

1 **Tertiary remnants and Holocene colonizers: genetic structure and phylogeography of**
2 **Scots pine reveal higher genetic diversity in young boreal than in relic**
3 **Mediterranean populations and a dual colonisation of Fennoscandia**

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29 Key words: *Pinus sylvestris* L., postglacial migration, hybrid zone, *nad1*, *nad7*, chloroplast
30 microsatellites

31 Running title: Range-wide diversity of Scots pine

32 Corresponding author: Monika Dering

33 Number of words in the Abstract: 299

34 Number of words: 6439

35 Number of references: 79

36

37 **Abstract**

38 **Aim:** Scots pine is one of the dominant tree species in forest ecosystems of the temperate and
39 boreal zones in Eurasia. Since the Tertiary it has persisted also in the Mediterranean region,
40 forming relic populations. In this study we investigate range – wide genetic diversity of Scots
41 pine, aiming to provide comprehensive information on the phylogeography and genetic
42 resources of the species.

43 **Location:** Europe and Asia

44 **Methods:** Thirteen paternally inherited chloroplast microsatellite (cpSSR) loci were used to
45 investigate the genetic structure of 62 populations from Fennoscandia and 30 populations
46 from the Mediterranean. We also surveyed variability of two maternally inherited
47 mitochondrial DNA (mtDNA) regions and additionally included literature data from 156
48 populations (248 populations in total).

49 **Results:** Scots pine retains an overall high genetic diversity in the chloroplast genome but,
50 unexpectedly, the historically younger populations from Fennoscandia showed statistically
51 higher mean intra – population diversity than the Mediterranean stands (0.807 and 0.750,
52 respectively). The latter also contained a lower number of private cpSSR haplotypes.

53 Population differentiation with respect to mtDNA was higher ($G_{ST} = 0.628$) than at cpSSR

54 ($G_{ST} = 0.015$) indicating an efficient pollen-mediated gene – flow among refugial population
55 of the species. One novel haplotype mtDNA was found, increasing the number of known
56 mitotypes for the species from 4 to 5. The geographic distribution of mitotypes was structured
57 into 4 groups corresponding to the main refugial areas of the species.

58 **Main conclusions:** Genetic impoverishment of the Mediterranean populations may enhance
59 their vulnerability to future environmental changes. The spatial distribution of two mitotypes
60 in Fennoscandia, featuring predominance of mitotype *a* in Norway and Sweden and *b* in
61 Finland, gives strong support for dual colonization of that region from south eastern (Finland)
62 and south – western (Sweden) directions. These results thus provide new insights into both
63 Holocene expansion of Scots pine and the present distribution of species' genetic resources.

64 **Introduction**

65 Phylogeographic studies demonstrated that the last Pleistocene glaciation and subsequent
66 Holocene migrations were important factors shaping the modern patterns of genetic diversity
67 found in trees of the Mediterranean, temperate and boreal regions in Europe (Magri *et al.*,
68 2006; Dering *et al.*, 2014; Sękiewicz *et al.*, 2015). Both palaeobotanical and molecular data
69 have provided evidence that southern margins of the European continent acted as refugia for
70 many European tree species in Late Pleniglacial (LPG; 24 –14.6 ka) during which the most
71 extreme glacial conditions prevailed and the Scandinavian Ice Sheet (SIS) attained its
72 maximal size. However, in the case of some species, a specific set of life – history traits may
73 have enabled them to maintain additional refugial populations sparsely distributed closer to
74 the ice margins (Bhagwat & Willis, 2008). This idea of northern refugia has been a subject of
75 vivid debate for over a decade, during which supporting arguments of enthusiasts and doubts
76 of sceptics have been colliding (Willis & van Andel, 2004; Parducci *et al.*, 2012; Tzedakis *et*
77 *al.*, 2013; de Lafontaine *et al.*, 2014). However, this concept serves as a reasonable - and
78 compelling in its simplicity – resolution of some peculiarities observed in phylogeographic

79 patterns. Principally, it helps address Reid's paradox concerning the mismatch between
80 current migration capacity of most tree species and that reconstructed from palaeobotanical
81 data (Clark *et al.*, 1998). Additionally, it explains departures from the commonly recognized
82 Northern Hemisphere 'southern richness and northern purity scenario' which manifests itself
83 in a lower level of variability in recently colonized areas in comparison with the sources of
84 colonisation (Hewitt, 2000).

85 Among all pine species, Scots pine (*Pinus sylvestris* L.) has the widest geographic
86 range. Along the west-east axis it spans the 14,000 km long area starting from mountain areas
87 of the Iberian Peninsula (8° W), throughout Caucasus, Altai Mts., Sayan Mts. up to Eastern
88 Siberia (135°E). Along the north – south axis the range is much narrower (ca. 2,700 km) and
89 runs from the northern part of the Scandinavian Peninsula (ca. 69° N) to Pontic Mts. (ca. 40°
90 S) in Asia Minor Peninsula but southernmost stand is located in Sierra Nevada Mts. in Spain
91 (ca. 37° S). Scots pine is a key component of many forest ecosystems in temperate and boreal
92 zones. It also plays an important role in the forests of the Mediterranean where it inhabits
93 isolated mountain ranges. According to the 'rear – edge populations' concept, these
94 southernmost populations represent valuable genetic resources (Hampe & Petit, 2005).

95 Scots pine is also of great economic value in Eurasian forestry. Thus, understanding
96 the spatio-temporal pattern of its genetic variation is of high priority for improved
97 management, especially in the context of ongoing climate changes. In terms of forecasting the
98 probable responses of forest tree species to global climate changes reconstruction of their
99 Pleistocene and Holocene demographic dynamics is of high relevance. Hence, the glacial and
100 postglacial history of Scots pine has been intensively investigated (Sinclair *et al.*, 1999;
101 Cheddadi *et al.*, 2006; Naydenov *et al.*, 2007; Pyhäjärvi *et al.*, 2008; Buchovska *et al.*, 2013).
102 The main Scots pine refugia were located on three European Peninsulas (Cheddadi *et al.*,
103 2006) and in Asia Minor (Naydenov *et al.*, 2007). However, the contribution of some of these

104 peninsular refugia to the Holocene colonisation was limited and some populations, e.g., those
105 from Asia Minor or the Iberian Peninsula, had been trapped in their refugia (Naydenov *et al.*,
106 2007).

107 According to species distribution models, Scots pine is a cold adapted tree species
108 with the ability to grow on permafrost, that could have persisted in mid – latitudes during
109 LPG (Svenning *et al.*, 2008). Palaeobotanical and genetic data do suggest its presence during
110 the LPG in the Hungarian Plains, the Circum – Alpine area, the Carpathians, Central Europe,
111 NW Russian Plains, the Ural Mts. (Willis & van Andel, 2004; Cheddadi *et al.*, 2006;
112 Naydenov *et al.*, 2007; Binney *et al.*, 2009) or even very close to SIS as in western and south-
113 western Norway (Parducci *et al.*, 2012). Presumably, during the colonization phase those
114 northernmost refugial populations were the most active in settling the current boreal part of
115 Scots pine range in Europe. The exact location of refugia and the precise colonization routes
116 leading to northern Scandinavia remain unclear, mostly due to limited sampling done in that
117 region. Investigations conducted in Fennoscandia by Naydenov *et al.* (2007) and Pyhäjärvi *et*
118 *al.* (2008) indicated geographically – dependent distribution of mitotypes *a* and *b* of
119 mitochondrial marker *nad7*: mitotype *a* prevailed in Sweden while mitotype *b* occurred in
120 especially high frequencies in Finland and Baltic countries. This peculiar distribution suggests
121 that this part of the continent might have become colonized independently from western and
122 eastern directions. This would imply the formation of a contact zone between distinct
123 colonization routes of Scots pine that should be located somewhere in northern Fennoscandia.

124 The current broad distribution of Scots pine implies that populations from the
125 southernmost latitudes and those located at northern latitudes have likely experienced
126 contrasting demographic processes during glacial and postglacial periods. Relatively stable
127 environmental conditions in southern Europe that lasted through the whole Quaternary
128 buffered the impact of numerous climate extremes that occurred in Europe and allowed Scots

129 pine to maintain an extended *in situ* persistence that likely promoted accumulation of genetic
130 diversity, including unique genetic variation (Taberlet *et al.*, 1998). Holocene warming
131 subsequently encouraged poleward expansion of the species from its refugia. Consistent with
132 theoretical models of migration, postglacial range expansion has likely entailed repeated
133 founding effects leading to loss of genetic diversity with increasing distance from refugial
134 populations. The so called ‘southern richness and northern purity scenario’ that refers to this
135 phenomenon has been indicated as one of the most important consequences of the ice-ages for
136 organization of intraspecific genetic diversity in European biota (Hewitt, 2000). Boreal
137 populations of Scots pine represent the descendants of post-glacial migrants – the leading
138 edge-populations – and occupy the main part of the species’ current range in Europe. Little is
139 known about the genetic resources stored in the northernmost populations of Scots pine
140 despite much discussion focusing on their future growth patterns and adaptation to changing
141 climate, in the context of economic value of the species for European forestry (Reich &
142 Oleksyn, 2008; Savolainen *et al.*, 2011; Zadworny *et al.*, 2016),

143 In the present study, we investigated genetic structure of the southernmost (rear –
144 edge) and northernmost (leading – edge) populations of Scots pine. We assessed variation in
145 maternally inherited mtDNA and paternally inherited cpSSR, aiming to answer the following
146 questions: (1) was Fennoscandia colonized by Scots pine from two independent directions?
147 (2) did the two fronts of colonisation meet and form a hybrid zone? And if so, (3) where is it
148 located? (4) does the distribution of cpSSR diversity across the species’ European range
149 follow the ‘southern richness and northern purity’ scenario? (5) do populations from the
150 southern limits exhibit unique genetic variation? Additionally, because plastid and
151 mitochondrial genomes in Pinaceae experience asymmetrical dispersal, they are well suited
152 for evaluation of the contribution of differential gene flow realized with seeds (genes located
153 in mitochondria) and pollen (genes located in chloroplasts) during postglacial range

154 expansion. We therefore asked (6) if the patterns of differentiation revealed with mtDNA and
155 cpSSR correspond to each other, or are they divergent?

156 **Methods**

157 *Sampling and genetic analysis*

158 Ninety – two natural populations of Scots pine were sampled and screened for their mtDNA
159 of which 73 were screened also for their cpSSR polymorphism. Needles were collected from
160 12-17 individuals per population (1384 individuals in total, Table 1). Genomic DNA was
161 extracted from needle tissue using a DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany).

162 Two mitochondrial DNA regions known for their polymorphism in Scots pine: *nad1*
163 intron b/c (Soranzo *et al.*, 2000) and *nad7* intron 1 were used in this study (Pyhäjärvi *et al.*,
164 2008; Naydenov *et al.*, 2007). Regions *nad1* and *nad7* were amplified with fluorescently –
165 labelled primers according to Soranzo *et al.*, (2000) and Buchovska *et al.*, (2013).

166 Fourteen chloroplast microsatellite markers were chosen for the genetic analysis:
167 Pt15169, Pt26081, Pt30204, Pt36480, Pt45002, Pt71936 (Vendramin *et al.*, 1996), PCP1289,
168 PCP26106, PCP30277, PCP36567, PCP41131, PCP4507, PCP87314 and PCP102652 (Provan
169 *et al.*, 1998). They were amplified in two PCR – multiplex reactions in total volume of 10 µl
170 using Qiagen Multiplex PCR Kits (Qiagen, Hilden, Germany) under conditions recommended
171 by the manufacturer.

