CM where it predominated. The most widespread haplotypes at the breeding grounds were found in the majority of the wintering individuals, but none of them showed the haplotype specific to the CM. We did not find differences in  $\delta^2\text{H}$  for the primary feathers among breeding populations, but rectrices of individuals wintering in the Pyrenees had considerably lower  $\delta^2\text{H}$  values: isotopic analysis could thus be useful to assign wintering birds to their Alpine breeding grounds. Further studies combining ringing and the analyses of intrinsic markers are an essential step in better appraising the species' metapopulation dynamics and guiding conservation.

**Keywords** Alpine environments · Bird ringing · Intrinsic markers · Migration · Population dynamics

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## Zusammenfassung

### Die Populationsstruktur des Schneesperlings *Montifringilla nivalis* in Westeuropa auf Grundlage von genetischen und Isotopenanalysen

Die Populationsstrukturen und saisonalen Wanderungen alpiner Vögel in Europa sind noch immer weitgehend unbekannt. Hochalpine Arten sehen sich akuten Risiken von Habitatverlust, Lebensraumschwund und lokaler Extinktion durch den gegenwärtigen und erwarteten Klimawandel gegenüber. Deswegen ist ein besseres Verständnis der räumlichen Struktur und des Austauschs zwischen Populationen europäischer alpiner Vögel sowohl unter ökologischen als auch naturschützerischen Gesichtspunkten wichtig. Der Schneesperling oder Schneefink *Montifringilla nivalis* ist ein Charaktervogel alpiner Habitats in Europa. Obwohl seine Brutvorkommen relativ gut bekannt sind, wissen wir wenig über die Populationsstruktur und Wanderungen der Art in Westeuropa. In dieser Arbeit untersuchten wir mittels zweier mitochondrialer Loci (Cytochrom *B* und Kontrollregion) und Wasserstoffisotopen ( $\delta^2\text{H}$ ) inwiefern Brutpopulationen in den Pyrenäen, Alpen, Kantabrischen Gebirge, und eine Winterpopulation in den östlichen Pyrenäen als Metapopulation fungieren. Wir charakterisierten zuerst die phylogenetischen Beziehungen der Unterart *nivalis* innerhalb des Artenkomplexes. Bei der Untersuchung der Populationsstruktur in Westeuropa wurden die meisten mitochondrialen Haplotypen in allen Brutpopulationen nachgewiesen. Lediglich im Kantabrischen Gebirge dominierte ein privater Haplotyp. Dieser wurde auch nicht in den überwinterten Vögeln gefunden, bei denen die sonst am weitesten verbreiteten Haplotypen überwiegen. Wir fanden keine Unterschiede im  $\delta^2\text{H}$  von Handschwingen zwischen den Brutgebieten, allerdings hatten in den Pyrenäen überwinterte Individuen deutlich niedrigere  $\delta^2\text{H}$  Werte in den Schwanzfedern. Dies deutet darauf hin, dass Isotopenanalysen es ermöglichen könnten überwinterte Tiere ihren alpinen Brutgebieten zuzuordnen. Bei zukünftigen Studien wäre insbesondere die kombinierte Analyse von Ringfunden und intrinsischen Markern ein wichtiger Schritt, um die Metapopulationsdynamik der Art besser zu verstehen und Hinweise für ihren Schutz zu liefern.

## Introduction

The population structure and seasonal movements of montane bird species in Europe are still largely unknown. What is certain, however, is that their alpine ecosystems,

and hence their species assemblages, are especially vulnerable to climate change (Beniston 2003; Huntley et al. 2007; La Sorte and Jetz 2010). Bird species occurring in high mountains and particularly adapted to cold climatic conditions now face high risks of habitat loss, range contraction and local extinction as a consequence of global warming (Huntley et al. 2007; Flousek et al. 2015). Due to the difficulties of studying them in such challenging environments, studies of alpine birds have been relatively scarce compared to those focused on farmland, forest or lowland species (Chamberlain et al. 2012). Therefore, there is a need for a better understanding of the spatial structuring and movements among populations of European mountain birds, a requisite to appraising the impacts of global warming on their population dynamics in order to guide conservation efforts (see Chamberlain et al. 2012; Lehikoinen et al. 2014; Flousek et al. 2015).

Most of our current knowledge on bird migration has been obtained by bird ringing, and this has provided invaluable information for a better understanding of the ecology, life history, behaviour and conservation of many species (Baillie 2001; Bairlein 2001; Marchant 2002). Despite the importance of this conventional method in deciphering the patterns of bird movements, ringing has the main limitation that recovery rates of individuals moving seasonally across large geographical areas are usually very low and can be affected by spatial heterogeneity (Norris et al. 2006; Korner-Nievergelt et al. 2014). Alternative ways of elucidating patterns of bird migration have rapidly emerged in the past decades and, nowadays, the use of intrinsic markers, either genetic or isotopic, constitutes a promising approach (Webster et al. 2002; Coiffait et al. 2009; Hobson 2011). Intrinsic markers are particularly useful because no prior capture of a given individual is required to infer migratory movements (Rubenstein and Hobson 2004; Hobson 2005).

