



HAL
open science

Gene flow and genetic divergence among mainland and insular populations across the south-western range of the Eurasian treecreeper (*Certhia familiaris*, Aves)

Jean-Marc Pons, Alice Cibois, Jérôme Fournier, Jérôme Fuchs, Georges Oliosio, Jean-Claude Thibault

► To cite this version:

Jean-Marc Pons, Alice Cibois, Jérôme Fournier, Jérôme Fuchs, Georges Oliosio, et al.. Gene flow and genetic divergence among mainland and insular populations across the south-western range of the Eurasian treecreeper (*Certhia familiaris*, Aves). *Biological Journal of the Linnean Society*, 2019, 126 (3), pp.447-461. 10.1093/biolinnean/bly200 . hal-02322911

HAL Id: hal-02322911

<https://hal.science/hal-02322911>

Submitted on 31 Oct 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Gene flow and genetic divergence among mainland and insular populations across the
2 southwestern range of the Eurasian treecreeper (*Certhia familiaris*, Aves)

3 Pons J.-M.^(1,2), Cibois A.⁽³⁾, Fournier J.⁽⁴⁾, Fuchs J.⁽¹⁾, Olioso G.⁽⁵⁾, Thibault J.-C⁽¹⁾.

4 Jean-Marc Pons⁽¹⁾

5 Institut Systématique, Evolution, Biodiversité (ISYEB)

6 Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE

7 57 rue Cuvier, CP50, 75005 Paris, France

8

9 Alice Cibois⁽³⁾

10 Department of Mammalogy and Ornithology

11 Natural History Museum of Geneva

12 CP 6434, 1211 Geneva 6, Switzerland

13

14 Jérôme Fournier⁽⁴⁾

15 Centre de Recherche sur la Biologie des Populations d'Oiseaux

16 Centre d'Ecologie et des Sciences de la Conservation (CESCO)

17 Muséum national d'Histoire naturelle, CNRS, Sorbonne Université

18 55 rue Buffon, CP51, 75005 Paris, France

19

20 Jérôme Fuchs⁽¹⁾

21 Institut Systématique, Evolution, Biodiversité (ISYEB)

22 Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE

23 57 rue Cuvier, CP50, 75005 Paris, France

24

25 Georges Olioso⁽⁵⁾

26 190 rue de l'industrie

27 11210 Port-La-Nouvelle

28 France

29

30 Jean-Claude Thibault⁽¹⁾

31 Institut Systématique, Evolution, Biodiversité (ISYEB)

32 Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE

33 57 rue Cuvier, CP50, 75005 Paris, France

34 France

35

36 Corresponding author⁽²⁾

37

38 Running title: population genetics of the Eurasian treecreeper

39

40

41

42

43 ABSTRACT

44 **The Eurasian treecreeper (*Certhia familiaris*) comprises two mitochondrial lineages that**
45 **diverged during the mid-Pleistocene. One paleoendemic lineage has an allopatric range**
46 **currently restricted to the Island of Corsica and the Caucasus region, whereas the**
47 **second one has a very large Eurasian range. Here we used microsatellites (N = 6) and**
48 **mitochondrial DNA (COI) to assess the genetic structure of insular and mainland**
49 **populations from Corsica, mainland France and Central Italy (N = 258) and the level of**
50 **mitochondrial and nuclear gene flow among these populations. Concordant with the**
51 **mtDNA signal, microsatellites results clearly demonstrate that the Corsican population**
52 **(*C. f. corsa*) is strongly divergent from nearby mainland populations (*C. f. macrodactyla*).**
53 **Microsatellite data also support significant divergence and low gene flow between the**
54 **Central Italian and mainland French populations. Our results suggest low nuclear gene**
55 **flow from the mainland into Corsica and no mitochondrial gene flow. Sporadic gene**
56 **flow from the nearby mainland may explain the presence of continental nuclear alleles**
57 **in the genome of 5% of sampled insular birds. Our study confirms the existence of an**
58 **endemic Corsican treecreeper lineage with important conservation value. Our results**
59 **also imply that Eurasian treecreepers from Central Italy constitute a distinct**
60 **management unit.**

61

62

63

64

65 **KEYWORDS:** Corsica, Mediterranean islands, microsatellites, mitochondrial DNA,
66 population structure, gene flow.

67

68 INTRODUCTION

69 The Eurasian treecreeper (*Certhia familiaris*) is a forest passerine found over a very large
70 Palaearctic range from the British Isles to Japan and northern China. Ten morphological
71 subspecies are currently recognized based on slight clinal variation in plumage colour
72 (Harrap, 2018). In a previous study, Pons *et al.* (2015) using **mitochondrial markers (COI,**
73 **Cyt b, ND2)** suggested that Eurasian treecreepers found in Corsica belong to a paleoendemic
74 lineage which has an allopatric range, restricted to this island and the Caucasus region.
75 Corsican and Caucasian treecreepers are currently assigned to two distinct morphological
76 subspecies, *C. f. corsa* E. J. O. Hartert, 1905 and *C. f. caucasica* Buturlin, 1907 respectively.
77 All other **sampled** subspecies (N = 6), including *C. f. macrodactyla* C. L. Brehm, 1831, which
78 is found in **mainland** France and Italy, belong to a more recent and widespread lineage
79 distributed over Eurasia (Pons *et al.*, 2015). Phylogeography of the Eurasian treecreeper was
80 thus more strongly influenced by Pleistocene climatic oscillations leading to population
81 contractions and expansions rather than by geography, and its current subspecific treatment
82 does not correctly reflect its evolutionary history. The phylogeographic pattern depicted for
83 the Corsican treecreeper (Pons *et al.*, 2015) differs from the one known for most Corsican
84 subspecies of forested passerines which are closely related to their nearest mainland
85 counterparts (i.e. Blue tit *Cyanistes caeruleus ogliastroae*, Kvist *et al.*, 2004; Great tit *Parus*
86 *major corsus*, Kvist *et al.*, 2003; Coal tit *Periparus ater sardus*, Pentzold *et al.*, 2013; Tritsch
87 *et al.*, 2018). In contrast, it is worth noting that the endemic Corsican nuthatch (*Sitta*
88 *whiteheadi*) shares with the Corsican treecreeper a similar phylogeographic pattern, its sister
89 species being the eastern Palaearctic Chinese *Sitta villosa*, while the genus *Sitta* is also
90 represented by several intervening species in the western Palaearctic (Pasquet *et al.*, 2014).
91 The importance of testing previously established hypotheses about population history and
92 genetic structure based on mitochondrial DNA (mtDNA hereafter) alone with independent

93 nuclear markers has been pointed out (see Ballard & Whitlock, 2004; Toews & Brelsford,
94 2012). Contrasting results across molecular markers having distinct mode of inheritance, such
95 as mtDNA and microsatellites, may be very useful to infer evolutionary relationships among
96 populations that recently diverged and for which gene flow may not be entirely interrupted
97 (Brito, 2007; Pons *et al.*, 2014; Tritsch *et al.*, 2018).

98 Phylogenetic relationships among *C. familiaris* subspecies obtained in Pons *et al.* (2015)
99 using three nuclear introns are generally poorly resolved with the exception of a well-
100 supported sister relationship between Corsican and western European populations. This result,
101 in agreement with geographic distances, is in strong conflict with the mtDNA inferences that
102 support a deeply divergent clade grouping Corsican and Caucasian treecreeper populations.
103 Such mito-nuclear discordance may be explained by several non-exclusive hypotheses
104 including retention of ancestral polymorphism from the ancestor of the Eurasian and Corsican
105 lineage, positive selection on mtDNA variants, male mediated gene flow with no consequence
106 for maternally inherited mtDNA, and hybrid sterility in the heterogametic female sex according
107 to Haldane's rule (see Ballard & Whitlock, 2004; Toews & Brelsford, 2012). Pons *et al.*
108 (2015) suggested that retention of ancestral polymorphism might be the most probable
109 hypothesis explaining mito-nuclear discordance in phylogenetic relationships of *C. f. corsa*
110 with its closest relatives. To obtain further insights on the evolutionary history of the Eurasian
111 treecreeper and the causes of this mito-nuclear discrepancy, we investigated in the present
112 study the relationships among mainland and insular populations of the Eurasian treecreeper
113 using much larger population samples than in Pons *et al.* (2015) and fast evolving nuclear
114 markers.

