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Genetic variation among Corsican and continental populations of the Eurasian treecreeper (Aves: *Certhia familiaris*) reveals the existence of a palaeoendemic mitochondrial lineage

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In this study we investigated the phylogenetics of the Eurasian treecreeper (*Certhia familiaris*), a forest passerine with a wide Palaearctic range including Corsica, using three mitochondrial genes and three nuclear introns, and its phylogeographic history using the COI gene. Our phylogenetic results, including eight of the ten sub-species currently recognized, support the monophyly of *C. familiaris* with respect to its Indo-Asian sister species *C. hodgsoni*. *C. familiaris* comprises two lineages that diverged during the mid-Pleistocene (*c.* 1 Myr): one palaeoendemic lineage has an allopatric range nowadays restricted to the Corsica island and the Caucasus region whereas the second one, more recent and widespread, is distributed over most of Eurasia and in northern China. The most likely scenario that may explain such a pattern is a double colonization of the western Palaearctic from the eastern range of the species. During the middle Pleistocene period, a first lineage expanded its range up into Europe but did not persist through glacial cycles except in Corsica and the Caucasus region. Later, during the upper Pleistocene, a second lineage began to diversify around 0.9 Myr, spreading towards the western Palaearctic from a unique refuge likely located in the eastern Palaearctic. Apart from *C. f. corsa*, our results do not suggest any distinct evolutionary history for other sub-species previously described on morphological grounds in Europe. Our study highlights the important conservation value of the Corsican treecreeper and emphasizes the major role of mature pine forests in the evolution of endemic bird taxa in Corsica. *Biological Journal of the Linnean Society*, 2015, ••, ••–••.

ADDITIONAL KEYWORDS: *Certhia* – COI – Corsica – island – Palaearctic – palaeoendemic lineage – Pleistocene – phylogeography.

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INTRODUCTION

The Pleistocene climatic oscillations characterised by the alternating of glacial and interglacial periods have a strong impact on the geographical distribution and present-day genetic structure of many temperate taxa (Taberlet *et al*., 1998; Avise, 2000; Hewitt, 2000). As a consequence of past climatic events, Palaearctic flora and fauna went through repeated phases of contraction–expansion of their geographical ranges that in many cases favoured interspecific genetic divergence or sub-species differentiation (Hewitt, 2004; Schmitt, 2007; Pons *et al*., 2011). In Europe, comparative genetic surveys highlighted three Mediterranean primary refugia not covered by ice masses: the Iberian Peninsula, the Italian Peninsula and the Balkans where populations persisted during cooling periods and were able to colonize northern areas during warming periods (Hewitt, 2000; Weiss & Ferrand, 2007). In addition, some studies suggested that Mediterranean islands (Kvist *et al*., 2004; Brambilla *et al*., 2008), Central Europe (Deffontaine *et al*., 2005; Magri *et al*., 2006) and the Caucasus region could have constituted glacial refugia for some Palaearctic taxa (Hewitt, 2004; Zink *et al*., 2008; Connor & Kvavadze, 2009; Hung, Drovetski & Zink, 2012).

Corsica is a continental Mediterranean island situated 60 km from the coast of Tuscany (Italy). Its surface totals about 8700 km^2 extending for 183 km. The centre of the island is made up of a single mountain chain that runs in a north to south direction and is cut by deep valleys. In Corsica, isolated from the European mainland at least during all of the Quaternary period, pre-Pleistocene endemics have been documented for a wide array of unrelated organisms from plants to invertebrate and non-flying vertebrate taxa, all of which have a low potential for long distance over-sea dispersal (Delaugerre & Cheylan, 1992; Pereira & Salotti, 2002; Jeanmonod, Schlüssel & Gamisans, 2011; Ketmaier & Caccone, 2013). However, endemic taxa of Pleistocene origin are also known for vertebrates with high dispersal abilities such as bats (Hulva *et al*., 2007; Puechmaille *et al*., 2012) or birds (Louchart, 2002; Förschler *et al*., 2009) with most of them showing a clear affinity to European or North African taxa. Yet, noticeable exceptions to this pattern are found among birds. For example, the closest relative of the Corsican nuthatch (*Sitta whiteheadi*) is the eastern Palaearctic Chinese nuthatch *Sitta villosa* (Pasquet *et al*., 2014) whose range is situated 4000 km away from Corsica, whereas the genus *Sitta* is represented by several intervening species in the western Palaearctic (e.g. *S. europeae*, *S. krueperi*). Such an unusual biogeographical pattern may result from a vicariant event dating back to around 1 Myr ago and be related to pine forest refugium to which *Sitta whiteheadi* is restricted in Corsica (Pasquet *et al*., 2014). More generally, it is worth noting that most endemic bird sub-species described on morphological grounds in Corsica and Sardinia are not Mediterranean or alpine taxa but forest birds (Prodon, Thibault & Dejaifve, 2002). One scenario that could explain this pattern is that only forest birds had the opportunity to shift their altitudinal range in response to Pleistocene climatic changes and thus were able to differentiate in Corsica (Prodon *et al*., 2002). The extant of the level of endemism achieved by Corsican land bird sub-species has been assessed using genetic markers for only a small number of taxa (e.g. Pasquet & Thibault, 1997; Kvist *et al*., 2004; Brambilla *et al*., 2008; Förschler *et al*., 2009). Hence, the evolutionary history of the Corsican terrestrial bird assemblage is thus still largely unknown.

The present study aims to understand the phylogeographic history of the Eurasian treecreeper (*Certhia familiaris* Linnaeus, 1758), a forest passerine found in Corsica and over the entire Palaearctic from the British Isles to eastern Russia, Japan and northern China. The genus *Certhia* includes nine species that share many common morphological attributes such as a medium–long stiffened tail, a decurved bill and a highly cryptic plumage. The genus occupies a very specific niche, gleaning small invertebrates from the trunk and branches of trees. This ecological specialization has constrained morphological divergence among species and sub-species, which are very uniform in appearance. New systematic arrangements based on vocal and genetic differences among taxa have been recently proposed (Tietze, Martens & Sun, 2006). The Brown treecreeper (*C. americana* Bonaparte, 1838) and the southern Asian Hodgson's treecreeper (*C. hodgsoni* W.E. Brooks, 1871) formerly treated as sub-species of *C. familiaris* are now elevated to the species level.

Ten sub-species are currently recognized for *C. familiaris* based on slight clinal variation in plumage colour (Harrap, 2008). Five out of the ten recognized sub-species occur in the western Palaearctic whereas the other five sub-species are distributed in the eastern Palaearctic. Among these ten sub-species, the most distinctive in plumage and morphology is the isolated Corsican sub-species (Harrap, 2008; Tietze & Martens, 2009). It has been suggested that Corsican treecreepers (*C. f. corsa* Hartert, 1905) might be most closely related to *C. f. macrodactyla* C. L., Brehm, 1831 whose range includes the Italian and Iberian peninsulae (Vaurie, 1959; Harrap, 2008). Tietze *et al*.(2006) proposed a phylogeny of the genus *Certhia* based on a short segment of the cytochrome *b* gene, but they did not

focus on the intraspecific differentiation within *C. familiaris*. Thus, the evolutionary history of *C. familiaris* has not been addressed, let alone using a multilocus approach.

In this study, we assessed the phylogenetic relationships among *C. familiaris* sub-species using multilocus DNA sequence data and propose a time frame for the evolutionary history of *C. familiaris*. We also aimed to clarify phylogeographic patterns that characterise the evolution of *C. familiaris* using the COI mitochondrial gene and population samples spread out over a large part of the geographical range of the species.

We specifically address the following questions: (1) Do morphological sub-species correspond to divergent genetic lineages? (2) Is the genetic variability geographically structured? (3) In which regions did the Eurasian treecreeper persist during the Quaternary ice ages? and (4) What is the level of genetic distinctiveness achieved by Corsican birds?

MATERIAL AND METHODS

Sampling

We obtained tissue samples, mainly feather ($N = 85$) but also blood ($N = 21$), from 106 individuals covering a large part of the Eurasian treecreeper's distribution (Fig. 1 and see Appendix for details of exact localities, institutions and collectors' names). Eight of the ten sub-species were included in the phylogenetic analyses. We were unable to obtain tissue samples from *C. f. japonica* Hartert, 1897 and *C. f. persica* Zarudny & Loudon, 1905. We selected 25 individuals to assess the phylogenetic relationships among the eight sub-species using multilocus markers and checked the monophyly of *C. familiaris* with respect to *C. hodgsoni*. We used *Certhia brachydactyla* as an outgroup according to a previous mitochondrial phylogenetic study performed on *Certhia* (Tietze *et al.*, 2006). Phylogeographic and population genetics analyses were performed on 109 individuals using the mitochondrial cytochrome *c* oxidase subunit I (COI). Eight GenBank sequences of Eurasian treecreepers from Russia were added into the analyses (GenBank accession numbers are listed in the Appendix).