172 Analysis of PCR products of mtDNA and cpSSR was performed on an ABI PRISM
173 3130 genetic analyzer with the GeneScan 500 – LIZ size standard and genotypes were scored
174 using GeneMapper vs. 4.0. (Applied Biosystems).

175 *Data analysis*

176 *Genetic diversity*

177 Mitotypes were defined according to Naydenov *et al.* (2007) and Pyhäjärvi *et al.*
178 (2008). For each population sampled, the effective number of mitotypes (N_E) and unbiased
179 Nei's gene diversity index (H_E) (Nei, 1973) were calculated.

180 The raw data on cpSSRs were processed with Raw2G software in order to bin alleles
181 according to the method of Idury & Cardon (1997). Based on least-squares minimization
182 procedures, this software enables the raw allele sizes to be assigned to their appropriate allelic
183 bins, which correspond to discrete alleles. This allowed us to define the rate of allelic
184 dispersion for each of the markers used. Substantial variation in allelic dispersion is expected
185 to generate genotyping errors, especially in cases of single nucleotide SSR. Loci for which
186 mean standard deviation was > 0.25 in allelic bins were excluded from the analysis.

187 We defined different haplotypes as unique combinations of size variants across the
188 cpSSR. Using HAPLOTYPE ANALYSIS 1.05 (Eliades & Eliades, 2009) number of different
189 haplotypes (A), number of private haplotypes (P_H), effective number of haplotypes, Nei's
190 gene diversity index of (H_E) estimated without bias and mean genetic distance between
191 haplotypes (D_{sh}^2) were calculated.

192 *Geographical patterns of diversity*

193 In range-wide analysis of the distributional pattern of mtDNA variability, additional
194 156 records of mtDNA mitotypes from natural populations of Scots pine were obtained from
195 the papers by Naydenov *et al.* (2007), Pyhäjärvi *et al.* (2008), Vidyakin *et al.* (2012), and
196 Buchovska *et al.* (2013) and included in our analyses (Appendix S1). Using PERMUT 2.0
197 (Pons & Petit, 1996) population differentiation based on mtDNA was evaluated using two
198 parameters, G_{ST} and N_{ST} . G_{ST} estimates population differentiation considering solely the
199 frequencies of the mitotypes while N_{ST} considers genetic relatedness among mitotypes.
200 Greater value of N_{ST} in comparison to G_{ST} means that closely related mitotypes tend to cluster
201 in the same area suggesting a phylogenetic structure. SAMOVA 2.0 (Dupanloup *et al.*, 2002)

202 was applied to infer the geographic population structure based on mtDNA and cpSSR. To
203 infer the most probable number of K groups, SAMOVA 2.0 was run with number of groups
204 from 2 up to 10 for mtDNA and from 2 to 30 for cpSSR and the result attaining the maximum
205 F_{CT} value was chosen as the indicator of the best grouping. Additionally, geographic structure
206 of cpSSR diversity was tested with a Bayesian approach implemented in BAPS 6.0 (Corander
207 *et al.*, 2008). We applied a spatial genetic mixture analysis to predefined groups of
208 individuals. The software uses Markov chain Monte Carlo simulation to group sampled
209 population into K clusters predefined by the user and the best partitioning is attained based on
210 the highest marginal log – likelihood. After the testing stage, the final analysis was conducted
211 for 10 replicates for K ranging from 3 to 19.

212 A hierarchical analysis of molecular variance (AMOVA) was performed for cpSSR
213 data with GenAlEx 6.2 by using the mtDNA grouping revealed by SAMOVA to test for
214 congruence between the mtDNA and cpDNA patterns of differentiations. AMOVA was also
215 conducted for two biogeographic regions, Mediterranean vs. Fennoscandia, that host Tertiary
216 and Holocene populations, respectively. Diversity parameters based on cpSSRs were
217 compared among SAMOVA mtDNA groups and between Mediterranean and Scandinavian
218 populations using multiple comparisons Wilcoxon for each pair and Wilcoxon – Kruskal –
219 Wallis tests.

220 **Results**

221 *mtDNA and cpSSRs diversity*

222 As a result of the analysis of 1384 individuals of Scots pine, a new combination of size
223 variants from both mitochondrial regions used allowed us to define a new haplotype,
224 producing a total of five haplotypes instead of the four described so far (Table 1). Among the
225 five mitotypes observed, the most abundant was mitotype *a* (63.4%), followed by *b* (17.1%),
226 *c* (10.6%), *d* (8.3%) and *e* (0.6%); the latter found exclusively in Turkish population TR7 and

227 consisting of size variants 248 bp of *nad1* and 268 bp of *nad7*. The mitotype diversity index
228 per population H_{MT} ranged from 0 to 0.533 with an average value of 0.131 (Table 1).
229 Comparison between the relic part of the Scots pine range (Mediterranean) and the Holocene
230 – derived populations from central and northern Europe indicated that the Mediterranean
231 region had significantly lower mitotype diversity (Wilcoxon test – $H_{Med} = 0.0728$: $H_{NCEur} =$
232 0.156 , $p = 0.0366$).

233 The allele binning procedure revealed substantial allelic dispersion that existed in most
234 of the cpSSR loci used in this study; thus only four cpSSR loci (PCP1289, PCP87314,
235 PCP102652 and Pt26081) were used for further analysis. A total of 6, 7, 5 and 6 size variants
236 were found at loci PCP1289, PCP87314, PCP102652 and Pt26081, respectively. Their
237 combinations yielded a total of 73 haplotypes, of which only 9 had relative frequencies above
238 1% (Table 2). Thirty six haplotypes (49.3%) were private and attained very low frequencies.
239 The majority of the private haplotypes were found in Scandinavia (28), followed by
240 populations on the Iberian Peninsula (6) and single private haplotypes in populations from
241 Turkey, France and Greece (Table 1). The high number of haplotypes detected was reflected
242 in generally high values of haplotypic diversity ranging from 0.971 (SW30) to 0.533 (FIN3).
243 The effective number of haplotypes ranged from 1.991 (FIN3) to 10.714 (SW30) and mean
244 genetic distance between individuals ranged from 0.176 (FIN3) to 3.062 (TR3).

245 *Geographic patterns of differentiation based on mtDNA and cpSSRs*

246 Population differentiation based on mtDNA data was high among the 248 populations
247 analysed, with G_{ST} and N_{ST} equalling 0.633 and 0.657, respectively, but the insignificant
248 difference between G_{ST} and N_{ST} ($p = 0.537$) did not support the existence of a formal
249 phylogeographic structure, which reflects the low polymorphism of the mitochondrial
250 markers. However, the visual inspection of spatial distribution of mitotypes suggests clear
251 geographic pattern (Fig. 1A; Appendix S2), supported further with SAMOVA. Examination

252 of clustering options produced values of F_{CT} ranging from 0.7849 ($K = 8$) to a maximal value
253 of 0.8202 ($K = 4$), which indicated number 4 to be the preferred number of genetically
254 homogenous clusters for the whole dataset (Table 3). A comparably high value of $F_{CT} =$
255 0.8214 was also obtained for $K = 7$ (Appendix S3). However, this partitioning was less
256 biologically justified because it resulted in separation of three additional clusters that
257 consisted of geographically remote populations. The four thus defined clusters contained the
258 following populations: (I) populations from Norway, Sweden, Russian Plains, Baltic region,
259 central, eastern and western Europe with some populations from the Balkan Peninsula, Asia
260 Minor Peninsula and Iberian Peninsula; (II) population from Finland, Russia and non –
261 coastal Baltic populations; (III) population from Turkey; and (IV) Spanish populations and
262 single Turkish population. The most abundant cluster I comprised mostly populations
263 carrying the most frequent mitotype *a* and some populations with low frequency of mitotype
264 *b*. This cluster included very geographically distant populations. Interestingly, Norwegian
265 populations were almost entirely fixed for the most common mitotype *a* (Fig. 1A). Cluster II
266 included populations in which mitotype *b* prevailed. Populations from the Iberian and Asia
267 Minor Peninsulas formed separate and homogenous clusters in which mitotype *c* and *d*
268 dominated, respectively. In one of the Greek populations, mitotype *d* specific to Turkish
269 stands was noted, and in one Turkish stand, size variant in the nad1 *b/c*, noted almost
270 exclusively in Iberian Peninsula (248 bp), was detected.

271 In contrast to mtDNA, differentiation of Scots pine populations with respect to cpSSR
272 was weak, with G_{ST} and R_{ST} equalling 0.015 and 0.045 which reflects high polymorphism of
273 the cpSSR markers. Nonetheless, the significant difference ($p = 0.03$) between G_{ST} and R_{ST}
274 indicated a phylogeographic structure, but SAMOVA analysis was not able to produce any
275 reasonable clustering of populations and resulted mainly in isolation of single populations.
276 Using Bayesian methods the best partition contained 13 clusters which contained populations

277 originating from different and sometimes geographically very remote regions (Table 4). A
278 clear geographic structure was not detected but some genetic discontinuity between
279 populations was noticeable, especially between the Iberian and Anatolian Peninsulas, where
280 mutually exclusive clusters were noted (Appendix S4). In contrast, populations from the
281 Scandinavian Peninsula shared clusters with many populations from southern Europe (Table
282 4; Appendix S4).

283 AMOVA was significant at all hierarchical levels in both configurations tested (Table
284 5). The cpSSR variation attributable to mtDNA clusters ($K = 4$) accounted for 1% of the total
285 variation noted ($\Phi_{RT} = 0.014$, $p = 0.003$) while 95% of the total variation was located
286 within populations. In comparison, the differentiation between Mediterranean and
287 Scandinavian populations was higher, accounting for 2% of total variation ($\Phi_{RT} = 0.017$, p
288 $= 0.001$). Significant differences were also revealed in the diversity parameters. Scandinavian
289 populations had higher number of haplotypes per population (A ; $p = 0.0226$), higher effective
290 number of haplotypes (N_E ; $p = 0.0099$) and haplotypic diversity (H_{CP} ; $p = 0.0120$), compared
291 with Mediterranean populations. With respect to SAMOVA clusters, significant differences
292 were noted in the number of alleles per population (A) between cluster I (mainly Sweden and
293 Central Europe) and cluster III (Asia Minor) ($p = 0.0337$) and between cluster III and IV
294 (Iberian Peninsula) ($p = 0.005$); in effective number of alleles (N_E) between cluster III and IV
295 ($p = 0.0208$) and in haplotypic diversity (H_{CP}) between cluster III and IV ($p = 0.0331$).

