



Where is the line? Phylogeography and secondary contact of western Palearctic coal tits (*Periparus ater*: Aves, Passeriformes, Paridae)

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ARTICLE INFO

Article history:

Received 31 January 2012

Received in revised form 23 October 2012

Accepted 23 October 2012

Available online 19 November 2012

Corresponding Editor: Julia A. Clarke.

Keywords:

Intraspecific diversification

Post-Pleistocene range expansion

Mitochondrial DNA

Mismatch distributions

Minimum spanning haplotype network

Genetic lineage

Molecular dating

Western Palearctic

ABSTRACT

In this study, a phylogeographic scenario of the coal tit (*Periparus ater*) was reconstructed based on a fragment of the mitochondrial control region, and within- and between-population genetic diversity was analysed with a focus on the western Palearctic breeding range. We inferred a first pan-European delimitation of a postulated secondary contact zone among coal tits from the north-eastern Palearctic *ater* subspecies group and those from the south-western Palearctic *abietum* group. Generally, between-population differentiation was greatest in the Mediterranean range, which was explained by a greater separation in multiple Pleistocene refuge areas compared to the lower differentiation across the north-eastern Palearctic range. Genetic diversity indices were lowest on Mediterranean island populations as compared to continental populations. Pairwise Φ_{ST} values were highest among island populations and the Eurasian continent on the one hand and among the continental north-eastern *ater* and south-western *abietum* group on the other. Local co-occurrence of *ater* and *abietum* haplotypes was found all across Germany and in one Greek population. Molecular dating suggested that these two major subspecies groups separated from each other and from two further North African and Middle Eastern coal tit lineages during the early to mid-Pliocene. Successively, the Mediterranean region remained a centre of mainly insular diversification until late Pleistocene times including a long period of steady population growth. At the same time, at least four distinct genetic lineages emerged in eastern Eurasia, the nominate *ater* subspecies group being one of them. Finally, during the Holocene extant wide-range secondary contact in Europe was established via rapid westward range expansion from an East Asian refuge and via northeastward expansion from Mediterranean refuges.

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1. Introduction

Despite an ongoing controversy about the late-Pleistocene origin (LPO) of bird species, there is good evidence for the Holarctic avifauna that mainly intraspecific genetic and morphological differentiation was triggered through Pleistocene range fragmentation (Klicka and Zink, 1997; Avise and Walker, 1998; Johnson and Cicero, 2004; Weir and Schluter, 2004). Post-glacial recolonization from refugial centres of origin has produced a number of secondary contact zones in the Palearctic with several hotspots of local sympatry among species (Aliabadian et al., 2004). Some of these contact zones

are located in mountain systems such as the Caucasus, the Alps or the Pyrenees, which apparently provided effective barriers to dispersal and enhanced allopatric speciation, such as in chiffchaffs *Phylloscopus collybita* (Vieillot, 1817) and *P. ibericus* Ticehurst, 1937 (Helbig et al., 1996, 2001) and green woodpeckers, *Picus viridis* Linnaeus, 1758 (Pons et al., 2011). Recently, the impact of geographic barriers on a restriction of gene flow in hybridizing sister species was shown in a secondary contact zone of the house sparrow *Passer domesticus* (Linnaeus, 1758) and the Italian sparrow *P. italiae* (Vieillot, 1817) in the Alps (Hermansen et al., 2011).

In Palearctic tits (Paridae) secondary contact and local hybridization among sister taxa was described from various Eurasian regions (map in Eck, 2006). In many cases of parapatric distribution, genetic studies revealed that contact areas were previously undetected or imprecisely described such as for Central Asian

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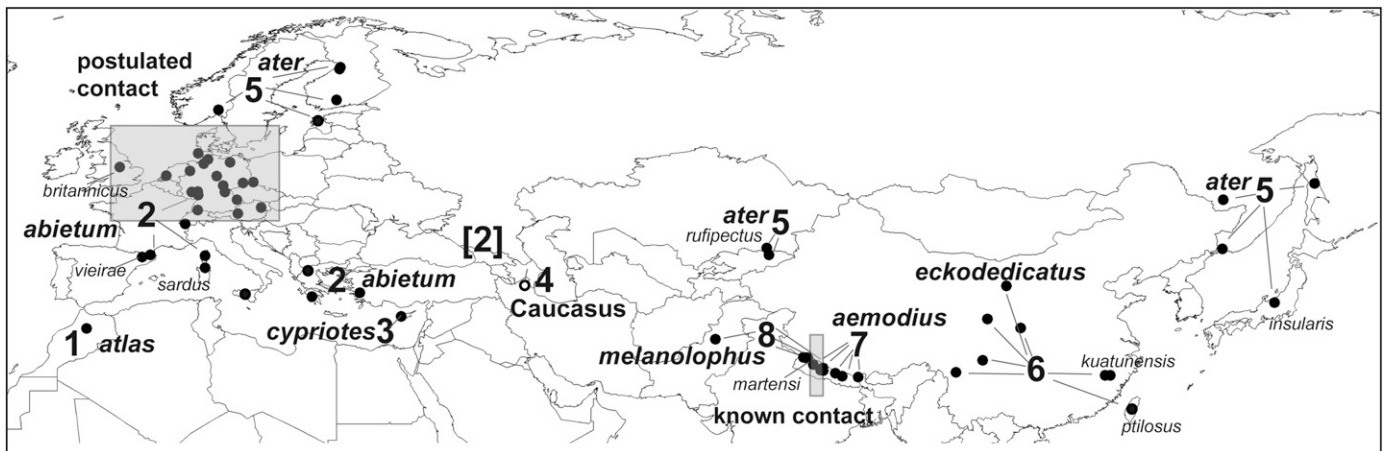


Fig. 1. Sampling locations (black dots) used for phylogenetic reconstructions and molecular dating; mitochondrial lineages (MiLis) according to Tietze et al. (2011) indicated at sampled regions (names of subspecies groups in bold; single subspecies analysed in this study except the nominate form of the respective cluster indicated at sampling sites; for the Caucasian lineage 4 only *cytb* data from *ssp. chorassanicus* and *ssp. gaddi* were included in the molecular dating analysis; open circle; [2] Crimean subspecies *moltchanovi* belonging to the *abietum* group not analysed in this study); two areas of secondary contact among adjacent subspecies groups roughly indicated for the Himalayas (well circumscribed by Martens and Eck, 1995) and Central Europe (hypothesized by Martens et al., 2006).

and Chinese great tit (*Parus major* Linnaeus, 1758) subspecies (Kvist and Rytkönen, 2006; Zhao et al., 2012) or just hypothesized as for European coal tit populations (Martens et al., 2006). The coal tit, *Periparus ater* Linnaeus, 1758, has a wide trans-Palaearctic breeding range from the British Isles and western Europe across the entire Palaearctic taiga belt to the Russian Far East and Japan in the North, to the Mediterranean region and North Africa in the Southwest as well as to the Levant region, the Middle East and the Caucasus. These populations are separated from a large allopatric Asian breeding area in the Himalayas (including the spot-winged tit *P. ater melanolophus* Vigors, 1831) extending to C and SW China and Taiwan (Del Hoyo et al., 2007). Thus, the overall pattern of differentiation and phylogenetic relationships in the *P. ater* group is far more complex than the currently accepted systematics implies. Based on mitochondrial markers eight separate mitochondrial lineages have been described to date (Tietze et al., 2011; see Fig. 1). Only two of these lineages correspond to single subspecies (MiLi 3 with *ssp. cypriotes* on Cyprus and MiLi 8 with *ssp. melanolophus* in the western Himalayas), whereas the remaining lineages comprise two (MiLi 1 with *ssp. P. a. atlas* Meade-Waldo, 1901 and *P. a. ledouci* Malherbe, 1845 in North Africa) or more subspecies (see Tietze et al., 2011 for affiliation of coal tit subspecies to mitochondrial lineages). Since many of these genetic lineages roughly correspond to taxonomic units such as those established by Harrap and Quinn (1996) we refer to them as “subspecies groups” or as genetic “lineages” or haplotype “clusters” when referring to genetic units in the following.

Focussing on the postulated contact among two larger genetic assemblages in western Europe we distinguish these as follows (according to molecular data by Tietze et al., 2011): (1) the *abietum* (subspecies) group comprises western Palaearctic populations of re-validated subspecies *P. a. abietum* Brehm, 1831 (Germany to Southern France and coastal Mediterranean European populations) along with *P. a. vieirae* Nicholson, 1906 (Iberian Peninsula), *P. a. hibernicus* Ogilvie-Grant, 1910 (Ireland), *P. a. britannicus* Sharpe & Dresser, 1871 (British Isles), *P. a. sardus* O. Kleinschmidt, 1903 (Corsica and Sardinia) and Middle Eastern *P. a. moltchanovi* Menzies, 1903 (Crimea; see Fig. 1 and Tietze et al., 2011); the *ater* (subspecies) group comprises northern Eurasian populations from Scandinavia to Far East Russia (all *P. a. ater* Linnaeus, 1758), Central Asian populations (*P. a. rufipectus* Severtsov, 1873) and those from Japan (*P. a. insularis* Hellmayr, 1902; see Fig. 1 and Tietze et al., 2011).

One secondary contact zone among genetically and morphologically distinct subspecies groups (MiLis 7 and 8; Fig. 1) is known from the Himalayas. With merely 80 km of width, probably even less, the extent of this contact zone is relatively narrow, but well circumscribed because the two forms *P. a. melanolophus* and *P. a. martensi* Eck, 1998 as well as their hybrids are morphologically highly distinctive (Martens, 1975; Martens and Eck, 1995). The situation in the western Palaearctic is completely different. Secondary contact among populations of the north-eastern *ater* subspecies group and the south-western *abietum* subspecies group in western Europe was hypothesized by Martens et al., 2006 (cf. Tietze et al., 2011), but any geographic circumscription of this contact zone failed so far because morphological distinctiveness of subspecies *ater* vs. *abietum* is in fact just subtle (see photographs of representatives of *P. a. ater* and *P. a. abietum* in Martens, 2012). Furthermore, unlike in other parid groups, differentiation of songs is not marked in the coal tit (Tietze et al., 2011) and therefore the use of molecular markers remains the sole method to infer information on the actual range of the genetically distinctive subspecies groups. Phylogenetic analyses conducted so far had a rather limited sampling, thus information on any local or regional contact of the two genetically distinct population groups is completely missing.

In this study we provide a first phylogeographic approach based on mitochondrial control region sequences in order to (a) assess the extent of secondary contact of north-eastern *ater* and south-western *abietum* coal tits in western Europe, (b) evaluate the genetic diversity and demographic history of sampled populations, and (c) reconstruct the time frame of lineage separation based on a set of two mitochondrial markers and Bayesian Skyline Plots.