Genetic markers frequently include the analysis of mitochondrial DNA (mtDNA), which may enable inferring the population structure and the phylogenetic history of species (Webster et al. 2002; Wennerberg et al. 2002; Dong et al. 2013). Moreover, if some specific haplotypes occur on geographically distant breeding grounds, then mtDNA can be used for tracing the breeding origin of migrating or wintering individuals (see Wennerberg 2001; Webster et al. 2002; Liu et al. 2012). The analysis of hydrogen stable isotopes [i.e. the ratio of the heavier ( $^2\text{H}$ ) vs. the lighter ( $^1\text{H}$ ) isotopes;  $\delta^2\text{H}$ ] in bird tissues can also inform us about avian migration (Hobson 2005; Hobson and Wassenaar 2008; Inger and Bearhop 2008; Hobson 2011). Values of  $\delta^2\text{H}$  in precipitation vary along a latitudinal and elevational gradient on most continents (Clark and Fritz 1997). As diet elements are transferred into bird tissues, they provide information about location at the time of tissue

growth (Rubenstein and Hobson 2004; Hobson 2008). In this regard, feathers are commonly used because species' moulting patterns are usually well known (Jenni and Winkler 1994). As feathers are metabolically inert tissues, they also inform us about the isotope ratios in the areas where they were grown (e.g. breeding grounds) (Hobson et al. 2004; Rubenstein et al. 2002; Rubenstein and Hobson 2004). Overall, by combining the use of different intrinsic markers ecologists can now try to unravel patterns of bird movements, even if distances covered are vast and/or bird locations remote (see Hobson and Wassenaar 2008).

The White-winged Snowfinch *Montifringilla nivalis* is a characteristic passerine of alpine habitats with seven subspecies that are widely distributed but scattered across the Palearctic mountain ranges, from Iberia to the Tibetan plateau (del Hoyo et al. 2009). The species inhabits barren rocky habitats next to alpine pastures situated above the tree line and up to glaciers. In Europe, the subspecies *Montifringilla nivalis nivalis* presents a patchy breeding distribution, ranging from the Cantabrian Mountains (CM) in north-western Spain throughout the Pyrenees, the Alps, Corsica and the Apennines east to Greece (Cramp and Perrins 1994). Breeding sites are usually above 2000 m a.s.l. and nests are constructed in cliff crevices but also in human-built infrastructure such as mountain buildings or ski-lift pylons (Heiniger 1991; Strinella et al. 2011a; Grangé 2008; del Hoyo et al. 2009). Outside the breeding season, White-winged Snowfinches are typically gregarious and can form large flocks that, depending on severity of weather, descend to lower elevations. Little is known, however, about the connections between breeding and wintering populations. Traditionally, White-winged Snowfinches in Europe have been considered to be resident, with mostly local, seasonal elevational movements (del Hoyo et al. 2009). Yet, based on a 10-year ringing capture-mark-recapture programme conducted on a wintering population of the Spanish Eastern Pyrenees, we found that some individuals breeding in the European Alps move to the Pyrenees for overwintering (authors' unpublished data). In particular, in 2006 we recovered one wintering individual in the Eastern Pyrenees that had been ringed as a juvenile in the previous breeding season in the Austrian Alps (distance ca. 1065 km). Moreover, three wintering individuals ringed in the Eastern Pyrenees in 2009 were recovered in the Swiss and Italian Alps (distances of ca. 501–760 km) between 2009 and 2012 (see Table 1). However, low recovery rates and spatial heterogeneity in ringing effort and recovery probability make it difficult to quantify long-distance movements so that the magnitude of the phenomenon remains unclear (see Cramp and Perrins 1994). The use of complementary approaches, such as the analysis of intrinsic markers, could thus enhance our ability to answer key questions

concerning the species' population structure and dynamics.

The main goal of our study was to assess the degrees of exchange and movement among different populations of White-winged Snowfinch across Western Europe. The specific objectives were:

1. To estimate the phylogenetic relationships of the White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) study populations within the Snowfinch complex.
2. To investigate the genetic structure of White-winged Snowfinch populations in Western Europe by analysing two mitochondrial loci, cytochrome *b* (*cytb*) and the control region (CR).
3. To determine the potential breeding origins of individuals from a wintering population in the Eastern Pyrenees by analysing  $\delta^2\text{H}$  values in feathers.

Our predictions were that if White-winged Snowfinches exchange among populations and gene flow occurs, then no clear genetic structure would be found either in their breeding or their wintering grounds. Otherwise, some kind of genetic structure would be indicative of populations being mainly resident and isolated. We also predicted a wide isotopic range for the wintering population in the Eastern Pyrenees due to the influx of breeding birds from the Alps. The latter were expected to be isotopically different from local birds in the Pyrenees.

## Methods

### Study area and data collection

Between 2009 and 2014, we captured, sampled and released a total of 104 White-winged Snowfinches during the breeding (July and August;  $n = 48$ ) and wintering (January–March;  $n = 56$ ) seasons. Sampled sites included three breeding areas: the CM (north-western Spain), the Spanish central Pyrenees (CP; northern Spain), and the Swiss Alps (SA; south-western Switzerland); and a single wintering location in the Spanish Eastern Pyrenees (EP; Fig. 1). During the breeding season, we captured 36 adults and fledglings in the CM, six adults in the CP and another six in the SA. Breeding grounds were located above the tree line in barren rocky habitats next to alpine pastures and snow surfaces, where the birds forage during the reproductive period (Heiniger 1991; Antor 1995; Strinella et al. 2007; Grangé 2008). In the CM and the CP, nests of captured individuals were constructed on inaccessible cliffs, whereas individuals captured in the SA were breeding on mountain buildings. All breeding birds were captured with mist nets. We further captured 56 wintering individuals of unknown age in the EP near a ski resort area at an elevation