115 Our present study compares mtDNA and microsatellite results on population structure,
116 genetic diversity and gene flow between insular and mainland populations of the Eurasian
117 treecreeper (*Certhia familiaris* Linnaeus, 1758) across its south-western range. More than 250

118 individuals from Corsica and nearby France and Italy were sampled to investigate population
119 genetics of south-western populations of the Eurasian treecreeper. In addition, we also
120 analysed morphological variation to assess whether the Corsican population differs from its
121 mainland counterparts, as was initially suggested.

122 We specifically aim to answer the following questions:

- 123 - Is endemism of the Corsican population highlighted by previous studies on the basis of
124 plumage variation (Harrap, 2018) and mtDNA (Pons *et al.*, 2015) confirmed by
125 microsatellite data?
- 126 - Are there differences in nuclear and mtDNA gene flow between Corsican and mainland
127 populations?
- 128 - Is genetic variation of Corsican and mainland French populations geographically
129 structured?
- 130 - Do mainland populations from France and Italy belong to the same nuclear genetic
131 cluster as suggested by their mtDNA relationships?

132 The mito-nuclear discordance highlighted in Pons *et al.* (2015) will be discussed taking
133 account of the present results. We will discuss the systematic status of the Corsican
134 treecreeper in the light of new information on gene flow and population divergence brought
135 by the present study and conservation issues will be evoked.

136

137 MATERIAL AND METHODS

138 **Sampling**

139 We obtained feather samples (**two secondary feathers per bird**) from 258 individuals. **All**
140 **birds were mist-netted from February to June between 2011 and 2016, except birds from**
141 **the Apennines sampled in 1992 by Guido Tellini during an independent field session.** The
142 male of the Eurasian treecreeper is an aggressively territorial bird, singing and approaching in

143 response to playback of conspecific songs. In this study, all birds were caught using male
144 song play-back experiments. **The presence of the cloacal protuberance was checked for**
145 **each bird. We mist-netted 258 males and 3 females which were not included in the**
146 **analyses.** Six discrete geographical populations were sampled in France (Alps, Jura,
147 Pyrenees, Massif Central, Eastern France, Western France; N = 122) and one isolated
148 population in Central Italy (Apennines, N = 26) (Figure 1). One individual sampled in the
149 Italian Alps near the French border was included in the “Alps” population. In Corsica (N =
150 109), the whole range of the subspecies has been sampled (Figure S1). More individuals were
151 sampled in Northern Corsica where most forest areas are located. Information on exact
152 localities and collectors’ names is reported in Table S1.

153 **Laboratory work**

154 *DNA extraction*

155 DNA was isolated from feathers using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA,
156 USA) following the standard QIAamp protocol.

157 *Microsatellite genotyping*

158 We genotyped 12 microsatellite loci, eight of which were originally developed for passerines
159 (SpuL5-22, SpuA6, SpuL6-16, Haas *et al.*, 2009; SS1-6, SS1-11, SS1-12, SS2-32, SS2-52,
160 SS2-80, SS2-106, SS2-130, SS3-42C, Rubenstein, 2005). Microsatellite fragments were
161 amplified using 12 fluorescent primers and multiplex PCRs, following standard protocols.
162 Genotyping was conducted on an ABI 3700 Genetic Analyzer (Applied Biosystem) using
163 GeneScan500 LIZ (Applied Biosystems) as internal lane size standard. Results were
164 visualized using Peak Scanner (Applied Biosystem) and analyzed with Geneious version 9.1.8
165 (<http://www.geneious.com>, Kearse *et al.*, 2012).

166 *COI amplification and sequencing*

167 The mitochondrial cytochrome c oxidase subunit 1 (COI) was amplified and sequenced using
168 primers COIext/FISH1R (Ward, Hanner & Hebert, 2009; Johnsen *et al.*, 2010). Standard
169 amplification and sequencing protocols were followed. COI sequences were aligned using
170 Bioedit version 7.0.9 (Hall, 1999). New sequences were deposited in Genbank with the
171 accession numbers MK125306-125323.

172 **Data analyses**

173 *Microsatellites*

174 We used MSA 4.05 (Dieringer & Schötterer, 2003), Arlequin 3.11 (Excoffier *et al.*, 2005),
175 and FreeNa 1.0 (Chapuis & Estoup, 2007) to quantify the genetic diversity of the
176 microsatellite loci for allelic richness, Garza-Williamson index (M-ratio test), observed and
177 expected heterozygosity, and to test for deviation from Hardy-Weinberg genotype frequency
178 equilibrium. PGDSpider 2.0.3.0 (Lischer & Excoffier, 2012) was a very useful tool for
179 converting our data set into different formats.

180 The F_{st} values between pairs of population were calculated in Arlequin, with sequential
181 Bonferroni corrections. We performed Bayesian analyses using STRUCTURE 2.3.4
182 (Pritchard *et al.*, 2000) to determine the optimum group structure from the microsatellite loci.
183 For the complete dataset, the number of K clusters was set from 2 to 10, with 10 iterations per
184 number of clusters, a burn-in of 100,000 followed by 900,000 iterations while allowing for
185 admixture. We also conducted analyses for the Corsican samples and for the populations from
186 continental France separately, to check for population structure within these areas. We used
187 Structure Harvester (Earl & von Holdt, 2012) to choose the best estimate of K based on the
188 $\ln P(D)$ and ΔK ad hoc statistics (Evanno *et al.*, 2005).

189 We estimated the nuclear migration rates among the three groups highlighted in
190 STRUCTURE (Corsica, mainland France, Central Italy) using two approaches that differ in
191 their time frames: BayesAss3.0.3 (Wilson & Rannala, 2003) that estimates recent (2-3 last

192 generations) migration rates between populations using Markov chain Monte Carlo (MCMC),
193 and MIGRATE 3.6 (Beerli, 2009) that uses a MCMC-based maximum-likelihood approach
194 based on an expansion of the coalescence model (from present to the most recent common
195 ancestor). In BayesAss, after a few preliminary runs a chain length of ten million iterations
196 with a burn-in of one million iterations and a thinning interval of 1000 was chosen to run the
197 program. Delta values (parameters a, f, m , defining the size of the proposed change to the
198 parameter values at each iteration) set to 0.3 in the final analyses were used for migration
199 rates. Two independent runs were performed, with unique random seeds, to assess
200 convergence. In MIGRATE, the runs consisted of 10 short chains (sampling 10 000 trees) and
201 three long chains (sampling 100 000 trees) with a burnin period of 10 000 trees. All runs were
202 repeated five times to verify consistency of results. To avoid applying a unique mutation rate
203 to the microsatellite loci, we inferred the number of immigrants per generation N_i by
204 multiplying the mutation-scaled effective population size Θ to the mutation-scaled effective
205 immigration rate M .
206 Nei Genetic distances among mainland and insular populations were calculated using
207 GENETIX (Belkhir *et al.*, 1996) and visualized with a neighbour joining network constructed
208 using PHYLIP (Felsenstein, 2005).

209 *Mitochondrial COI*

210 We used the McDonald-Kreitman test (MK) (McDonald & Kreitman, 1991), as implemented
211 in DnaSP ver. 5.0 (Librado & Rozas, 2009), to test if selection was acting on the COI protein-
212 coding gene used to infer population genetic patterns. MK tests were performed between the
213 insular and the mainland lineages.