2. Materials and methods

2.1. Sampling

DNA samples from several localities across Eurasia and the Mediterranean region (Figs. 1 and 2 and Table A1) were obtained from live birds such as feathers and blood/tissue samples and from study skins (toe pads). We had a regional sampling focus in Europe particularly in Germany because of the hypothesized range overlap of *ater* and *abietum* populations in this region. Because the north-eastern *ater* subspecies group is presumably more closely related to

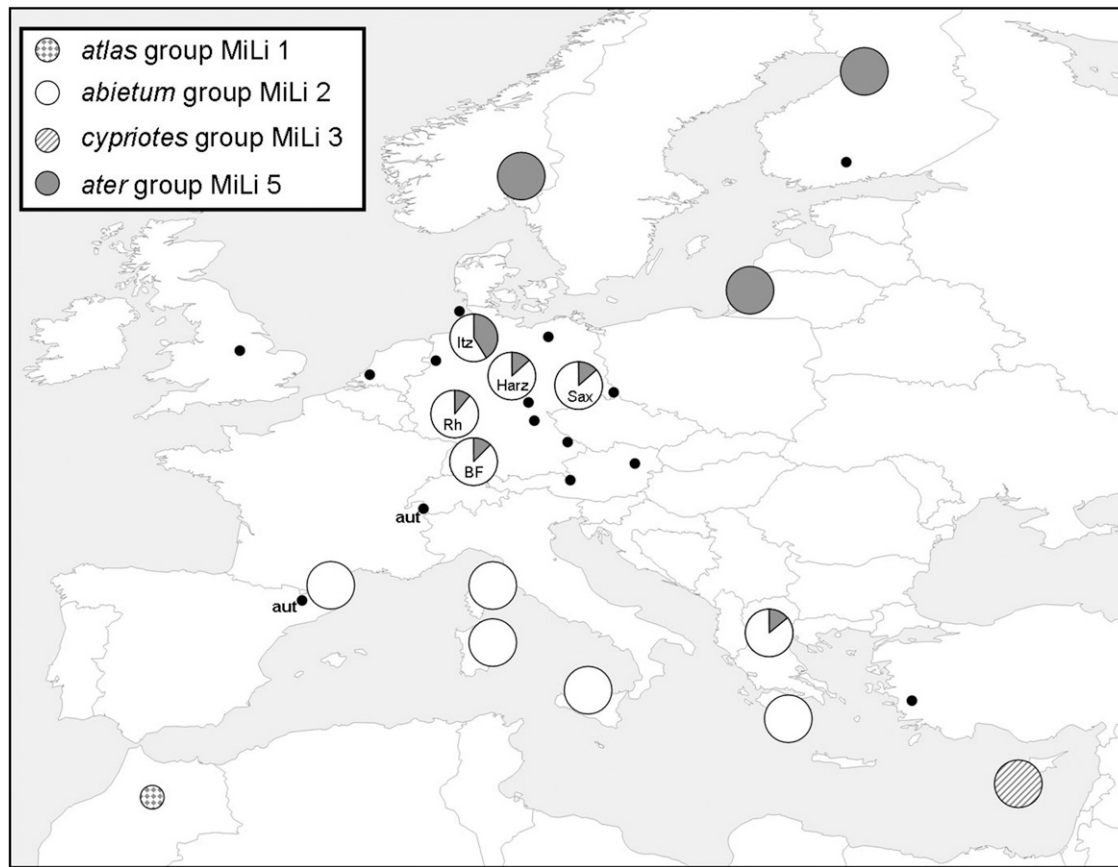


Fig. 2. Distribution of mitochondrial control region lineages (MiLi) in western European and Mediterranean coal tit populations; MiLi numbering corresponds to Tietze et al. (2011; see Fig. 1); pie charts show the percentage of individuals from different haplotype groups (MiLis) for populations with $n > 7$, affiliation to groups indicated by shades; German mixed populations: BF = southern Black Forest, Rh = Rhineland, Sax = Saxony, Harz = Harz Mountains, Itz = Itzehoe; sampled populations with $n < 5$ indicated by black dots (except North African *P. ater atlas* marked by shade because of its strong genetic distinctiveness); aut = autumn/migration populations from Spain and Switzerland.

the allopatric coal tit populations of the Himalayas and China than to those of the western Palearctic (Tietze et al., 2011), we included samples from Asian populations in our data set in order to cover the entire genetic diversity of Eurasian coal tits. Altogether 271 samples were used for analysis. For details see Table A1.

2.2. DNA extraction

Total genomic DNA was isolated in a chloroform-isoamyl alcohol extraction method or with DNA extraction kits depending on the sample type. DNA from feathers was extracted using AGOWA® sbeadex Forensic Kit according to the manufacturer's instructions (AGOWA genomics). In order to avoid contamination, DNA extraction from feathers was carried out in a separate clean laboratory. The innuPREP DNA Mini Kit (Analytik Jena, biosolutions) protocol was applied for DNA extraction from muscle tissue after a small piece was cut into a tube. Blood samples were first centrifuged and DNA was extracted from the pellet using the protocol of peqGOLD Blood DNA Mini Kit (peqLab Biotechnologie GmbH).

2.3. PCR settings and sequencing

A fragment of approximately 600 bp covering the first and parts of the second domain of the mitochondrial control region (CR) was amplified from total genomic DNA. We chose this particular marker because in some parid species the substitution rate for CR was shown to be considerably higher than the rate of cytochrome-*b* (Ruokonen and Kvist, 2002; Päckert et al., 2007), thus allowing for a better phylogenetic resolution of relatively recent separation

events. Therefore the primers L16700 (5'-ATC ATA AAT TCT CGC CGG GAC TCT-3') and H636 (5'-ATG AGG AGT ATT CAA CCG AC-3') (Kvist et al., 1998, 1999) were used. Polymerase chain reactions were performed using a 25 μ l template DNA extract containing 5 pmol of each primer, 200 μ M dNTPs, 1 \times PCR buffer (25 mM MgCl₂) and 1 unit of DFS-*Taq* DNA polymerase (Bioron International). The amplification protocol was: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation of 94 °C for 1 min, annealing at 51 °C for 1 min and final extension at 72 °C for 1 min. Cycle sequencing reactions were performed in a total volume of 10 μ l containing template DNA, 0.5 pmol of the primer H636 or Perip.CRev2 (5'-TTT CAT GCT TTA CAG GGT ATG CA-3'), 0.5 μ l 2.5 \times reaction mix and 1 \times BigDye Sequencing Buffer (following BigDye® Terminator Cycle Sequencing Kit Protocol, Applied Biosystems). All samples were sequenced in forward and reverse orientation. PCR products were purified in an enzymatic reaction using ExoSAP-IT (GE Healthcare). The cycle sequencing protocol consisted of 25 cycles with 10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C. The purified sample was diluted in 10–20 μ l 0.1 mM EDTA and applied to a 16-column automatic capillary sequencer (ABI 3130xl, Applied Biosystems) using POP-7 as a polymer.

In addition, we checked barcoding sequence data sets for local records with unusually high intraspecific sequence variation in coal tits (Kerr et al., 2009; Johnsen et al., 2010; Lee et al., 2010). For comparison with GenBank barcoding sequences a small subset of our own samples was barcoded by standard sequencing of cytochrome oxidase (COI) to analyse whether haplotypes from either *ater* and/or *abietum* group were present in these populations (for origin and COI GenBank accession numbers of these samples see Table A1).

2.4. Sequence analysis

Sequence ambiguities were edited by eye using Chromas Lite Version 2.01 (Technelysium Pty Ltd) and carefully aligned with MEGA 4.0.2 (Tamura et al., 2007). A minimum spanning network was constructed including all sequences obtained using TCS version 1.21 (Clement et al., 2000). For populations with $n > 7$ we estimated nucleotide diversity π (Nei, 1987), haplotype diversity \hat{h} (Nei, 1987), theta θ ($=2N_e\mu$ where N_e is the effective population size and μ the mutation rate) from the number of polymorphic sites per nucleotide (Tajima, 1996) and Tajima's D (Tajima, 1989) (Librado and Rozas, 2009). Mismatch distributions were inferred under the model of population growth and decline (Rogers and Harpending, 1992; Rogers, 1995). Expected distributions were calculated by a priori estimating theta initial, and tau from the original sequence data for each population separately by letting theta final as infinite. Genetic variation within and among populations was estimated via analysis of molecular variance (AMOVA) and pairwise Φ_{ST} -statistics with Arlequin ver. 3.11 (Excoffier et al., 2005), using Tamura-Nei's distances. Significance was tested with 1000 permutations. We formed five groups for AMOVA based on populations of four subspecies groups and the overlap zone (1) *P. a. cypriotes*, from Cyprus, (2) *P. a. sardus* from Corsica and Sardinia, (3) south-western *abietum* group, continental southern European populations from Greece, Sicily and the French Pyrenees (*P. a. abietum* and *P. a. vieirae*, plus samples from the UK, *P. a. britannicus*, and from Turkey; see Fig. 1), (4) all German mixed populations, (5) north-eastern *ater* group, north-eastern Eurasian samples from Norway, Finland, Far East Russia (all *P. a. ater*, Kyrgyzstan/Kazakhstan (*P. a. rufipectus*) and Japan (*P. a. insularis*)). Pairwise Φ_{ST} values were calculated only for populations with sample size greater than 5. The two autumn (migration) populations from the Spanish Pyrenees and from Switzerland were excluded from AMOVA and from pairwise Φ_{ST} statistics.

A concatenated alignment of 1165 bp of cytochrome-*b* (*cytb*) and CR sequences was used for molecular dating with BEAST version 1.4.8 (Drummond and Rambaut, 2007; we used all *cytb* sequences from Martens et al. (2006; GenBank accession numbers DQ217844–DQ217901) and a few from Tietze et al. (2011; GenBank accession numbers GQ331066, GQ331068, GQ331074, GQ331078, GQ331093, GQ331094, GQ331096) plus the corresponding CR sequences from the same individuals produced in this study and a few available from the previous study by Päckert et al., 2007. Times to the most recent common ancestor (t_{MRC}) were estimated in a relaxed uncorrelated lognormal clock approach as implemented in the BEAST version 1.4.8 package. The length of the Markov chain was set to 10,000,000 generations, log parameters were sampled every 1000th generation and the "auto optimize" option was activated. A linearized consensus tree including posterior probabilities was inferred from the tree output files (concatenated sequence data sets) using TREEANNOTATOR version 1.4.8 (as implemented in the BEAST package) with the burn-in parameter set to 3000. Time estimates of lineage splits and mean substitution rates were inferred from the log output files using TRACER version 1.4 (Rambaut and Drummond, 2007). The input sequence data were manually partitioned according to two different gene fragments in the XML file generated with BEAUTI. The GTR and the HKY models, respectively, were a priori assigned to each partition according to the estimates with MRMODELTEST. For molecular dating, two independent runs with BEAST were performed: (1) the *cytb* partition was assigned a fixed mean substitution rate of 0.0105 according to Weir and Schluter (2008); (2) fixed node ages were assigned to some outgroup nodes of Canary blue tits (*Cyanistes* Kaup, 1829) and North American chickadees (*Poecile* Kaup, 1829) setting the t_{MRC} priors to a uniform range of age estimates for paleogeographic events corresponding to the respective lineage splits (e.g. volcanic

island ages, opening of the Bering Strait, cf. Päckert et al., 2007, 2009a).