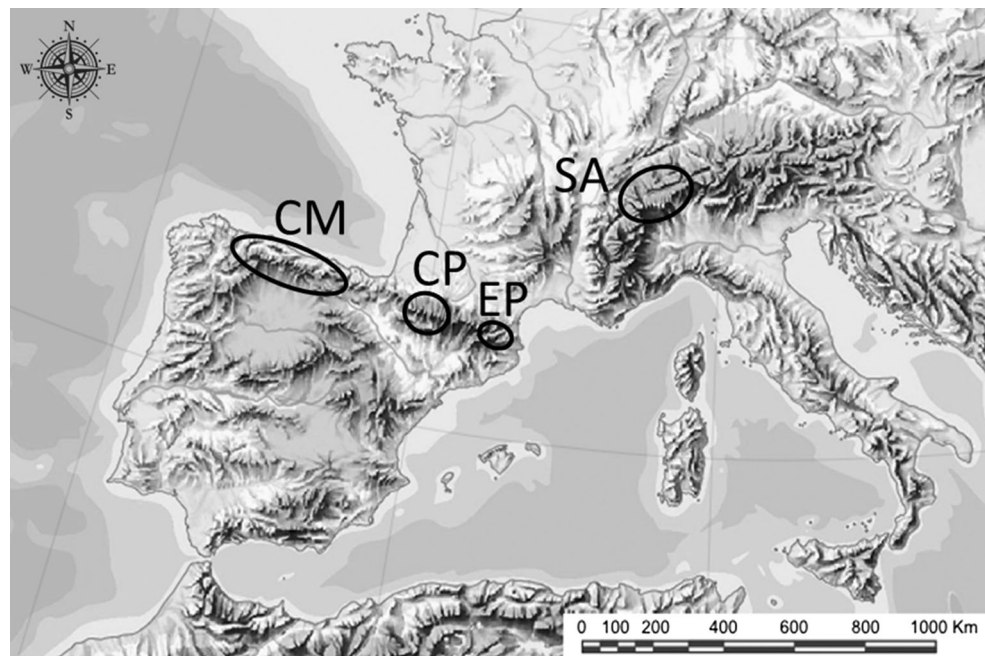
**Table 1** Ring recoveries of Snowfinches within a 10-year ringing capture-mark-recapture programme conducted on a wintering population of the Spanish Eastern Pyrenees (Toses, Catalonia)

Ring n°	Place ringed	Coordinates	Date ringed	Age (Euring)	Place recovered	Coordinates	Date recovered	Distance (km)	Time (days)
DK01459	Austrian Alps	47°31N, 13°25E	3 June 2005	First year (3)	Eastern Pyrenees	42°19N, 02°01E	14 January 2006	1065	225
2A186745	Eastern Pyrenees	42°19N, 02°01E	2 January 2009	Unknown (4)	Swiss Alps	46°40N, 09°19E	10 March 2011	760	797
2A186716	Eastern Pyrenees	42°19N, 02°01E	2 January 2009	Unknown (4)	Italian Alps	44°22N, 07°05E	07 August 2012	501	1313
2A234827	Eastern Pyrenees	42°19N, 02°01E	24 January 2009	Unknown (4)	Swiss Alps	46°09N, 07°28E	07 March 2009	612	42

All birds were recovered alive and released in good physical condition

The information provided includes: Ring number, Place ringed and Coordinates, Date ringed, Age (Euring code) by ringing, Place recovered and Coordinates, Date recovered, Distance (km) and Time (days) from ringed to recovered place. All birds were recovered alive and released in good physical condition

**Fig. 1** Map of the study area. The monitored White-winged Snowfinch breeding populations included those of the Cantabrian Mountains (CM), the Spanish central Pyrenees (CP) and the south-western Swiss Alps (SA), which are the westernmost breeding grounds of the species' European distribution. The monitored wintering population was in the Spanish Eastern Pyrenees (EP)



of ca. 1850 m a.s.l. Wintering birds were attracted by setting feeders with grain for the whole winter season and they were captured by using a whoosh net. All captured individuals were marked with a conventional numbered metal ring on the tarsus, and either the innermost primary (P1) or the outermost rectrix (R6) (in few cases both) was collected for subsequent genetic and isotopic analyses (see below). Feathers were stored in sealed plastic or paper bags at  $-20\text{ }^{\circ}\text{C}$  until analysed.

#### DNA extraction and sequencing

DNA was extracted from the feather calamus following a standard phenol–chloroform protocol (Green and

Sambrook 2012). Subsequent polymerase chain reactions (PCRs) were performed in reaction volumes of  $25\text{ }\mu\text{L}$  in a GeneAmp PCR System 9700 (Applied Biosystems) to amplify the mitochondrial *cytb* and the CR. The *cytb* was amplified by using the primer pair O-L14851 and O-H16065 (Weir and Schluter 2007). PCR conditions with Promega GoTaq G2 DNA Polymerase were the following: initial denaturation at  $94\text{ }^{\circ}\text{C}$  for 5 min, followed by 30 cycles of denaturation at  $94\text{ }^{\circ}\text{C}$  for 45 s, annealing at  $56\text{ }^{\circ}\text{C}$  for 45 s, extension at  $72\text{ }^{\circ}\text{C}$  for 1.5 min, and a final extension step at  $72\text{ }^{\circ}\text{C}$  for 10 min. The CR was amplified by using the primer pair F304 and H1261 (Marshall and Baker 1997), and PCR conditions were the following: denaturation at  $94\text{ }^{\circ}\text{C}$  for 5 min, subsequent 35 cycles of

denaturation at 93 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. All amplified fragments were checked for correct size on a 1.5% agarose gel by comparing them with a 100-base pair (bp) ladder (Fermentas), and they were cleaned for cycle sequencing PCR with the GenElute PCR clean-up kit (Sigma). The purified PCR products were diluted with 40 µL distilled H<sub>2</sub>O. Cycle sequencing reaction was carried out by using the Terminator Ready Reaction Mix Big Dye (version 3.1; Applied Biosystems) and the amplification primers in a volume of 10 µL. The sequencing reaction conditions included an initial denaturation at 96 °C for 5 min, 30 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 10 s, and extension at 60 °C for 4 min and 30 s. The PCR products were purified using a sodium-acetate precipitation protocol, and then separated and detected on an ABI PRISM 3130 Genetic Analyser (Applied Biosystems).