DNA Extraction, Amplification and Sequencing

DNA was isolated from blood samples using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA). For feathers we used the QIAamp DNA Micro Kit (Qiagen). We obtained sequences data from three

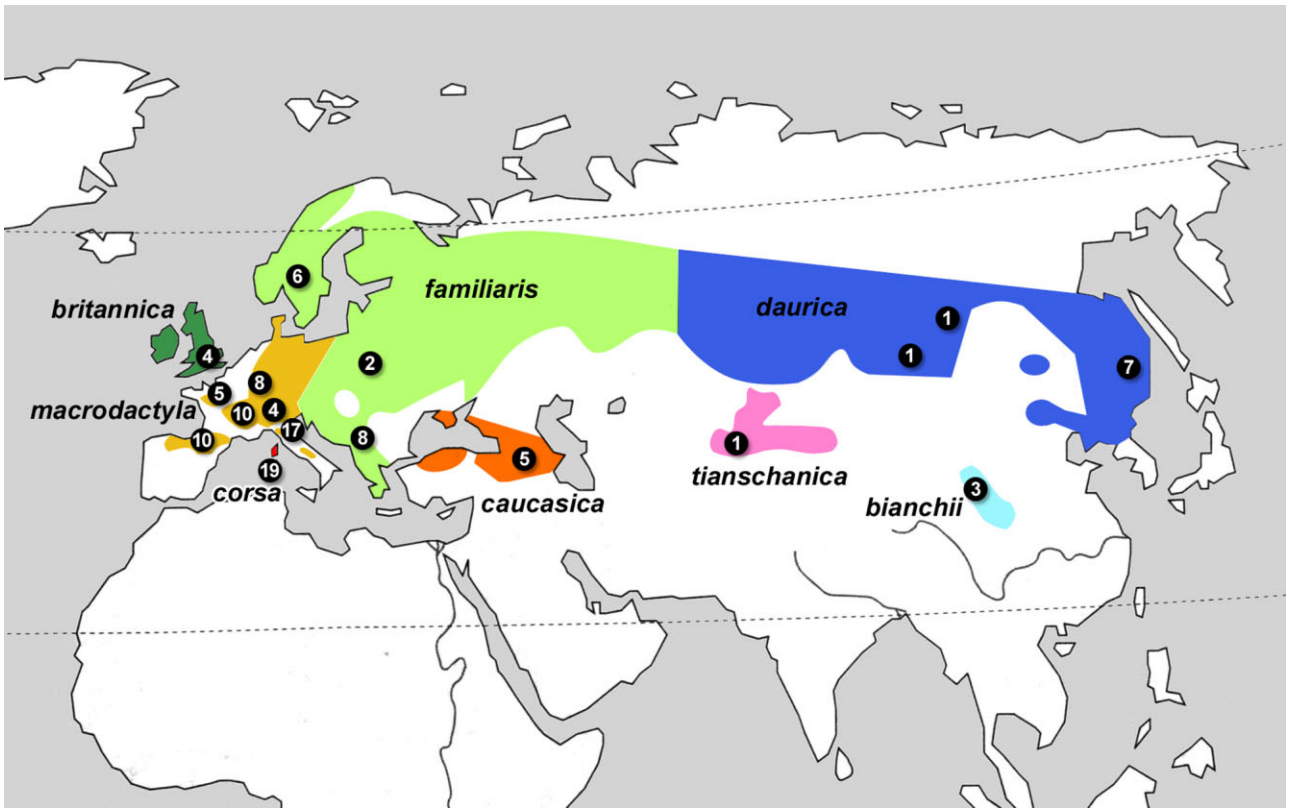

Figure 1. Geographical ranges of the eight *Certhia familiaris* sub-species included in the present study. Black dots give sample size for each sub-species. Precise sampling localities are reported in the Appendix.

mitochondrial markers [ND2, ATP6 and COI], two autosomal nuclear markers [LDH and TGFb2] and one Z-linked marker [ACOI]. Nuclear markers were amplified and sequenced using the following primers: LDH [B1/b4 (Helbig *et al*., 2005)]; TGFb2 [tgf5/tgf6 (Bures, Dvornik & Saetre, 2002)]; ACOI [ACO19F/ACO19R (Kimball *et al*., 2009)]. ND2, ATP6 and COI were amplified and sequenced using primers L5219/H6313 (Sorenson *et al*., 1999), L9245/H9947 (Eberhard & Bermingham, 2004), COIext/FISH1R (Ward, Hanner & Hebert, 2009; Johnsen *et al*., 2010) respectively. Standard amplification and sequencing protocols were followed. Sequences were aligned and heterozygotes sites were checked by eye using BioEdit version 7.0.9 software (Hall, 1999). Sequences were deposited in GenBank with the accession numbers KP282464 to KP282613.

Analyses

Selection on the mitochondrial loci

We used the McDonald–Kreitman test (MK) (McDonald & Kreitman, 1991), as implemented in DnaSP v. 5.0 (Librado & Rozas, 2009) to test whether selection was acting on the three mitochondrial protein-coding genes used to infer phylogeny and population genetics in *C. familiaris*. MK tests were performed between *C. familiaris* and its sister species *C. hodgsoni* as well as between *corsa–caucasica* and the Palaearctic lineages within *C. familiaris*.

Phylogenetic reconstruction

We used PHASE v2.1.1 (Stephens, Smith & Donnelly, 2001), as implemented in DNASP 5.0 (Librado & Rozas, 2009), to infer the alleles for each nuclear locus. Three runs were performed and results were compared across runs. We used the recombination model and ran the iterations of the final run ten times longer than for the initial runs. We considered insertion and deletions events in the nuclear loci as informative mutational events. Sequence files were modified to take into account this form of genetic variation by replacing the missing value with a nucleotide that would induce a mutation; for example a deletion at a site where only an A was present was modified to a G. When the insertion–deletion event involved more than a nucleotide, we considered it as a single event; for example a TGT deletion was considered a single event and modified in the data set by using AGT.

Gene tree reconstructions of the unique haplotypes were performed using Bayesian inferences (BI), as implemented in MRBAYES 3.2 software (Ronquist *et al*., 2012). Substitution models and partitioning strategy were selected using PartitionFinder v1.1.1 software (Lanfear *et al*., 2012).We divided the mitochondrial data set into nine putative partitions (by locus and codon position) and for the nuclear data into three partitions (each intron). Four Metropolis-coupled Markov chain Monte Carlo (MCMC) iterations (one cold and three heated) were run for 5×10^6 iterations with trees sampled every 1000 iterations. The first 500 000 iterations (5000 trees) were discarded ('burn-in' period) and the posterior probabilities were estimated for the remaining sampled generations. Two independent Bayesian runs initiated from random starting trees were performed. We ensured that the potential scale reduction factor approached 1.0 for all parameters and that the average standard deviation of split frequencies converged towards zero. We also used the program TRACER v1.5 (Rambaut & Drummond, 2009) to check that we reached convergence for the posterior distributions of the parameter estimates and that our effective sample size of the underlying posterior distribution was large enough (> 200) for a meaningful estimation of parameters.

We estimated the species tree using the species tree approach implemented in BEAST v. 1.7.2 (Drummond *et al*., 2006; Drummond & Rambaut, 2007;*BEAST, Heled & Drummond, 2010). Species tree approaches implement the coalescent to estimate a species tree based on the different gene trees; this approach has been shown to outperform the traditional concatenation approaches in that incomplete lineage sorting is taken into account (Edwards, 2009). We assumed a strict molecular clock model for all loci and used the best fit model for each partition, as determined using PartitionFinder; each locus was specified with its own clock rate and the corresponding inheritance mode. We ran the chains for 50 million iterations.

Divergence times

We used BEAST 1.8 (Drummond *et al*., 2006; Drummond & Rambaut, 2007) with a strict molecular clock model and a speciation yule tree prior to estimate the divergence times among the primary lineages. We compared the divergence time estimates obtained using: (1) the neutral four-fold rate from Subramanian *et al*. (2009); and (2) the substitution rates proposed by Lerner *et al*. (2011) for the ATP6 and ND3 loci. Subramanian *et al*. (2009) estimated the rate of molecular evolution at four-fold degenerated sites from complete mtDNA sequences of Adelie penguins (*Pygoscelis adeliae*) to be 0.073 substitutions per site per lineage per million year ($s\ s^{-1}\ l^{-1}$/Myr) (95% HPD: 0.025–0.123). More recently, Lerner *et al*. (2011), using complete mtDNA genomes from the honeycreepers (Passeriformes, Drepanididae) and calibration points based on the age of volcanic islands in the Hawaiian archipelago, proposed new substitution rates for ND2 (0.029 s/s/l/Myr; 95% HPD: 0.024–

0.033 s/s/l/Myr), ATP6 (0.026 s/s/l/Myr; 95% HPD: 0.021–0.031 s/s/l/Myr), and COI (0.016 s/s/l/Myr; 95% HPD: 0.014–0.019 s/s/l/Myr).