296 **Discussion**

297 *Genetic diversity and differentiation*

298 Our results confirmed that Scots pine harbours high genetic diversity stored in its
299 chloroplast genome ($H_{CP} = 0.786$), albeit our estimates are somewhat lower than those
300 obtained in previous regional studies for Scottish ($H_{CP} = 0.991$) (Provan *et al.*, 1998), Italian

301 ($H_{CP} = 0.92$) (Scalfi *et al.*, 2009) and Spanish populations ($H_{CP} = 0.978$) (Robledo-Arnuncio
302 *et al.*, 2005). The only known lower value comes from the latest study conducted in Romania,
303 that included managed populations ($H_{CP} = 0.44$) (Bernhardsson *et al.*, 2016). Our study is
304 based on very wide representation of populations from different regions, and some of these
305 populations were characterized by very low levels of genetic diversity that affected the
306 average value reported here (e.g., SP12: $H_{CP} = 0.543$, SR: $H_{CP} = 0.571$). Contrary to our
307 expectations on the expected decrease of cpSSR diversity in northern direction, Scots pine
308 populations from Fennoscandia have displayed statistically higher ($p = 0.012$) haplotypic
309 diversity (0.807) than the south European populations (0.750) (Table 1), that represent the
310 oldest pool of species genetic diversity and the persistence of which likely predates
311 Quaternary glaciations (Sinclair *et al.*, 1999). Populations from Fennoscandia also contained
312 more private cpSSR haplotypes compared to the southern populations. Similar trends have
313 also been reported for other species (Hathaway *et al.*, 2009; Soubani *et al.*, 2014) and such
314 trend is in opposition to the frequently noted phenomenon of ‘southern richness and northern
315 purity’. In general, areas of the greatest diversity are assumed to be the origins of postglacial
316 migration. Such departures from this distribution of diversity may result from peculiarities of
317 postglacial history of the species such as mixing of different refugial lineages or cryptic
318 northern refugia (Tzedakis *et al.*, 2013; Havrdová *et al.*, 2015) (see below).

319 We found that levels of differentiation estimated on the basis of mtDNA and cpSSR
320 are incongruent. Significant mtDNA differentiation among populations ($G_{ST} = 0.633$) was
321 comparable to previous studies (Naydenov *et al.*, 2007; Pyhäjärvi *et al.*, 2008). In contrast, the
322 differentiation assessed with cpSSR was very weak ($G_{ST} = 0.015$). Such discordance is
323 expected in gymnosperms if markers from mitochondrial and chloroplast genomes are used. It
324 is due to the contrasting modes of inheritance of cytoplasmic genomes (maternally transmitted
325 mitochondria and paternally transmitted chloroplasts), higher polymorphism of cpSSR and to

326 the greater potential for dispersion of chloroplast genes by pollen (Gérardi *et al.*, 2010; Cinget
327 *et al.*, 2015; Bernhardsson *et al.*, 2016). The contrasting levels of genetic differentiation
328 documented here exemplify the high potential of wind – dispersed pollen to erode the
329 population genetic structure based on the seed – dispersed mtDNA. Wind – dispersal of
330 pollen has been shown to be accountable for among – refugial interglacial population gene
331 flow in *Abies alba* (Liepelt *et al.*, 2002), *Pinus banksiana* (Godbout *et al.*, 2010) and *Picea*
332 *mariana* (Gérardi *et al.*, 2010).

333 *Geographic pattern and its implications for glacial and postglacial history of the species*
334 SAMOVA using mtDNA reflected strong spatial genetic structure within the Scots pine
335 range, involving four distinct groups (Table 3) well related to the LPG refugial areas
336 previously defined for this species (Sinclair *et al.*, 1999; Naydenov *et al.*, 2007; Pyhäjärvi *et*
337 *al.*, 2008). The two most spatially restricted clusters correspond to Scots pine refugia located
338 on the Iberian and Asia Minor Peninsulas. They represent the valuable relic genetic resources
339 that are of high conservation priority, although they appear to have played no role in
340 postglacial recolonization (Naydenov *et al.*, 2007; Pyhäjärvi *et al.*, 2008). Two other clusters
341 included populations with high prevalence of either mitotype *a* or *b*, both of which have much
342 wider distribution.

343 The phylogeographic resolution achieved in this study on the basis of cpSSR markers
344 was low due to the very high polymorphism of these markers and their high pollen-mediated
345 potential for dispersal. Based on four cpSSR loci, 13 clusters were defined, some of which
346 were heterogeneous and consisted of geographically distinct populations inhabiting the
347 Iberian, Anatolian and Scandinavian Peninsulas (Table 4; AppendixS4). This result
348 exemplifies the particularly high rate of pollen – mediated gene flow in wind pollinated
349 species which has a homogenizing effect on genetic structure. However, the patterns of
350 genetic differentiation based on mtDNA and cpSSR were not entirely decoupled. Despite its

351 weakness, the cpSSRs differentiation partly retained the signal of ancient divergence apparent
352 in mtDNA lineages (Table 5). Significant level (1%) of differentiation inferred with cpSSR
353 among SAMOVA clusters suggests partial concordance between both sets of data. Yet higher
354 divergence was reported between the Holocene – derived populations located in Fennoscandia
355 and the Tertiary remnants from the Mediterranean (2%). All together, these results indicate
356 that the gene pool of Scots pine is not fully homogenic and some weak structuring does exist
357 which likely is the consequence of the species' wide range and variety of selective regimes
358 operating in occupied habitats.

359 Our extensive set of populations showed that variants of mtDNA previously thought to
360 be strictly confined to some geographic areas are also noted at low frequencies beyond those
361 locations. The size variant 248 bp of *nadI* b/c noted previously mostly in Spanish
362 populations, was detected in one Turkish and one Greek stand, and Naydenov *et al.* (2008)
363 also reported it from Bulgaria. Recently, Bernhardsson *et al.* (2016) has found this variant in
364 one Romanian stand. Mitotype *c*, restricted to Turkey, was also detected in a single Greek
365 stand. Occurrence of spontaneous mutation, as suggested by Bernhardsson *et al.* (2016) is
366 possible, but a more parsimonious explanation considers low – frequency pre – existence of
367 these variants in other parts of the species' southern range. A similar situation was observed
368 for a *Fagus sylvatica* haplotype noted in Spanish and Slovenian stands (Magri *et al.*, 2006).
369 This would mean that the polymorphism at investigated mtDNA regions arose prior to the last
370 glacial cycle.

371 Bilgen & Kaya (2007) have attributed the genetic isolation of the easternmost
372 population of Scots pine in the Pontic Mts. to the Anatolian diagonal, a series of mountain
373 ranges extending from the Anti Taurus Mts. toward to the eastern Black Sea, that has been
374 recognized as an important biogeographic barrier (Ansell *et al.*, 2011). The morphological
375 distinctiveness of Scots pine populations from the Pontic Mts. has been recently reported by

376 Jasińska *et al.* (2014). It has been hypothesized that the Pontic Mts. may have been colonized
377 by Scots pine from the Caucasus (Bilgen & Kaya, 2007) which gains no support in the light of
378 the results presented here. The single Caucasus population included in this study (Fig. 1A;
379 Georgia – from Buchovska *et al.*, 2013) was composed of mitotypes *a* and *b* – the former
380 widely distributed in the whole of Europe including Turkey, and the latter present at a
381 particularly high frequency in Finland, Central Europe and NW Russia (Fig. 1A); the
382 mitotype *d* specific to Turkey was not present in the Caucasus population. Thus, based on
383 these limited data there remains uncertainty regarding whether glacial refugia for Scots pine
384 existed in the Caucasus but were not active during re – populating, similar to some other
385 species (Petit *et al.*, 2002; Skrede *et al.*, 2006) or whether this region was colonized from
386 northern direction, as for example in case of common juniper (*Juniperus communis* L.)
387 (Hantemirova *et al.*, 2016).

388 In comparison with previous studies, better resolution was attained with regard to the
389 distribution of mitotype *b*. This mitotype reaches high frequencies in north-eastern Europe,
390 especially in Finland, but it was also noted in more remote geographic locations such as the
391 Georgia, Czech Republic, Slovakia, Bulgaria, or Asiatic part of Russia (112.00° E) (Fig. 1A).
392 A recent study by Bernhardsson *et al.* (2016) confirms the occurrence of this mitotype in
393 Romania and Hungary, as well. However, the presence of this mitotype as far west as France
394 (single population) may be of non – autochthonous origin. Historical data on the development
395 of the French Navy since the 16th to 19th century indicate that the Scots pine from north-
396 eastern Europe, i.e. Baltic countries, Ukraine and Poland, was especially valued in mast
397 construction (Daszkiewicz & Oleksyn, 2005). Scots pine wood for this purpose was imported
398 from the port of Riga and for that reason the imported Scots pine timber was known in
399 western Europe as ‘the Riga pine’. Historical sources also contain information on attempts to
400 cultivate ‘Riga pine’ in France made, among others, by the botanist Henri – Louis Duhamel

401 du Monceau (1700 – 1782), in order to achieve independence from the eastern sources of
402 wood (Daszkiewicz & Oleksyn, 2005).

403 Our study focuses especially on the extensive collection of Scots pine populations
404 inhabiting Fennoscandia with the aim to reconstruct the possible colonization routes and
405 explain the distribution of mitotype *b* in this part of Europe, which is hypothesized to be
406 related to some northern refugium (Pyhäjärvi *et al.*, 2008; Naydenov *et al.*, 2007; Buchovska
407 *et al.*, 2013). However, the direct correspondence of the distributional pattern of mitotype *b* to
408 the location of the northern refugium is impossible to deduce from our data, mainly due to the
409 widespread occurrence of mitotype *b*. The apparent increase in frequency of mitotype *b* from
410 north-eastern Europe, especially from the NW Russian Plain and Baltic countries toward
411 Finland (Fig. 1A), favours the concept of northern refugia located somewhere in the NW
412 Russian Plain (Buchovska *et al.* 2013; Tzedakis *et al.*, 2013), in the NE Russian Plain
413 (Väliranta *et al.*, 2011) or maybe also in the Ural Mts. (Naydenov *et al.*, 2007). Genetically
414 distinct refugial populations in NW Russia existed for example for Norway spruce (Tollefsrud
415 *et al.*, 2008; Parducci *et al.* 2012; Tollefsrud *et al.* 2015) and mountain avens (*Dryas*
416 *octopetala* L.) (Skrede *et al.*, 2006) strengthening the hypothesis of refugia for Scots pine in
417 that region. Considering the geographic distribution of mitotype *b* (Fig. 1A) it is probable that
418 this NW Russian refugium, apart from Fennoscandia, could also be the source for
419 colonisation of Eastern and Central Europe (Fig. 1B), as previously suggested by Buchovska
420 *et al.* (2013). On the other hand, the contribution to the European part of the species' range
421 the system of eastern refugia located in Western Siberia, Central Siberia, Altai (Western
422 Mongolia) or Yakutia proposed by some authors (Sannikov & Petrova, 2012; Sannikov *et al.*,
423 2014; Semerikov *et al.*, 2014) seems rather limited. Definitely, wider sampling in east
424 European and Asiatic parts of the range is needed for a more precise reconstruction of
425 postglacial migration routes of Scots pine in that region.