Past population dynamics were inferred from mismatch distributions estimated with DnaSP version 5.10 and from Bayesian Skyline Plots (Drummond et al., 2005) using BEAST 1.4.8. Bayesian skyline analyses were run under the coalescent tree prior (Bayesian Skyline) with the piecewise constant model applied and the number of groups set to ten. We inferred a mutation rate for our CR fragment using the same clock calibration as described for the concatenated mitochondrial DNA (mtDNA) data above. For CR sequences alone a rate of 0.01156 resulted from the calibration and this rate was applied in the Bayesian Skyline analysis. The length of the Markov chain was set to 30,000,000 generations, log parameters were sampled every 1000th generation and the "auto optimize" option was activated. Bayesian Skyline Plots BSP were generated with TRACER version 1.4.

3. Results

3.1. Phylogeography

The 271 sequences of the mitochondrial control region yielded a 438 bp long alignment including 76 polymorphic sites of which 55 were parsimony informative. Among the 271 analysed individuals 86 haplotypes were found. The minimum spanning network (Fig. 3) indicated a strong phylogeographic structure: The two postulated mitochondrial lineages in continental Europe and northern Eurasia (*ater* group and *abietum* group) were well reflected by CR sequences, and haplotypes between the two groups differed by at least ten base substitutions (between-group *p*-distances of 0.038). Within the *abietum* group 46 haplotypes were found, of which the most frequent one (*abietum* 1) was shared among 27 individuals originating from the Pyrenees, Greece, Switzerland, Sicily and Germany. Two further common haplotypes (*abietum* 2 and 3, equally widely distributed as *abietum* 1) were shared by 21 and 19 individuals, respectively, and differed from haplotype *abietum* 1 by two and three substitutions, respectively. Haplotype *abietum* 2 was considered an evolutionary centre of the network since it had the biggest outgroup weight. All other haplotypes were represented by fewer individuals (including several private haplotypes) and differed by up to seven substitutions from haplotype 1 (mean within-group distances *abietum* cluster exclusive of Corsica $pdist = 0.009$; Corsica vs. remaining *abietum* cluster $pdist = 0.014$). Along with all samples of subspecies *abietum* this haplotype cluster comprised individuals of *P. a. britannicus* from the British Isles, *P. a. vieirae* from the Iberian Peninsula and *P. a. sardus* from Corsica and Sardinia. Strikingly, the two latter islands were represented by two different haplotype lineages, each differing by at least four substitutions from the central haplotype (Fig. 3).

The south-western Palearctic network was separated from a second Eurasian network including the north-eastern Palearctic *ater* group and three separate clusters from the western Himalayas, the eastern Himalayas and from China (Fig. 3). In the north-eastern Palearctic *ater* cluster itself 21 different haplotypes were found, among which the most frequent one (*ater* 1) was shared by 24 individuals from Japan, Far Western and Far Eastern Russia, Kyrgyzstan, Kazakhstan, Finland, Norway, the Czech Republic, Austria and Germany. Hence, this haplotype was widespread across the entire northern Palearctic. The second most frequent haplotype differed by only one substitution step from *ater* 1 and was shared by fourteen individuals from Scandinavia, Far Eastern Russia, Japan and only a few from German mixed populations. All other *ater* haplotypes were shared by fewer individuals and differed by up to five substitutions from haplotype *ater* 1. Along with individuals of the nominate subspecies *ater* this haplotype cluster comprised

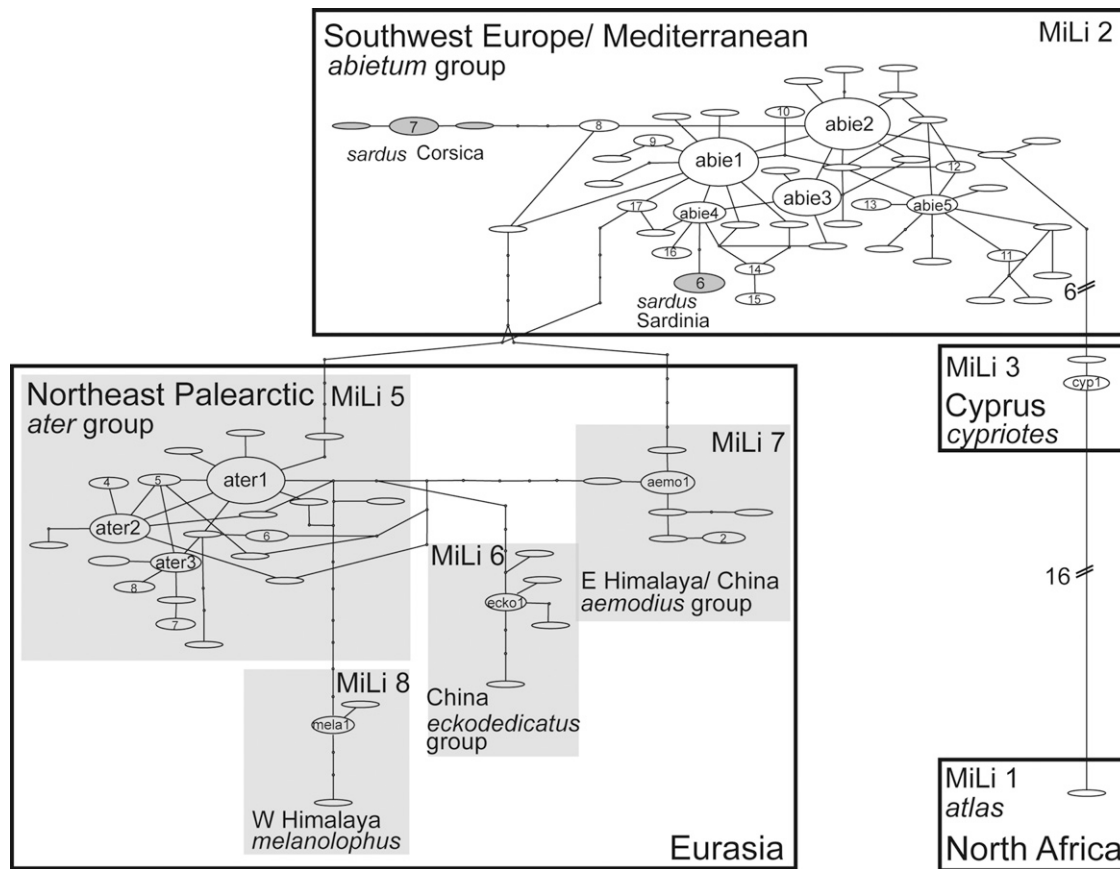


Fig. 3. Minimum spanning network of Palearctic *Periparus ater* lineages based on 438bp of the mitochondrial control region ($n=271$) with gaps coded as a fifth state; numbering of mtDNA lineages (MiLi) corresponds to Tietze et al. (2011; see Fig. 1), but no CR data for MiLi4 samples were available; haplotypes from Corsica and Sardinia (subspecies *sardus*) nested in the south-western European *abietum* cluster are grey-shaded.

samples of *P. a. insularis* from Japan and *P. a. rufipectus* from Central Asia. Birds from Cyprus (*P. a. cypriotes*) represented a separate lineage and differed by at least six substitutions from the most closely connected *abietum* haplotype. On Cyprus two haplotypes were present, one of them found in six individuals. A highly distinct haplotype was inferred from one sample from Northwest Africa (*P. a. atlas*) with a difference of 16 substitutions from the most closely connected haplotype from Cyprus.

Using a small subset of our own samples, we verified single COI haplotypes from GenBank having a far northern distribution in Norway and a far eastern distribution on southern Crimea as belonging to the *abietum* clade (COI tree provided as Supplementary Fig. S1).

3.2. Genetic diversity

In general, genetic diversity within populations was considerably lower in the south-western Palearctic range (Table 1). Haplotype and nucleotide diversities were lowest on Sardinia (one single haplotype found) and on Cyprus (0.286 and 0.0064 respectively) and highest in German populations where both *ater* and *abietum* haplotypes were present throughout the German breeding range ($h > 0.87$, $\pi > 0.011$; Table 1). Island populations from Cyprus, Corsica and Sardinia showed lowest haplotype diversities. The Pyrenean breeding population showed surprisingly high haplotype diversity. Nucleotide diversity varied from 0.00064 in the Cypriot population (and Sardinia where $\pi = 0$) to 0.01986 in northern Germany (Itzehoe, Schleswig-Holstein). In general, nucleotide diversity was the lowest in all southern island and continental populations (most values below 0.001) and only

somewhat higher in the northern populations from Russian Far East, Norway and Finland (values range from 0.003 to 0.004). In contrast, nucleotide diversity was highest in German populations (values range from 0.0115 to 0.0198, Table 1). For most populations Tajima's *D* values were negative, but not significant in any case. In the two autumn/migration populations (Spanish Pyrenees and Switzerland) haplotype diversity was relatively high, whereas nucleotide diversity and theta were considerably higher in the Swiss Col de Bretolet population (Alpine ringing station) than in the Spanish Pyrenees population (Col de Pal).

Mismatch distributions for breeding populations from Russia (both East and West), Norway, Finland, the Pyrenees, Corsica and Cyprus were unimodal, indicating that only one mitochondrial lineage was present in these populations (Figs. 2 and 4; *ater* in the North, *abietum* and *cypriotes* in the South, respectively). German populations as well as the two pooled Greek populations showed a bimodal distribution indicating that both, carriers of *abietum* and of *ater* haplotypes were present there (Fig. 4; right-skewed peak at 10–15 substitutions corresponds to pairwise comparisons among *ater* and *abietum* haplotypes; compare map in Fig. 2). However, even in the two northernmost mixed populations from the Harz Mountains and Itzehoe (both Germany), deviating from our expectations, *ater* haplotypes were notably underrepresented during the breeding season. The population from Sicily also showed a slightly bimodal distribution with a second peak at five substitutions indicating a relatively high local variation of *abietum*-group haplotypes only (Fig. 4).