214 Mean K2P pairwise genetic distances among populations were estimated by MEGA6 (Tamura
215 *et al.*, 2013). Standard diversity indices (haplotype diversity, nucleotide diversity, number of
216 polymorphic sites) were calculated using ARLEQUIN 3.5 (Excoffier & Lisher, 2010). We

217 used Fu's F_s and Tajima' D tests (1000 replicates) to detect signatures of population
218 expansion. These two tests were initially developed as selection tests; it has been shown that
219 they are in fact very sensitive to demographic fluctuations, with significant negative values
220 being a signature of population expansion (Fu, 1997). Hence, in the absence of selection, as
221 evidenced by the MK test, Fu's F_s and Tajima' D represent reliable indicators as to whether a
222 population experienced a population size change. We computed pairwise F_{st} for all pairs of
223 populations to assess the level of geographical structuring of the genetic variability.
224 We generated a median-joining network with NETWORK 4.6.1.2 (Bandelt, Forster & Rohl,
225 1999) to visualize relationships among COI haplotypes and to check for the possible presence
226 of insular haplotypes in mainland populations and vice versa.

227 **Morphometric differentiation**

228 *Biometric data*

229 Four biometric variables were included in the analyses to assess morphological variations
230 among the French, Italian and Corsican populations. **All Measurements were made**
231 **according to Eck *et al.* (2011) and Demongin (2016).** Wing-length (WL) was measured to
232 the nearest 0.5 mm using a ruler (see **Figure 13, page 76; Eck et al., 2011**), bill length at the
233 nostrils (BLN), tarsus length (TL) and hind claw length (HCL) to the nearest 0.1 mm using a
234 calliper.

235 *Morphometric analyses*

236 **All statistics were performed in R (R development Core Team, 2018). Normality of**
237 **quantitative variables was first checked using the one sample Kolmogorov-Smirnov test**
238 **and homoscedasticity using the Bartlett test. Biometric data were compared using one-**
239 **way parametric analysis of variance (ANOVA) or Kruskal-Wallis non-parametric test if**
240 **data were not normally distributed. We used the Tukey test to perform multiple**
241 **comparisons among populations or multiple non-parametric tests using the package**

242 **pgirmess (Giraudoux et al., 2018). A standardized Principal component analysis (PCA)**
243 **using a correlation matrix was performed with the package FactoMineR (Husson et al.,**
244 **2018) to visualize how individuals from different geographic origins and admixed**
245 **Corsican treecreepers were distributed in multivariate space. As biometric**
246 **measurements were obtained from several ringers, we statistically controlled potential**
247 **measurement biases using a linear mixed-effects model with the package lme4 (LME4**
248 **Authors, 2018) and we also performed a PCA using covariance matrix (following the**
249 **recommendation of Perktas & Gosler, 2010). PC1 scores were compared using an**
250 **ANOVA with “ringer” considered as a random factor and “region” as fixed factor.**

251

252 RESULTS

253 Microsatellites

254 Six non-polymorphic loci (SpuA6, SS1-6, SS2-32, SS2-52, SS2-106, SS2-130) were removed
255 from the analysis. The genetic diversity for the six remaining microsatellite loci in each
256 population is summarized in the supplementary material (Table S2). The allelic richness
257 varied from 1.4 to 7.9. Deviation from HWE ($\alpha = 0.05$) was found in two cases (Corsica for
258 locus 2, and France-East for locus 6), but null allele frequency estimated by Free Na for these
259 loci and populations were inferior to 0.15, suggesting that the presence of null alleles for these
260 loci should have little effect on the outcome of Bayesian assignment analyses (Carlsson,
261 2008). The values of the Garza-Williamson index are close to 1 (average of 0.84), indicating
262 that recent bottlenecks cannot be detected in the populations, although the power of this test
263 might be impeded by the low number of loci used (Perry *et al.*, 2012). Based on the expected
264 heterozygosity (HE), the genetic variation appeared moderate and fairly constant between the
265 samples (0.5-0.7; Table S2) excepting Corsica and Central Italy which has the lowest HE
266 (0.3-0.4).

267 *Population differentiation and genetic structure*

268 Pairwise F_{st} were highly significant after Bonferroni correction for 13 pairs of populations, all
269 involving the populations located in Corsica and Central Italy (mean F_{st} Corsica vs France =
270 0.27; F_{st} Corsica vs Central Italy = 0.30, mean F_{st} France vs Central Italy = 0.17; Table 1). By
271 contrast, no significant differentiation was observed among the populations located in France
272 (15 pairwise comparisons). The neighbour joining network (Figure 2) clearly shows that
273 Corsican, Central Italian and mainland French populations form three separated groups, the
274 Corsican population being the farthest one.

275 Results from STRUCTURE indicate that the Corsican and Italian populations were
276 significantly differentiated from all populations from France, with $K=3$ as the most likely
277 number of genetic clusters when all populations were analysed as a single dataset (Figure 3,
278 Figure S2). No further genetic substructure was observed within Corsica or within continental
279 France (Figure S2). Five individuals sampled in Corsica (numbers 266, 284, 1010, 1040,
280 1064) showed significant admixture, i.e. a non-Corsican inferred ancestry with a probability $>$
281 50%, suggesting potential genotypic introgression from the continental populations. These
282 five individuals were sampled in different localities in Corsica (Rospa Sorba, Tova, Fratte,
283 Albertacce and Bastelica). Similarly, 34 individuals sampled in continental France also
284 showed significant admixture, whereas no samples from Central Italy present another inferred
285 ancestry.

286 *Contemporary gene flow among populations*

287 The effective sample size for all parameters were all above 200 and the two independent
288 BayesAss+ runs gave very similar results regarding migration rates among the three primary
289 populations (Table S3), suggesting that our analyses reached convergence.
290 Estimates for the fraction of the Corsican individuals being migrants from others populations
291 were very low (0.7%) from mainland France and 0.4% from Italy suggesting that migration

292 from the mainland is very rare (Table S3). Estimates for the fraction of the Italian individuals
293 being migrants from others populations were slightly higher but still very low (1.44% from
294 Corsica; 2.2% from mainland France) suggesting that migration into Italy from Corsica or
295 mainland France is rare. Estimates for the fraction of the mainland France individuals being
296 migrants from Corsica was less than 0.5%. Overall these results suggest that contemporary
297 gene flow between insular and mainland lineages is very low. As confidence intervals of gene
298 flow estimates are very large and overlapping, our results do not allow any detailed
299 comparisons of the contemporary gene flow among populations (Table S3).

300 *Historical gene flow*

301 Migrate results suggested asymmetrical past gene flow between the three groups (Figure 4),
302 mainland France providing the largest number of immigrants to Corsica ($N_i = 10.67$) and to
303 Central Italy ($N_i = 6.17$). Gene flow between Corsica and Central Italy is low but not null
304 (close to 1 immigrant per generation). This asymmetry was also reflected in the estimation of
305 population size, which is six times larger in mainland France ($\Theta = 9.24$) than in Corsica ($\Theta =$
306 1.49) and Central Italy ($\Theta = 1.7$). Additionally, we enforced a more realistic migration model
307 in which Corsica is treated as a sink, with no migrants to mainland France or Central Italy.
308 Using this model, the migration to Corsica remained three times more important from
309 mainland France ($N_i = 8.07$) than from Central Italy ($N_i = 2.85$).

310 **COI**

311 *Genetic variation*

312 The MK tests did not detect any significant evidence of selection in the COI gene when
313 comparing the insular lineage with the mainland lineage (Fischer's exact test, $P = 1$). Our
314 results do not support any clear patterns of population expansion except for populations from
315 eastern France and the Alps for which both Tajima's D and Fu's tests are significant (Table
316 2). Genetic diversity parameters (H , π) varied from moderate to low values depending on

317 geographical populations (Table 2). Our results did not suggest any differences in genetic
318 diversity between the insular and mainland populations. The most striking result is a complete
319 lack of genetic variability for the Italian population. In France, haplotype diversity was
320 highest in mountainous populations (Alps, Jura and Pyrenees).

321 *Genetic divergence*

322 K2P genetic distances among mainland populations from France and Central Italy were very
323 low, varying from 0.03% to 0.06% (Table S4). By contrast, the K2P genetic distances
324 between the insular population from Corsica and mainland populations from France and
325 Central Italy were much higher, varying from 2.3% to 2.4 %. Accordingly, pairwise
326 population F_{st} values between the Corsican population and mainland populations were highly
327 significant and close to 1 (Table 3), indicating a near complete geographical partitioning of
328 the genetic variation. On the other hand, among mainland populations our results show a lack
329 of genetic differentiation except for the Pyrenean population which is slightly **but**
330 **significantly** differentiated.