426 The peculiar distribution of mitotype *a* and *b* in Fennoscandia – the former prevailing
427 in Norway and Sweden and the latter in Finland – strongly supports the hypothesis of dual
428 colonisation of that region, an idea that has appeared in earlier studies (Sinclair *et al.*, 1999;
429 Pyhäjärvi *et al.*, 2008; Savolainen *et al.*, 2011; Buchovska *et al.*, 2013). Comparative study of
430 postglacial recolonization routes in Europe pointed to possible immigration of biota into
431 Fennoscandia from both north-eastern and south-western directions (Taberlet *et al.*, 1998).
432 However, up to now, there has been no strong evidence supporting this scenario for Scots
433 pine. Melting of the Scandinavian ice sheet caused an uplift of the Scandinavian Peninsula
434 leading to the development of Ancylus Lake ca. 10.7 ka cal BP, which filled the Baltic basin
435 and much of the current Baltic coastal areas (Björck, 1995). Culmination of the Ancylus Lake
436 transgression at 10.3 ka cal BP involved not only the large body of fresh water, but also the
437 existence of remnants of the ice cap in northern Sweden (Ojala *et al.*, 2005), that would have
438 hampered the colonisation of southern Sweden from the eastern direction. Early release of the
439 coastal part of south – western Sweden, and southern Sweden in general, from the ice cap (up
440 to 59°N until 13.4 ka cal BP; Lundqvist & Wohlfarth, 2001; Stroeve *et al.* 2016; Hughes *et*
441 *al.*, 2016), also affecting central Norway (Paus *et al.*, 2011; Hughes *et al.*, 2016), created the
442 opportunity for colonisation of Scandinavian peninsula from the western direction *via* Danish
443 Straits that at that time formed a land – bridge (Fig. 8 in Ojala *et al.*, 2005). The immigration
444 of Scots pine into Sweden directly across the Baltic Sea is also likely (Fig. 1B) (Buchovska *et*
445 *al.* 2013) as the island of Bornholm could have been deglaciated as early as 18 ka BP (Hughes
446 *et al.*, 2015). The colonisation routes outlined here for Scots pine correspond well to routes
447 proposed previously for other plant species, e.g., *Quercus* ssp. (Petit *et al.*, 2002), *Betula nana*
448 (Jadwiszczak, 2012), *Cerastium alpinum* (Berglund & Westerbergh, 2001) or animals
449 (Seddon *et al.*, 2001; Bray *et al.*, 2013).

450 Our results demonstrate a near fixation of the Norwegian population with the mitotype
451 *a*, commonly distributed in western European (Spain, France and Germany) and Scottish
452 populations of the species; only 4 trees carrying mitotype *b* were noted in Norway (Table 1,
453 Fig. 1A). These findings have important implications for reconstruction of the migratory
454 history of Scots pine in north – western Europe. Based on these observations we propose the
455 following colonization routes in north – western Europe: (1) one running from western
456 Europe across the English Channel into Scotland *via* the terrestrial corridors between
457 continental Europe and Britain and second (2) running from western Europe and passing
458 through Jutland and Danish Straits into Norway. We also hypothesize additional immigration
459 route into Norway running through currently submerged land – bridge located in southern
460 North Sea (Coles, 2000; Ward *et al.*, 2006) (Fig. 1B).

461 Sinclair *et al.* (1998) reported a distinct character the western – most Scottish
462 populations of *P. sylvestris* using *coxI* marker and postulated a possible dual immigration of
463 Scots pine into Scotland from (1) continental Europe and (2) refugium located in Ireland.
464 However, due to the extinction of the last Irish Scots pine populations ca. 2,000 BP
465 (Bradshaw & Browne, 1987) genetic arguments are not available and palynological data alone
466 are not conclusive with respect to the postulated refugium in Ireland. According to Mitchell
467 (2006), the Irish Sea constituted an impermeable barrier during migration and thus most of the
468 tree species that colonized Ireland from continental Europe arrived from southern direction
469 *via* the Celtic Sea, rather than from the Britain. This contradicts the idea of immigration of
470 Scots pine into Scotland from Ireland, especially if the latest reconstructions of the extent of
471 British – Irish ice sheet are considered. They clearly show that Ireland was totally covered by
472 the ice up to 21 ka BP while southern Britain was never covered by ice sheet during the last
473 glaciation (Hughes *et al.*, 2016).

474 In terms of possible colonization of Norway, the most likely routes include Danish
475 Straits, southern Sweden and further along the coast into Norway (Fig. 1B). The source
476 population would be located somewhere in Central Europe in which mitotype *a* and *b* are
477 present. The genetic homogeneity observed in the Norwegian populations would be then the
478 result of density – dependent processes (i.e. founder effect and/or gene surfing) invoked
479 recently as factor partly responsible for structuring genetic diversity (Waters *et al.*, 2013).
480 However, the genetic pattern found in Norway might reflect also the direct colonization from
481 western Europe (in where mitotype *a* dominates; Fig. 1A) throughout Jutland (Fig. 1B). This
482 route would assume also the existence of a land – bridge in the area of the current southern
483 North Sea that could have emerged due to a lowering of the sea level. Such land bridge would
484 have extended from southern Britain to Jutland and is referred to as Doggerland (Coles,
485 2000). There are palynological data suggesting the presence of tree species in this area
486 (*Betula*, *Pinus*) as early as 9 ky PB and numerous archaeological findings indicate human
487 occupation of Doggerland (Ward *et al.*, 2006). The existence of Doggerland is currently
488 considered to be significant for Mesolithic (early Holocene) dynamics of hunter – gatherers’
489 migrations (Peeters & Momber, 2014). Currently, due to uncertainty in the glacial history of
490 North Sea the role of Doggerland land – bridge in postglacial migrations of biota including
491 Scots pine is elusive but worth consideration in future studies.

492 The colonisation of Fennoscandia from more than one source implies mixing of
493 previously isolated gene pools that led to formation of a hybrid zone (Hewitt, 2001). The most
494 widely recognized feature of hybrid zones is an increased level of genetic diversity (Rius &
495 Darling, 2014; Havrdová *et al.*, 2015) and that was noted for Scots pine populations from
496 Fennoscandia in our data. Hence, based on these data we postulate that a hybrid zone for
497 Scots pine is located in the northern Scandinavian Peninsula and is formed by merging of two
498 colonisation routes. However, this contact zone is atypical when compared to others that have

499 been described (Dering & Lewandowski, 2009; Gömöry *et al.*, 2012). First, the hybrid zone
500 characterized with mitochondrial marker *nad1* b/c reflects the merging of different
501 colonisation fronts and not divergent genetic lineages that evolved in isolated refugia. This
502 result can be due to low resolution of the mtDNA marker used, that does not allow detection
503 of divergent mitochondrial refugial lineages of each colonisation wave. Considering the likely
504 wide and continuous occurrence of Scots pine during LPG (Svenning *et al.*, 2008), gene flow
505 could have been strong enough to prevent divergence in the case of refugial populations from
506 mid – latitudes that were the main sources during colonization of the northernmost areas. This
507 hypothesis is supported by the low genetic differentiation assessed with pollen – dispersed
508 cpSSR and the lack of geographic structure. Secondly, the boundary between the colonisation
509 waves forming the hybrid zone is quite sharp. This in turn suggests that, in northern part of
510 Scots pine range, seed dispersal into already colonized areas was limited.

511

512 **Concluding remarks**

513 The most striking result of this study is the discovery that the Tertiary relic
514 populations of Scots pine store lower amounts of genetic diversity in comparison to the
515 northern populations established in the LG period and the Holocene. Accordingly, special
516 attention should be paid to those rear – edge populations. They *per se* deserve protection in
517 the context of global biodiversity preservation (Hampe & Petit, 2005). Moreover, those
518 populations represent the oldest part of the species' gene pool that has evolved under the
519 dynamically changing Mediterranean environment, which produced notable diversity of that
520 region (Fady & Conord, 2010). This gene pool can be highly relevant for future performance
521 of Scots pine under ongoing environmental alterations driven by current global climate
522 changes. Although there is no consensus on how these changes will affect individual species
523 or whole ecosystems (Carnicer *et al.*, 2014), the widest spectrum of diversity should be

524 preserved because the rate of adaptation is driven by the amount of available additive genetic
525 variance and selection strength. Accordingly, the rapid adaptation of Scots pine to habitats of
526 the northern latitudes likely relied on standing genetic diversity (Savolainen *et al.*, 2011). In
527 the face of the climate predictions for the Mediterranean region, populations of Scot pine
528 present there may be seriously endangered by progressive contraction, which will pose a risk
529 that the unique gene pool may be lost. In fact, those relic populations contain lower numbers
530 of unique haplotypes in comparison to the northernmost populations. According to predictions
531 made by Benito Garzón *et al.* (2008), based on modelling of the future range of *Pinus*
532 *sylvestris*, a 95% decrease in the current species distribution in Spain is expected by the year
533 2080. Currently, the Iberian Scots pine populations are experiencing decline due to dieback
534 and low level of recruitment principally caused by summer drought (Gea – Izquierdo *et al.*,
535 2015). Accordingly, the performance of rear – edge populations of the species should be the
536 focus of long – term research aimed at monitoring of population dynamics.

537 With regard to the northernmost populations of Scots pine (the leading – edge
538 populations) their large amount of intraspecific diversity invites more complex thinking about
539 the evolution of the Scots pine range after glaciation. A high number of private cpDNA
540 haplotypes found in northernmost populations implies that the process of colonisation can be
541 a possible impulse for the generation of new genetic variants, at least for neutral genetic
542 diversity. With respect to adaptive variation, the situation is undoubtedly more complex, but
543 the impacts of range expansion and co – acting evolutionary processes are important as well
544 (Savolainen *et al.*, 2011; Alberto *et al.*, 2013). We found strong evidence for the existence of
545 a contact zone, which is a notable feature in the diversity pattern of Scots pine for two
546 reasons: (1) it has not yet been detected elsewhere in the species range and (2), due to general
547 evolutionary significance of hybrid zones for adaptive potential of species (Rius & Darling,
548 2014). Mixing of previously isolated lineages may produce novel allelic combinations that

549 may bring beneficial results for the species in newly colonized areas or under new
550 environmental conditions.

551 Based on the data presented here and investigations of Cheddadi *et al.* (2006), we
552 argue that apart from southernmost areas, Scots pine likely formed a set of patchily
553 distributed populations at mid – latitudes that were connected with gene flow extensive
554 enough to prevent strong genetic divergence. These populations were probably mostly active
555 recolonization. Hence, the classic concept of refugia worked out for thermophilous species is
556 not valid for Scots pine. Deeper examination of the mitochondrial genome could shed more
557 light on the phylogeography of Scots pine in central and north-eastern Europe.

558

559 Acknowledgments

560 We thank A. Chojnicka, A.K. Jasińska and M. Łuczak , J. Kamczyc, S. Marek, T. Ok, M.
561 Sękiewicz, D. Tomaszewski, K. Sobierajska, K. Ufnalski and B. Nihlgard for help in
562 collecting the material and laboratory works. The Authors thank A. Stroeven and B. Reinardy
563 for their helpful comments on the glacial history of Europe. This study was supported by the
564 National Science Centre (2011/02/A/NZ9/00108) and Institute of Dendrology, Polish
565 Academy of Sciences.

566 Biosketch

567 The authors are scientists interested in phylogeography, ecology and distribution of genetic
568 diversity of trees. Our multidisciplinary team investigates also the relationships between plant
569 traits and the changing climate in order to understand key plant controls of ecosystem
570 functioning, such as nutrient cycling and productivity.

571

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824 Fig. 1. Geographic distribution (A) of European populations of Scots pine (92 originally
825 sampled in this study and others for which data were obtained from Naydenov *et al.*
826 (2007), Pyhäjärvi *et al.* (2008), Vidyakin *et al.* (2012) and Buchovska *et al.* (2013),
827 see Appendix 1). Pie charts represent mitotypic composition of each population and
828 the frequency of the mitotypes. Each colour represents one of the five mitotypes
829 detected (green – mitotype *a*, yellow – mitotype *b*, red – mitotype *c*, blue – mitotype *d*
830 and light blue – mitotype *e*). The species' range is shaded. The distribution map of
831 Scots pine was provided by Euforgen, (B) reconstruction of possible migration routes
832 of Scots pine from its refugial areas located in middle latitudes. White arrows
833 represent the migration routes that need further support.