AMOVA showed that 58.5% of the overall genetic variation was explained by variation among groups compared to only 8.2% by variation among populations within groups, the remaining 33.3%

Table 1

Population genetic diversity values and neutrality test estimated from the control region sequences (438 bp) of European, Mediterranean and Eurasian coal tits. Grey-shaded lines indicate mixed populations with northern *ater* and southern *abietum* haplotypes present; Tajima's D was not significant for any of the populations.

Population	N ind.	N haplotypes	Haplotype diversity	Nucleotide diversity	Tajima's D	Theta per site
<i>ater</i> group	50	10	0.802	0.00275	-1.01633	0.00358
Russian Far East	11	6	0.873	0.00325	-1.14785	0.00457
Western Russia	10	5	0.844	0.00250	0.09646	0.00162
Norway	13	6	0.859	0.00409	-0.19127	0.00432
Finland	9	5	0.861	0.00372	-0.39837	0.00411
Contact area Germany	82	32	0.926	0.01986	-0.83816	0.02614
N Germany (Itzehoe)	17	9	0.890	0.01925	0.85037	0.01588
C Germany (Harz Mountains)	15	9	0.905	0.01148	-0.83753	0.01442
W Germany (Rhineland)	9	8	0.972	0.01610	-1.17750	0.02113
S Germany (Black Forest)	8	7	0.964	0.01164	-1.49745	0.01636
E Germany (Saxony)	20	10	0.879	0.01443	-0.59298	0.01711
<i>abietum</i> group	64	22	0.925	0.01285	-0.83212	0.01627
<i>abietum</i> group (excl. Cyprus)	57	20	0.915	0.01026	-1.05558	0.01041
Greece (pooled)	13	8	0.859	0.00094	-1.60894	0.01507
Greece Makedonia	7	5	0.857	0.01392	-1.42234	0.01864
Greece Peloponnes	6	4	0.867	0.00472	-0.31466	0.00500
France, E Pyrenees, breeding	13	9	0.936	0.00468	-0.71425	0.00574
Spain, Pyrenees, migration	15	8	0.905	0.00551	-0.74164	0.00685
Corsica	10	3	0.378	0.00089	-1.40085	0.00157
Sardinia	9	1	0.000	0.00000	-	-
Cyprus	7	2	0.286	0.00064	-1.00623	0.00091
Sicily	10	5	0.756	0.003388	-0.17819	0.00390
SW Switzerland, migration	18	9	0.902	0.01527	0.09214	0.01493

were explained by variation within populations. Fixation indices were high for among population variation Φ_{ST} (0.667, $p < 0.001$) as well as for among groups Φ_{CT} (0.585, $p < 0.01$) and lower, but still significant for among populations within groups Φ_{SC} (0.197, $p < 0.001$). Pairwise comparisons among populations with sample size of $n > 7$ yielded highest fixation indices for most among-group population pairs (see Table 2 for pairwise Φ_{ST} values): (a) the Cypriot population vs. all others (Φ_{ST} between 0.58 and 0.99), (b) the *sardus* population from Corsica vs. all others (Φ_{ST} between 0.52 and 0.98), (c) between all northern Eurasian *ater* group populations vs. all southern continental *abietum* group populations and mixed populations from Germany (Φ_{ST} between 0.70 and 0.92). Low genetic variation within groups as inferred from AMOVA was reflected by low pairwise Φ_{ST} values for within-group comparisons of (a) southern continental European populations (*abietum* group: Φ_{ST} between -0.01 and 0.36), and (b) north-eastern *ater* group populations (Φ_{ST} between -0.02 and 0.27; Table 2). Highest Φ_{ST}

values within the continental *abietum* group resulted for pairwise comparisons of the population from Sicily vs. populations from the Pyrenees and from Greece. Strikingly, pairwise comparisons of German mixed populations with southern *abietum* populations yielded much lower Φ_{ST} values comparable to those from within-group comparisons, whereas comparisons with north-eastern *ater* group populations yielded higher values comparable to among-group comparisons.

3.3. Molecular dating and past demography

The dated phylogeny for all Palearctic coal tits and Asian relatives of genus *Periparus* is shown in Fig. 5. 95% Highest Posterior Density (HPD) intervals from the two calibrations were largely overlapping at strongly supported nodes within *P. ater*. However, not all internal nodes of the coal tit clade were equally supported in both calibrations. Particularly for deep splits among parid genera,

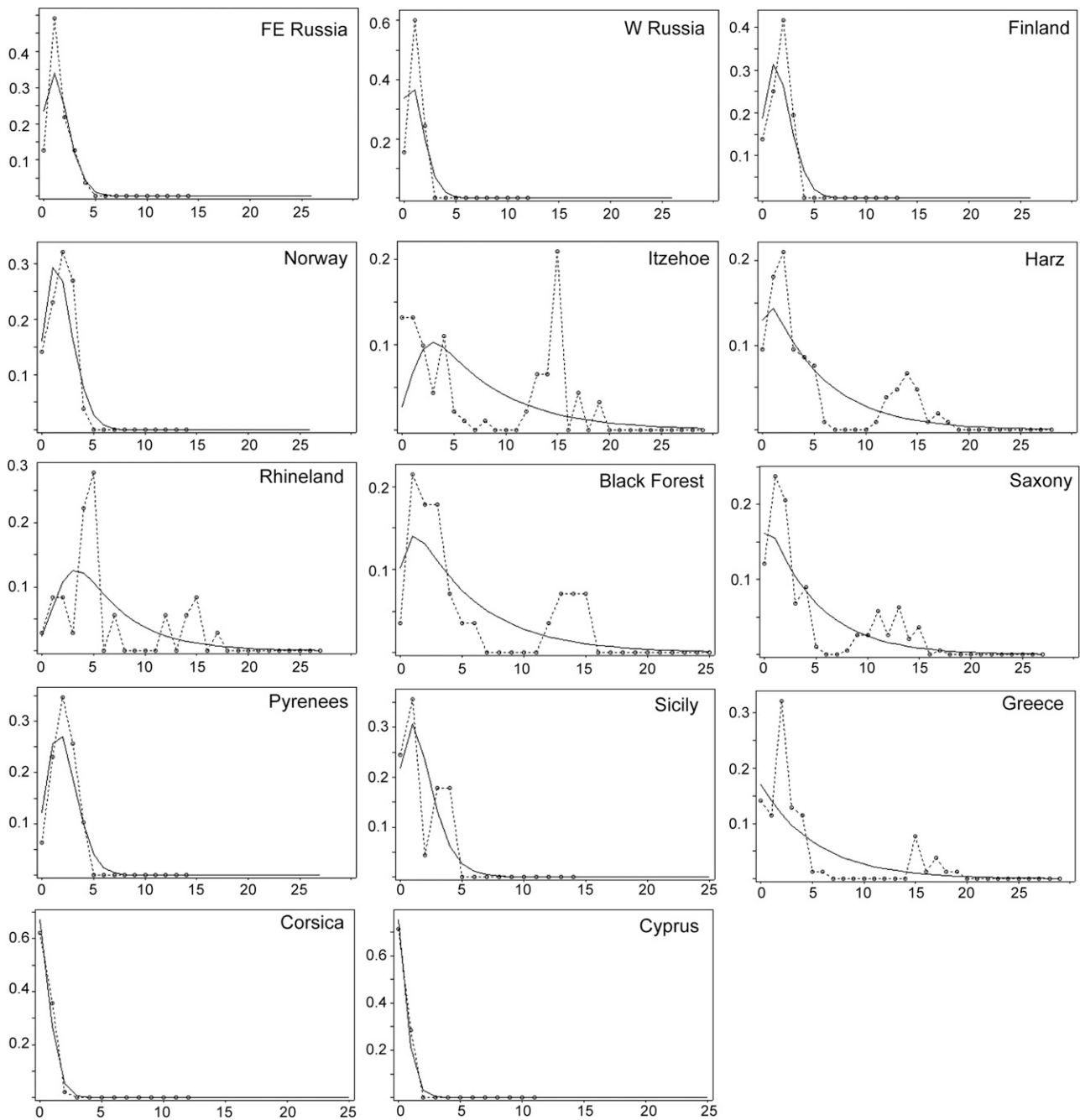


Fig. 4. Mismatch distributions for 14 European coal tit populations, observed (dashed line) and expected (continuous line) frequencies of pairwise sequence differences (x-axis) under a population growth-decline model; mixed populations characterized by bimodal distributions with a peak at 10–15 substitutions denoting pairwise differences among haplotypes from the north-eastern *ater* and the south-western *abietum* lineages.

age estimates based on a fixed cytochrome-*b* substitution rate were younger than those based on fixed node ages. The North African *atlas* lineage and the Middle Eastern *phaenotus* lineage appeared as the earliest mid-Pliocene offshoots of the coal tit clade originating from a basal split at about 4 Ma ago (Fig. 5). The remaining Eurasian lineages were separated into two major groups: Clade A comprised all south-western European lineages with subspecies *P. a. cypriotes* being sister to a terminal clade of the continental *abietum* lineage and the insular *sardus* lineage from Corsica. Clade B comprised all sequences of the north-eastern *ater* lineage firmly nested in a strongly supported clade with three further Asian lineages from the Himalayas and China (Fig. 5).

Basal splits within each of the two major Palearctic clades A and B were dated to the Pliocene/Pleistocene boundary. Within-clade

differentiation still traceable in extant haplotype diversity was dated to the late Pleistocene and was most apparent within the northern Eurasian *ater* clade (Fig. 5).

Mismatch distributions for both the entire *ater* lineage and the entire *abietum* lineage matched the expected distribution for past range expansion under a growth-decline model (Fig. 6a and b). According to Bayesian Skyline Plots BSPs the extant genetic differentiation within both groups accumulated during approximately the last 200,000 years, which is considerably younger than mean t_{MRCA} estimates for both lineages with BEAST (Fig. 5). However, the corresponding BSPs for both lineages show some marked differences. Within-clade differentiation in the southern *abietum* group started considerably earlier than in the northern *ater* group and included a relatively large period of steady increase in effective

Table 2
Pairwise Φ_{ST} -values coal tits from AMOVA; significant values in bold ($p < 0.05$); dark grey = within group pairwise comparisons (continental *abietum* subspecies group, German mixed populations and northeastern *ater* subspecies group), light grey = pairwise comparisons among southwestern *abietum* group and German mixed populations.