331 *Median joining network*

332 In accordance with above genetic results, the median joining network (Figure 5) shows that
333 Corsican treecreepers do not share any haplotypes either with mainland treecreepers sampled
334 in France or Italy. Likewise, no Corsican haplotypes were found among mainland populations
335 from France and Central Italy. The lack of geographic mixing of insular and mainland
336 haplotypes clearly suggests that female-mediated gene flow across the Mediterranean Sea is
337 non-existent or so low that much larger sample sizes would be necessary to detect it.

338 **Morphometry**

339 *Univariate analyses*

340 One-way analyses indicated that there were highly significant differences between the insular
341 and mainland populations for most morphological variables. In particular, the insular

342 population had a longer bill length at nostrils (BLN) and a shorter hind claw length (HCL)
343 (Table S5) than both Italian and French mainland populations.

344 *Multivariate analyses*

345 More than 68% of the total variance was explained by the first two principal components
346 (Figure 6). **Component loadings of the variables are reported in Table S6.** Bill length at
347 nostrils (BLN), tarsus length (TL) and HCL were highly correlated to the first axis (**PC1**)
348 which can be interpreted as a size axis. **ANOVA using a linear mixed effects model with**
349 **“ringer” as a random factor performed on the first axis of the covariance matrix PCA**
350 **showed highly significant differences among the 3 populations ($F_{(2, 240)} = 17.63$, $P <$**
351 **0.0001).** **On the other hand, there was no clear separation among the three populations**
352 **according to PC2 (Figure 6).** Insular birds from Corsica formed a morphological cluster
353 fairly well separated from French and Italian clusters despite some overlap. Confidence
354 ellipses of each geographical population were clearly separated from each other (Figure 6),
355 the Italian population occupying an intermediate position between French and Corsican.
356 Clearly more samples from Central Italy would be necessary to soundly assess morphological
357 variation of the population of the Apennines. Nevertheless our results clearly suggest that the
358 Corsican population is morphologically divergent from its nearby mainland populations.
359 Among the five admixed Corsican treecreepers possessing more than 50% of continental
360 alleles, three were located with other “pure” Corsican birds. Two admixed individuals
361 (individual number 266, 1064, dark blue and red squares respectively, Figure 6), were close to
362 mainland Italian and French birds. However, the lack of a clear gap between insular and
363 continental morphological clusters only allows us to conclude that admixed birds are probably
364 morphologically more similar to Corsican than to mainland treecreepers.

365

366

367 DISCUSSION

368 *The Corsican population*

369 In this study, we used genetic data from both microsatellites and mtDNA to examine the
370 levels of divergence and diversity within and among insular and mainland populations of the
371 Eurasian treecreeper. Our mtDNA results based on a five-fold increase in sample size of
372 Corsican treecreepers when compared with Pons *et al.* (2015), confirm the high genetic
373 distinctiveness of the Corsica population with respect to mainland populations from France
374 and Central Italy ($DK2P > 2\%$, Pairwise $F_{st} > 0.90$). In accordance with mtDNA, our
375 microsatellites results also support high genetic divergence ($0.31 < F_{st} > 0.16$) consistent with
376 an endemic Corsican lineage. Bayesian inferences of population structure based on
377 microsatellites support the presence of three genetic clusters (Figure 3). The first cluster
378 includes all individuals from France, the second includes all treecreepers from Central Italy
379 and the third one all insular individuals from Corsica.

380 Overall, our results strongly support the endemism of the Corsican population which
381 possesses private and highly differentiated mtDNA haplotypes as well as microsatellite alleles
382 never found in nearby mainland populations. Interestingly, five Corsican treecreepers (i.e
383 nearly 5% of our sample) possessed more than 50% of continental nuclear alleles suggesting
384 slight genetic admixture. This result highlights the importance of sample size in detecting low
385 levels of gene flow between populations.

386 While no mitochondrial gene flow across the Mediterranean Sea was detected in the present
387 study (no mixing of insular and mainland populations in any sampled populations), our
388 microsatellites results obtained with MIGRATE suggest the existence of moderate gene flow
389 from continental France into Corsica. Gene flow from mainland populations into the Corsican
390 population is further supported by the detection of five Corsican treecreepers possessing both
391 insular mtDNA haplotypes and microsatellites alleles mostly of continental origin. Such mito-

392 nuclear discordance can arise from several non-exclusive mechanisms (reviewed in Toews &
393 Brelsford, 2012). Sex-biased dispersal is often considered as a possible mechanism that may
394 explain mito-nuclear discordance in gene flow (Petit & Excoffier, 2009; Pons *et al.*, 2014). In
395 birds, several recent studies suggest that female biased dispersal is not a general rule contrary
396 to what was classically suggested (Dobson, 2013; Li & Merilä, 2010; Both, Robinson & van
397 der Jeugd, 2012). If males are the main long-distance dispersers in the Eurasian treecreepers,
398 this may explain the introgression of mainland microsatellite alleles in the Corsican
399 population while at the same time no introgression of mtDNA haplotypes is expected.
400 Unfortunately, information on sex-specific dispersal distances is currently lacking for the
401 Eurasian treecreepers. Another possible explanation is selection against hybrids of the
402 heterospecific sex (female in birds) as expected if the Haldane's rule applies to the Eurasian
403 treecreeper. If hybrid females are counter-selected while hybrid males are able to backcross
404 with treecreepers belonging to the insular lineage, this would solely result in the introgression
405 of continental nuclear alleles into the Corsican population. Furthermore, incomplete lineage
406 sorting of microsatellite alleles due to longer coalescent times of nuclear markers compared to
407 mtDNA (Zink, 2010) cannot be excluded and might explain the presence of some continental
408 alleles in the genetic pool of the Corsican populations. Finally, the slight mixed genetic
409 composition of the Corsican treecreeper population might be the result of both incomplete
410 lineage sorting and recent gene flow from occasional arrivals in Corsica of a small number of
411 continental dispersers Pons *et al.* (2015) found that the main conflict between mitochondrial
412 and nuclear phylogenetic trees was the sister relationship of the Corsican population with
413 nearby mainland populations of Western Europe recovered in the nuclear species tree
414 obtained with three nuclear introns whereas the Corsican and the geographically distant
415 Caucasian lineages formed a strongly supported monophyletic group in the mtDNA tree. Pons
416 *et al.* (2015) favoured the sharing of ancestral polymorphism as the most probable process

417 explaining mito-nuclear phylogenetic discordance and dismissed the dispersal hypothesis.
418 New information brought by the present study also supports occasional gene flow from the
419 continent as a possible explanation for the presence of continental nuclear alleles in the
420 genome of insular birds. The Eurasian treecreeper is known to occur as a rare vagrant to the
421 Channel Islands, Mallorca and the Faroe Islands (Cramp & Perrins, 1993; Harrap & Quinn,
422 1996); it is thus plausible that long-distance dispersers may occasionally reach Corsica.
423 We did not detect any significant genetic structure within Corsica for the Eurasian
424 treecreeper; this situation is in contrast with what has been found for *Sitta whiteheadi*
425 (Thibault *et al.*, 2016), another forested passerine endemic to Corsica. Such a difference
426 between both species might be explained by their distinctive habitat requirements, the
427 Corsican nuthatch occurring only in mature scattered forested patches of *Pinus nigra laricio*
428 (Thibault *et al.*, 2016) whereas the Eurasian treecreeper occupies a larger variety of tree
429 species and is more continuously distributed over Corsica, although still in mature forests
430 (Thibault *et al.*, unpublished results); moreover, birds regularly disperse outside their breeding
431 range into secondary habitats.