### Sequence analyses

Sequences from *cytb* and CR were aligned separately by using the ClustalW algorithm (Thompson et al. 1997) implemented in BioEdit 7.2.5 (Hall 1999). Sequences were compared visually to the original chromatograms to avoid reading errors. First, for the phylogenetic analysis, we created a data set with 74 of our *cytb* sequences (including both breeding and wintering individuals; GenBank Accession nos. KX369044.1–KX369117.1) together with the 96 published *cytb* sequences available in GenBank for the genera *Montifringilla*, *Pyrgilauda* and *Onychostruthus* (see Qu et al. 2006), one White-winged Snowfinch (*M. nivalis* subsp. *nivalis*; GenBank Accession no. DQ244058.1), one White-winged Snowfinch (*Montifringilla nivalis* subsp. *groumgrzimali*; JX236394.1), one Tibetan Snowfinch *Montifringilla henrici* (DQ244059.1), nine Black-winged Snowfinches *Montifringilla adamsi* (FJ624139.1–FJ624146.1, DQ244060.1), nine Blanford's Ground Sparrow *Pyrgilauda blanfordi* (FJ624113.1–FJ624120.1, DQ244063.1), one Small Ground Sparrow *Pyrgilauda davidiana* (EF530034.1), 34 Rufous-necked Ground Sparrows *Pyrgilauda ruficollis* (AY825303.1–AY825329.1, AY961957.1–AY961963.1) and 40 White-rumped Ground Sparrows *Pyrgilauda* (= *Onychostruthus*) *taczanowskii* (EU382121.1–EU382137.1, EU382139.1–EU382160.1, DQ244061.1).

Then, based on 340-bp sequences, we only considered different haplotypes both within our samples ( $n = 5$  haplotypes, originating from all sampled populations) and those collected from GenBank: White-winged Snowfinch (*M. nivalis* subsp. *nivalis*;  $n = 1$ ), White-winged Snowfinch (*M. nivalis* subsp. *groumgrzimali*;  $n = 1$ ), Tibetan Snowfinch ( $n = 1$ ), Black-winged Snowfinch ( $n = 4$ ),

Blanford's Ground Sparrow ( $n = 6$ ), Small Ground Sparrow ( $n = 1$ ), Rufous-necked Ground Sparrow ( $n = 20$ ) and White-rumped Ground Sparrow ( $n = 22$ ). One sequence from the House Sparrow *Passer domesticus* (AY495393.1) was included as an outgroup to root the tree. Phylogenetic relationships were determined by Bayesian inference (BI) implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and by Maximum Likelihood (ML) algorithms implemented in MEGA 6 (Tamura et al. 2013). We selected the general time-reversible model with a  $\gamma$ -shaped distribution of rates across sites (GTR+G) as the optimal nucleotide substitution model based on the Bayesian Information Criterion (BIC) implemented in jModelTest 2.1.7 (Darriba et al. 2012). The Bayesian analyses were run four times for 10 million generations, and the ML analyses were run by bootstrapping with 1000 replicates to infer the phylogenetic trees. The topology of the BI tree was similar to the ML tree, which is the one presented showing both bootstrap and Bayesian posterior probabilities for the main clades. Net average genetic distances (Tamura 3-parameter model with  $\gamma$  correction) between phylogenetic lineages (i.e. species and subspecies) were also calculated in MEGA. Second, revised sequences from both mitochondrial markers (*cytb* and CR) were concatenated into a single 501-bp sequence to assess the population genetic structure by haplotype network analysis. The haplotype network was visualised with the median-joining method in PopART version 1.7 (Leigh and Bryant 2015).

### Stable isotope analysis

A total of 103 feathers were included for  $\delta^2\text{H}$ : 34 from the CM, five from the CP and six from the SA during the breeding season, and 58 from the EP during the wintering season. All samples were cleaned using a 2:1 chloroform-methanol solvent rinse and air dried in a fume hood. We corrected for exchangeable hydrogen by conducting the hydrogen isotope analyses with the comparative equilibration method described by Wassenaar and Hobson (2003), thus using three calibrated keratin hydrogen isotope reference materials (CBS  $-197\text{‰}$ , SPK  $-121\text{‰}$ , KHS  $-54.1\text{‰}$ ). Approximately 0.35 mg of the feather material was weighed into a silver cup, crushed, and then loaded into a zero-blank carousel. Pyrolytic combustion of samples under He flow (1350 °C) produced H<sub>2</sub> gas that was separated in an elemental analyser (Eurovector, Milan, Italy) and analysed using continuous-flow isotope-ratio mass spectrometry (Isoprime, Manchester, UK). Isotope measurements are expressed in the typical  $\delta$  notation as part per 1000 (‰) relative to Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation. Within-run analytical precision was estimated to be  $\pm 2\text{‰}$  based on five replicate measurements from each of the three keratin

reference standards for every 84 sample unknowns. Measurements of  $\delta^2\text{H}$  were performed at the Stable Isotope Hydrology and Ecology Laboratory of Environment Canada in Saskatoon, Canada.

