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1 **Mitochondrial DNA haplotypes indicate two post-glacial re-colonization routes of the**  
2 **spruce bark beetle *Ips typographus* through northern Europe to Scandinavia**

3  
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17  
18 **Keywords:** spruce bark beetle, expansion, phylogeography, glacial refugium

19

20 **Abstract**

21 Species in northern Europe re-colonized the region after the last glacial maximum via several  
22 routes, which could have lingering signatures in current intra-specific trait variation. The spruce  
23 bark beetle, *Ips typographus*, occurs across Europe, and biological differences have been found  
24 between southern and northern Scandinavian populations. However, the post-glacial history of *I.*  
25 *typographus* in Scandinavia has not been previously studied at a fine geographical scale.  
26 Therefore we collected specimens across northern Europe and analyzed the genetic variation of a  
27 quite large mitochondrial fragment (698 bp). A high genetic diversity was found in some of the  
28 most northern populations, in the Baltic States, Gotland and central Europe. Detected genetic and  
29 phylogeographic structures suggest that *I. typographus* re-colonized Scandinavia via two  
30 pathways, one from the northeast and one from the south. These findings are consistent with the  
31 re-colonization history of its host plant, *Picea abies*. However, we observed low haplotype and  
32 nucleotide diversity in southern Scandinavian populations of *I. typographus*, indicating that  
33 (unlike *P. abies*) it did not disperse across the Baltic Sea in multiple events. Further, the  
34 divergence among Scandinavian populations was shallow, conflicting with a scenario where *I.*  
35 *typographus* expanded concurrently with its host plant from a “cryptic refugium” in the  
36 northwest.

37 **Résumé**

38 *Les variations de l'ADN mitochondrial suggèrent l'existence de deux routes postglaciaires du*  
39 *Nord de l'Europe vers la Scandinavie chez le scolyte de l'épicéa Ips typographus*

40  
41 Après le dernier maximum glaciaire, les espèces européennes ont recolonisé les régions du Nord  
42 de l'Europe via différentes routes de migration, ce qui a pu conduire aux variations intra-  
43 spécifiques actuellement observées pour un caractère biologique. Le typographe, *Ips*  
44 *typographus*, est présent dans toute l'Europe et des différences biologiques ont été identifiées  
45 entre les populations du Sud et du Nord de la Scandinavie. Cependant, l'histoire postglaciaire d'*I.*  
46 *typographus* dans le Nord de l'Europe n'a, jusqu'à présent, pas été étudiée à une échelle  
47 géographique fine. Dès lors, nous avons récolté des échantillons sur l'étendue de l'Europe du  
48 Nord et avons analysé la diversité génétique d'un relativement long fragment de gène  
49 mitochondrial (698 pb). Une plus forte diversité génétique a été observée dans certaines des  
50 localités situées le plus au Nord ainsi que dans les pays baltes, dans le Gotland et en Europe  
51 centrale. La présence d'une structure génétique et phylogéographique suggère qu'*I. typographus*  
52 a recolonisé la Scandinavie via deux routes de migration différentes: à partir du Nord-Est et à  
53 partir du Sud-Est. Ces résultats sont en accord avec l'histoire de recolonisation identifiée chez la  
54 plante hôte *Picea abies*. Cependant, nous avons observé une faible diversité haplotypique et une  
55 faible diversité nucléotidique chez les populations d'*Ips typographus* du Sud de la Scandinavie,  
56 ce qui indique que (contrairement à *P. abies*) il n'a pas traversé la Mer Baltique à plusieurs  
57 reprises. Enfin, la très faible divergence entre toutes les populations scandinaves ne soutient pas  
58 un scénario d'expansion simultanée d'*I. typographus* et de sa plante hôte depuis "un refuge  
59 cryptique" du Nord-Ouest.