432 The lower **microsatellite** variability of the Corsican population **found here might result**
433 **from** its geographical isolation and its smaller effective population size compared to mainland
434 European populations allowing genetic drift and lineage sorting to be more effective. Low
435 genetic variation was found in a wide range of insular taxa (Frankham, 1997) and in several
436 small insular bird populations (Pons *et al.*, 2016).

437 *The Apennine population*

438 Mitochondrial and nuclear markers provide different patterns of genetic structure for the
439 Italian treecreeper population from the Apennines. The sampled population was characterized
440 by a lack of mitochondrial variation, only possessing the most widespread, and thus probably
441 the most ancestral haloptype found all over Europe. Consequently, mtDNA pairwise F_{st}

442 values with the northern French populations were low and most often not significant. By
443 contrast, our microsatellites results showed significant genetic divergence ($F_{st} = 0.16$), the
444 Italian population being assigned to a specific genetic cluster with STRUCTURE. Sex-biased
445 gene flow as a possible explanation of mito-nuclear discordance in the Apennine population is
446 not supported by our data because maternal gene flow would have also favoured nuclear
447 genetic admixture, yet most Italian treecreepers are not admixed (Figure 3). The high mutation
448 rate of microsatellites compared to mtDNA, associated with low demographic size of the
449 Apennine population, may have permitted the detection of a genetic cluster of too recent
450 origin to be detected by mtDNA alone. In the Apennine Mountains, the Eurasian treecreeper
451 occupies a small geographic range, isolated from northern Alpine and southern Abruzzi
452 populations (Meschini & Frugis, 1993), with numbers estimated at only a few hundred birds
453 (Tellini *et al.*, 1997). Due to small geographic range and founder effect, the Eurasian
454 treecreeper population from the Apennines had most probably a small effective population
455 size allowing microsatellite differentiation despite longer coalescence time of nuclear markers
456 compare to mtDNA (Zink & Barrowclough, 2008).

457 **Both lack of mitochondrial variability and low microsatellites diversity** of the **Apennine**
458 population may be explained if we consider that it was founded from a subset of the ancestral
459 genetic lineage (Hewitt, 1996). Under such a scenario, Central Italy was colonized from
460 northern European populations that would have been rapidly expanding from a unique refuge
461 located in the Eastern part of the *C. familiaris* range (Pons *et al.*, 2015), and **thus** did not play
462 any role as a glacial refuge for the Eurasian treecreeper. This biogeographic scenario is further
463 supported by our STRUCTURE microsatellite results suggesting that most treecreepers from
464 Central Italy are not admixed and harbour less genetic diversity than the more northern
465 French populations. More samples covering a larger geographical area in Italy would
466 nevertheless be required to firmly test this phylogeographic pattern. **Another**

467 **phylogeographic scenario in which the Apennine population would have diverged in the**
468 **Italian peninsula during the Pleistocene may be possible. Under this scenario, low**
469 **genetic diversity of the extant Apennines population might result from one or several**
470 **bottlenecks that would have occurred in Italy during cold periods. Indeed, in Europe,**
471 **several comparative genetic surveys highlighted three Mediterranean primary refugia**
472 **not covered by ice masses: the Iberian Peninsula, the Italian Peninsula and the Balkans,**
473 **where populations persisted during cooling periods and were able to colonize northern**
474 **areas during warming periods (Hewitt, 2000; Weiss & Ferrand, 2007). Several studies**
475 **report genetic divergence of Italian vertebrates lineages dating back to the Pleistocene**
476 **(Brito, 2005; Lo Brutto *et al.*, 2011; Ruedi *et al.*, 2008). However, our genetic data do not**
477 **give strong credit to such a phylogeographic scenario for the Eurasian treecreeper,**
478 **because in the case of ancient bottleneck events, we would expect at least some mtDNA**
479 **differentiation.**

480 *The mainland French populations*

481 The six geographically isolated populations found in France are not genetically differentiated,
482 except the Pyrenean population which is significantly divergent based on our mtDNA results
483 ($0.06 < F_{st} < 0.13$). This lack of genetic divergence was expected for treecreeper populations
484 found in the Alps, Jura and Eastern France which are geographically close to each other and
485 to very large populations found across northern and eastern Europe (Cramp & Perrins, 1993;
486 Nissa & Muller, 2015). As these populations are more or less connected, their genetic pools
487 are most probably continuously homogenized by ongoing gene flow. The western population
488 has been discovered only in the late 1970's (Nissa & Muller, 2015). **Our genetic results do**
489 **not favour the hypothesis of a small and old isolated population that would have been**
490 **overlooked by local ornithologists. Lack of genetic divergence of this small population may**
491 **be explained both by its probable very recent foundation and ongoing gene flow with eastern**

492 populations. The latter seems particularly likely given that westwards range expansion of
493 north-eastern populations is currently observed in France (Nissa & Muller, 2015). Similarly,
494 the Rhone valley is probably not an effective dispersal barrier, and therefore gene flow
495 between the Massif Central and Alpine populations is likely sufficient to prevent genetic
496 differentiation. The most distinctive population found in the Pyrenees is also the most isolated
497 one. The Pyrenean population is situated at the south-western limit of the Eurasian treecreeper
498 range, thus probably limiting the occurrence of gene flow with other northern populations
499 sampled in France.

500 *Systematic issues*

501 Hartert (1905) described the subspecies *corsa* on the basis of its slightly larger size than the
502 mid-European form, its long bill and more distinct markings on the upperparts. Based on
503 mtDNA, *C. f. corsa* belongs to a lineage that is also found in the Caucasus region, and that
504 most probably disappeared from the rest of Europe during the mid-Pleistocene (c. 1 Mya).
505 According to Pons *et al.* (2015), *C. f. corsa* does not share a common recent evolutionary
506 history with the nearby mainland treecreepers populations that all belong to a widespread
507 lineage that probably arrived recently in Western Europe. Due to its spatio-temporal isolation
508 and insularity, the Corsican population evolved distinct phenotypic and genetic characters that
509 warrant its subspecific rank. The present study based on an expanded sampling of individuals
510 and additional nuclear markers confirms the genetic distinctiveness of Corsican treecreepers
511 (mtDNAK2P ~ 2 %, nuclear F_{st} ~ 0.28) and morphological differentiation (longer bill, shorter
512 hind claw) with respect to nearby mainland populations currently assigned to *C. f.*
513 *macroductyla* (see **Tietze & Martens, 2009**). *C. f. corsa* also differs from all continental
514 subspecies by vocal characters (Tietze *et al.*, 2008). From an evolutionary perspective,
515 sporadic gene flow from the mainland supported by the present study did not prevent the
516 Corsican population from acquiring specific characters that may result from insular selection

517 pressures and/or phenotypic plasticity. It thus makes sense to question whether assigning a
518 specific rank to this insular population would not be a better taxonomic option. We
519 nevertheless suggest keeping the current systematic arrangement assigning a subspecific rank
520 to the Corsican treecreeper population because of its fairly recent splitting and lack of
521 information on the efficiency of pre and post mating isolating barriers with mainland
522 treecreepers. Most importantly, it would be crucial to assess the level of nuclear genetic
523 divergence achieved by the Caucasus population with respect to both northern European
524 populations and *corsa* before proposing any systematic arrangement.