Groups		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1 Cyprus																	
2	2 Corsica	0.98																
3	3 Sardinia	0.99	0.98															
4	4 Pyrenees	0.86	0.79	0.66														
	5 Greece S	0.89	0.83	0.78	-0.06													
	6 Greece N	0.74	0.65	0.52	0.07	-0.01												
	7 Sicily	0.89	0.84	0.83	0.36	0.26	0.25											
4	8 Black Forest	0.73	0.65	0.51	0.02	-0.07	-0.08	0.26										
	9 Rhineland	0.67	0.60	0.51	0.12	0.00	0.00	0.02	0.02									
	10 Saxony	0.67	0.57	0.41	0.04	-0.01	-0.08	0.24	-0.06	0.08								
	11 Itzehoe	0.58	0.52	0.41	0.25	0.18	0.01	0.31	0.07	0.14	0.09							
	12 Harz Mts	0.69	0.59	0.46	0.02	-0.05	-0.07	0.17	-0.07	0.00	-0.03	0.10						
5	13 Norway	0.93	0.93	0.93	0.87	0.88	0.74	0.89	0.77	0.74	0.71	0.43	0.73					
	14 Finland	0.94	0.94	0.95	0.87	0.88	0.71	0.89	0.75	0.71	0.69	0.40	0.71	0.03				
	15 C Asia	0.99	0.98	1.00	0.89	0.92	0.70	0.92	0.74	0.68	0.67	0.36	0.69	0.27	0.07			
	16 FE Russia	0.94	0.94	0.95	0.88	0.89	0.73	0.89	0.76	0.73	0.70	0.41	0.72	0.03	0.04	0.15		
	17 W Russia	0.95	0.95	0.95	0.88	0.89	0.73	0.90	0.77	0.73	0.70	0.41	0.73	-0.03	-0.02	0.25	-0.03	

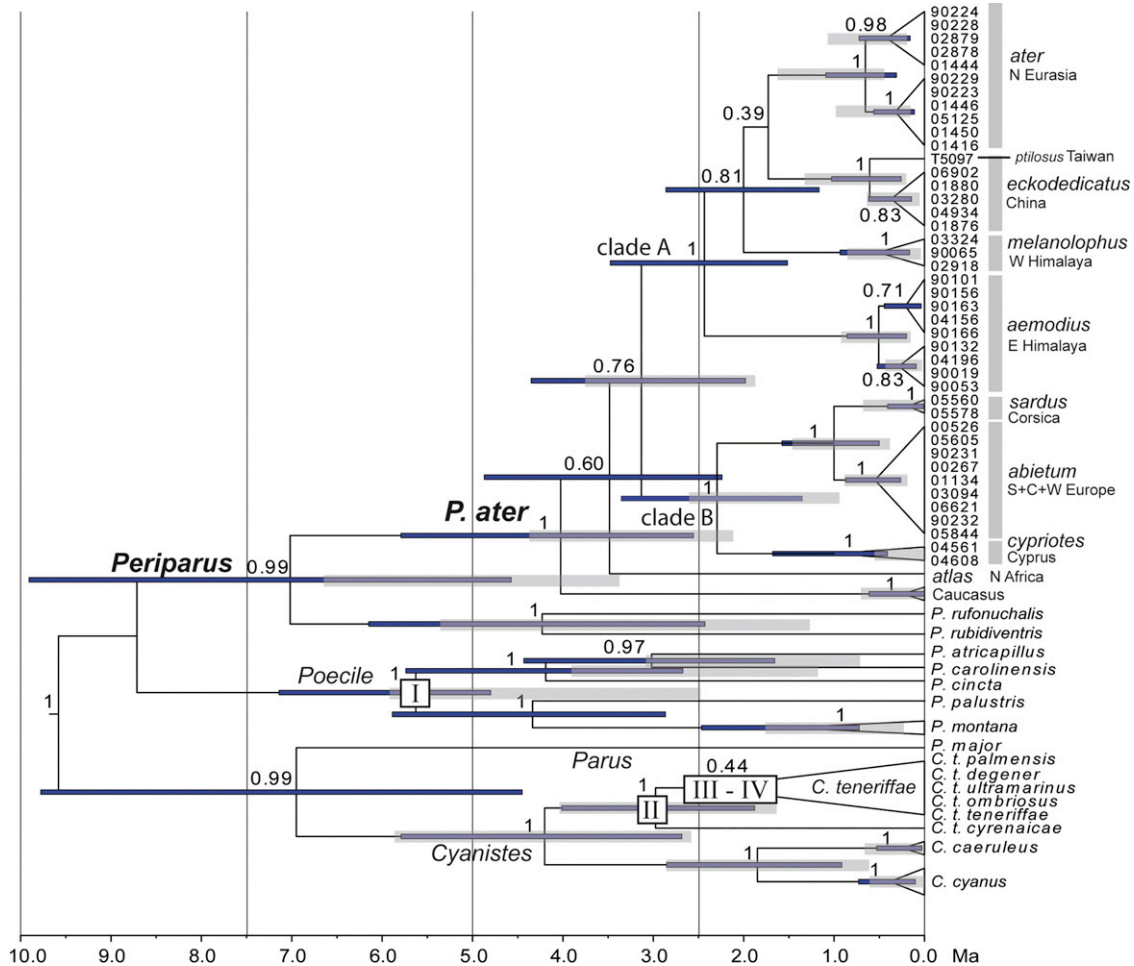


Fig. 5. Dated mitochondrial phylogeny (1165 bp of control region and partial cytochrome-*b*; 47 *Periparus* sequences) for coal tits, *Periparus ater*, and allies; individual sample numbers are indicated at terminal clades of each coal tit lineage, Caucasus clade represented by *P. a. chorassanicus* and *P. a. gaddi* (cytochrome-*b* only; GenBank numbers GQ331066 and GQ331078); tree topology corresponds to the run with fixed-node ages applied; 95% Highest Posterior Density (HPD) intervals from the two independent calibrations given at nodes with posterior probabilities greater than 0.5; dark blue bars = fixed node ages; grey bars = fixed substitution rate (cytochrome-*b* only; $r = 0.0105$; missing for some nodes with posterior probabilities < 0.5); fixed node ages for calibration: I = Holarctic faunal interchange until Pliocene opening of Bering Strait [14.0–4.8 Ma ago], II = Messinian Salinity Crisis [5.96–0 Ma ago] (upper t_{MRCA} bound only), III = age of La Palma [3.4–0 Ma ago] (upper bound), IV = age of El Hierro [1.1–0 Ma ago] (upper bound). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

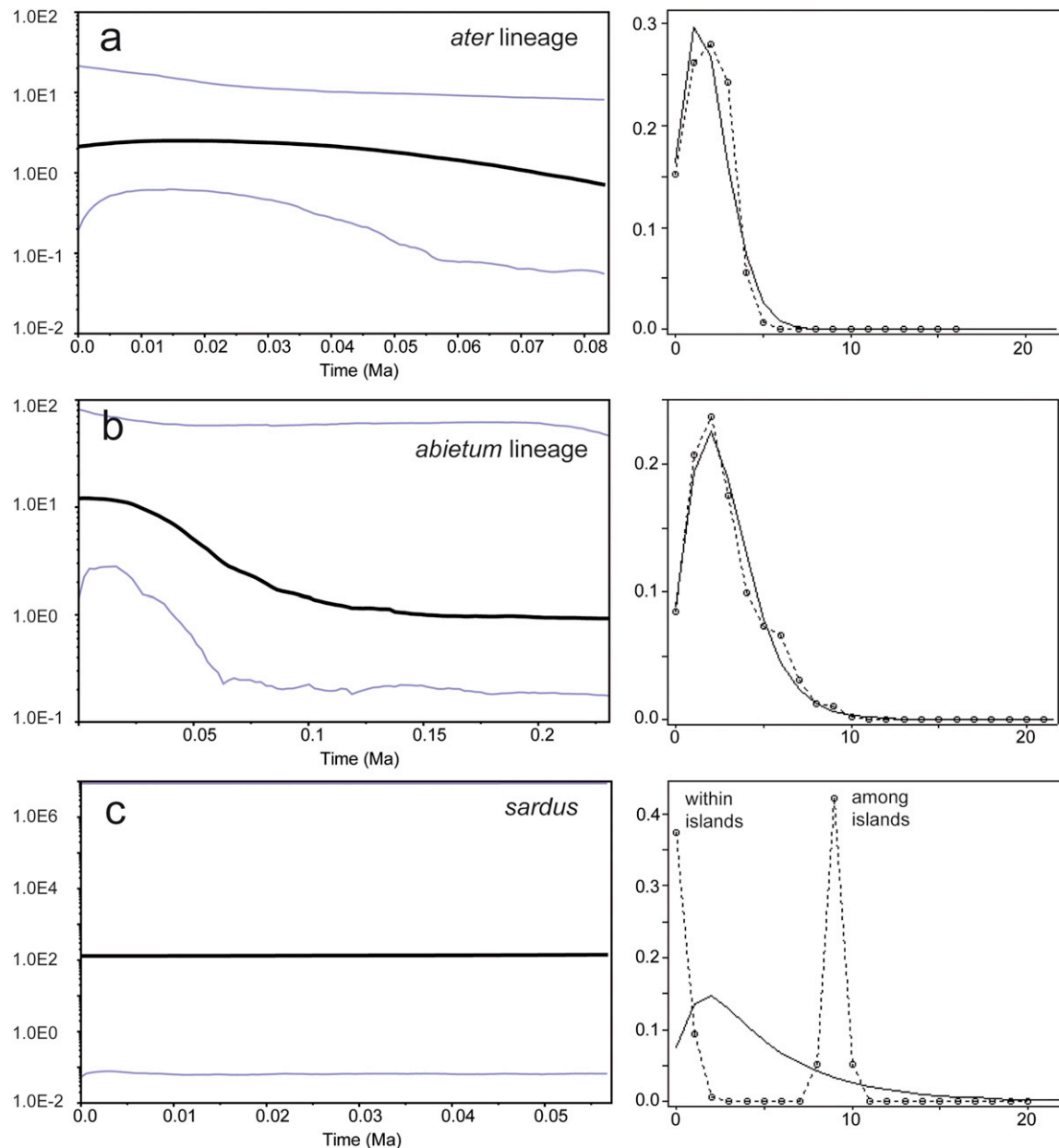


Fig. 6. Bayesian Skyline Plots (BSP; left) and mismatch distributions (right) for three coal tit subgroups; changes in mean effective female population size (N_{ef}) over time as inferred for the three partial mtDNA data sets indicated in BSPs by black lines with 95% Highest Posterior Density intervals indicated by blue lines: (a) northern Palearctic *ater* lineage ($n = 66$), (b) south-western European *abietum* lineage ($n = 147$), and (c) subspecies *sardus*, pooled individuals from Corsica and Sardinia ($n = 19$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

population size (Fig. 6a and b). Though absolute pairwise differences range at same magnitudes BSPs of the *abietum* group as a whole and subspecies *sardus* alone differ considerably. The bimodal mismatch distribution for all pooled subspecies *sardus* samples was effectively due to the large differences of within-island variation compared to among island variation (Fig. 6c). Unlike in the two continental subspecies groups no changes in effective population size of insular subspecies *sardus* could be inferred from BSPs (Fig. 6c).