We incorporated the uncertainty in the molecular evolution rates by modelling the rate as a normal distribution with mean and standard deviation corresponding to the values reported in Subramanian *et al*. (2009) and Lerner *et al*. (2011). For the four-fold degenerated sites, our settings differ slightly from the original settings of Subramanian *et al*. (2009) in that we used a speciation tree prior (instead of coalescent constant) and a TrN substitution model instead of a GTR. These changes are justified by the fact that: (1) our analyses include multiple species whereas those of Subramanian *et al*. (2009) were focused on *Pygoscelis adeliae*; and (2) some substitution types were not represented in enough numbers to allow parameter identifiability. We ran the MCMC for 10^8 generations and sampled trees and parameters every 1000 generations. The first 10% of the samples were removed as the burn-in period; this fraction of the total number of samples is very conservative as stabilization of the parameters values occurred earlier. We used the program TRACER v1.5 (Rambaut & Drummond, 2009) to check that our effective sample size of the underlying posterior distribution was large enough (> 200) for a meaningful estimation of parameters.

Genetic variation and network analysis

The substitution model that best fit our COI data was selected with TOPALi v.2.5 (Milne *et al*., 2004). Mean pairwise genetic distances within and among subspecies were estimated by MEGA6 (Tamura *et al*., 2013). Standard diversity indices (haplotype diversity, nucleotide diversity, number of polymorphic sites) were calculated using Arlequin 3.5 (Excoffier & Lischer, 2010). We used Fu's Fs and Tajima' D tests (1000 replicates) to detect signatures of population expansion. We computed pairwise Φst for all pairs of taxa to assess the level of geographical structuring of the genetic variability. For *C. f. macrodactyla* (France $N = 37$; Italy $N = 17$) and *C. f. familiaris* (Scandinavia $N = 6$, Balkans $N = 8$), we assessed whether there was any genetic divergence between northern and southern populations. The significance of variance components was tested with 110 permutations. As only one tissue sample was available for *C.f. tianschanica* E. J. O. Hartert, 1905, this sub-species was removed from the data set before performing population genetic analyses.

We generated a median-joining network to visualize relationships among COI haplotypes ($N = 111$) and ATP6 and ND2 haplotypes for a subsample of 27 individuals with NETWORK 4.6.1.2 (Bandelt, Forster & Rohl, 1999).

bGYMC

We used the Bayesian implementation of the general mixed Yule-coalescent model (bGMYC 1.0; Reid & Carstens, 2012) to delimitate putative species using our molecular data. This implementation is an extension of the GMYC model (Pons *et al*., 2006) that incorporates gene tree uncertainty by sampling over the posterior distribution of sampled gene trees. We used the posterior distribution of ultrametric gene trees obtained from the analyses of the unique 29 *Certhia* mitochondrial haplotypes using BEAST v1.8 (Drummond & Rambaut, 2007) under a strict clock model with each locus having its own specific rate (Lerner *et al*., 2011). We ran MCMC for 10^7 iterations with sampling of parameters and trees every 10^3 iterations. The first 10% of the samples were removed as the burn-in period. We analyzed 100 trees sampled randomly from the posterior distribution and used the default setting in bGMYC. We ran the MCMC chains for 5×10^4 iterations, with a burn-in of 4×10^4 iterations, and sampled parameters every 100^{th} iterations.

RESULTS

PHYLOGENETIC RELATIONSHIPS

Selection on the mitochondrial loci (COI, ATP6, ND2)

The MK tests did not detect any significant evidence of selection in the mitochondrial DNA (mtDNA) genes when comparing *C. familiaris* with *C. hodgsoni* (Fischer's exact test, $P_{minimum} > 0.27$). In the same way, there was no indication of selection when MK tests were performed between the *corsa-caucasica* and the Palaearctic lineage comprising all other *C. familiaris* sub-species (Fischer's exact test, $P_{minimum} > 0.5$)

Mitochondrial phylogeny

The mitochondrial tree (Fig. 2) was well resolved ($0.99 < PP = 1.0$ for all nodes) and strongly supported the monophyly of *C. familiaris* with respect to *C. hodgsoni*. Two reciprocally monophyletic divergent lineages were highlighted within *C. familiaris*. One group included individuals belonging to *C.f. caucasica* Buturlin, 1907 and *C. f. corsa*, two disjunct subspecies with limited ranges, and the second one clustered individuals belonging to the six remaining sub-species. Within this group, three subgroups were further highlighted by our mitochondrial data. The first subgroup, widely distributed over the western Palaearctic, comprised sub-species found from the British Isles up to the Kyrgyzstan (*C. f. macrodactyla*, *C. f. familiaris*, *C. f. britannica* Ridgway, 1882, *C. f. tianschanica*). The two remaining groups have an eastern Palaearctic distribution and corresponded to *C. f. daurica* Domaniewski, 1922 and *C. f. bianchii* E.

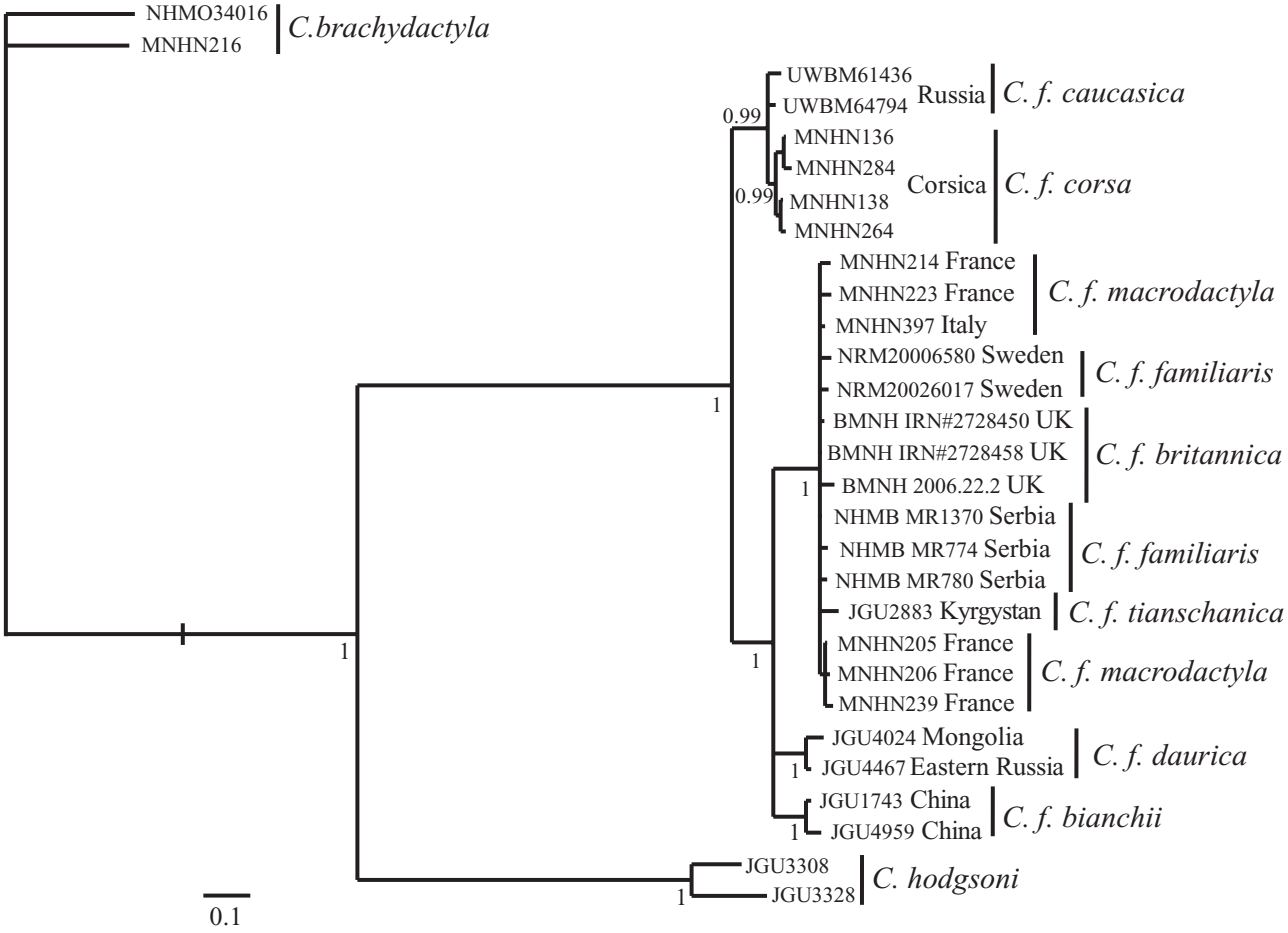

Figure 2. Fifty per cent majority-rule consensus tree obtained from the Bayesian analyses of the three concatenated mitochondrial markers (2409 bp, COI, ND2, ATP6). Only unique haplotypes from 32 individuals were included in the matrix. Values close to nodes represent Bayesian posterior probabilities. *Certhia brachydactyla* was used as outgroup.

J. O Hartert, 1905 respectively. The tree branching order among these three lineages could not be resolved.

Nuclear gene trees

The monophyly of *C. familiaris* with respect to *C. hodgsoni* was strongly supported in the phylogenetic tree based on the Z-linked locus ACOI (Fig. S1). Phylogenetic relationships among subspecies within *C. familiaris* remained unresolved. The autosomal marker TGFB supported a sister relationships between *C. familiaris* and *C. hodgsoni* with respect to *C. brachydactyla* whereas LDH failed to recover any phylogenetic structure among taxa (Figs S2 and S3). Allele sharing among *familiaris* subspecies was very common and may be due to recent divergence events, slower lineage sorting, may be influenced by lower mutation rate and by larger effective population size for nuclear DNA.