## Results

### Phylogenetic analysis

The phylogenetic relationships of our samples within the Snowfinch complex are consistent with current taxonomic classification (Fig. 2). The different genera formed a monophyletic group with four well-supported clades of mitochondrial haplotypes: first Rufous-necked Ground Sparrow; second Blanford's Ground Sparrow and Small Ground Sparrow; third White-rumped Ground Sparrow; and fourth Black-winged Snowfinch, Tibetan Snowfinch and White-winged Snowfinch (*M. nivalis* subsp. *groumgrzimaili* and *M. nivalis* subsp. *nivalis*). Within this last group, the five haplotypes obtained from our samples clustered with a haplotype of a White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) obtained from GenBank that was also found in our sequences. Monophyly of the White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) haplotypes was highly supported in contrast to other relationships between *Montifringilla* taxa such as White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) vs. (*M. nivalis* subsp. *groumgrzimaili*) or Black-winged Snowfinch vs. Tibetan Snowfinch (see Fig. 2). Overall, net average genetic distance was shortest between Blanford's Ground Sparrow and Small Ground Sparrow (0.2%) and largest between White-rumped Ground Sparrow and White-winged Snowfinch (*M. nivalis* subsp. *groumgrzimaili*) (16.4%). Distances between White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) and the other taxa were all relatively large: Black-winged Snowfinch (5.9%), Tibetan Snowfinch (7.4%), White-winged Snowfinch (*M. nivalis* subsp. *groumgrzimaili*) (8.4%), Blanford's Ground Sparrow (12.1%), Small Ground Sparrow (12.5%), Rufous-necked Ground Sparrow (12.9%) and White-rumped Ground Sparrow (14.3%).

### Population genetic structure

We identified 11 haplotypes in the concatenated sequence data from 104 White-winged Snowfinches (*M. nivalis* subsp. *nivalis*) sampled at breeding or wintering sites (Fig. 3). One haplotype was specific to the CM, where it predominated (24 out of 36 individuals), but none of the 56 wintering individuals in the EP had this haplotype. The other four most common haplotypes were widespread among breeding sites, although one was not detected in the

CM and another one was not detected in the CP. These four haplotypes, however, were all present in the wintering individuals. Six additional haplotypes were found in one or two wintering individuals each. Overall, with ten of 11 haplotypes, the wintering birds in the Pyrenees harboured almost all mitochondrial diversity detected in the White-winged Snowfinches (*M. nivalis* subsp. *nivalis*) in this study (see Fig. 3).

### Stable hydrogen isotopes

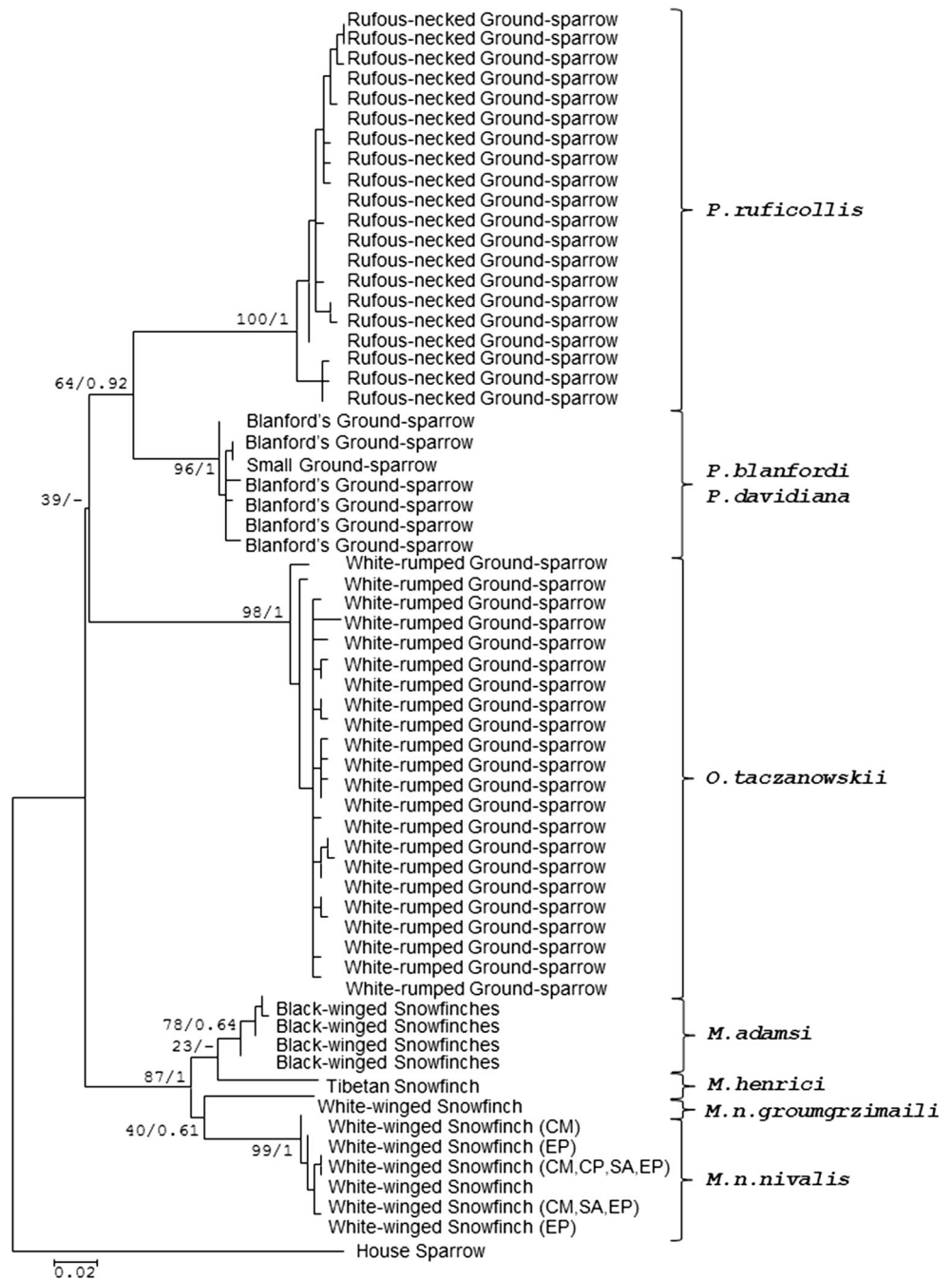
There were no differences in feather  $\delta^2\text{H}$  values between individuals from breeding (CM, CP and SA) and wintering (EP) populations for P1 (Fig. 4). Therefore, we could not determine the breeding origin of wintering individuals by analysing  $\delta^2\text{H}$  values in their primaries. Nevertheless, the analysis of  $\delta^2\text{H}$  values in R6 showed that wintering individuals had considerably more negative values than those from the breeding populations in the CM and the CP (rectrices from the SA were not available; see Fig. 4).