4. Discussion

4.1. Phylogeography

With at least three mitochondrial lineages in the western Palearctic, coal tits show a much more diverse phylogeographic pattern than for example great tits *P. major* or willow tits *Poecile*

montana (von Baldenstein, 1827) (Kvist et al., 2001, 2003). Although the species is not present on the Canary Islands, the strong differentiation among western Palearctic coal tit populations in the South rather parallels the situation in European and Canary Islands *Cyanistes* blue tit species (Kvist et al., 2005; Dietzen et al., 2008; Illera et al., 2011). Accordingly, for coal tits the Mediterranean region was a diversification centre from the mid-Pliocene on, with initial separation of a North African lineage (including *P. a. atlas* and *P. a. ledouci*; cf. Tietze et al., 2011) and successive colonisations of the islands of Cyprus, Corsica and Sardinia during the Pleistocene (*P. a. cypristes* and *P. a. sardus*). At the same time four lineages separated in the eastern Palearctic, the north-eastern Eurasian *ater* lineage being one of them (inclusive of support from nuclear intron sequences; Tietze et al., 2011).

Based on mitochondrial sequence data, Kvist et al. (2004) suggested three European Pleistocene refugia of blue tits (the

Iberian Peninsula, the Balkan Peninsula and Corsica) as ancestral areas of a post-Pleistocene northward recolonization. Circum-Mediterranean radiation resulting in strong phylogeographic structure was also suggested for other bird species such as southern grey shrikes *Lanius meridionalis* Temminck, 1820 (Klassert et al., 2008), great grey shrikes *L. excubitor* Linnaeus, 1758, (Olsson et al., 2010) and the subalpine warbler *Sylvia cantillans* (Pallas, 1764) (Brambilla et al., 2008). Despite a lack of continental differentiation among south-western coal tit populations (*abietum* subspecies group) this species might have occupied similar refuges in North Africa, on at least two Mediterranean islands (see below) and in the Middle East (Caucasus subspecies group sensu Harrap and Quinn, 1996 except ssp. *moltchanovi*; cf. Tietze et al., 2011).

4.2. Insular differentiation

Low genetic diversity values found in island coal tit populations illustrate the extreme contrast between large among-island divergence and low within-island genetic variation. Such decrease of genetic variation in small isolated populations through drift and bottlenecks was also found among other bird species such as South Island robins *Petroica australis* (Sparrman, 1788) (Boessenkool et al., 2007), several endangered New Zealand endemics (Jamieson, 2009), but also in mammals such as wolves *Canis lupus* Linnaeus, 1758 (Frankham, 1997). In addition, geographic isolation often restricts the number of settlement events, which in turn causes genetic homogeneity within a population (Jaenike, 1973). This could explain why coal tit populations on islands showed lower genetic diversity than mainland populations. However, due to rather small local sampling sizes on Mediterranean islands and only one sampling site on Sardinia these results have to be interpreted with particular caution.

Nevertheless, regardless of within-island genetic diversity high among-group Φ_{ST} values in the South indicate that more than one Mediterranean Pleistocene refuge led to a high diversity of genetic lineages and this is also supported by higher diversity indices of acoustic and morphological markers in the southern coal tit range (Tietze et al., 2011). Apart from continental refuges in Europe (Kvist et al., 2004) and North Africa (Martens et al., 2006), Corsica, Sardinia and Cyprus can be suggested as further Pleistocene refuge areas of ancestors of extant *P. a. sardus* and *P. a. cypriotes*. Once established these populations possibly remained rather stable without any considerable population size changes. This is in concordance with Marra (2005), who considered these regions as completely isolated at least during the Pleistocene. The Cypriot avifauna comprises some further endemics that are genetically and behaviourally distinct: the Cyprus wheatear *Oenanthe [pleschanka] cypriaca* (Homeyer, 1884) with a currently debated taxonomic status (Randler et al., 2010a,b, 2012; Pentzold et al., 2011) or the short-toed treecreeper *Certhia brachydactyla dorotheae* Hartert, 1904 (Tietze et al., 2006; Förschler and Randler, 2009). Like in coal tits, other Corsican passerine populations represent private haplotype lineages closely linked to a European haplotype cluster, such as in blue tits (*C. caeruleus ogliastrae* Hartert, 1905; Kvist et al., 2004), great tits (*P. major corsus* Kleinschmidt, 1903; Kvist et al., 2003) and goldcrests (*Regulus regulus interni* Hartert, 1906; Päckert et al., 2009b). One exception was found in the genetically distinct Corsican citril finch subspecies *Carduelis citrinella corsicana* (Koenig, 1899), which nevertheless seems to have evolved quite recently (Förschler et al., 2009).

Contrasting the strong phylogeographic pattern in the Mediterranean, extant mtDNA variation of the northern *ater* group probably originated from a recent bottleneck during a rapid post-Pleistocene westward expansion from one or two eastern refuges across the boreal taiga belt. A similar scenario of a rapid post-Pleistocene range expansion in the northern Palearctic was proposed for other

tit species such as willow tits, great tits (Kvist et al., 2001, 2003) and for long-tailed tits *Aegithalos caudatus* (Linnaeus, 1758) (Zink et al., 2008; Päckert et al., 2010).

4.3. Continental European secondary contact zone

Possible secondary contact of two distinct coal tit forms in western and central Europe was never seriously considered, mainly because of the rather limited morphological distinctiveness of the subspecies *abietum* populations vs. the northern European *ater* populations. Strikingly, the high genetic differentiation of the strongly allopatric Mediterranean and North African populations matches their greater morphological distinctiveness (bright yellowish plumage of *P. a. ledouci* from Algeria and Tunisia; dark brownish mantle, dark belly and rufous-brown flanks in *P. a. cypriotes*; Vaurie, 1959; Harrap and Quinn, 1996; Tietze et al., 2011).

For western Europe a morphological cline towards a more olive-washed mantle and more deeply buff-tinged flanks in the South was already discussed by Vaurie (1959; see also Cramp & Perrins, 1993; Glutz von Blotzheim & Bauer, 1993). Based on these differences in plumage colouration along with a clinal increase of bill length towards southern German and pan-Alpine populations some authors argued in favour of the validity of subspecies *abietum* (Niethammer, 1943; Wolters, 1968; Martens, 2012). There are analogies of morphological clines and wide-range intergradation in the European nuthatch (*Sitta europaea caesia* Wolf, 1810 vs. *S. e. europaea* Linnaeus, 1758) and in long-tailed tits *A. caudatus europaeus* (Herrmann, 1804) and *A. c. caudatus* (Linnaeus, 1758); for both examples see Nazarenko, 1982; Harrap and Quinn (1996). In the far eastern Palearctic the intermediate morphology of *P. ater amurensis* with a small crest suggests intergradation with the north-eastern Chinese *P. a. pekinensis* (Vaurie, 1959).

However, based on their genetic distinctiveness, the question has been raised where the south-western and the north-eastern coal tit subspecies groups actually have come into local or regional secondary contact (Martens et al., 2006). According to our genetic data, mixed populations of *ater* and *abietum* birds were found all across Germany but single *ater* haplotypes were also distributed far south (one *ater* in Greece), as well as *abietum* haplotypes confirmed by our CR and COI sequences were found far North in Norway (Johnsen et al., 2010) and far East in south-western Turkey and on southern Crimea (Kerr et al., 2009; Tietze et al., 2011, ssp. *moltchanovi*). Based on these scattered records the secondary contact zone of northern Eurasian and south-western European coal tits seems relatively wide, but it might have been overlooked so far because *P. a. abietum* and *P. a. ater* phenotypes are morphologically quite similar. The situation in the Himalayas is different, because there even mitochondrial introgression among *P. a. melanolophus* and *P. a. martensi* could be detected based on the distinct parental and hybrid phenotypes (Martens and Eck, 1995; Martens et al., 2006). The lack of behavioural barriers such as song differences (Tietze et al., 2011) might furthermore facilitate wide-range mixing and mitochondrial introgression in European coal tit populations. However, as our results are solely based on mitochondrial DNA sequences only limited conclusions on gene flow among the two genetic clusters can be drawn here. Whether European coal tits are freely panmictic (particular in mixed German populations) remains to be tested on the basis of nuclear genetic markers. However, large range intergradation at the subspecies level would be expected from the clinal pan-European morphological variation of the coal tit and would contrast those narrow contact zones where birds show distinct song types and/or morphology as shown for chiffchaffs (Martens, 1982; Helbig et al., 2001), great tits (Päckert et al., 2005; Kvist and Rytönen, 2006) and greenish warblers *Phylloscopus trochiloides* (Sundevall, 1837) (Irwin et al., 2001, 2005). A few cases have demonstrated that mitochondrial introgression can

trespass a contact zone among distinct parental phenotypes across large distances, as in crows (Haring et al., 2007), and swamp the big picture as found for the yellowhammer *Emberiza citrinella* Linnaeus, 1758 and pine bunting *E. leucocephalos* Gmelin, 1771 (Irwin et al., 2009).

Acknowledgements

We are particularly grateful to all colleagues who provided samples for molecular analyses: J. Broggi, T. Dolich, M. Fischer (Naturkundemuseum Erfurt, Germany), H. von Hirschheydt (Vogelwarte Sempach, Switzerland) with the ringers S. Althaus, M. Thoma and F. Steiner (on Col de Bretolet, Switzerland), A. Johnsen (Natural History Museum Oslo, Norway), T. Lubjuhn, P. Lymberakis (Natural History Museum of Crete, Greece), S. Martens (Itzehoe), C.W. Moeliker (Natuurhistorisch Museum Rotterdam, The Netherlands),

A. Ostastshenko, F. Schramm and H. Zang B. Martens and H. Pieper helped on various field trips. We are also grateful to F. Spina for providing collection permits in Italy and on Sardinia. B. Martens helped on various field trips. For further supporters see Martens et al. (2006) and Tietze et al. (2011). Travel and research funds for field trips by J.M. were granted by the Deutsche Ornithologen-Gesellschaft and the Gesellschaft für Tropenornithologie (various grants), by Feldbausch-Stiftung and Wagner-Stiftung (at Fachbereich Biologie, University of Mainz, Germany). This project was substantially funded by the State Ministry of Science and Arts of Saxony (Staatsministerium für Wissenschaft und Kunst Sachsen, AZ 4-7531.50-02-621-09/1).

Appendix A.