60 **Introduction**

61 Climatic oscillations during the Quaternary (the last 2 million years) have driven repeated  
62 extensions and contractions of ice sheets in the Northern Hemisphere. This has been  
63 accompanied by migrations of populations to lower latitudes and isolation in refugia (Taberlet et  
64 al. 1998; Hewitt 2000), followed by expansion from their refugia and re-colonization of formerly  
65 glaciated areas. These contraction and expansion cycles have strongly shaped current spatial  
66 distributions of species' lineages (Avice 2000; Hewitt 1999). However, identifying key features  
67 of the historical processes, such as refugial sites and migration routes, is challenging due to the  
68 diversity of ecological and evolutionary forces acting upon species (Avice 2008).

69  
70 Here we address the phylogeography and evolutionary history of the European spruce bark  
71 beetle, *Ips typographus* L. (Coleoptera: Scolytinae), the most serious insect pest of Norway  
72 spruce (*Picea abies* L. Karst). It has killed substantial numbers of spruce trees in Europe  
73 (Grégoire and Evans, 2004), especially during the last decades of the 20<sup>th</sup> century when strong  
74 storms triggered outbreaks of the pest across large areas of Scandinavia (Långström et al. 2009;  
75 Kärvemo and Schroeder 2010).

76  
77 The phylogeography of *I. typographus* has been previously investigated at a large geographic  
78 scale by analyzing mitochondrial, internal transcribed spacer (ITS) or microsatellite markers in  
79 samples collected from locations across Europe (Stauffer et al. 1999; Sallé et al. 2007; Bertheau  
80 et al. 2013). These large-scale analyses have provided valuable insights into effects of specific  
81 life-history and evolutionary traits on the species' genetic variation. Notably, very weak genetic  
82 structure has been detected in microsatellite sequences and was attributed to strong gene flow  
83 between populations (Sallé et al. 2007; Gugerli et al. 2008). Accordingly, both field observations  
84 (Botterweg 1982; Byers 1995; Franklin and Grégoire 1999) and laboratory experiments (Forsse  
85 and Solbreck 1985) indicate that the beetle has high dispersal capacities. In addition, *I.*  
86 *typographus* appears to have very recent (late Pleistocene) origins in Europe, as all mtDNA  
87 haplotypes are poorly differentiated (Bertheau et al. 2013).

88  
89 Due to the high gene flow and recent origin of *I. typographus* in Europe, detecting any genetic  
90 structure (and thus potentially important indications of its recent population history) is

91 challenging. Use of mitochondrial (mtDNA) markers alone is often insufficient to resolve the  
92 complex history of a species, for two main reasons. Firstly, they only provide access to the  
93 matrilineal history, due to the uniparental inheritance of mtDNA. Secondly, selection (Dowling et  
94 al. 2008), introgression (Ballard and Whitlock 2004) or pseudogenes (Buhay 2009) may bias the  
95 inferred history. Nevertheless, as universal mitochondrial primers for specific taxonomic groups  
96 are readily available (e.g. the sets compiled for insect analyses by Simon et al. 1994), mtDNA  
97 provides convenient material for rapid investigations of matrilineal genealogies, thereby assisting  
98 reconstruction of the evolutionary history of populations in specific regions. Furthermore,  
99 mtDNA mutates rapidly in animals, thus mtDNA markers are valuable for detecting potential  
100 structures in species with recent origins (Avice 2000), particularly highly dispersive species, in  
101 which intense gene flows can rapidly reduce genetic differentiation among populations.

102

103 Recently, mtDNA analysis of intensively sampled populations at a fine geographic scale has  
104 provided valuable new insights into the population history of *I. typographus* in south-eastern  
105 Europe. Whereas large-scale approaches identified a single refugium for the whole of southern  
106 Europe (Stauffer et al. 1999; Sallé et al. 2007; Bertheau et al. 2013), the fine-scale approach  
107 detected at least one additional refugium in the Carpathians during the last glacial maximum  
108 (Krascsenitsová et al. 2013). These findings also highlight congruence in the history of local  
109 populations of this insect and its host plant, as distinct genetic differentiation between western  
110 and south-eastern Carpathian populations of *P. abies* has been detected (Tollefsrud et al. 2008).