## Discussion

By combining two mitochondrial loci (*cytb* and CR) and stable hydrogen isotopes ( $\delta^2\text{H}$ ), the analyses of intrinsic markers allowed us to assess for the first time the phylogeny, population structure and winter movements of the White-winged Snowfinch in Western Europe. In particular, our study examined past exchange among breeding populations in the CM, the CP and the SA, and current winter movements of Alpine birds to the Pyrenees. Our results not only are important because they provide novel information about the species' population ecology, but especially because they improve our understanding of the population dynamics of European alpine birds. That knowledge is essential if we aim to assess the potential impacts of climate change on alpine bird communities (La Sorte and Jetz 2010; Chamberlain et al. 2012).

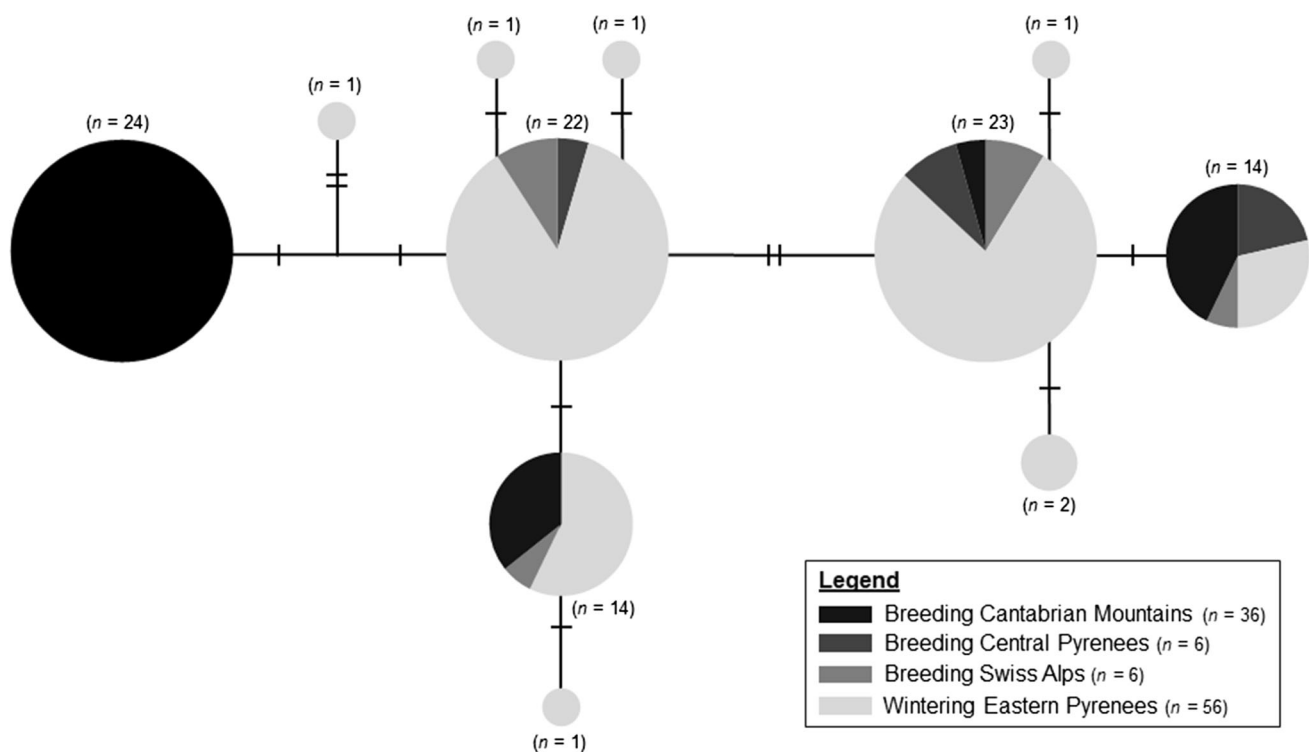
The phylogenetic analysis lay the basis for our study by showing the phylogenetic relationships of our European samples from White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) within the Snowfinch complex, including all recognised species except the Afghan Ground Sparrow *Pyrgilauda theresae*, for which *cytb* sequences were not available. We found four well-supported clades, but relatively close relationships between Rufous-necked Ground Sparrow and Blanford's Ground Sparrow and Small Ground Sparrow (64% bootstrap support and 0.92 Bayesian posterior probabilities). The three main clades correspond to the genera *Pyrgilauda*, *Onychostruthus* and *Montifringilla* (see Gebauer and Kaiser 1994; Qu et al. 2006). Blanford's Ground Sparrow and Small Ground Sparrow