Nuclear species trees

Using the species tree approach, *C. familiaris* was recovered as monophyletic with respect to *C. hodgsoni* with high posterior probability support (Fig. 3, PP = 0.99). Most of the other relationships among lineages did not receive strong support. The Chinese sub-species *bianchii* was the first to branch off followed by the Caucasian population which was not sister to the Corsican sub-species. The latter formed a monophyletic group with other western Palaearctic taxa which received a good PP support (PP = 0.97).

Mitochondrial DNA Divergence Times

Our divergence time estimates slightly varied according to the mtDNA marker used. ND2 time estimates tended to be more recent than estimates derived from the COI analysis, whereas ATP6 provided intermediate dates. Our combined data divergence time estimates using the neutral four-fold rate and passerine

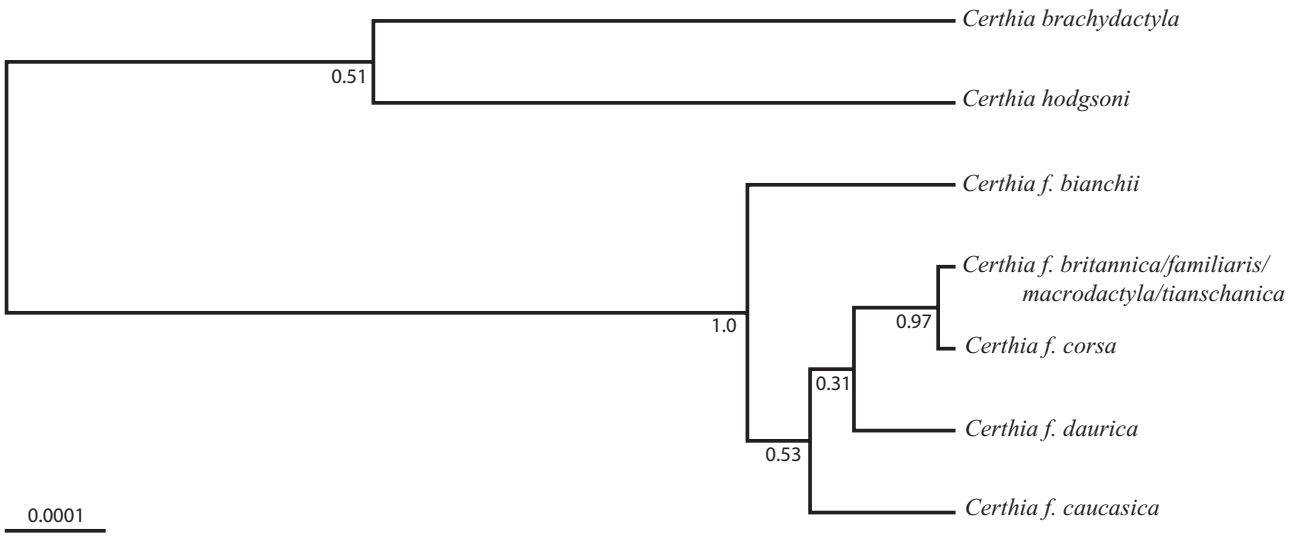

Figure 3. Species tree obtained from two autosomal nuclear introns (LDH, TGFB) and one Z-linked intron (ACOI) using the coalescent approach implemented in *BEAST. Twenty-five individuals were included in the analyses. Values given next to the nodes represent posterior probabilities.

bird substitution rates (Lerner *et al*., 2011) were largely concordant. Neutral four-fold 95% HPD were larger than those obtained from DNA substitution rates (Table 1). *C. hodgsoni* split from *C. familiaris* during the early Pleistocene around 2.2 million years ago (Table 1) and *C. familiaris* started to diversify around 0.35 Myr (middle Pleistocene), the *corsa-caucasica* lineage being the first to branch off. The divergence among *C. familiaris* western sub-species (*familiaris*, *macrodactyla*, *britannica*, *tianschanica*) with respect to eastern sub-species (*daurica*, *bianchii*) occurred around 0.26 Myr. The Corsican lineage (*corsa*) diverged from its Caucasian counterpart (*caucasica*) around 0.086 Myr. In the same way, western *C. familiaris* sub-species also started to diverge during the upper Pleistocene (0.099 Myr) from each other.

Phylogeographic Patterns

Median-joining network

Median-joining networks based on COI sequences ($N = 111$ Eurasian treecreepers) and on ATP6 and ND2 markers ($N = 27$) are represented on Figures 4, S4 and S5 respectively. Nineteen COI haplotypes were detected that clustered in three sub-networks corresponding to the three main lineages highlighted in the phylogenetic analyses (Fig. 4). The *corsa–caucasica* sub-network was separated from the eastern Palaearctic sub-network and the western Palaearctic network by 9 and 14 mutations, respectively. The western Palaearctic sub-network displayed a star-like shape with a common central haplotype having a wide geographical distribution at the centre of the network and derived haplotypes weakly differentiated radiating from the ancestral haplotype. Such a pattern is commonly observed in the case of recent population expansion. In the western sub-network, three sub-species (*macrodactyla*, *familiaris*, *britannica*) shared the same ancestral haplotype and were weakly differentiated. The unique *C. f. tianschanica* haplotype from the Kyrgyzstan region included in the network diverged from the most common western haplotype by only one mutation step. All individuals from the southern Balkan region ($N = 8$) but one shared the most common haplotype. Such a result does not support a refugium glacial scenario for this region. In the same way, 17 individuals from the Apennines (Italy) included in our network were identical, all of them possessing the most common haplotype found everywhere in western Europe. *C. f. britannica* had much higher genetic diversity indices than other sub-species ($H = 0.83$; $\pi = 0.001$, see Table 2) and two of the four British haplotypes were not found on the Continent. These results may suggest that *britannica* may have begun to differentiate from its continental counterparts. However a larger sample size for British treecreepers would be necessary to more soundly address this point. As expected the phylogeographic structure recovered with ATP6 and ND2 (Figs S4 and S5) using a reduced number of individuals is similar to the one obtained with COI and the complete data set.

Table 1. Divergence time estimates in million years (95% Highest Posterior Density) obtained using a neutral four-fold-degenerated site rate and DNA substitutions rate estimated for each mitochondrial marker by Lerner *et al.* (2011)

	C. familiaris/ *C. hodgsoni*	*corsa*, *caucasica*/other *familiaris* sub-species	*corsa*/*caucasica*	*bianchii*, *daurica*/ western sub-species	*C. f. familiaris*, *macrodactyla*, *britannica*
Four-fold degenerated	2.24 (1.03–3.88)	0.351 (0.147–0.622)	0.0857(0.0219–0.173)	0.257 (0.110–0.460)	0.0985 (0.0383–0.183)
Combined rates	2.40 (1.91–2.95)	0.420 (0.313–0.536)	0.0938 (0.0523–0.145)	0.278 (0.205–0.359)	0.0936 (0.058–0.131)
ATP6	2.30 (1.59–3.04)	0.399 (0.265–0.541)	0.0886 (0.0452–0.141)	0.265 (0.176–0.366)	0.089 (0.05–0.130)
COI	2.93 (1.95–3.93)	0.511 (0.334–0.708)	0.113 (0.0556–0.178)	0.34 (0.219–0.478)	0.115 (0.066–0.172)
ND2	1.83 (1.32–2.36)	0.316 (0.220–0.426)	0.0698 (0.0357–0.108)	0.211 (0.144–0.287)	0.0706 (0.0413–0.103)

Genetic variation (COI)

The mtDNA diversity parameters (H, π) were low and presented similar values among most sub-species whatever their geographical range (Table 2). Two taxa, namely *C. f. britannica* and *C. f. bianchii*, had higher diversity parameters. Evidence of population expansion was highlighted for *C. f. familiaris* and *C.f. macrodactyla* for which we found significant negative Fu's Fs and Tajima's D values (Table 2). We did not detect any sign of population expansion neither for *C. f. corsa* nor for *C. f. caucasica*. We also performed population expansion tests at the level of *caucasica*/*corsa* and *macrodactyla*/*familiaris* which constituted highly supported mtDNA clades. Results strongly suggest a pattern of population expansion in case of the *macrodactyla*/*familiaris* clade (Tajima's D = –2; $P = 0.002$ and Fu's D = –8.79; $P < 0.0001$) whereas no clear sign of population expansion was detected for the *caucasica/corsa* clade (Tajima's D = –1.26; $P = 0.07$ and Fu's D = –1.89; $P = 0.03$).