525 *Conservation issues*

526 **Based on significant genetic distinctiveness and low genetic diversity highlighted in this**
527 **study, as well as small population size and isolated geographic distribution, we suggest**
528 **that the Eurasian treecreeper population from the Apennines warrants treating as a**
529 **distinct management unit (sensu Moritz, 1994). Information on genetics of the**
530 **population located in the Abruzzi is also much needed, in order to assess the**
531 **conservation status of the Eurasian treecreeper in Central and southern Italy.**

532 Several recent studies have highlighted the existence of endemic genetic lineages in Corsica
533 and other western Mediterranean islands even for highly mobile organisms like birds (i.e.
534 *Sylvia cantillans*, Brambilla *et al.*, 2008; *Carduelis corsicana*, Pasquet *et al.*, 1997; *Burhinus*
535 *oedicephalus*, Mori *et al.*, 2017; *Sitta whiteheadi*, Pasquet *et al.*, 2014; *Muscicapa striata*, Pons
536 *et al.*, 2016; *Periparus ater*, Tritsch *et al.*, 2018). For these species an important part of the
537 genetic variability is located in Mediterranean islands. Therefore these islands harbour an
538 important part of Mediterranean biodiversity to be conserved. The present study adds further
539 support to the originality of the Corsican avifauna that requires specific management
540 decisions. Among the insular endemic lineages mentioned above, only the Corsican nuthatch
541 and the Corsican treecreeper lineages are strictly limited to Corsica (Pons *et al.*, 2015). The

542 breeding habitat of the Corsican treecreeper is mainly restricted to the mature and dense
543 forests of Corsican pines (*Pinus nigra laricio*), Holm oaks (*Quercus ilex*) and several
544 deciduous trees (*Castanea sativa*, *Fagus sylvatica*) only found in mountainous areas of the
545 island (Thibault & Bonnacorsi, 1999; Thibault *et al.*, unpublished results.). Its relatively small
546 breeding population size estimated at 5000-10 000 pairs (Thibault, unpublished results)
547 should be managed independently of the continental populations which have an extremely
548 large range encompassing a large part of Eurasia and comprise 40 000 000-80 000 000
549 individuals (Birdlife international, 2018).

550 The Corsican population is currently subject to several threats such as fire and logging that are
551 major causes of reduction and fragmentation of its habitat. Due to this adverse ecological
552 context, its unique evolutionary history, small insular range and breeding population size, we
553 argue that the Corsican treecreeper should be registered on the Annex 1 of the European bird
554 directive that lists endangered species and subspecies in Europe.

555

556

557

558

559

560

561

562

563

564

565

566

567 ACKNOWLEDGEMENTS

568 We thank all the ringers (P. Aubry, R. Bussi re, N. Cochard, E. Dupoux, F. H mery, L.
569 Jomat, J.-F. Le Bas, C. Lebihan, P. Legay, J. M rot, P. Mulot, P.-Y. Perroi, W. Raiti re, X.
570 Rozec, C. Senechal, V. Ternois, P. Urbina-Tobias and T. Williamson) from the “Centre de
571 Recherches sur la Biologie des Populations d’Oiseaux, CRBPO, Mus um national d’Histoire
572 naturelle” for collecting feathers in mainland France under the national program concerning
573 the polymorphism of the Eurasian treecreeper directed by J. Fournier. The present study was
574 conducted under the program 647 « End misme et biog ographie des oiseaux de Corse » of
575 the CRBPO, Mus um national d’Histoire naturelle. We are grateful to G. Tellini for sending
576 feathers from the Apennines and to N. Baccetti, I. Cecere, M. Zenatello (Istituto Superiore per
577 la Protezione e la Ricerca Ambientale, Italy) and G. Boano (Museo di Storia Naturale di
578 Milano, Zoologia dei Vertebrati) who helped with the Italian samples in various ways. We
579 also thank J. Piacentini who participated to the field work in Corsica. **We thank four**
580 **anonymous reviewers for their helpful comments. We are grateful to B. Warren for his**
581 **help with the English editing.** We also acknowledge the staff of UMS MNHN/CNRS 2700
582 Outils et M thodes de la Syst matique Int grative (OMSI), Mus um National d’Histoire
583 Naturelle, and especially to J. Lambourdi re for her invaluable help with the laboratory work.
584 This work was partly financed by the “Direction R gionale de l’Environnement, de
585 l’Am nagement et du Logement” of Corsica, for which B. Recorbet provided invaluable
586 support.

587

588

589

590

591

592 REFERENCES

- 593 **Ballard JWO, Whitlock MC. 2004.**The incomplete natural history of mitochondria.
594 *Molecular ecology* **13**: 729-744.
- 595 **Bandelt HJ, Forster P, Rohl A. 1999.** Median-joining networks for inferring intraspecific
596 phylogenies. *Molecular Biology and Evolution* **16**: 37-48.
- 597 **Beerli P. 2009.** How to use migrate or why are markov chain monte carlo programs difficult
598 to use? In: G Bertorelle, MW Bruford, HC Haue, A Rizzoli, C Vernesi (eds). Population
599 Genetics for Animal Conservation, *Conservation Biology* **17**: 42-79. Cambridge University
600 Press, Cambridge UK.
- 601 **Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996-2004.** GENETIX 4.05,
602 logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome,
603 Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier
604 (France).
- 605 **BirdLife International. 2018.** Species factsheet: *Certhia familiaris*. Downloaded from
606 <http://www.birdlife.org> on 17/09/2018.
- 607 **Both C, Robinson RA, van der Jeugd HP. 2012.** Long-distance dispersal in migratory pied
608 flycatchers *Ficedula hypoleuca* is relatively common between the UK and the Netherlands.
609 *Journal of avian biology* **4**: 193-197.
- 610 **Brambilla M, Vitulano S, Spina F, Baccetti N, Gargallo G, Fabbri E, Guidali F, Randi**
611 **E. 2008.** A molecular phylogeny of the *Sylvia cantillans* complex: Cryptic species within the
612 Mediterranean basin. *Molecular Phylogenetics and Evolution* **48**: 461-472.
- 613 **Brito PH. 2005.** The influence of Pleistocene glacial refugia on tawny owl genetic diversity
614 and phylogeography in western Europe. *Molecular Ecology* **14**: 3077-3094.

615 **Brito PH. 2007.** Contrasting patterns of mitochondrial and microsatellite genetic structure
616 among Western European populations of tawny owls (*Strix aluco*). *Molecular Ecology* **16**:
617 3423-3437.

618 **Carlsson J. 2008.** Effects of Microsatellite Null Alleles on Assignment Testing. *Journal of*
619 *Heredity* **99**: 616-623.

620 **Chapuis MP, Estoup A. 2007.** Microsatellite null alleles and estimation of population
621 differentiation. *Molecular Biology and Evolution* **24**: 621-631.

622 **Cramp S, Perrins CM. 1993.** *The birds of Western Palearctic Vol. VII.* Oxford University
623 Press, Oxford, New York, 577 p.

624 **Demongin L. 2016.** *Identification Guide to Birds in the Hand.* Crexeco.

625 **Dieringer D, Schötterer C. 2003.** Microsatellite analyser (MSA): a platform independent
626 analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**: 167-169.

627 **Dobson FS. 2013.** The enduring question of sex-biased dispersal: Paul J. Greenwood's (1980)
628 seminal contribution. *Animal Behaviour* **85**: 299-304.

629 **Earl DA, von Holdt BM. 2012.** STRUCTURE HARVESTER: a website and program for
630 visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
631 *Genetics Resources* **4**: 359-361.

632 **Eck S, Fiebig J, Fiedler W, Heynen I, Nicolai B, Töpfer T, van den Elzen R, Winkler R,**
633 **Woog F. 2011.** *Measuring birds. Vögel vermessen.* Deutsche Ornithologen-Gesellschaft.
634 Christ Media Natur, Minden.

635 **Evanno G, Regnault S, Goudet J. 2005.** Detecting the number of clusters of individuals
636 using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611-2620.

637 **Excoffier L, Lischer HEL. 2010.** Arlequin suite ver. 3.5: A new series of programs to
638 perform population genetics analyses under Linux and Windows. *Molecular Ecology*
639 *Research* **10**: 564-567.

640 **Excoffier L, Laval G, Schneider S. 2005.** Arlequin ver. 3.1: An integrated software package
641 for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47-50.

642 **Felsenstein J. 2005.** PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the
643 author. Department of Genome Sciences, University of Washington, Seattle.

644 **Frankham R. 1997.** Do island populations have less genetic variation than mainland
645 populations? *Heredity* **78**: 311-327.

646 **Fu YX. 1997.** Statistical tests of neutrality of mutations against population growth,
647 hitchhiking and background selection. *Genetics* **147**: 915-925.

648 **Giraudoux P, Antonietti JP, Beale C, Pleydell D, Treglia M. 2018.** Package ‘pgirmess’.
649 CRAN-R.