**Fig. 2** Phylogenetic relationships within the Snowfinch complex based on 340-base pair mitochondrial DNA cytochrome *b* (*cytb*) sequences. The total number of sequences included 74 White-winged Snowfinch *M. nivalis* subsp. *nivalis* individuals captured in our study (considering both breeding and wintering populations), and 96 sequences from related taxa published in GenBank. Only different haplotypes are shown in the phylogenetic tree ( $n = 62$ ). The topology of the tree is based on maximum likelihood algorithms, and both the percentage of bootstrap support (1000 repeats) and Bayesian posterior probabilities are shown next to the main clades. For the haplotypes identified from our samples we indicate the population(s) (CM, CP, SA, EP) where they were found. For other abbreviations, see Fig. 1



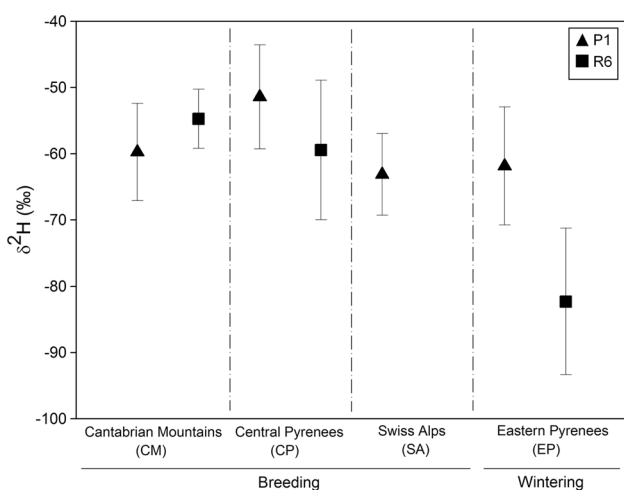
showed the shortest genetic distance, even sharing one haplotype. However, when we checked our sequences beyond the 340 bp that could be included in our phylogenetic analysis, we found that some nucleotides differed between the haplotypes from the two species. White-rumped Ground Sparrow was clearly distinct from all other species within the Snowfinch complex (see Qu et al. 2004). The clade involving Black-winged Snowfinch, Tibetan Snowfinch and White-winged Snowfinch (*M. nivalis* subsp. *gromgrzimali* and *M. nivalis* subsp. *nivalis*) was strongly

supported in the phylogenetic analysis. This is in line with the traditional placement of all these species in the genus *Montifringilla* (Gebauer and Kaiser 1994; Qu et al. 2006). The analyses of Qu et al. (2006) suggested a closer relationship between White-winged Snowfinch and Black-winged Snowfinch than White-winged Snowfinch and the Tibetan Snowfinch. This is consistent with the genetic distances we found between these taxa, although the only very weakly supported topology within *Montifringilla* may indicate a different grouping. More extensive sampling of



**Fig. 3** Haplotype network based on the concatenated sequences from *cytb* and CR in the White-winged Snowfinch (*M. nivalis* subsp. *nivalis*). Each circle represents a different haplotype ( $n = 11$ ). Individuals sampled in the CM (black), the CP (dark grey) or the SA (grey) during the breeding season, and the EP (light grey) during

the wintering season (see legend). Circle size represents the relative frequency of given haplotypes (the number of individuals exhibiting the corresponding haplotype is also provided). Haplotypes are connected by straight lines and each breaking node indicates a single-step mutation. For abbreviations, see Figs. 1 and 2



**Fig. 4** Stable hydrogen isotopes ( $\delta^2\text{H}$ ; ‰) mean and SD (error bars) measured in feathers of White-winged Snowfinch (*M. nivalis* subsp. *nivalis*) collected in the CM ( $n = 34$ ;  $P1 = 30$  and  $R6 = 4$ ), the CP ( $n = 5$ ;  $P1 = 3$  and  $R6 = 2$ ) and the SA ( $n = 6$ ) during the breeding season, and the Spanish EP during the wintering season ( $n = 58$ ;  $P1 = 53$  and  $R6 = 5$ ). The analysed feathers were either the innermost primary ( $P1$ ; triangles) or the outermost rectrix ( $R6$ ; squares); in a few cases both feathers were from the same individual. Rectrices from the SA were not available. For other abbreviations, see Figs. 1 and 2

Tibetan Snowfinch and White-winged Snowfinch (*M. nivalis* subsp. *groumgrzimaili*) would be useful for a better resolution of these relationships.

As expected, the two subspecies of White-winged Snowfinch grouped within the same clade, although the genetic distance is relatively large. Seven subspecies have been described for the White-winged Snowfinch. They are widely distributed but scattered across the Palearctic mountain ranges, from Iberia to the Tibetan plateau (del Hoyo et al. 2009). Whereas the *M. nivalis* subsp. *nivalis* is present from Western Europe to Greece (Cramp and Perrins 1994), the *M. nivalis* subsp. *groumgrzimaili* has its distribution in the Tibetan plateau, being one of the easternmost subspecies. Thus, relatively large genetic distances could be expected between these geographically distant subspecies and potentially also among the others. Unfortunately, comparisons with the other five subspecies were not possible because they have not been sequenced yet. Further studies on the population structure and genetic differences involving all White-winged Snowfinch subspecies would be interesting.

When assessing the genetic structure in our European White-winged Snowfinch populations, the most common haplotypes, except one, were widespread among breeding



populations. The genetic data alone, however, are not informative of whether movements between populations still occur because the patterns we observe currently reflect past exchanges among breeding populations. For instance, incomplete lineage sorting is still a possibility. That is, these shared haplotypes may represent ancestral polymorphism maintained since the original divergence of the populations, or they may exhibit this configuration since the last event of introgression between these populations. Gene flow could still occur if some birds from the Alps wintering in the Pyrenees (ring recoveries) from time to time stayed in the Pyrenees for breeding, although this is speculative. It is worth mentioning that even low gene flow (i.e. low frequency of female exchange among populations) may hamper population genetic structuring (Ewens 2004; Rousset 2004; Liu et al. 2011). We found, however, one haplotype specific to and predominant in the CM. The fact that the most common haplotypes were also found in the CM may indicate an asymmetry in gene flow, with individuals from other breeding populations (e.g. the Pyrenees) moving to the CM, but less likely the other way round.