834

835 Supporting Information

836 Appendix S1

837 Data sources for mtDNA analysis.

838

839 Appendix S2

840 Geographic distribution of 248 European and Asiatic population of Scots pine (92 originally
841 sampled in this study and others for which data were obtained from Naydenov *et al.* (2007),
842 Pyhäjärvi *et al.* (2008), Vidyakin *et al.* (2012) and Buchovska *et al.* (2013), see Appendix 1)
843 analyzed in this study and mitotypes detected. Pie charts represent mitotypic composition of
844 each population and the frequency of the mitotypes. Each colour represents one of the five
845 mitotypes detected (green – mitotype *a*, yellow – mitotype *b*, red – mitotype *c*, blue –
846 mitotype *d* and light blue – mitotype *e*). The species' range is shaded. The distribution map of
847 Scots pine was provided by Euforgen.

848

849 Appendix S3

850 Alternative spatial population structure of 248 populations Scots pine at $K=7$ inferred with
851 SAMOVA based on mtDNA mitotypes.

852

852 Appendix S4

853 Geographic distribution of 13 Scots pine population clusters inferred with BAPS based on
854 four cpSSR used; each colour denotes one of the 13 clusters.

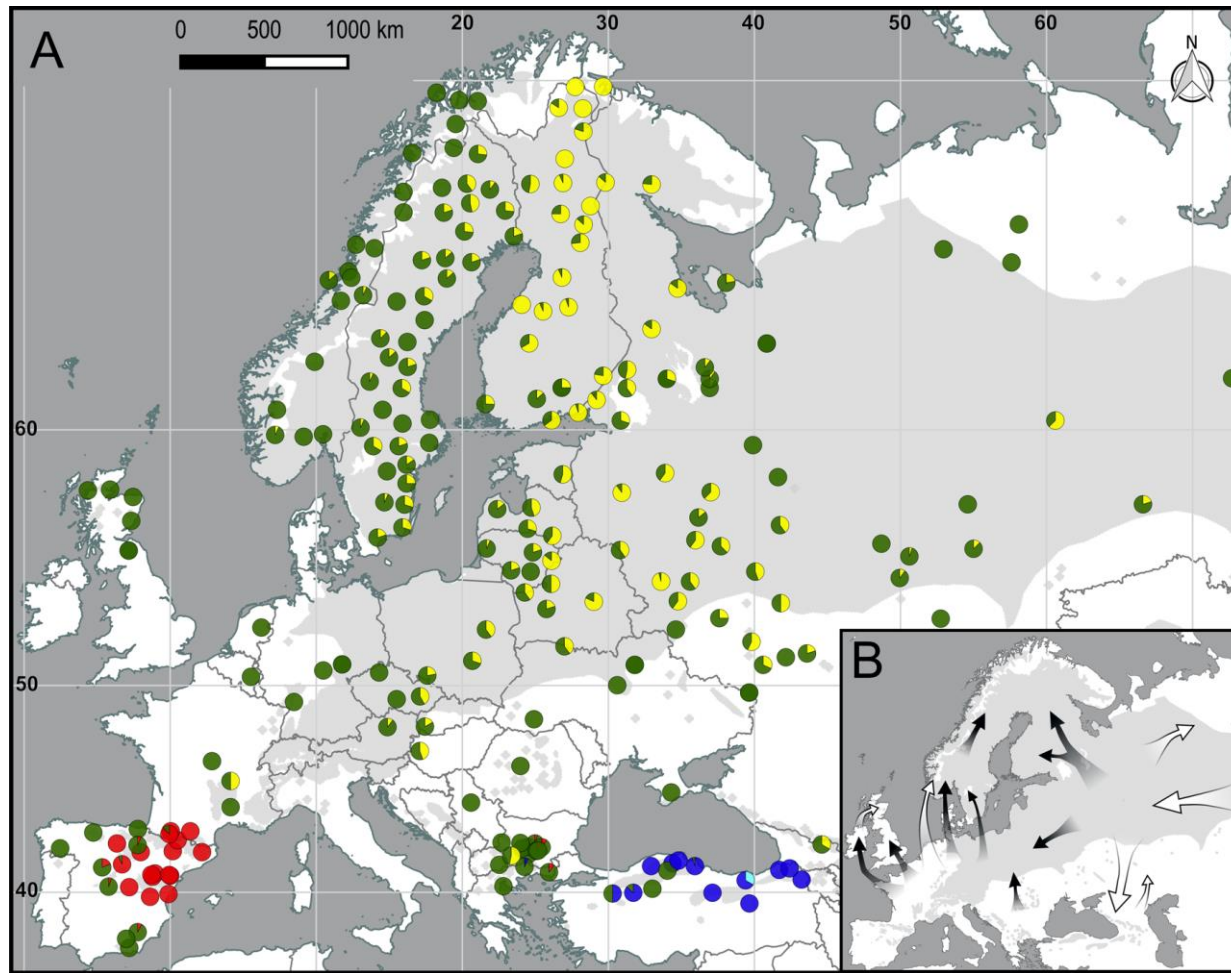


Fig. 1. Geographic distribution (A) of European populations of Scots pine (92 originally sampled in this study and others for which data were obtained from Naydenov *et al.* (2007), Pyhäjärvi *et al.* (2008), Vidyakin *et al.* (2012) and Buchovska *et al.* (2013), see Appendix 1). Pie charts represent mitotypic composition of each population and the frequency of the mitotypes. Each colour represents one of the five mitotypes detected (green – mitotype *a*, yellow – mitotype *b*, red – mitotype *c*, blue – mitotype *d* and light blue – mitotype *e*). The species' range is shaded. The distribution map of Scots pine was provided by Euforgen, (B) reconstruction of possible migration routes of Scots pine from its refugial areas located in middle latitudes. White arrows represent the migration routes that need further support.

Table 1. Locations of natural Scots pine populations sampled in this study, along with frequencies of mtDNA haplotypes and statistical characteristics of cpSSR haplotypes for each population (Norwegian populations not included). Regional and range-wide summaries are also shown.

Pop. code	Population name	Lat.	Long.	mt haplotypes					cpSSRs haplotypes						
				<i>N</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>H_{MT}</i>	<i>A</i>	<i>P</i>	<i>N_E</i>	<i>H_{CP}</i>	<i>D²_{sh}</i>
SW1	Sweden. Övraryd	56.423	14.218	15	12	3				0.343	8	1	3.169	0.733	1.067
SW2	Sweden. Dalem, Askersund	58.857	14.864	15	15					0	11	2	7.759	0.933	1.924
SW3	Sweden. Ekopark Kilsbergen	59.424	15.036	15	12	3				0.343	8	0	4.592	0.838	0.829
SW4	Sweden. Spånsfäbodarna	60.364	15.181	15	15					0	7	0	3.082	0.724	0.610
SW5	Sweden. Furundal, Dalbyn	61.095	15.257	15	10	5				0.476	8	2	5.488	0.876	1.062
SW6	Sweden. Näsberg	61.791	15.647	15	12	3				0.343	7	0	3.689	0.781	0.686
SW7	Sweden. Ånge	62.587	15.631	15	15					0	11	1	9.000	0.952	1.571
SW8	Sweden. Mo	63.270	16.820	15	15					0	8	0	3.814	0.790	0.862
SW9	Sweden. Ö.Gafsele	64.002	17.401	15	10	5				0.476	11	0	7.759	0.933	1.057
SW10	Sweden. Bratten	64.529	18.336	15	13	2				0.247	5	1	3.358	0.752	0.800
SW11	Sweden. Järvträsk	65.127	19.466	15	13	2				0.247	8	1	3.169	0.733	1.019
SW12	Sweden. Maskosel	65.881	19.544	15	11	4				0.419	10	1	6.818	0.914	1.333
SW13	Sweden. Jokkmokk	66.662	19.947	15	8	7				0.533	5	0	4.091	0.810	0.557
SW14	Sweden. Koskullskulle	67.187	20.956	15	9	6				0.514	8	0	5.233	0.867	0.748
SW15	Sweden. Jukkasjärvi	67.969	20.460	15	11	4				0.419	5	0	3.358	0.752	0.367
SW16	Sweden. Abisco	68.350	18.801	15	15					0	9	1	4.787	0.848	1.157
SW17	Sweden. Angesan	66.704	22.334	15	11	4				0.419	9	2	5.488	0.876	0.981
SW18	Sweden. Kamlunga	65.998	22.925	15	12	3				0.343	8	0	5.233	0.867	0.714
SW19	Sweden. Örbyhus	60.336	17.808	15	15					0	5	0	2.103	0.562	0.205
SW20	Sweden. Hornö	59.539	17.136	15	15					0	5	1	3.689	0.781	0.667
SW21	Sweden. Kloten	59.902	15.297	15	15					0	3	0	2.528	0.648	0.262
SW22	Sweden. Rottneros	59.763	13.047	15	14	1				0.134	9	0	7.258	0.924	1.019
SW23	Sweden. Vålberg	59.430	13.295	15	10	5				0.476	7	0	4.245	0.819	0.771
SW24	Sweden. Berg	57.075	14.689	15	14	1				0.134	6	0	4.412	0.829	0.900
SW25	Sweden. Kvikjokk I	66.894	18.746	15	12	3				0.343	8	2	4.592	0.838	1.038

SW26	Sweden. Kvikjokk II	67.081	18.617	15	15		0	7	1	4.412	0.829	0.886
SW27	Sweden. Stensele	65.068	17.237	15	12	3	0.247	7	0	4.245	0.819	0.657
SW28	Sweden. Jörn	65.003	20.035	15	15		0	10	0	7.258	0.924	1.238
SW29	Sweden. Stromsund	63.837	14.893	15	15		0	8	0	5.233	0.867	0.762
SW30	Sweden. Asarna	62.670	14.379	15	13	2	0.247	12	4	10.714	0.971	1.890
SW31	Sweden. Fulunas	61.307	13.058	15	14	1	0.134	7	1	5.233	0.867	0.986
FIN1	Finland. Loppi	60.740	24.497	15	13	2	0.247	7	0	4.412	0.829	0.876
FIN2	Finland. Ähtäri	62.546	23.963	15	5	10	0.476	7	0	4.245	0.819	1.071
FIN3	Finland. Leppälahti	63.542	24.932	15	1	14	0.134	3	0	1.991	0.533	0.176
FIN4	Finland. Kestilä	64.561	26.219	15	1	14	0.134	7	2	4.592	0.838	1.519
FIN5	Finland. Metsäla	65.566	27.470	15	4	11	0.419	6	0	2.922	0.705	0.471
FIN6	Finland. Posio	66.073	27.704	15	2	13	0.247	6	0	2.143	0.571	0.610
FIN7	Finland. Paloperä	66.583	28.173	15		15	0	7	1	3.082	0.724	0.700
FIN8	Finland. Näätämö	69.634	29.052	15		15	0	6	0	2.528	0.648	0.367
FIN9	Finland. Inarijärvi	69.107	27.643	15		15	0	7	1	3.082	0.724	0.710
FIN10	Finland. Alajärvi	68.538	27.687	15	3	12	0.343	8	0	4.592	0.838	0.719
FIN11	Finland. Vajunen	67.844	26.416	15		15	0	8	1	6.081	0.895	1.919
FIN12	Finland. Raudanjoki	67.209	26.294	15	1	14	0.134	8	1	6.818	0.914	1.400
FR1	France. Col de la Croix de Morand	45.150	03.350	16	8	7	0.533	8	1	3.459	0.758	1.108
FR2	France. Col de la Croix de Bor	44.760	03.530	15	15		0	7	0	5.769	0.886	0.848
NOR1	Norway. Skibotn I	69.313	20.389	15	15		0	-	-	-	-	-
NOR2	Norway. Skibotn II	69.290	20.438	15	15		0	-	-	-	-	-
NOR3	Norway. Vikran	69.485	18.875	15	15		0	-	-	-	-	-
NOR4	Norway. Rundhaug	68.950	18.937	15	15		0	-	-	-	-	-
NOR5	Norway. Innhøvet	67.988	15.971	15	15		0	-	-	-	-	-
NOR6	Norway. Evensgård	66.971	15.369	15	15		0	-	-	-	-	-
NOR7	Norway. Bleiknes	66.971	15.365	15	15		0	-	-	-	-	-
NOR8	Norway. Svenningdal	65.410	13.378	15	15		0	-	-	-	-	-
NOR9	Norway. Trofors	65.511	13.375	15	15		0	-	-	-	-	-
NOR10	Norway. Snåsa	64.296	12.599	15	14	1	0.134	-	-	-	-	-
NOR11	Norway. Namsos I	64.481	11.623	15	13	2	0.247	-	-	-	-	-
NOR12	Norway. Namsos II	64.485	11.700	15	15		0	-	-	-	-	-
NOR13	Norway. Namsos II	64.485	11.623	15	15		0	-	-	-	-	-
NOR14	Norway. Salsnes	64.653	11.445	15	15		0	-	-	-	-	-