Table A1

Information on *Periparus ater* samples used for molecular analysis. Museum acronyms: MTD=Senckenberg Natural History Collections, C=Senckenberg Natural History Collections, Museum of Zoology Dresden, Germany; BMNH= British Museum of Natural History, Bird Group Tring, UK; ZMUO=Zoological Museum University of Oslo, Norway; NMR: Natuurhistorisch Museum Rotterdam, The Netherlands; NRO=Zoological Museum University of Oulu, Finland; GenBank accession numbers listed for CR sequences, from samples with two GenBank numbers additional COI barcoding sequence is available; if not stated otherwise "sample" refers to the tissue collection of J. Martens [MAR] at Mainz University, Germany; haplotype cluster: all haplotypes shared by two or more individuals listed by successive numbers, samples without haplotype number and only the taxon name of cluster indicated carried private CR haplotypes; autumn and winter birds that were excluded from AMOVA, but included in haplotype network reconstructions marked with an asterisk following the "voucher/sample" number.

Sample	State	Region	Locality	Haplotype cluster	GenBank No.
Wien260	Austria		Wien	ater 1	JX170038
Wien201	Austria		Wien	ater 1	JX170039
3097	Czech Republic	Bohemia, Šumava	Pekna	ater 1	JX170083
3094	Czech Republic	Bohemia, Šumava	Slunecna	abietum	JX170081
2571	Czech Republic	Bohemia, Šumava	Pekna	abietum 9	JX170082
Finland 2	Finland	Haukipudas	Oinaansuo	ater	JX170062
NRO T15008	Finland		Heinola	ater	JX170061
90224	Finland		Lammi	ater 1	JX170056
90225	Finland		Lammi	ater 6	JX170057
90222	Finland		Liminka	ater 3	JX170054
90223	Finland		Liminka	ater 2	JX170055
NRO T14839	Finland		Oulu	ater 6	JX170059
NRO T14861	Finland		Oulu	ater 1	JX170060
NRO T12789	Finland		Siikajoki	ater 1	JX170058
5560	France	Corsica	Cirque de Bonifato	abietum	JX169876
5563	France	Corsica	Cirque de Bonifato	abietum	JX169877
5565	France	Corsica	Cirque de Bonifato	abietum 7	JX169878
5566	France	Corsica	Cirque de Bonifato	abietum 7	JX169879
5567	France	Corsica	Cirque de Bonifato	abietum 7	JX169880
5568	France	Corsica	Cirque de Bonifato	abietum 7	JX169881
5572	France	Corsica	Evisa	abietum 7	JX169882
5578	France	Corsica	Evisa	abietum 7	JX169883
5600	France	Corsica	Evisa	abietum 7	JX169884
5586	France	Corsica	Restonica valley	abietum 7	JX169885
32	France	Pyrénées-Orientales	Serralongue	abietum	JX169895
34	France	Pyrénées-Orientales	Serralongue	abietum 3	JX169896
46	France	Pyrénées-Orientales	Serralongue	abietum 5	JX169897
526	France	Pyrénées-Orientales	Serralongue	abietum 9	JX169898
527	France	Pyrénées-Orientales	Serralongue	abietum 1	JX169899
532	France	Pyrénées-Orientales	Serralongue	abietum 16	DQ466203
1114	France	Pyrénées-Orientales	Serralongue	abietum 3	JX169901
1118	France	Pyrénées-Orientales	Serralongue	abietum 3	JX169902
1119	France	Pyrénées-Orientales	Serralongue	abietum 16	JX169903
JX508787					
1120	France	Pyrénées-Orientales	Serralongue	abietum 1	DQ466204
1121	France	Pyrénées-Orientales	Serralongue	abietum 8	JX169905
1122	France	Pyrénées-Orientales	Serralongue	abietum 2	JX169906
1134	France	Pyrénées-Orientales	Serralongue	abietum 4	JX169907
					JX508790
267	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum 14	DQ466202
268	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	ater 1	JX169957
269	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum 3	JX169951
270	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum 2	JX169952
271	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum	JX169953
279	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum 2	JX169954
285	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum 1	JX169955
7318	Germany	Baden-Württemberg	S Black Forest (Grafenhausen)	abietum	JX169956
3315	Germany	Bavaria	Bayreuth	abietum 3	JX170084
5125	Germany	Mecklenburg-Vorpommern	Serrahn	ater 2	JX170040

Table A1 (Continued)

Sample	State	Region	Locality	Haplotype cluster	GenBank No.
C2003R	Germany	North Rhine Westphalia	Holwickede	<i>abietum</i> 1	JX169976
R6155178K	Germany	Lower Saxony	Brillit	<i>abietum</i>	JX169975
7114	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>abietum</i> 1	JX170024
7977	Germany	Lower Saxony	Harz Mountains (Bad Harzburg)	<i>abietum</i> 2	JX170027
7978	Germany	Lower Saxony	Harz Mountains (Bad Harzburg)	<i>abietum</i> 3	JX170028
7976	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>abietum</i> 1	JX170026
7975	Germany	Lower Saxony	Harz Mountains (Brettingerrode)	<i>abietum</i> 3	JX170025
5604	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>abietum</i> 3	JX170017
5605	Germany	Lower Saxony	Harz Mountains (Bad Harzburg)	<i>abietum</i> 1	JX170018
6257	Germany	Lower Saxony	Harz Mountains (Eckertal)	<i>ater</i>	JX170019
6258	Germany	Lower Saxony	Harz Mountains (Eckertal)	<i>abietum</i> 2	JX170020
6621	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>abietum</i>	JX170023
6619	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>ater</i> 1	JX170021
90230	Germany	Lower Saxony	Emsland (Lingen)	<i>abietum</i> 3	JX170029
90231	Germany	Lower Saxony	Emsland (Lingen)	<i>abietum</i> 17	JX170030
90232	Germany	Lower Saxony	Emsland (Lingen)	<i>abietum</i> 11	JX170031
6620	Germany	Lower Saxony	Harz Mountains (Braunlage)	<i>abietum</i>	JX170022
313	Germany	Rhineland Palatinate	Mainz-Bretzenheim	<i>ater</i>	JX169958
2531	Germany	Rhineland Palatinate	Mainz-Bretzenheim	<i>abietum</i> 1	JX169961
1*	Germany	Rhineland Palatinate	Oberolmer Wald, Mainz	<i>abietum</i>	DQ466198
2*	Germany	Rhineland Palatinate	Oberolmer Wald, Mainz	<i>ater</i>	JX169959
7971	Germany	Rhineland Palatinate	Soonwald	<i>abietum</i> 4	JX169963
7972	Germany	Rhineland Palatinate	Soonwald	<i>abietum</i>	JX169964
4072*	Germany	Rhineland Palatinate	Worms	<i>ater</i>	JX169962
RLP 008	Germany	Rhineland Palatinate	Hanhofen	<i>abietum</i> 13	JX169968
RLP 009	Germany	Rhineland Palatinate	Hanhofen	<i>abietum</i>	JX169969
RLP 010	Germany	Rhineland Palatinate	Hanhofen	<i>abietum</i>	JX169967
RLP 005	Germany	Rhineland Palatinate	Hanhofen	<i>abietum</i> 5	JX169971
RLP 006	Germany	Rhineland Palatinate	Hanhofen	<i>abietum</i>	JX169965
RLP 007	Germany	Rhineland Palatinate	Hanhofen	<i>ater</i>	JX169966
RLP 011	Germany	Rhineland Palatinate	Harthausen	<i>abietum</i> 13	JX169970
993	Germany	Saxony	Crottendorf	<i>abietum</i> 1	JX169982
MTD C59468	Germany	Saxony	Dresden	<i>abietum</i> 2	JX169979
988	Germany	Saxony	Dresden	<i>abietum</i>	JX169977
7486	Germany	Saxony	Görlitz	<i>abietum</i> 2	JX169972
7488	Germany	Saxony	Görlitz	<i>abietum</i> 4	JX169974
7487	Germany	Saxony	Görlitz	<i>abietum</i> 2	JX169973
MTD C59476	Germany	Saxony	Kraupa	<i>abietum</i> 3	JX169980
MTD C60967	Germany	Saxony	Liegau Augustusbad	<i>abietum</i> 17	JX169978
992	Germany	Saxony	Moritzburg	<i>abietum</i>	JX169981
994	Germany	Saxony	Neudorf	<i>abietum</i> 3	JX169983
7505	Germany	Schleswig-Holstein	Amrum Island	<i>ater</i> 9	JX170000
7506	Germany	Schleswig-Holstein	Amrum Island	<i>ater</i> 9	JX170001
7513	Germany	Schleswig-Holstein	Amrum Island	<i>ater</i> 9	JX170002
ITZ 2010 A	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 2	JX170007
ITZ 2010 B	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 14	JX170008
ITZ 2010 D1	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 1	JX170009
ITZ 2010 E1	Germany	Schleswig-Holstein	Itzehoe	<i>ater</i> 3	JX170003
ITZ 2010 F	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 4	JX170010
ITZ 2010 G	Germany	Schleswig-Holstein	Itzehoe	<i>ater</i> 4	JX170004
7951	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 2	JX170013
7954	Germany	Schleswig-Holstein	Itzehoe	<i>ater</i> 1	JX170005
7955	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 2	JX170014
7958	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 1	JX170012
7959	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 2	JX170011
7960	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i> 2	JX170016
7964	Germany	Schleswig-Holstein	Itzehoe	<i>abietum</i>	JX170015
7969	Germany	Schleswig-Holstein	Itzehoe	<i>ater</i> 4	JX170006
3840	Germany	Thuringia	Rudolstadt	<i>abietum</i>	JX169994
65/00 A	Germany	Saxony	Erzgebirge	<i>abietum</i> 1	JX169986
78/01 A	Germany	Saxony	Erzgebirge	<i>abietum</i> 1	JX169984
78/02 A	Germany	Saxony	Erzgebirge	<i>ater</i>	JX169988
78/02 C	Germany	Saxony	Erzgebirge	<i>abietum</i> 1	JX169985
78/03 A	Germany	Saxony	Erzgebirge	<i>abietum</i>	JX169991
78/03 B	Germany	Saxony	Erzgebirge	<i>ater</i> 7	JX169990
117/03 A	Germany	Saxony	Erzgebirge	<i>abietum</i>	JX169992
117/03 B	Germany	Saxony	Erzgebirge	<i>abietum</i> 3	JX169987
117/03 C	Germany	Saxony	Erzgebirge	<i>abietum</i> 3	JX169993
8024	Greece	Peloponnes	Karitena	<i>abietum</i> 5	JX169923
8025	Greece	Peloponnes	Karitena	<i>abietum</i>	JX169924
8026	Greece	Peloponnes	Karitena	<i>abietum</i> 1	JX169925
8027	Greece	Peloponnes	Karitena	<i>abietum</i> 3	JX169926
8028	Greece	Peloponnes	Karitena	<i>abietum</i> 3	JX169927
8029	Greece	Peloponnes	Karitena	<i>abietum</i> 1	JX169928
Sard11	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169886
					JX508788

Table A1 (Continued)