650 **Haas S, Smith J, Kimball R, Clark AM. 2009.** Isolation and characterization of
651 polymorphic microsatellite markers for the brown-headed nuthatch (*Sitta pusilla*).
652 *Conservation Genetics* **10**: 1393-1395.

653 **Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and
654 analysis program for Windows 95/98/NT. *Nuclear Acids Symposium Series* **41**: 95-98.

655 **Harrap S. 2018.** Eurasian Treecreeper (*Certhia familiaris*). In: delHoyo J., Elliott A, Sargatal
656 J, Christie DA, de Juana E. (eds.). *Handbook of the Birds of the World Alive*. Lynx Edicions,
657 Barcelona.

658 **Harrap S and Quinn D. 1996.** *Tits, Nuthatches and Treecreepers*. Christopher Helm. p.
659 177–195.

660 **Hartert E. 1905.** *Die Vögel der paläarktischen Fauna*. Berlin, **Heft 3**: 1-832 (description of
661 *Certhia familiaris corsa* p. 320).

662 **Hewitt GM. 1996.** Some genetic consequences of ice ages, and their role in divergence and
663 speciation. *Biological Journal of the Linnean Society* **58**: 247-276.

664 **Hewitt GM. 2000.** The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907-913.

665 **Huson F., Josse J., Le S., Mazet J. 2018.** Package 'FactoMineR'. CRAN-R.

666 **Johnsen A, Rindal E, Ericson PGP, Zuccon D, Kerr KCR, Stoeckle MY, Lifjeld D. 2010.**
667 DNA barcoding of Scandinavian birds reveals divergent lineages in trans-Atlantic species.
668 *Journal of Ornithology* **151**: 565-578.

669 **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S. 2012.** Geneious
670 Basic: an integrated and extendable desktop software platform for the organization and
671 analysis of sequence data. *Bioinformatics* **28**: 1647-1649.

672 **Kvist L, Higuchi H, Nazarenko AA, Vlachuk OP, Orell M. 2003.** Evolution and genetic
673 structure of the great tit (*Parus major*) complex. *Proceedings of the Royal Society B:*
674 *Biological Sciences* **270**: 1447-1454.

675 **Kvist L, Viiri K, Dias PC, Rytönen S, Orell M. 2004.** Glacial history and colonization of
676 Europe by the blue tit *Parus caeruleus*. *Journal of Avian Biology* **35**: 352-359.

677 **Li MH, Merilä J. 2010.** Genetic evidence for male-biased dispersal in the Siberian jay
678 (*Perisoreus infaustus*) based on autosomal and Z-chromosomal markers. *Molecular Ecology*
679 **19**: 5281-5295.

680 **Librado P, Rozas J. 2009.** DnaSP v5: a software for comprehensive analysis of DNA
681 polymorphism data. *Bioinformatics* **25**: 1451–1452.

682 **Lischer HEL, Excoffier L. 2012.** PGDSpider: An automated data conversion tool for
683 connecting population genetics and genomics programs. *Bioinformatics* **28**: 298-299.

684 **LME4 Authors. 2018.** Package 'lme4'. Linear Mixed-Effects Models using 'Eigen' and S4,
685 version 1.1-17. CRAN-R. URL: [https://github.com/lme4/lme4/http://lme4.r-forge.r-](https://github.com/lme4/lme4/http://lme4.r-forge.r-project.org/)
686 [project.org/](https://github.com/lme4/lme4/http://lme4.r-forge.r-project.org/)

687 **Lo Brutto S, Sara M, Arculeo M. 2010.** Italian Peninsula preserves an evolutionary lineage
688 of the fat dormouse *Glis glis* L. (Rodentia: Gliridae). *Biological journal of the Linnean*
689 *Society*, **102**: 11-21.

690 **McDonald JH, Kreitman M. 1991.** Adaptive protein evolution at the Adh locus in
691 *Drosophila*. *Nature* **351**: 652–654.

692 **Meschini E, Frugis S. 1993.** Atlante degli uccelli nidificanti in Italia. *Suppl. Ric. Biol.*
693 *Selvaggina* **20**: 1-344.

694 **Mori A, Giunchi D, Rodríguez-Godoy F, Grasso R, Baldaccini NE, Baratti M. 2017.**
695 Multilocus approach reveals an incipient differentiation process in the Stone-curlew, *Burhinus*
696 *oediconemus* around the Mediterranean basin. *Conservation genetics* **18**: 197-209.

697 **Moritz C. 1994.** Defining ‘evolutionarily significant units’ for conservation. *Trends in*
698 *ecology & evolution* **9**: 373-375.

699 **NatureServe and IUCN (International Union for Conservation of Nature) 2018.** *Certhia*
700 *familiaris*. *The IUCN Red List of Threatened Species*. Version 2018.1
701 <http://www.iucnredlist.org>. Downloaded on 18 September 2018.

702 **Nissa N, Muller Y (eds). 2015.** *Atlas des oiseaux de France métropolitaine - Nidification et*
703 *présence hivernale*. Delachaux et Niestlé.

704 **Pasquet E, Barker FK, Martens J, Tillier A, Cruaud C, Cibois A. 2014.** Evolution within
705 the nuthatches (*Sittidae*: Aves, Passeriformes): molecular phylogeny, biogeography, and
706 ecological perspectives. *Journal of Ornithology* **155**: 755-765.

707 **Pasquet E, Thibault JC. 1997.** Genetic differences among mainland and insular forms of the
708 Citril Finch *Serinus citrinella*. *Ibis* **139**: 679-684.

709 **Pentzold S, Trisch C, Marten J, Tietze DT, Giacalone G, Lo Valva M, Nazarenko AA,**
710 **Kvist L, Päckert M. 2013.** Where is the line? Phylogeography and secondary contact of
711 western Palearctic coal tits (*Periparus ater*: Aves, Passeriformes, Paridae).
712 *ZoologischerAnzeiger* **252**: 367-382.

713 **Perktas U, Gosler AG. 2010.** Measurement error revisited: its importance for the analysis of
714 size and shape of birds. *Acta Ornithologica* **45**: 161-172.

715 **Petit RJ, Excoffier L. 2009.** Gene flow and species delimitation. *Trends in Ecology &*
716 *evolution* **24**: 386-393.

717 **Pons JM, Sonsthagen S, Dove C, Crochet PA. 2014.** Extensive mitochondrial introgression
718 in North American Great Black-backed Gulls (*Larus marinus*) from the American Herring
719 Gull (*Larus smithsonianus*) with little nuclear DNA impact. *Heredity* **112**: 226.

720 **Pons JM, Thibault JC, Aymí R, Grussu M, Muntaner J, Olioso G, Sunyer JR, Touihri**
721 **M, Fuchs J. 2016.**The role of western Mediterranean islands in the evolutionary
722 diversification of the spotted flycatcher *Muscicapa striata*, a long-distance migratory
723 passerine species. *Journal of Avian Biology* **47**: 386-398.

724 **Pons JM, Thibault JC, Fournier J, Olioso G, Rakovic M, Tellini Florenzano G, Fuchs J.**
725 **2015.** Genetic variation among Corsican and continental populations of the Eurasian
726 treecreeper (Aves: *Certhia familiaris*) reveals the existence of a palaeoendemic mitochondrial
727 lineage. *Biological journal of the Linnean Society* **115**: 134-153.

728 **Pritchard JK, Stephens M, Donnelly P. 2000.** Inference of population structure using
729 multilocus genotype data. *Genetics* **155**: 945-959.

730 **R Development Core Team.2008.** R: A language and environment for statistical computing.
731 R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL
732 <http://www.R-project.org>.

733 **Rubenstein DR. 2005.** Isolation and characterization of polymorphic microsatellite loci in the
734 plural cooperatively breeding superb starling, *Lamprotornis superbus*. *Molecular Ecology*
735 *Notes* **5**: 739-744.

736 **Ruedi M, Walter S, Fischer MC, Scaravelli D, Excoffier L, Heckel G. 2008.** Italy as a
737 major Ice Age refuge area for the bat *Myotis myotis* (Chiroptera: Vespertilionidae) in Europe.
738 *Molecular Ecology* **17**: 1801-1814.