Genetic divergence (COI)

The substitution model that best fit our COI data set was HKY85. We performed pairwise population comparisons (Φst) to assess the level of genetic divergence among taxa (Table 3). *C. f. corsa* was significantly different from all other taxa ($0.96 < \Phi st < 0.98$) except for *C. f. caucasica* ($\Phi st = 0.03$; $P > 0.10$). As expected from the network, there was no significant difference between *C. f. macrodactyla* and *C. f. familiaris* ($\Phi st = 0.008$; $P > 0.10$). This result highlights the absence of partitioning of the genetic variability between these two sub-species. The pairwise Φst value between *C. f. britannica* and *C. f. familiaris* was not significant whereas genetic divergence between *C. f. britannica* and *C. f. macrodactyla* was marginally significant ($\Phi st = 0.21$; $P = 0.04$). We assessed geographical trends in genetic variation within *C.f. macrodactyla* by comparing French ($N = 37$) and Italian populations ($N = 17$). The southerly Italian population was monomorphic whereas low genetic variation was detected in the French population (H = 0.34, $\pi = 0.0003$). Φst value between these two geographical populations was not significant ($P = 0.54$). In the same way for *C. f. familiaris*, we failed to detect any geographical structuring of the genetic variation between the northern Scandinavian population and the population sampled in the Balkans. Genetic diversity indices were similar (Scandinavia: $N = 6$, H = 0.33, $\pi = 0.0005$; Balkans: $N = 8$, H = 0.25, $\pi = 0.0004$) and there was no geographical structure of the genetic variation ($\Phi st = 0.06$, $P = 0.70$).

Within taxa, mean Tamura-Nei genetic distances did not exceed 0.1%. Among taxa mean Tamura-Nei genetic distances ranged from 0.1% to 2.4% (Table 3). The genetic distance between *C. f. corsa* and other

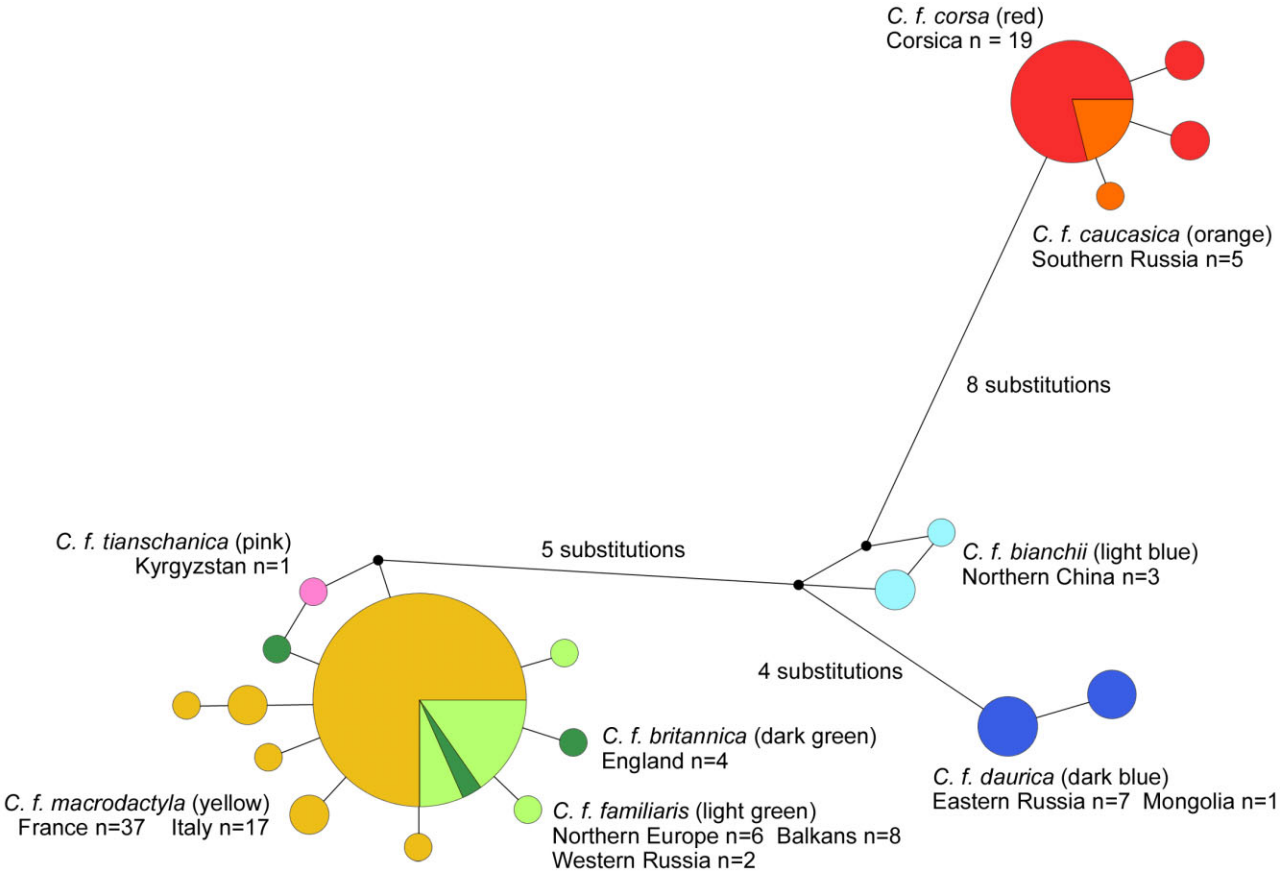

Figure 4. Median-joining network showing relationships among COI haplotypes for *Certhia familiaris* sub-species. The size of each circle is proportional to haplotype frequency. The small black circles correspond to extinct or unsampled haplotypes.

European sub-species (*macrodactyla*, *familiaris*, *britannica*) was 2.4% whereas it was only 0.1% with the farthest *C. f. caucasica*.

Species Delimitations

Analyses performed under the Generalized Mixed Yule-coalescent model (Pons *et al.*, 2006) and a random set of a hundred trees from the posterior distribution of the mitochondrial haplotypes using bGMYC (Reid & Carstens, 2012) indicated that the number of lineages that could be recognized as species is two: *Certhia hodgsoni* and *Certhia familiaris*. Notably, the two primary mitochondrial lineages within *C. familiaris* are not significantly differentiated ($P = 0.18$)

DISCUSSION

Phylogenetic Relationships

Tietze *et al.*(2006) proposed to elevate to species rank *C. hodgsoni*, the eastern populations belonging to a Sino-Himalayan group based on well marked differences in vocal and molecular characters with respect to the other Eurasian *C. familiaris*. In the present study, we used several mitochondrial and nuclear molecular markers and added three sub-species (*britannica*, *corsa*, *caucasica*) not previously included in Tietze *et al.*(2006). Both mitochondrial and nuclear phylogenetic results obtained in the present study support the monophyly of *C. familiaris* with respect to *C. hodgsoni* and favour the taxonomic treatment proposed by Tietze *et al.*(2006).

Phylogenetic relationships among nuclear alleles were most often not recovered with high PP support probably because they split too recently. Accordingly, nuclear lineage sorting is still in the process of occurring, and new mutations are too rare to retrieve a clear phylogenetic signal from a low number of nuclear genes. The main discrepancy between mitochondrial and nuclear gene trees is the sister relationship between the Corsican and the Palaearctic lineages recovered in the nuclear species tree (Fig. 3, PP = 0.97) whereas *corsa* and *caucasica* formed a

Table 2. Number of haplotypes, haplotype diversity (H), nucleotide diversity (π), Tajima's D and Fu's statistics of selective neutrality obtained for each taxon of *Certhia familiaris* using the mitochondrial gene COI (659 bp)

	corsa	*caucasica*	*britannica*	*familiaris*	*macrodactyla*	*daurica*	*bianchii*
Number of individuals	19	5	4	15	54	8	3
Number of haplotypes	3	2	3	3	6	2	2
Number of polymorphic sites	2	1	2	2	5	1	1
H	0.37 ± 0.13	0.4 ± 0.23	0.83 ± 0.22	0.26 ± 0.14	0.24 ± 0.007	0.54 ± 0.12	0.67 ± 0.31
π	0.0006 ± 0.0007	0.0006 ± 0.0007	0.001 ± 0.001	0.0004 ± 0.0005	0.0004 ± 0.0005	0.0008 ± 0.0008	0.001 ± 0.001
Tajima D's	−0.73 (NS)	−0.81 (NS)	−(1)	−1.149*	−1.75*	−0.02 (NS)	–
Fu's Fs	−0.67 (NS)	0.09 (NS)	–	−1.55*	−5.21**	0.87 (NS)	–

(1): Population expansion tests were not performed when sample size was less than five individuals.
NS ($P > 0.05$), *($P < 0.05$), **($P < 0.001$).

strongly supported group in the mtDNA tree (Fig. 2, PP = 1.0). There are several non-exclusive hypotheses that may explain such a discrepancy between mitochondrial and nuclear gene trees: (1) the retention of an ancient polymorphism from the ancestor of the Palaearctic and Corsican lineages; (2) male mediated gene flow with no consequence for the maternally inherited genome; (3) positive selection on mtDNA variants; and (4) hybrid sterility in the heterogametic female sex according to the Haldane's rule (Haldane, 1922). Additional studies would be necessary to firmly validate one or several of these alternative hypotheses. Nevertheless, according to our phylogenetic results (poor resolution of the nuclear species tree, no evidence of selection on mtDNA), the current knowledge on the dispersal behaviour of birds (female is the most dispersing sex in many passerines species, Greenwood, 1980) and the evolution of hybrid sterility in female birds, a long process often exceeding the time for speciation to be efficient (Price & Bouvier, 2002), we suggest that the retention of ancestral polymorphism (Fahey, Ricklefs & Dewoody, 2014) might be the most probable explanation to the mitochondrial-nuclear discrepancy regarding the genetic relationship of *C. f. corsa* with its closest relatives.