The genetic specificity of other mountain bird populations inhabiting the CM has been documented. For instance, the Western Capercaillie *Tetrao urogallus* has an isolated population in the CM which is considered as a different subspecies, *Tetrao urogallus cantabricus*, and this mountain range was part of the Iberian glacial refugia for the species (Duriez et al. 2007; Segelbacher and Pieltney 2007). In the case of the Grey Partridge *Perdix perdix*, the subspecies *Perdix perdix hispaniensis* inhabiting the CM, the Pyrenees and the Iberian System also differs genetically from other Eurasian populations (Martin et al. 2003). Identifying populations with a special genetic composition is important because they may serve as a long-term store of species' genetic diversity, and should therefore be of specific conservation concern (see Hampe and Petit 2005). Long-term population isolation may also promote changes in individuals' morphometry, behaviour and ecology that can promote evolutionary change (Crandall et al. 2000; Huber et al. 2007; Santamaría and Mendez 2012). When considering the wintering individuals in the EP, most of them had the same common widespread haplotypes found in the breeding populations. Wintering White-winged Snowfinches in the EP may thus consist of individuals from different breeding origins which, based on our ring recoveries, could include breeding grounds in the Alps and in the Pyrenees. However, no wintering individual in the EP showed the specific haplotype from the CM, and this supports the hypothesis of restricted movements of individuals from the CM to the other studied populations. The highest haplotype diversity found in the wintering individuals at the EP is expected if birds from different breeding regions of Europe meet on the same wintering

grounds (see Ewens 2004; Liu et al. 2011, 2012). Overall, while our results suggest an absence of clear genetic structure in the populations investigated, the population in the CM seems to be more isolated as indicated by its specific and predominant haplotype.

No differences in  $\delta^2\text{H}$  values from P1 were found among breeding populations. This means that by analysing  $\delta^2\text{H}$  values in the primaries, we could not assign wintering individuals in the EP to particular breeding grounds. Nevertheless, the analyses of  $\delta^2\text{H}$  values in R6 of wintering individuals showed considerably lower  $\delta^2\text{H}$  values compared to those from breeding birds at the CM or the CP (unfortunately, we did not have rectrices from breeders at SA). The differences in  $\delta^2\text{H}$  values among P1 and R6 could be explained by the species' moulting pattern. White-winged Snowfinches perform a complete post-juvenile and post-breeding moult at their breeding grounds, and the moulting sequence starts with the innermost primaries (Winkler and Winkler 1985; Strinella et al. 2011b). For instance, adults and fledglings in the CM started moulting their innermost primaries in late July to mid-August, whereas rectrix moult never commenced before late August ( $n = 262$ ). Once chicks have fledged, family groups of White-winged Snowfinches progressively move to higher elevations so that P1 is probably moulted at lower elevations than R6. Our isotope results are consistent with this because  $^2\text{H}$  in precipitation becomes more and more depleted with increasing elevation (Clark and Fritz 1997; Poage and Chamberlain 2001).

The analysis of  $\delta^2\text{H}$  values in feathers has proven to be useful to track elevational movements in birds (Hobson et al. 2003). A decrease in  $\delta^2\text{H}$  of surface water at a rate of  $-1.5\text{‰}/100\text{ m}$  has been shown along an elevational gradient in the Alps (Flaim et al. 2013). Because of a different elevational range between the Alps (up to 4800 m a.s.l.) and the CM (2650 m) or the Pyrenees (3400 m), we would expect a higher range and considerably more negative  $\delta^2\text{H}$  values in rectrices of birds who moulted in the Alps, compared to those from the Iberian mountain ranges. This could explain our findings in the wintering population at the EP, suggesting that those birds with the most negative  $\delta^2\text{H}$  values in their rectrices (i.e. between  $-86$  and  $-94\text{‰}$ ) may come from breeding grounds in the European Alps. Indeed, such wintering movements have been shown by ring recoveries, proving that wintering birds are more mobile than previously thought. In fact, these values are in line with the precipitation and feather  $\delta^2\text{H}$  isoscape estimates for the Alpine region (see Bowen et al. 2005). Nevertheless, we cannot discount the fact that such negative  $\delta^2\text{H}$  values also occur in rectrices from White-winged Snowfinches breeding in the Pyrenees (see Arizaga et al. 2014). Therefore, further isotopic analyses involving larger sample sizes and more localities are essential for a more certain interpretation.

Overall, understanding White-winged Snowfinch population structure and seasonal movements represents an essential step forward. The potential for a bird species to adapt to climate change will partly depend on its elevational movements and its capacity to move among mountain ranges in order to find the most suitable conditions for breeding and overwintering. In this regard, in northern Europe it has been shown that montane birds migrating long distances declined less on average than residents and short-distance migrants (Lehikoinen et al. 2014; but see Flousek et al. 2015). Further studies combining ringing and the analyses of intrinsic markers (e.g. microsatellites for a deeper understanding of the genetic structure or  $\delta^2\text{H}$  analyses for deciphering the magnitude of birds moving between breeding grounds in the Alps and wintering sites in the Pyrenees) are essential for a better understanding of the population structure and dynamics of the White-winged Snowfinch in Western Europe. That would represent an essential step for better appraising the species' metapopulation dynamics and guiding conservation efforts.

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