Sample	State	Region	Locality	Haplotype cluster	GenBank No.
Sard12	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169887
Sard16	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169888
Sard17	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169889
Sard18	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169890
Sard20	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169891
Sard26	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169892
Sard27	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169893
Sard37	Italy	Sardinia	Monte Limbara	<i>abietum</i> 6	JX169894
90228	Japan	Hokkaido	Nagano	<i>ater</i> 1	JX170079
90229	Japan	Hokkaido	Nagano	<i>ater</i> 2	JX170080
1608	Kazakhstan	Almaatinka valley		<i>ater</i> 1	JX170063
2878	Kyrgyzstan	Issyk-Kul		<i>ater</i> 1	DQ466207
2879	Kyrgyzstan	Issyk-Kul		<i>ater</i> 1	JX170065
2880	Kyrgyzstan	Issyk-Kul		<i>ater</i> 1	JX170066
2881	Kyrgyzstan	Issyk-Kul		<i>ater</i> 1	JX170067
4008	Morocco	Cèdre Goureaud		<i>atlas</i>	DQ466213
					JX508789
NMR9989-2272	Netherlands	Flendland		<i>abietum</i> 1	JX169997
NMR9989-2276	Netherlands	Rotterdam		<i>abietum</i> 10	JX169998
NMR9989-2279	Netherlands	Rotterdam		<i>abietum</i> 11	JX169999
ZMUO29 665	Norway	Akershus	Dæli	<i>ater</i> 6	JX170051
ZMUO29 676	Norway	Oslo	Brenna	<i>ater</i> 3	JX170053
ZMUO29 614	Norway	Oslo	Sinober	<i>ater</i>	JX170044
ZMUO29 617	Norway	Oslo	Sinober	<i>ater</i> 2	JX170045
ZMUO29 618	Norway	Oslo	Sinober	<i>ater</i> 8	JX170046
ZMUO29 619	Norway	Oslo	Sinober	<i>ater</i> 3	JX170047
ZMUO29 621	Norway	Oslo	Sinober	<i>ater</i> 2	JX170048
ZMUO22 326	Norway	Oslo	Sinober	<i>ater</i> 8	JX170041
ZMUO29 672	Norway	Oslo	Tangen	<i>ater</i> 3	JX170052
ZMUO22 327	Norway	Telemark	Skien	<i>ater</i> 1	JX170042
ZMUO29 557	Norway	Telemark	Skien	<i>ater</i> 2	JX170043
ZMUO29 632	Norway	Telemark	Skien	<i>ater</i> 1	JX170049
ZMUO29 636	Norway	Telemark	Skien	<i>ater</i> 2	JX170050
692*	Austria	Salzburg	Mühlbach am Hochkönig	<i>ater</i>	JF304544
693*	Austria	Salzburg	Mühlbach am Hochkönig	<i>ater</i> 1	JX170037
1471	Russia	Evreyskaya avtonomnaya oblast	Londoko	<i>ater</i> 1	DQ466211
1444	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 1	JX170069
1445	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 2	JX170070
1446	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 2	DQ466205
1447	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 8	JX170072
1448	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 1	JX170073
1449	Russia	Primorskiy kray	Vladivostok	<i>ater</i>	JX170074
1450	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 5	JX170075
1451	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 2	JX170076
1452	Russia	Primorskiy kray	Vladivostok	<i>ater</i> 5	JX170077
1416	Russia	Sakhalinskaya oblast	Island of Sachalin, Chaplanovo	<i>ater</i>	DQ466210
jb 503701*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 2	JX169908
jb 839635*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 1	JX169913
jb 839659*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 4	JX169914
jb 839670*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 3	JX169915
jb 850166*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 2	JX169916
jb 931207*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 15	JX169909
jb 931213*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 4	JX169917
jb 931214*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 3	JX169918
jb 931216*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 8	JX169919
jb 931250*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 15	JX169910
jb 931283*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 3	JX169920
jb 931305*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 1	JX169911
jb 931317*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 10	JX169921
jb 931319*	Spain	Pyrenees	Coll de Pal	<i>abietum</i> 3	JX169922
jb 931344*	Spain	Pyrenees	Coll de Pal	<i>abietum</i>	JX169912
7121	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 5	JX169936
7122	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 2	JX169937
7123	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 2	JX169938
b 294208*	Switzerland	Valais	Col de Bretolet	<i>abietum</i>	JX169939
b 294209*	Switzerland	Valais	Col de Bretolet	<i>ater</i> 1	JX170032
b 294211*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 4	JX169940
b 294244*	Switzerland	Valais	Col de Bretolet	<i>abietum</i>	JX169941
b 294246*	Switzerland	Valais	Col de Bretolet	<i>ater</i> 3	JX170033
b 294255*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 4	JX169942
b 294256*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 1	JX169943
b 294259*	Switzerland	Valais	Col de Bretolet	<i>ater</i> 3	JX170034
b 294273*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 2	JX169944
b 294274*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 3	JX169945
b 294275*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 4	JX169946
b 294278*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 1	JX169947
b 294590*	Switzerland	Valais	Col de Bretolet	<i>abietum</i> 1	JX169948

Table A1 (Continued)

Sample	State	Region	Locality	Haplotype cluster	GenBank No.
b 294614*	Switzerland	Valais	Col de Bretolet	ater 3	JX170035
b 294661*	Switzerland	Valais	Col de Bretolet	abietum 2	JX169949
5537	Turkey	W Akbuk		abietum 4	JX170085
NHM 2006.22.1	United Kingdom	Nottinghamshire	Nottingham, Treswell Wood	abietum 2	JX169995
NHM 2006.21.1	United Kingdom	Nottinghamshire	Nottingham, Treswell Wood	abietum 1	JX169996
4561	Cyprus		chapel Stavros tis Psokas	cypristes 1	JF304546
4567	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169870
4568	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169871
4596	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169872
4604	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169873
4605	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169874
4608	Cyprus		chapel Stavros tis Psokas	cypristes 1	JX169875
Grec80416749	Greece	Makedonia	Dramas, Rodopi	abietum 1	JX169930
Grec80416759	Greece	Makedonia	Dramas, Rodopi	abietum 2	JX169934
Grec80416721	Greece	Makedonia	Dramas, Rodopi	abietum	JX169929
Grec80416726	Greece	Makedonia	Dramas, Rodopi	abietum	JX169935
Grec80416754	Greece	Makedonia	Dramas, Rodopi	abietum 1	JX169931
Grec80416760	Greece	Makedonia	Dramas, Rodopi	abietum 1	JX169933
Grec80416758	Greece	Makedonia	Dramas, Rodopi	ater 7	JX169932
Sic8413	Italy	Sicily	Mt Etna, Mareneve N Linguaglossa	abietum 5	JX170088
Sic8414	Italy	Sicily	Mt Etna, Mareneve N Linguaglossa	abietum 5	JX170089
Sic8415	Italy	Sicily	Mt Maletto S Randazzo	abietum 5	JX170090
Sic8433	Italy	Sicily	Mt Maletto S Randazzo	abietum 5	JX170091
Sic8437	Italy	Sicily	Mt Nebrodi, Floresta	abietum 5	JX170092
Sic8412	Italy	Sicily	Mt Nebrodi, Floresta	abietum 12	JX170087
Sic8432	Italy	Sicily	Mt Etna, Mareneve	abietum 12	JX170093
Sic4	Italy	Sicily	Madonie	abietum	JX474804
Sic6	Italy	Sicily	Madonie	abietum 1	JX474805
Sic39	Italy	Sicily	Madonie	abietum	JX474803
Rus1403	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 3	JX474792
Rus2387	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 5	JX474793
Rus2403	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 5	JX474794
Rus2417	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 1	JX474795
Rus2441	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 2	JX474796
Rus2460	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater	JX474797
Rus2461	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater	JX474798
Rus5289	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 1	JX474799
Rus5299	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 2	JX474800
Rus6170	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 2	JX474801
Rus7057	Russia	Oblast Kaliningrad	Kurschskaja kossa	ater 6	JX474802
<i>Himalayan/Chinese populations</i>					
1876	China	Gansu	Lianhua Shan	eckodedicatus	DQ466206
1880	China	Gansu	Lianhua Shan	eckodedicatus	JX474769
6902	China	Ningxia	Helan Shan	eckodedicatus 1	JX474780
6903	China	Ningxia	Helan Shan	eckodedicatus 1	JX474781
4934	China	Shaanxi	Qangqing Panda Reserve	eckodedicatus	JX474775
3280	China	Sichuan	Wawu Shan	eckodedicatus 1	DQ466212
5724	China	Jiangxi	Huanggang Shan	eckodedicatus	JX474778
4155	Nepal	Rasuwa District	Somdang, W of Syabrubesi	aemodius 1	JX474773
4156	Nepal	Rasuwa District	Somdang, W of Syabrubesi	aemodius 1	DQ466214
4195	Nepal	Rasuwa District	Somdang, W of Syabrubesi	aemodius 1	JX474774
4196	Nepal	Rasuwa District	Somdang, W of Syabrubesi	aemodius 2	JX474776
4222	Nepal	Rasuwa District	Somdang, W of Syabrubesi	aemodius 2	JX474777
90018	Nepal	Sindhu Palchok District	Dadar Danda, Kalinchok	aemodius	JX474782
90019	Nepal	Sindhu Palchok District	Dadar Danda, Kalinchok	aemodius	DQ466215
90028	Nepal	Sindhu Palchok District	Dadar Danda, Kalinchok	aemodius	JX474783
90046	Nepal	Sindhu Palchok District	Dadar Danda, Kalinchok	aemodius 2	JX474784
90053	Nepal	Sindhu Palchok District	Dadar Danda, Kalinchok	aemodius	DQ466216
3681	Nepal	Taplejung District	Gunsa	aemodius 1	JX474772
90132	Nepal	Mustang District	Purano Marpha	aemodius 2	DQ466218
90137	Nepal	Mustang District	Purano Marpha	aemodius 1	JX474785
90101	Nepal	Parbat District	Between Chitre and Deorali	aemodius	DQ466217
2913	Nepal	Humla District	Valley below Chala	melanolophus 1	JX474770
2918	Nepal	Bajhang District	Saipal	melanolophus 1	JF304545
2921	Nepal	Humla District	West of Simikot	melanolophus 1	JX474771
6615	Nepal	Jumla District	Jagdula Lekh Valley	melanolophus	JX474779
3324	Nepal	West Nepal	Chucho Khola	melanolophus	DQ466209
<i>Hybrid population</i>					
90155	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474786
90156	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474787
90157	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474788
90163	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474789
90164	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474790
90165	Nepal	Myagdi District	Upper Myagdi Khola	melanolophus 1	DQ466219
90166	Nepal	Myagdi District	Upper Myagdi Khola	aemodius 1	JX474791

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcz.2012.10.003>.

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