NOR15	Norway. Dovre	61.958	09.280	15	15		0	-	-	-	-	-
NOR16	Norway. Kinsarvik	60.374	06.709	15	15		0	-	-	-	-	-
NOR17	Norway. Tyssedal	60.118	06.576	15	14	1	0.134	-	-	-	-	-
NOR18	Norway. Brunkeberg	69.444	08.453	15	15		0	-	-	-	-	-
NOR19	Norway. Passebekk	59.527	09.589	15	15		0	-	-	-	-	-
	Average											
	Central and northern Europe						0.156	7.44	0.622	4.700	0.807	0.914
BG	Bulgaria. Pirin. Bansko-Razlog	41.880	23.360	15	7	8	0.533	4	0	2.848	0.695	0.538
GR1	Greece. Pieria Ori (Elatothori)	40.310	22.200	15	15		0	7	0	3.571	0.771	0.567
GR2	Greece. Ano Vrandou	41.310	23.650	15	14		0.134	6	1	3.261	0.743	0.619
SR	Serbia. Divcibare Mts.	44.100	19.990	15	15		0	6	0	2.143	0.571	0.490
A	Andora. St.Miguel d'Engolasters	42.520	01.570	14		15	0	8	0	5.158	0.868	0.780
SP1	Spain. Pyrennees	42.670	00.770	15		15	0	6	1	3.261	0.743	0.933
	Spain. Sierra de Gudar. Puerto de San Rafael	40.360	-00.720	15		15	0	8	0	6.818	0.914	1.281
SP3	Spain. Sierra de Gudar. Valldelinares	40.380	-00.610	15		15	0	7	1	3.082	0.724	1.986
SP4	Spain.Sierra de Neila	42.050	-03.010	15		15	0	8	2	5.233	0.867	1.195
SP5	Spain. Sierra de Cebollera. Lago Negro	42.070	-02.630	15	1	14	0.134	6	0	3.814	0.790	0.510
SP6	Spain. Pyreneese. San Juan de la Pena	42.510	-00.660	15	2	13	0.247	9	1	4.787	0.848	1.133
SP7	Spain. Sierra de Urbion	41.970	-02.830	15	14	1	0.134	4	0	2.368	0.619	0.233
SP8	SpainVirgala Menor. Vitoria-Gasteiz	42.780	-02.810	15	15		0	7	0	3.082	0.724	0.500
SP9	Spain. Puerta de Navafria	40.980	-03.810	15	1	14	0.134	7	0	3.571	0.771	0.500
SP10	Spain. Cuenca. Una	40.220	-01.980	15		15	0	7	0	3.689	0.781	0.762
SP11	Spain. Cuenca. Vega del Codoro	40.440	-01.900	16		16	0	8	1	4.741	0.842	1.365
SP12	Spain. Sierra de Baza	37.370	-02.850	15	15		0	4	0	2.027	0.543	0.229
P	Portugal. Sierra de Geres	41.800	-08.130	15	15		0	5	0	3.689	0.781	0.943
TR1	Turkey. Gerede-K	40.640	32.410	15	15		0	7	0	3.082	0.724	0.886
TR10	Turkey. Kars-Erzurm	40.180	42.630	15		15	0	7	0	3.429	0.773	0.708
TR2	Turkey. Tokat--Y	39.960	36.52	15		15	0	6	0	3.462	0.762	2.390
TR3	Turkey. Catack	39.960	31.110	15	2	13	0.247	7	0	3.571	0.771	3.062
TR4	Turkey. Tosya Ilgaz	41.120	34.060	15	15		0	8	0	3.169	0.733	2.919
TR5	Turkey. Bayabat-Sinop	41.640	34.830	15		15	0	6	1	3.814	0.790	2.100
TR6	Turkey. Sakaltutan Gecidi	39.870	39.050	15		15	0	6	0	2.528	0.648	0.771
TR7	Turkey. Gumushane. Limni Golu	40.610	39.410	15		10	0.476	6	0	3.000	0.714	0.538

TR8	Turkey. Artvin	41.150	41.760	15			15	0	4	0	3.169	0.733	0.333
TR9	Turkey. Savsat-Ardahan	41.230	42.430	17			17	0	6	0	3.568	0.765	0.610
	Mediterranean							0.0728	6.428	0.286	3.569	0.750	1.031
Total	All populations			878	237	148	116	5		36			
Mean								0.176	7.055		4.267	0.786	0.959
Std									1.77		1.68	0.099	0.572

N – sample size, *H_{MT}* – mitotype diversity, *A* – number of haplotypes, *P* – number of private haplotypes, *N_E* – effective number of haplotypes, *H_{CP}* – haplotype diversity, *D²_{sh}* – mean genetic distance between individuals

Table 2. Nine most common haplotypes detected in 73 natural populations of Scots pine analysed in this study with 4 cpSSR loci.

Label	Haplotype	Counts	Frequency
H1	105/112/117/110	459	0.419
H2	105/112/117/109	111	0.101
H3	106/112/117/110	87	0.079
H4	105/113/117/110	70	0.063
H5	104/112/117/110	53	0.048
H6	105/112/117/111	52	0.047
H7	104/113/117/110	27	0.025
H8	105/111/117/110	23	0.021
H9	104/112/117/109	22	0.020
Rare haplotypes		191	0.174

Table 3. Spatial population structure of 248 Scots pine populations inferred with SAMOVA based on mtDNA mitotypes at $K = 4$ ($F_{CT} = 0.8202$). The literature data included in this analysis are listed below the names population sampled in this study.

K - groups	Populations	Country	F_{CT}
$K = 4$			$0.8202, p < 0.001$
I	SW1-SW31, NOR1-NOR19, FIN1, FIN2, FR1, FR2, GR1, GR2, BG, SR, SP8, SP7, SP12, P, TR1,TR4 Naydenov et al. 2007: 1-13, 20-29, 32-51, 53 Pyhäjärvi et al. 2008: 3, 5, 7-9, 11-13, 15, 17-29, 35-37 Vidyakin et al. 2012 : 1-15 Buchovska et al. 2013 : 1-12, 14,15,17-32, 34, 35, 37-44, 46-48, 50-54	Sweden, Norway, Finland France, Spain, Greece Bulgaria, Turkey, Russia, Czech Republic, Poland, Lithuania, Belarus, Ukraine	
II	FIN3-FIN12 Naydenov et al. 2007: 17-19, 30, 31 Pyhäjärvi et al. 2008: 1, 2, 4, 6, 10, 14, 16 Buchovska et al. 2013 : 16, 36, 45, 49	Finland, Russia	
III	TR2, TR5-TR10 Naydenov et al. 2007: 15, 16	Turkey	
IV	A, SP1-SP6, SP9-SP11, TR3, Naydenov et al. 2007: 14, 52, 54 Pyhäjärvi et al. 2008: 30-32, 34	Spain, Andorra, Turkey	

Table 4. Spatial population structure of 73 Scots pine populations inferred with BAPS on the basis of 4 cpSSR loci.

Cluster number	Populations	Geographic origin
I	SW10, SW15, SW21	Sweden
II	SW9, SW22, SW23, SW26, SW28, SW29, FIN1	Sweden, Finland
III	SW5, SW6, SW17, SW25, SW31, FIN6, TR4, TR6, SP11	Sweden, Finland, Turkey, Spain
IV	SW2, SW12	Sweden
V	SW24, SW27, FIN4, SP3	Sweden, Finland
VI	SW4, SW11, SW19, FIN7-FIN10, A, P, SP6, SP8, SP12, TR10	Sweden, Finland, Portugal, Andora, Spain, Turkey
VII	SW13, FIN3, FIN5, FR1, GR1, TR2	Sweden, Finland, France, Greece, Turkey
VIII	SW1, SW3, SW7, SW8, SW16, SW18, SP2, SP5, SP7, SP9, FR2, BG, GR2, TR1	Sweden, Spain, France, Bulgaria, Greece, Turkey
IX	SP10	Spain
X	SW14, FIN2, FIN12, TR3, TR5, TR7, TR8, TR9	Sweden, Finland, Turkey
XI	SW20, FIN11, SP4	Sweden, Finland, Spain
XII	SW30, SP1	Sweden, Spain
XIII	SR	Serbia

Table 5. Hierarchical analysis of molecular variance (AMOVA) based on four cpSSRs for 73 Scots pine populations.