739 **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013.** MEGA6: Molecular
740 Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.

741 **Tellini FG, Baccetti N, Arcamone E, Meschini A, Sposimo P. 1997.** *Atlante de gliuocelli*
742 *nidificanti e svernanti in Toscana (1982–1992)*. Provincia di Livorno e Centro Ornitologico
743 Italiano. Quaderni del Museo Provinciale di Storia Naturale di Livorno, Monografie **1**.

744 **Thibault JC, Bonaccorsi G. 1999.** *The Birds of Corsica. An Annotated Check-list*. No**17**.
745 British Ornithologists Union, Tring.

746 **Thibault JC, Cibois A, Prodon R, Pasquet E. 2016.** Quaternary history of an endemic
747 passerine bird on Corsica island: glacial refugium and impact of recent forest regression.
748 *Quaternary Research* **85**: 271-278.

749 **Tietze DT, Martens J, Sun YH, Päckert M. 2008.** Evolutionary history of treecreeper
750 vocalisations (Aves: *Certhia*). *Organisms, Diversity Evolution* **8**: 305-324.

751 **Tietze DT, Martens J. 2009.** Morphometric characterisation of treecreepers (genus *Certhia*).
752 *Journal of Ornithology* **150**: 431-457.

753 **Toews DP, Brelsford A. 2012.** The biogeography of mitochondrial and nuclear discordance
754 in animals. *Molecular Ecology* **21**: 3907-3930.

755 **Tritsch C, Stuckas H, Martens J, Pentzold S, Kvist L, Lo Valvo M, Giacalone G, Tietze**
756 **DT, Nazarenko AA, Päckert M. 2018.** Gene flow in the European coal tit, *Periparus ater*
757 (Aves: Passeriformes): low among Mediterranean populations but high in a continental
758 contact zone. *Biological Journal of the Linnean Society* **124**: 319-338.

759 **Ward RD, Hanner R, Hebert PDN. 2009.** The campaign to DNA barcode all fishes, FISH-
760 BOL. *Journal of Fish Biology* **74**: 329-356.

761 **Weiss S, Ferrand N. 2007.** *Phylogeography of the Southern Europe Refugia – Evolutionary*
762 *perspectives on the origins and conservation of European biodiversity*. Springer, Dordrecht,
763 The Netherlands. 390p.

764 **Wilson GA, Rannala B. 2003.** Bayesian inference of recent migration rates using multilocus
765 genotypes. *Genetics* **163**: 1177–1191.

766 **Zink RM. 2010.** Drawbacks with the use of microsatellites in phylogeography: the song
767 sparrow *Melospiza melodia* as a case study. *Journal of Avian Biology* **41**: 1-7.

768 **Zink RM, Barrowclough GF. 2008.** Mitochondrial DNA under siege in avian
769 phylogeography. *Molecular ecology* **17**: 2107-2121.

770

TABLES

Table 1: Microsatellites pairwise F_{st} averaging the variance components over loci. Values in bold differ significantly from zero after Bonferroni correction. MC = Massif Central, PY= Pyrenees, WE = Western France, ES= Eastern France, JU = Jura, AL = Alps

	FRANCE (Mainland)							
	CORSICA	ITALY	AL	WE	ES	JU	MC	PY
CO	0							
IT	0.30	0						
AL	0.29	0.17	0					
WE	0.31	0.22	0.00	0				
ES	0.22	0.13	0.01	0.02	0			
JU	0.28	0.16	-0.04	-0.02	-0.03	0		
MC	0.24	0.18	0.00	0.00	-0.00	-0.01	0	
PY	0.22	0.16	0.03	0.03	0.02	0.01	0.02	0

Table 2: Number of haplotypes, haplotype diversity (H), nucleotide diversity (π), Tajima's D and Fu's statistics of selective neutrality obtained for insular and mainland populations from Corsica, France and Central Italy (N = 257) using the mitochondrial gene COI (667bp). Ni = number of individuals, Nh = number of haplotypes, Np = number of polymorphic sites. MC= Massif Central, PY= Pyrenees, WE = Western France, ES = Eastern France, JU= Jura, AL= Alps. In bold significant values supporting population expansion. Fu's Fs statistic was considered as significant if P-value is below 0.02 (Excoffier and Lischer 2010).

	FRANCE (Mainland)							
	CORSICA	MC	PY	WE	ES	JU	AL	ITALY
Ni	108	27	23	21	20	9	23	26
Nh	3	3	3	2	4	4	4	1
Np	2	3	2	1	3	3	3	0
H	0.29	0.15	0.49	0.18	0.28	0.58	0.32	0
π	0.0005	0.0003	0.0008	0.0003	0.0005	0.001	0.0005	0
Tajima's D	- 0.29	- 1.73	-0.017	-0.617	- 1.72	- 1.51	- 1.48	0
p-value	0.41	0.02	0.42	0.23	0.03	0.06	0.05	1
Fu's Fs	-0.24	- 1.49	0.024	- 0.14	- 2.75	- 1.89	- 2.32	0
p-value	0.36	0.03	0.43	0.20	0.0001	0.008	0.01	N.A.

Table 3: mtDNA pairwise populations F_{st} among insular and mainland populations from Corsica, France and Central Italy (N = 257). F_{st} values significant at 5% level are in bold. MC = Massif Central, PY= Pyrenees, WE = Western France, ES = Eastern France, JU = Jura, AL = Alps.

		FRANCE (Mainland)					
	CORSICA	MC	PY	WE	ES	JU	AL
CO	0						
MC	0.98	0					
PY	0.98	0.09	0				
WF	0.98	0.01	0.10	0			
EF	0.98	0.002	0.08	0.02	0		
JU	0.98	0.05	0.07	0.07	0.03	0	
AL	0.98	0.02	0.08	0.03	0.01	0.03	
ITALY	0.98	0.001	0.13	0.06	0.01	0.13	0.03

LEGENDS OF FIGURES

Figure 1: Map showing sampling localities and the distribution of the Eurasian treecreeper.

Mainland France; WE = western France, ES = eastern France, MC = Massif Central, JU = Jura, AL = Alps, PY = Pyrenees; Central Italy, AP = Apennines. Distribution was made using the IUCN distributions (NatureServe and IUCN 2018).

Figure 2: Network based on microsatellites showing genetic distances among insular and mainland populations of *Certhia familiaris*. AL = Alps, WE = Western France, ES = Eastern France, JU = Jura, MC = Massif Central, PY = Pyrenees, C. Italia = Central Italy (Apennines).

Figure 3: Bayesian clustering analysis of microsatellite data with individual assignment probabilities for $K = 3$. Five individuals sampled in Corsica (numbers 266, 284, 1010, 1040, 1064) showed significant admixture, i.e. a non-Corsican inferred ancestry with a probability $> 50\%$. AL = Alps, WE = Western France, ES = Eastern France, JU = Jura MC = Massif Central, PY = Pyrenees.

Figure 4: Migrate results of past gene flow estimations among insular and mainland French and central Italian populations (Apennines). Numbers refer to the inferred number of immigrants per generation between the three groups.

Figure 5: Median joining network showing relationships among COI haplotypes for mainland and insular populations of *Certhia familiaris* ($n = 257$, 667 bp). The size of each circle is proportional to haplotype frequency. AL = Alps, WE = Western France, ES = Eastern France, PY = Pyrenees, MC = Massif Central, Central Italy (Apennines) = IT, JU = Jura. There is no mixing of insular and mainland haplotypes.

Figure 6: Scatter plot of the first two principal components resulting from a PCA performed on four biometric variables (Wing length, WL; Tarsus length, TL; Bill length at nostrils, BLN; Hind claw length, HCL). Five admixed Corsican individuals that possessed more than

50% of continental alleles are represented by colored squares in Dark blue, Light blue, Green, Black and Red. Confidence ellipses show significant differences between the three main populations; Grey circle = France; Orange circle = Central Italy; Pink circle = Corsica.