The split between the Eurasian *C. familiaris* and the Sino-Himalayan *C. hodgsoni* mitochondrial lineages dated back to the early Pleistocene, around 2 Myr, whereas *C. familiaris* lineages began to differentiate more recently around 0.35 Myr. Our mitochondrial results further highlight the evolutionary history of the Eurasian *C. familiaris* which comprises two main divergent lineages: one previously unknown cryptic and rather old lineage with an allopatric, restricted range including the Corsica island and the Caucasus region; and a second more recent, widespread Eurasian lineage that covers most of Eurasia and northern China. Our mitochondrial phylogenetic tree failed to unravel the branching order within the Eurasian clade that further differentiated into three sub-clades. These are one western Eurasian sub-clade which includes the following sub-species *C. f. britannica*, *C. f. macrodactyla*, *C. f. familiaris*, *C. f. tianschanica*, and two eastern sub-clades corresponding to *C. f. daurica* and *C. f. bianchii*, respectively. *C. f. japonica* could not be included in this study but recently published COI sequences in BOLD (Barcode Of Life Data, http://www.boldsystems.org) are identical or differ by only one mutation from sequences found in *C. f. daurica* from eastern Russia.

Biogeographical Issues

Pleistocene climatic oscillations

The Pleistocene has played a major role in the differentiation of several Palaearctic bird species, in par-

Table 3. Genetic distances (Tamura–Nei average distance and ± standard error, below the diagonal) and pairwise population comparisons (FSTs, above the diagonal) between *Certhia* sub-species

	corsa	*caucasica*	*macrodactyla*	*familiaris*	*britannica*	*bianchii*	*daurica*
corsa	–	0.029 (NS)	0.98***	0.98***	0.97***	0.96***	0.97***
caucasica	0.1 ± 0	–	0.98***	0.98***	0.95***	0.95***	0.96***
macrodactyla	2.4 ± 0.6	2.4 ± 0.6	–	0.008 (NS)	0.21*	0.95***	0.97***
familiaris	2.4 ± 0.6	2.4 ± 0.6	0	–	0.15 (NS)	0.96***	0.93***
britannica	2.4 ± 0.6	2.4 ± 0.6	0.1 ± 0.1	0.1 ± 0.1	–	0.89**	0.90***
bianchii	1.5 ± 0.5	1.5 ± 0.5	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.4	–	
daurica	2.1 ± 0.5	2.1 ± 0.5	1.4 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	0.9 ± 0.3	–

NS ($P > 0.05$), *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$).

ticular of forest birds like the Eurasian treecreeper (e.g. Kvist *et al*., 2004; Brito, 2005; Hung *et al*., 2012). Repeated changes in habitat distribution caused by alternating cycles of cold and warm periods over the last 2 Myr has most probably been involved in driving divergence of main lineages that characterise present-day genetic structure of many forest taxa. The reduction of forest patches during glacial maxima may have isolated populations in small refugia and favoured allopatric divergence at the intraspecific level.

Our genetic survey reveals a phylogeographic pattern for the Eurasian treecreeper that was not previously described in other European bird species studied to date. The Corsican treecreepers belong to a palaeoendemic mitochondrial lineage that disappeared from the European mainland, except in the Caucasus region, during a glacial period that predated the last glacial maximum (LGM, 0.02 Myr). This Corsican/Caucasian lineage most probably split from a widespread lineage around 0.4 Myr that further diversified more recently around 0.3 Myr according to our divergence time estimates, into one eastern Palaearctic lineage (*bianchii*, *daurica*) and one western Palaearctic lineage (*macrodactyla*, *familiaris*, *britannica*). The most likely scenario supported by our results to explain the phylogeographic pattern of the Eurasian treecreeper is the double colonization of the western Palaearctic from the eastern range of the species. During the middle Pleistocene period, a first lineage expanded its range up into Europe but did not persist through glacial cycles except in Corsica and the Caucasus region. Later, during the upper Pleistocene, a second lineage began to diversify around 0.09 Myr spreading towards the western Palaearctic from a unique refuge likely located in the eastern Palaearctic which was less affected by cold climate during glacial periods (Hewitt, 1996). For instance, a large part of the Asian Palaearctic was ice-free during the last glacial maximum while most of Europe was covered by ice (Adams, 1997). The recent spatial expansion of the *macrodactyla*/*familiaris* lineage was clearly suggested by negative values of Tajima's D and Fu's Fs statistics ($P < 0.05$).

A range expansion from glacial refugia located in southern Europe (e.g. Iberian Peninsula, Italy), often documented for diverse European taxa (Hewitt, 2004), is not favoured by the very low genetic diversity values of treecreeper populations from Italy and the Balkans region sampled in this study. Such a lack of genetic diversity is not in accordance with several studies of European taxa in which genetic variation was mainly recovered from populations located within southern glacial refugia (Brito, 2005; Provan & Benett, 2008; Pons *et al*., 2011). The absence of northward decreasing in genetic diversity in Europe, the lack of genetic structure in Europe as well as the mitochondrial sister relationship of the western Palaearctic lineage with sub-species from East Siberia and Northwest China advocate in favour of a recent expansion from a unique refuge located in the eastern part of the *C. familiaris* range. As an alternative explanation, it is possible that southern European populations were subjected to repeated bottlenecks or selection that may have erased a major part of their genetic diversity. Selection is not supported by the MK tests. Additional analyses with nuclear markers and more individuals would be necessary to properly test the bottleneck hypothesis.

Avian endemism and glacial refugia

Our results support the persistence of a palaeoendemic mitochondrial lineage for the Eurasian treecreeper which is currently restricted to two disjunct areas, Corsica and the Caucasus region. Accordingly, Corsica likely acted as a refuge zone (*s. l.*) for this species during several late Pleistocene glacial periods that pre-dated the LGM (0.02 Myr) although the Corsican population had not served as a source for the colonization of the neighbouring continental regions. The alternative hypothesis, one or

several dispersal events between Corsica and the Caucasus region, two disjunct regions located about 2700 km from each other, seems highly unlikely knowing the poor over water dispersal abilities of the Eurasian treecreeper (Harrap, 2008) and the absence of *corsa-caucasica* haplotypes elsewhere in southern Europe. A third hypothesis would be that mtDNA did not evolve neutrally in the Eurasian treecreeper as it has been suggested for its relative the Eurasian nuthatch (*Sitta europaea*, Zink, Drovetski & Rohwer, 2006), the *corsa–caucasica* mitochondrial lineage being submitted to positive selection. Although we did not detect any evidence of selection with the MK tests, more data and statistical analyses would be necessary to dismiss the selection hypothesis (see Garvin *et al*., 2015 for a review).

A global phylogeographic pattern emerges from the studies that assessed the level of genetic endemism in Corsican forest birds, characterised by private haplotype lineages closely linked to a European linage whose range encompasses neighbouring continental regions. Such a pattern has been documented in the Goldcrest (*Regulus regulus*, Päckert, Martens & Severinghaus, 2009), the blue tit (*Cyanistes caeruleus ogliastrae*, Kvist *et al*., 2004), the great tit (*Parus major corsus*, Kvist *et al*., 2003), the coal tit (*Periparus ater sardus*, Pentzold *et al*., 2013), the long-tailed tit (*Aegithalos caudatus*, Päckert, Martens & Sun, 2010) and the Mediterranean citril finch (*Carduelis corsicana*, Förschler *et al*., 2009). This phylogeographic pattern does not apply to either the Corsican treecreepers or to the Corsican nuthatch, two passerine taxa whose habitat is currently restricted to the mature forests of Corsica, mainly the Corsican pine forest. Evolutionary history of these two insular taxa cannot be explained by the geographic distribution of their continental counterparts.

Taxonomic Issues

A lack of concordance between morphological subspecies and genetic lineages has been reported for many Palaearctic bird taxa (e.g. Pavlova *et al*., 2003; Koopman *et al*., 2005). From a taxonomic point of view, and in line with conservation concerns, it has been advocated that a given geographical population should be ranked at the sub-species rank only if it possesses a distinct evolutionary history from its closest relatives (Zink, 2004). Such an evolutionary distinctiveness most often can be characterised by a set of congruent phenotypic and genetic characters used by taxonomists to delimit and name taxa. In this section, we discuss the validity of the sub-species status assigned to Eurasian treecreepers populations found in Europe in line with their evolutionary history.