Source of variation	Degrees of freedom	Sum of squares	% of total variation	Phi	<i>p-value</i>
SAMOVA clusters					
Among clusters	3	14.624	1	0.012	0.002
Among pop. within groups	69	122.628	4	0.039	0.001
Within-pop.	1022	1262.405	95	0.051	0.001
Mediterranean. vs. Fennoscandia					
Among regions	1	12.584	2	0.017	0.001
Among pop. within groups	71	124.704	4	0.038	0.001
Within-pop.	1022	1125.153	95	0.055	0.001

Appendix 1 – Data sources for mtDNA analysis

Pyhäjärvi et al. 2008				Mitotype			
Population	N	Latitude	Longitude	a	b	c	d
1. Finland, Utsojki	23	69.850	27.005	0	23	0	0
2. Finland, Inari	19	69.005	27.189	3	16	0	0
3. Finland, Kolari	34	67.173	24.008	16	18	0	0
4. Finland, Salla	15	67.174	29.171	2	13	0	0
5. Sweden, Lejporova	10	67.005	21.185	9	1	0	0
6. Finland, Rovaniemi	12	66.341	26.680	3	9	0	0
7. Russia, Usinsk	15	66.014	57.500	15	0	0	0
8. Russia, Ust-Chilma	15	65.341	52.336	15	0	0	0
9. Russia, Archangiel region	9	64.666-61.170	38.014-46.500	7	2	0	0
10. Finland, Kalvia	29	63.680	24.014	0	29	0	0
11. Sweden, Hede flygplats	7	62.671	13.355	6	1	0	0
12. Russia, Karelia region	5	61.669-62.836	30.017-36.666	3	2	0	0
13. Russia, Surgut	16	61.347	73.333	16	0	0	0
14. Russia, St. Petersburg region	19	61.000-58.669	28.516-33.500	2	17	0	0
15. Finland, Uusikaupunki	35	60.838	21.333	26	9	0	0
16. Finland, Lapinjarvi	17	60.519	26.022	6	11	0	0
17. UK, Eastern Scotland	10	57.008	03.184	10	0	0	0
18. UK, Scotland, Achnashellah	3	57.503	05.519	3	0	0	0
19. Sweden, Norra-Gullabo	30	56.355	15.847	21	9	0	0
20. Russia, Moscow region	20	56.333-54.844	35.336-39.003	8	12	0	0

21. Lithuania, Nemencine	14	54.833	25.500	12	2	0	0
22. Holland, Gruppenvorst	30	52.017-52.500	05.525-06.352	30	0	0	0
23. Belgium, Sant-Custine, Harre	20	49.852-50.333	04.844'-05.522	20	0	0	0
24. Germany, Teisendorf, Bavaria	18	50.250	10.500	18	0	0	0
25. Poland, Radom	30	50.668	20.014	21	9	0	0
26. Slovakia	30	47.677-49.319	16.833-22.511	25	5	0	0
27. France, Hagenau	30	48.835	07.838	30	0	0	0
28. Austria, Oberlroidorf	18	47.346	16.358	8	10	0	0
29. Spain, Baza	26	37.338	-02.833	24	0	2	0
30. Spain, Borau	21	42.667	-00.511	0	0	21	0
31. Spain, Bronchales	7	40.500	-01.525	0	0	7	0
32. Spain, Gudar	22	40.347	-00.669	0	0	22	0
33. Spain, Javierregay	29	42.514	-00.347	0	0	29	0
34. Spain, Morrano	22	42.178	-00.017	0	0	22	0
35. Spain, Publa de Lillo	8	43.003	-05.181	8	0	0	0
36. Spain, Valsain	16	40.691	-04.005	13	0	3	0
37. Turkey, Kalabak	30	39.352	30.192	15	0	0	15
Naydenov et al. 2007							
				Mitotype			
Population	N	Latitude	Longitude	a	b	c	d
1. Bulgaria, Bansko	16	41.688	23.500	16	0	0	0
2. Bulgaria, Borovo	18	41.500	23.847	18	0	0	0
3. Bulgaria, Chehliovo	19	41.841	23.847	19	0	0	0
4. Bulgaria, Chiroka Laka	20	41.684	24.514	20	0	0	0

5. Bulgaria, Dospat	10	41.667	24.500	9	0	1	0
6. Bulgaria, Laki	20	41.850	24.833	18	0	2	0
7. Bulgaria, Nevestino	20	42.017	22.665	20	0	0	0
8. Bulgaria, Pechtera	20	42.008	24.338	19	0	1	0
9. Bulgaria, Simitli	20	41.841	23.170	20	0	0	0
10. Bulgaria, Smolian	20	41.500	25.333	18	0	2	0
11. Bulgaria, Velingrad	20	42.014	24.000	20	0	0	0
12. Austria, Grosser Priel	18	47.672	14.189	18	2	0	0
13. Austria, Merkenstein	19	48.855	16.022	19	0	0	0
14. Turkey, Ilgaz Gakdake	18	41.005	33.519	0	0	0	18
15. Turkey, Eskipazan	20	40.841	32.333	0	0	0	20
16. Turkey, Ulupihar	20	40.841	35.333	1	0	0	19
17. Russia, Zelenoborsk	17	67.170	32.341	4	13	0	0
18. Russia, Sosnovec	20	64.500	34.681	3	17	0	0
19. Russia, Suchozero	20	63.000	32.341	3	17	0	0
20. Russia, Shala	19	61.686	36.000	17	2	0	0
21. Russia, Sortovala	15	61.684	30.684	7	8	0	0
22. Romania, Cugir seed orchard	14	45.838	23.341	14	0	0	0
23. Czech Republic, Kuřim Tisov	12	49.500	16.500	7	5	0	0
24. Czech Republic, Lužná Olešná	11	50.170	13.700	11	0	0	0
25. France, Murat	9	45.017	02.184	9	0	0	0
26. UK, Balnagowan Wood	14	57.187	03.025	14	0	0	0
27. UK, Morayshire	20	57.508	03.358	20	0	0	0
28. Sweden, Hallestad District	13	58.684	15.514	11	2	0	0
29. Sweden, Rumusulla District	20	57.670	15.514	15	5	0	0

30. Finland, Kiuruvesi	19	63.667	26.667	1	18	0	0
31. Finland, Vehkalahti	20	60.514	27.333	1	19	0	0
32. Russia, Voronezh	19	50.500	40.000	13	6	0	0
33. Russia, Orlovsk	20	52.500	37.000	15	5	0	0
34. Lithuania, Kaunas	20	54.681	24.014	20	0	0	0
35. Latvia, Riga	20	56.841	24.022	11	9	0	0
36. Russia, Krasnoyarsk	20	60.000	90.000	20	0	0	0
37. Russia, Sprinsk	20	54.000	81.000	20	0	0	0
38. Lithuania, Vilnus	20	54.522	25.355	10	10	0	0
39. Russia, Krasnoe	20	54.000	86.333	20	0	0	0
40. Lithuania, Rokiskis	20	55.689	25.508	8	12	0	0
41. Russia, Kaluzhkaya Ob.	20	54.000	35.000	12	8	0	0
42. Russia, Tatarskaya Ob.	20	55.000	50.000	19	1	0	0
43. Lithuania, Dainava	20	53.847	23.667	12	8	0	0
44. Russia, Novosibirsk	20	55.014	82.681	16	4	0	0
45. Ukraine, Kievskaya Ob.	20	50.000	30.000	20	0	0	0
46. Belarus, Groendal	18	50.833	42.170	18	0	0	0
47. China, Baiyina-Heilongjiang	17	52.336	125.667	17	0	0	0
48. China, Jilin Prov.	17	43.000	126.000	17	0	0	0
49. China, Sung-Hua-Chiang	17	46.000	127.000	17	0	0	0
50. China, Heilongjiang Prov.	20	47.000	127.000	20	0	0	0
51. Spain, Sierras Peribeticas	18	37.333	-02.833	18	0	0	0
52. Spain, Montes Universales	19	40.333	-01.833	0	0	19	0
53. Spain, Guadarrama	19	40.681	-05.014	18	0	1	0
54. Spain, Alto Tago	18	40.678'	-02.170	0	0	18	0

Buchovska et al. 2013							
				Mitotype			
Population	N	Latitude	Longitude	a	b	c	d
1. Belarus, Gardin	10	53.347	25.184	8	2	0	0
2. Estonia	11	58.170	26.355	5	6	0	0
3. Lithuania, Kazlų Rūda	10	54.681	23.514	8	2	0	0
4. Lithuania, Ignalina	5	55.187	25.684	4	1	0	0
5. Lithuania, Mažeikiai	7	57.184	22.667	6	1	0	0
6. Lithuania, Neringa	31	55.503	21.017	29	2	0	0
7. Latvia	10	56.672	25.170	7	3	0	0
8. Czech Republic, V. Chvojno	9	50.008	16.858	7	2	0	0
9. Poland, Białystok	10	52.005	21.008	6	4	0	0
10. Ukraine, Lviv	9	48.019	24.500	9	0	0	0
11. Ukraine, Cherkasy	5	49.352	39.008	5	0	0	0
12. Georgia, Boržomsk	9	42.000	44.000	6	3	0	0
13. Russia, Smolensk	10	54.000	33.000	7	3	0	0
14. Russia, Kalinin	9	57.681	36.667	6	3	0	0
15. Russia, Moscow	8	55.667	37.170	3	5	0	0
16. Russia, Vladimir	10	56.336	41.184	6	4	0	0
17. Russia, Kostroma	8	58.000	41.000	8	0	0	0
18. Russia, Riazan	9	54.667	39.681	4	5	0	0
19. Russia, Briansk	10	53.333	34.184	4	6	0	0
20. Russia, Uljanovsk	10	54.181	49.514	9	1	0	0
21. Russia, Tatarstan	6	55.833	48.025	6	0	0	0

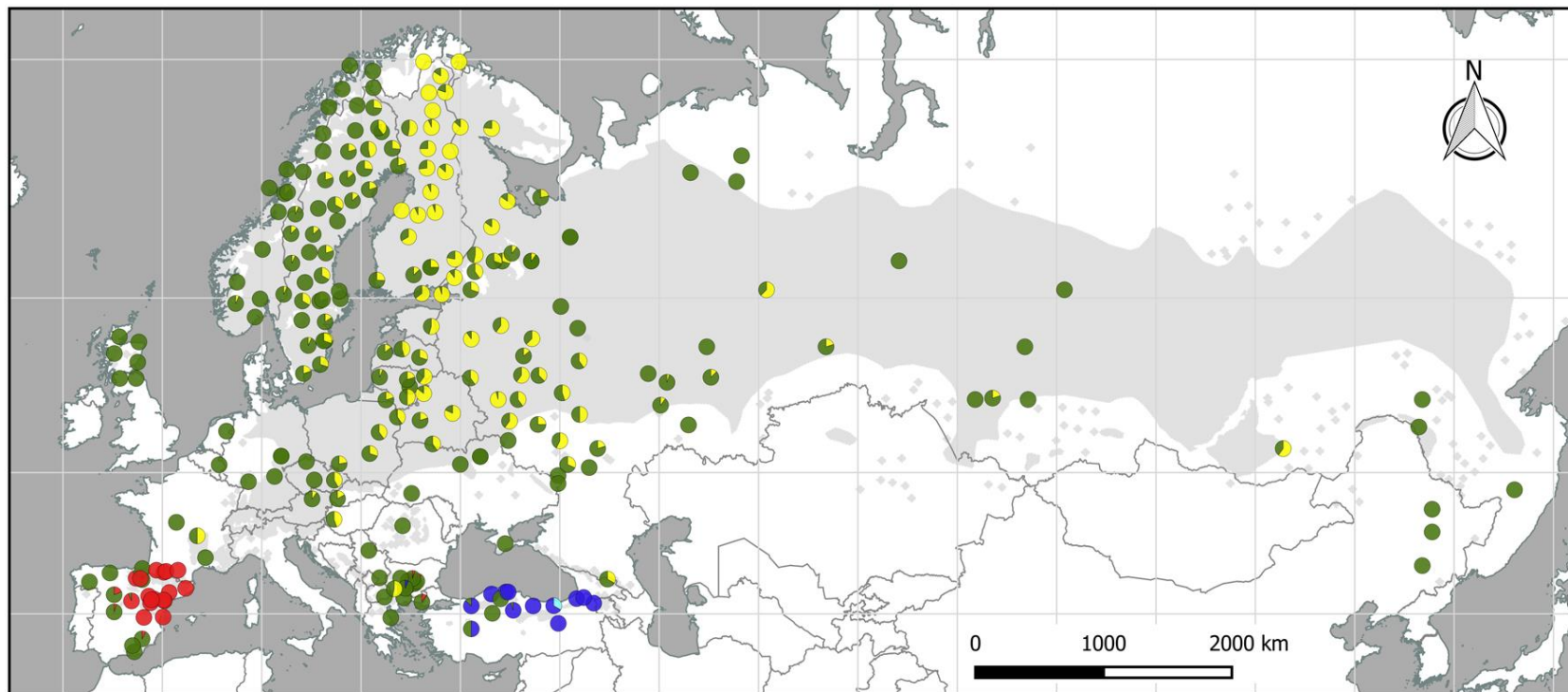
22. Russia, Tver	7	56.833	35.850	6	1	0	0
23. Russia, Udmurtia	9	57.000	54.000	9	0	0	0
24. Russia, Bashkiriya	16	55.500	54.667	14	2	0	0
25. Russia, Orenburg	8	52.686	52.184	8	0	0	0
26. Russia, Karpinsk	8	60.000	60.000	3	5	0	0
27. Russia, Tiumensk	5	57.000	66.000	4	1	0	0
28. Russia, Chararovsk	10	48.508	135.355	10	0	0	0
29. Russia, Tygdinsk	8	54.000	126.000	8	0	0	0
30. Russia, Tuganski	6	57.000	86.000	6	0	0	0
31. Russia, Ingodinsk	5	51.000	112.00	2	3	0	0
32. Russia, Kozvinsk	3	65.000	57.000	3	0	0	0
33. Russia, Tuusula	8	61.173	26.333	6	2	0	0
34. Russia, Karelia	10	61.667	33.667	7	3	0	0
35. Russia, Karelia	9	61.833	33.355	2	7	0	0
36. Russia, Karelia	10	61.667	36.508	9	1	0	0
37. Russia, Leningrad	10	60.000	30.347	7	3	0	0
38. Russia, Archangesk	10	62.54	40.344	10	0	0	0
39. Russia, Volodga	10	59.184	39.500	10	0	0	0
40. Russia, Tambov	10	53.175	41.333	5	5	0	0
41. Russia, Voronezh	7	51.833	39.333	3	4	0	0
42. Russia, Michailovsk	5	51.000	43.000	4	1	0	0
43. Russia, Magiliov	11	53.192	28.667	2	9	0	0
44. Ukraine, Kijev	7	50.833	31.333	7	0	0	0
45. Ukraine, Sumsk	10	52.003	34.003	10	0	0	0
46. Ukraine, Rovno	10	51.500	26.667	6	4	0	0