Our mitochondrial genetic survey reveals that *C. f. corsa* is not closely related to the continental *C. f. macrodactyla* as previously suggested (Vaurie, 1959; Harrap, 2008). Unexpectedly, *C. f. corsa* belongs to a palaeoendemic lineage also found in the Caucasus region which probably disappeared from the rest of Europe during the late Pleistocene. In addition to its genetic distinctiveness, *C. f. corsa* differs from all other continental sub-species by several morphological (bill and wing length) and vocal characters (Tietze *et al*., 2008; Tietze & Martens, 2009). Although Tietze *et al*. (2008) were able to analyze only a few recordings of males from Corsica, they highlighted some differences in Corsican song characteristics. In addition, song play-black experiments showed that whereas *C. f. macrodactyla* individuals from Central Europe weakly responded to the song of *C. f. corsa* (response to the Corsican song was less than half the intensity of that to the control playback), they more strongly reacted to *C. f. familiaris* playback (Tietze, 2007). *C. f. corsa* does not share a common evolutionary history with the continental treecreepers populations that are geographically distributed in Europe up to the Caucasus region and possesses several diagnostic characters (mtDNA, vocals, morphometry). Accordingly *C. f. corsa* fully deserves to be ranked as sub-species according to Zink's recommendations (2004). Moreover, knowing that insular taxa are often under peculiar ecological selective pressures, strong drift and founder effect that may lead to 'rapid' evolution and, given the fact that Corsican treecreepers are forest passerine birds with low dispersal ability isolated from continental populations by the Mediterranean Sea, it could be suggested that *C. f. corsa* might be considered at the species level in the framework of the Phylogenetic Species Concept. From a genetic point of view, such a taxonomic treatment is nevertheless not supported by our results. Our coalescent-based analyses using bGMYC revealed that the two primary mitochondrial lineages are probably not distinct enough to be recognized as species, even though this method is among the most liberal in splitting lineages (e.g. Miralles & Vences, 2013; Satler, Carstens & Hedin, 2013).

Our genetic results show that treecreepers from western Europe assigned to *C. f. macrodactyla* and those from eastern and northern Europe assigned to the *familiaris* sub-species on the basis of slight and clinal variation in plumage colouration (Harrap, 2008) are not genetically divergent. Most of them share the same ancestral haplotype found across Europe, except in the Corsica and Caucasus regions. Accordingly we failed to detect any geographical structure in the genetic variation of these two subspecies which intergrade in the Germany–Poland region (Harrap, 2008). From an evolutionary point of

view, our genetic results do not support the current taxonomic treatment of mainland European treecreepers as two different sub-species. The unique treecreeper individual included in our analyses assigned to the *tianschanica* sub-species, which closely resembles to nominate sub-species in plumage, belongs to the *macrodactyla/familiaris* lineage. More individuals are needed to rigorously assess the validity of this sub-species whose range extends from Kazakhstan to western China. Similarly, a larger sample size would also be recommended to assess the phylogenetic status of British treecreepers which differ vocally from continental birds (Tietze *et al*., 2008) and display slight differences in plumage colouration (Harrap, 2008). Of the four British treecreepers included in our analyses, two of them hold private haplotypes that were not found on the mainland ($\Phi st_{\text{britannica/macrodactyla}} = 0.21, P < 0.05$). Such a significant proportion of private haplotypes, if confirmed with a larger sample size would suggest that British birds have begun to differentiate from their mainland counterparts and would support the validity of *britannica* as a phylogenetic sub-species.

Conservation Issues

Our study emphasizes the high conservation value of the Corsican treecreeper sub-species which has a distinct evolutionary history and possesses several diagnostic characters (morphology, vocals). In addition *C. f. corsa* is only distributed over a restricted insular range geographically separated from the rest of the species range by the Mediterranean Sea. From a conservation perspective, all these evolutionary and ecological specificities show that the Corsican treecreeper is of major importance. It is restricted to the mature and dense forest habitats of Corsican pine (*Pinus nigra laricio*), Holm oak (*Quercus ilex*) and several deciduous trees (mainly *Castanea sativa* and *Fagus sylvatica*) (Thibault & Bonaccorsi, 1999). The Corsican pine forests also constitute the main habitat type of the endemic and 'Vulnerable' (IUCN category) Corsican nuthatch (Thibault *et al*., 2006).The present study adds new information emphasizing the crucial role of mature Corsican pine forests in the evolution of these two endemic bird taxa. Accordingly, our study advocates for increasing the conservation efforts devoted to this unique island's forest.

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APPENDIX

LIST OF TISSUE SAMPLES AND LOCALITIES INCLUDED IN THE MOLECULAR ANALYSES. COI SEQUENCES WERE OBTAINED FOR ALL INDIVIDUALS. ND2, ATP6, LDH, TGFB2 AND ACOI SEQUENCES WERE OBTAINED FOR A SUBSAMPLE OF INDIVIDUALS BELONGING TO THE DIFFERENT *C. FAMILIARIS* SUB-SPECIES, *C. HODGSONI* AND *C. BRACHYDACTYLA*

Species	Locality, Region	Country	Reference	Collector	COI	ATP6	ND2	LDH	TGFB	ACOI
C. brachydactyla mauretanica	Ifrane, Middle Atlas	Morocco	NHMO34016	Copete JL	X	X	X	X	X	X
C. brachydactyla megarhynchos	Saint Aubin du Cormier, Bretagne	France	MNHN216	Fournier J	X	X	X	X	X	X
C. familiaris bianchii	Shaanxi, Taibai Shan, above Houzhenzi	China	JGU1743	Martens, J	X	X	X	X	X	X
C. familiaris bianchii	Shaanxi, Taibai Shan, Xianbansi	China	JGU4911	Martens J, Sun YH	X	X	X	X	X	X
C. familiaris bianchii	Gansu, Zhuoni	China	JGU4959	Martens J, Sun YH	X	X	X	X	X	X
C. familiaris britannica	England	UK	IRN#2728450	BNHM, Roberts M	X	X	X	X	X	X
C. familiaris britannica	England	UK	IRN#2728458	BNHM, Roberts M	X	X	X	X	X	X
C. familiaris britannica	Mame, Yorkshire	UK	BNHM2006.22.2	Du Feu C	X	X	X			
C. familiaris britannica	Catterick, Yorkshire	UK	BNHM2006.22.3	Du Feu C	X					
C. familiaris caucasica	Krasnodarskiy, Kray	Russia	UWBM61436	Drovetski SV	X	X	X	X	X	X
C. familiaris caucasica	Krasnodarskiy, Kray	Russia	UWBM61534	Drovetski SV	X					
C. familiaris caucasica	Krasnodarskiy, Kray	Russia	UWBM64646	Drovetski SV	X					
C. familiaris caucasica	Krasnodarskiy, Kray	Russia	UWBM64668	Drovetski SV	X					
C. familiaris caucasica	Krasnodarskiy, Kray	Russia	UWBM64794	Drovetski SV	X	X	X	X	X	X
C. familiaris corsa	Maison forestière d'Aïtone, Corse	France	MNHN136	Thibault JC, Pons JM	X	X	X	X	X	X
C. familiaris corsa	Maison forestière d'Aïtone, Corse	France	MNHN138	Thibault JC, Pons JM	X	X	X	X	X	X
C. familiaris corsa	Maison forestière d'Aïtone, Corse	France	MNHN139	Thibault JC, Pons JM	X					
C. familiaris corsa	Maison forestière d'Aïtone, Corse	France	MNHN140	Thibault JC, Pons JM	X					

APPENDIX *Continued*

Species	Locality, Region	Country	Reference	Collector	COI	ATP6	ND2	LDH	TGFB	ACOI
C. familiaris corsa	Forêt d'Aïtone, Corse	France	MNHN142	Thibault JC, Pons JM	X					
C. familiaris corsa	Forêt d'Aïtone, Corse	France	MNHN145	Thibault JC, Pons JM	X					
C. familiaris corsa	Forêt Valdo-Niello, Corse	France	MNHN156	Thibault JC, Pons JM	X					
C. familiaris corsa	Asco, Corse	France	MNHN169	Thibault JC, Pons JM	X					
C. familiaris corsa	Asco, Corse	France	MNHN170	Thibault JC, Pons JM	X					
C. familiaris corsa	Tova, FT Tova, Corse	France	MNHN177	Thibault JC, Pons JM	X					
C. familiaris corsa	Melaja, Corse	France	MNHN188	Thibault JC	X					
C. familiaris corsa	Vizzavona Casavi, Corse	France	MNHH263	Thibault JC	X					
C. familiaris corsa	Vizzanova, Corse	France	MNHH264	Thibault JC	X	X	X			
C. familiaris corsa	FT Stella, Corse	France	MNHH265	Thibault JC	X					
C. familiaris corsa	FT Rospa Sorda, Corse	France	MNHH266	Thibault JC	X					
C. familiaris corsa	FT Tova, Tour 11, Corse	France	MNHN284	Thibault JC	X	X	X			
C. familiaris corsa	Valdoniellu-Albertacce, Corse	France	MNHN419	Thibault JC	X					
C. familiaris corsa	Valdoniellu-Albertacce, Corse	France	MNHN423	Thibault JC	X					
C. familiaris corsa	FT Melaja, Corse	France	MNHN609	Thibault JC	X					
C. familiaris daurica	Tes gol	Mongolia	JGU4024	Martens J, Stubbe M	X	X	X	X	X	X
C. familiaris daurica	Primorskiykray, Ussuri Valley, Oblachnaya	Russia	JGU90351	Martens J, Päckert M	X	X	X	X	X	X
C. familiaris daurica	Chitinskaya Oblast, Is. Olchon, Lake Baikal	Russia	JGU4467	Gamauf, A	X	X	X	X	X	X
C. familiaris familiaris	Märsta, Uppland	Sweden	NRM20006580	NRM	X	X	X	X	X	X
C. familiaris familiaris	Örebro, Äson, Närke	Sweden	NRM20026017	NRM	X	X	X	X	X	X
C. familiaris familiaris	Gräso, Örskär, Uppland	Sweden	NRM20026382	NRM	X					
C. familiaris familiaris	Jokkmokk, Kabdalis, Luovare, Lulelappmark	Sweden	NRM20036539	NRM	X					
C. familiaris familiaris	Skanör, Skane	Sweden	NRM20046286	NRM	X					
C. familiaris familiaris	Bredaryd, As, Smaland	Sweden	NRM20066559	NRM	X					
C. familiaris familiaris	Stara mt., Arbinje	Serbia	NHMB,MR1140	Rakovic M	X					
C. familiaris familiaris	Maljen mt, Divcibare	Serbia	NHMB,MR1370	Rakovic M	X	X	X	X	X	X
C. familiaris familiaris	Maljen mt, Divcibare	Serbia	NHMB,MR1371	Rakovic M	X					
C. familiaris familiaris	Stara mt., Arbinje	Serbia	NHMB,MR1372	Rakovic M	X					
C. familiaris familiaris	Stara mt, Babinzub	Serbia	NHMB,MR364	Rakovic M	X					
C. familiaris familiaris	Valjevo, Maljen mt, Divcibare	Serbia	NHMB,MR516	Rakovic M	X					
C. familiaris familiaris	Durmitor National Park	Montenegro	NHMB,MR774	Rakovic M	X	X	X	X	X	X
C. familiaris familiaris	Kopaonik National Park	Serbia	NHMB,MR780	Rakovic M	X	X	X			
C. familiaris macrodactyla	Sauxillanges, Auvergne	France	MNHN187	Fournier J	X					

APPENDIX *Continued*

Species	Locality, Region	Country	Reference	Collector	COI	ATP6	ND2	LDH	TGFB	ACOI
C. familiaris macrodactyla	Midi-Pyrénées	France	MNHN204	Clouet M	X					
C. familiaris macrodactyla	Midi-Pyrénées	France	MNHN205	Clouet M	X	X	X	X	X	X
C. familiaris macrodactyla	Arudy, Midi-Pyrénées	France	MNHN206	Legay P	X	X	X	X	X	X
C. familiaris macrodactyla	Arudy, Midi-Pyrénées	France	MNHN207	Legay P	X					
C. familiaris macrodactyla	Arudy, Midi-Pyrénées	France	MNHN208	Legay P	X					
C. familiaris macrodactyla	Saint Aubin du Cormier, Bretagne	France	MNHN209	Fournier, J	X					
C. familiaris macrodactyla	Saint Aubin du Cormier, Bretagne	France	MNHN210	Raitière W	X					
C. familiaris macrodactyla	Saint Aubin du Cormier, Bretagne	France	MNHN211	Raitière W	X					
C. familiaris macrodactyla	Midi-Pyrénées	France	MNHN212	Clouet M	X					
C. familiaris macrodactyla	Andelot-Blancheville, Champagne-Ardenne	France	MNHN213	Ternois V	X					
C. familiaris macrodactyla	Chaumont, Champagne-Ardenne	France	MNHN214	Ternois V	X	X	X			
C. familiaris macrodactyla	Roches-Bettaincourt, Champagne-Ardenne	France	MNHN215	Ternois V	X					
C. familiaris macrodactyla	Saint Aubin du Cormier, Bretagne	France	MNHN218	Fournier J	X					
C. familiaris macrodactyla	Basville, Limousin	France	MNHN219	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Basville, Limousin	France	MNHN220	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Crocq, Limousin	France	MNHN221	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Flayat, Limousin	France	MNHN222	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Saint-Oradoux-de-Chipouze, Limousin	France	MNHN223	Dupoux E, Williamson T	X	X	X			
C. familiaris macrodactyla	Saint-Oradoux-de-Chipouze, Limousin	France	MNHN224	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Saint-Martial-le-Vieux, Limousin	France	MNHN225	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Saint-Martial-le-Vieux, Limousin	France	MNHN226	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Flayat, Limousin	France	MNHN227	Dupoux E, Williamson T	X					
C. familiaris macrodactyla	Fsac, Bretagne	France	MNHN228	Fournier J	X					
C. familiaris macrodactyla	Les Voirons, Rhône-Alpes	France	MNHN229	Thibault JC	X					
C. familiaris macrodactyla	Les Voirons, Rhône-Alpes	France	MNHN230	Thibault JC	X					
C. familiaris macrodactyla	Les Voirons, Rhône-Alpes	France	MNHN231	Thibault JC	X					
C. familiaris macrodactyla	Les Voirons, Rhône-Alpes	France	MNHN232	Thibault JC	X					
C. familiaris macrodactyla	Bolquère, Midi-Pyrénées	France	MNHN239	Olioso G, Pons JM	X	X	X			
C. familiaris macrodactyla	Bolquère, Midi-Pyrénées	France	MNHN240	Olioso G, Pons JM	X					
C. familiaris macrodactyla	Angoustine, Midi-Pyrénées	France	MNHN250	Olioso G, Pons JM	X					
C. familiaris macrodactyla	La Llagonne, Midi-Pyrénées	France	MNHN253	Olioso G, Pons JM	X					

APPENDIX *Continued*

Species	Locality, Region	Country	Reference	Collector	COI	ATP6	ND2	LDH	TGFB	ACOI
C. familiaris macrodactyla	Andelot-Blancheville, Champagne-Ardenne	France	MNHN392	Perroi PY	X					
C. familiaris macrodactyla	Liffol-le-Petit, Champagne-Ardenne	France	MNHN393	Perroi PY	X					
C. familiaris macrodactyla	Liffol-le-Petit, Champagne-Ardenne	France	MNHN394	Perroi PY	X					
C. familiaris macrodactyla	Liffol-le-Petit, Champagne-Ardenne	France	MNHN395	Perroi PY	X					
C. familiaris macrodactyla	La Fauche, Champagne-Ardenne	France	MNHN396	Perroi PY	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN397	Tellini G	X	X	X	X	X	X
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN398	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN399	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN400	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN401	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN402	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN403	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN404	Tellini G	X	X	X	X	X	X
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN405	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN406	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN407	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN408	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN409	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN410	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN411	Tellini G	X					
C. familiaris macrodactyla	Foreste Casentinesi, Parco Nazionale	Italy	MNHN412	Tellini G	X					
C. familiaris macrodactyla	Alboin, Casteldelfino	Italy	MCCI 2588	Zuccon D	X					
C. familiaris tianschanica	Ysyk-Köl, Ananyevo	Kyrgyzstan	JGU2883	Martens J, Ostastshenko A	X	X	X	X	X	X
C. hodgsoni khamensis	Yaoji, Sichuan	China	JGU3308	Martens J, Tietze DT	X	X	X	X	X	X
C. hodgsoni mandellii	bei Simikot, Chucho Khola, Humla district	Nepal	JGU3328	Fischer M, Grimm H	X	X	X	X	X	X

Sequences retrieved from GenBank			
C. f. familiaris	Smolensk, western Russia	Russia	GQ481543
C. f. familiaris	Near Moscow, western Russia	Russia	GQ481542
C. f. daurica	Eastern Russia	Russia	GQ481545
C. f. daurica	Eastern Russia	Russia	GQ481544
C. f. daurica	Eastern Russia	Russia	GQ481540
C. f. daurica	Eastern Russia	Russia	GQ481539
C. f. daurica	Eastern Russia	Russia	CQ481538
C. f. daurica	Eastern Russia	Russia	CQ481541

BNHM: Natural History Museum, Tring; JGU: Johannes Gutenberg-Universität, Mainz; MNHN: Muséum National d'Histoire Naturelle, Paris; NHMB: Natural History Museum, Belgrade; NHMO: Natural History Museum of Oslo; NRM: Swedish Museum of Natural History, Stockholm; UWBM: University of Washington, Burke Museum, Seattle.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Bayesian phylogenetic tree resulting from the analyses of ACOI. ACOI gene tree supports the monophyly of *C. familiaris* with respect to *C. hodgsoni*.
Figure S2. Bayesian phylogenetic tree resulting from the analyses of TGFB.
Figure S3. Bayesian phylogenetic tree resulting from the analyses of LDH.
Figure S4. Median-joining network showing relationships among ATP6 haplotypes for *Certhia familiaris* sub-species. The size of each circle is proportional to haplotype frequency. The small red circles correspond to extinct or unsampled haplotypes.
Figure S5. Median-joining network showing relationships among ND2 haplotypes for *Certhia familiaris* sub-species. The size of each circle is proportional to haplotype frequency. The small red circles correspond to extinct or unsampled haplotypes.