47. Belarus, Vitebsk	10	55.347	30.333	6	4	0	0
48. Russia, Pskov	10	57.43	30.503	1	9	0	0
49. Russia, Novograd	10	58.184	33.355	4	6	0	0
50. Sweden, Kalmar	10	57.505	15.681	7	3	0	0
51. Germany, Erfurt	9	50.838	11.189	9	0	0	0
52. UK, Scotland	7	55.341	03.678	7	0	0	0
Vidyakin et al. 2012							
				Mitotype			
Population	N	Latitude	Longitude	a	b	c	d
1. Kirov Region, Kirsinsk forestry	22	59.173	52.352	22	0	0	0
2. Kirov Region, Pischalsk forestry	22	58.000	48.841	22	0	0	0
3. Kirov Region, Nizhne-Ivkino forestry	22	58.170	49.850	22	0	0	0
4. Kirov Region, Mukhinsk forestry	22	58.017	51.017	22	0	0	0
5. Kirov Region, Ezhihinskii forestry	22	58.000	47.667	22	0	0	0
6. Chuvash Republic, Kirovsk forestry	24	55.025	46.847	24	0	0	0
7. Mari El Republic, Kokshaisk forestry	24	56.187	47.688	24	0	0	0
8. Kirov Region, Lalsk forestry	21	60.675	47.338	21	0	0	0
9. Komi Republic, Kadzherom forestry	21	64.675	55.670	21	0	0	0
10. Arkhangelsk Region, Obozersk forestry	22	63.352	40.195	22	0	0	0
11. Arkhangelsk Region, Shulakushskii forestry	24	62.181	40.192	24	0	0	0
12. Arkhangelsk Region, Konoshskii forestry	23	60.847	40.689	23	0	0	0
13. Vologda Region, Vologda forestry	21	59.178	40.000	17	4	0	0

14. Arkhangelsk Region, Pravodvinsk forestry	23	61.850	45.841	23	0	0	0
15. Kostroma Region, Pyschug forestry	23	58.850	45.522	23	0	0	0

Appendix S2

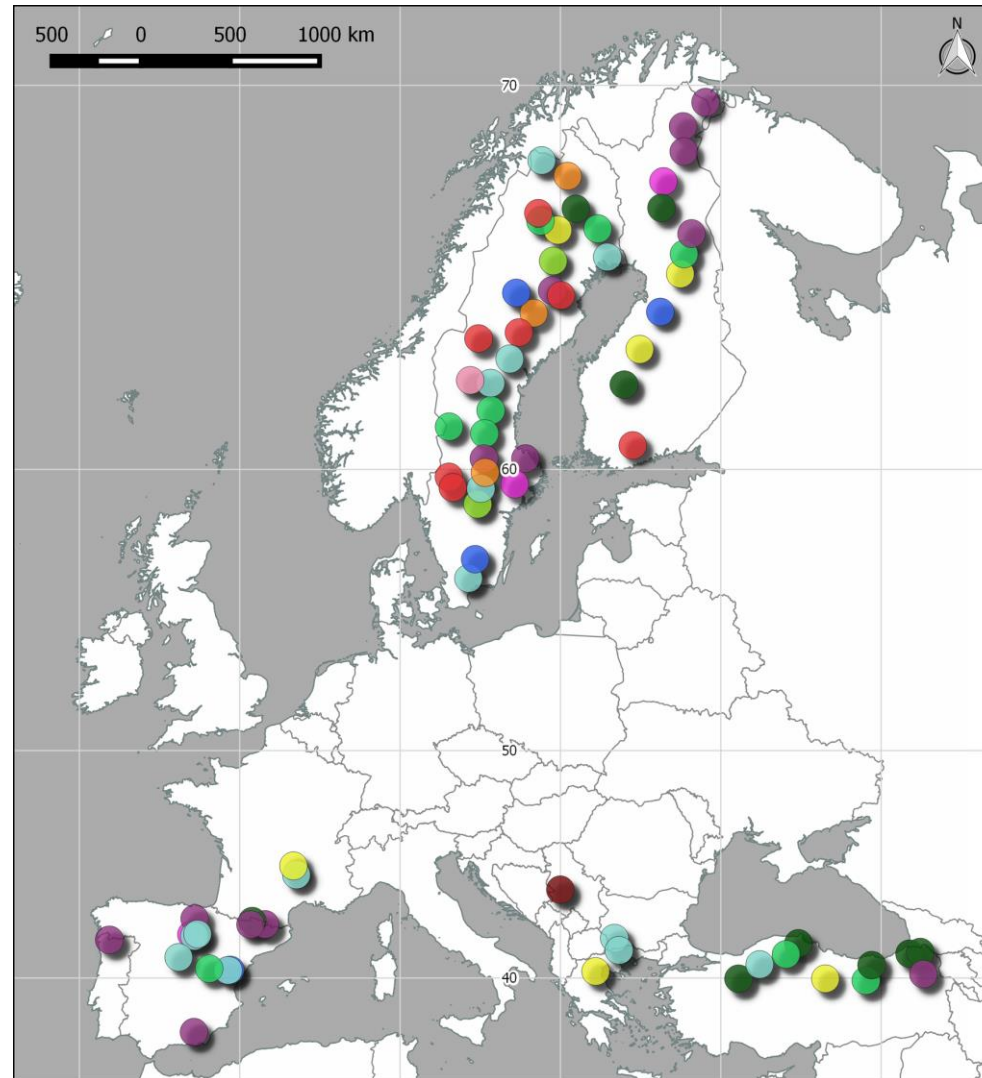
Geographic distribution of 248 European and Asiatic population of Scots pine (92 originally sampled in this study and others for which data were obtained from Naydenov *et al.* (2007), Pyhäjärvi *et al.* (2008), Vidyakin *et al.* (2012) and Buchovska *et al.* (2013), see Appendix 1) analyzed in this study and mitotypes detected. Pie charts represent mitotypic composition of each population and the frequency of the mitotypes. Each colour represents one of the five mitotypes detected (green – mitotype *a*, yellow – mitotype *b*, red – mitotype *c*, blue – mitotype *d* and light blue – mitotype *e*). The species' range is shaded. The distribution map of Scots pine was provided by Euforgen.



Appendix S3. Alternative spatial population structure of 248 populations Scots pine at $K=7$ inferred with SAMOVA based on mtDNA mitotypes.

K-groups	Populations	Country	Fct
K= 7			0.8214, $p<0.001$
I	NOR 1-NOR13, NOR15, NOR17-NOR19, SW2-SW8, SW10-SW12, SW14-SW31, FIN1, SP7, SP8, SP12, P, GR1, GR2, RS, TR1, TR4 Naydenov et al. 2007: 1-13, 20, 22 -29, 32-34, 36, 37, 39, 42, 44-46, 48-51, 53 Pyhäjärvi et al. 2008: 5, 7, 8, 11, 13, 15, 17-18, 21-27, 29, 35, 36 Vidyakin et al. 2012 : 1-15 Buchovska et al. 2013: 2, 5-8, 10, 14, 16-18, 21, 23-25, 28-32, 34, 35, 37, 38, 40, 42, 41, 44, 46, 47, 48, 53, 54	Norway, Sweden, Finland, Austria, Greece, Russia, Holland, Belgium, Spain, Turkey, Poland, Slovakia, Romania, Ukraine, Czech Republic, Estonia, Belarus, United Kingdom,	
II	FIN3-12 Naydenov et al. 2007: 17-19, 30, 31 Pyhäjärvi et al. 2008: 1, 2, 4, 6, 10, 14 Buchovska et al. 2013: 36, 45, 49	Finland, Russia (Karelia), Belarus	
III	SW1, SW21 Naydenov et al. 2007: 47 Buchovska et al. 2013: 3, 22, 39	Sweden, China, Belarus, Russia	
IV	TR2, TR3, TR5 -TR10 Naydenov et al. 2007: 14-16	Turkey	

V	<p>SP1-SP6, A, SP10-SP11</p> <p>Naydenov et al. 2007: 52, 54</p> <p>Pyhäjärvi et al. 2008: 30-32, 34</p>	<p>Spain, Andorra</p>
VI	<p>NOR14, NOR16</p> <p>Buchovska et al. 2013: 4, 9, 12, 26, 52</p> <p>Pyhäjärvi et al. 2008: 136</p>	<p>Norway, Russia, Lithuania, Ukraine, Sweden</p>
VII	<p>SW9, SW13, FIN2, FR, BG</p> <p>Naydenov et al. 2007: 21, 35, 38, 40, 41, 43</p> <p>Pyhäjärvi et al. 2008: 3, 12, 16, 19, 20, 28, 37</p> <p>Buchovska et al. 2013 : 1, 11, 15, 19, 20, 27, 42, 43, 50, 51</p>	<p>Sweden, Finland, Poland, Lithuania, Estonia, Latvia, Belarus, Russia, Austria, Turkey, France, Bulgaria,</p>



Appendix S4. Geographic distribution of 13 Scots pine population clusters inferred with BAPS based on four cpSSR used; each colour denotes one of the 13 clusters.