111

112 In northern Europe, *P. abies* apparently survived during the last glaciation in a large refugium in  
113 the Russian plains (Schmidt-Vogt 1977; Lagercrantz and Ryman 1990; Tollefsrud et al. 2008)  
114 and possibly “cryptic” refugia in the vicinity of the Norwegian coast (Kullman 2002; 2008;  
115 Parducci et al. 2012). In contrast, large-scale molecular studies have provided no evidence that  
116 the spruce bark beetle expanded concurrently with its host plant from refugia in these northern  
117 regions (Stauffer et al. 1999; Sallé et al. 2007; Bertheau et al. 2013). However, phenotypic  
118 differences have been reported between southern and northern Scandinavian populations of *I.*  
119 *typographus*, prompting speculation that they may have different geographic origins. For  
120 example, in experiments reported by Komonen et al. (2011) beetles from southern Sweden  
121 generally hibernated under spruce bark while those from central Sweden moved to the ground.

122 Similarly, size fluctuation of the beetle's populations in the south and north of mid-Norway are  
123 poorly synchronized, and the lack of synchrony is not clearly related to climatic variables  
124 (Økland and Bjørnstad 2003).

125  
126 In the presented study we assess the spatial genetic structure and diversity of *I. typographus*  
127 maternal lineages in northern Europe. Based on the history of the host plant in Scandinavia  
128 inferred from previous paleo-ecological (Kullman 2008; Tollefsrud et al. 2008) and molecular  
129 analyses (Lagercrantz and Ryman 1990; Tollefsrud et al. 2008; 2009; Parducci et al. 2012), and  
130 the patterns for *I. typographus* detected in large-scale investigations (Stauffer et al. 1999; Sallé et  
131 al. 2007; Bertheau et al. 2013), we identified three historical scenarios to guide our analysis:

132 (1) If the scenario inferred from large-scale studies of *I. typographus* is valid, there should be  
133 little genetic structure among its populations across Scandinavia, but genetic diversity  
134 should decrease from the south to the north, reflecting a single re-colonization route from  
135 central Europe across the Baltic Sea into southern Scandinavia (Stauffer et al. 1999;  
136 Bertheau et al. 2013).

137 (2) Alternatively, if *I. typographus* followed its host plant during its putative expansion from  
138 a Russian refugium, northern and southern Scandinavian populations should be  
139 genetically distinct, reflecting two re-colonization routes: one from the south (as above)  
140 and one from the north-east via Finland (Lagercrantz and Ryman 1990; Tollefsrud et al.  
141 2008; 2009).

142 (3) Finally, if *I. typographus* expanded concurrently with its host plant from cryptic refugia in  
143 mid-Norway (Kullman 2008; Parducci et al. 2012), there should be signs of an ancient  
144 phylogeographic divergence, indicative of prolonged isolation of spruce bark beetle  
145 populations in this region as it was only reached by the main re-colonization front of *P.*  
146 *abies* 3000–2000 BP (Tollefsrud et al. 2008).

147  
148 We tested the three proposed scenarios at fine-scale by collecting samples across most parts of  
149 northern Europe, then analyzing the genetic variation in a 698 bp fragment of a mitochondrial  
150 gene both within populations and between regions.

151

152 **Materials and Methods**

153

154 *Sampling and DNA extraction*

155 We collected 359 adult *I. typographus* from under the bark of standing or felled *P. abies* trees or  
156 pheromone traps in 44 northern European sites and three central European sites (Figure 1,  
157 Supporting information Table S1). The central European sites were included as references for this  
158 region. Beetles were stored at -20°C or in 96% ethanol at room temperature. Genomic DNA was  
159 extracted from the entire body of fresh or up to one-year-old specimens using a QIAGEN  
160 DNeasy Blood & Tissue kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's  
161 protocol. Each extracted DNA sample was eluted and stored in 100 µl of AE buffer (QIAGEN).  
162 Reference material is available at the Department of Ecology, Swedish University of Agricultural  
163 Sciences and the Laboratory of Biological Control and Spatial Ecology, Université Libre de  
164 Bruxelles.

165

166 *DNA amplification and sequencing*

167 We sequenced a 789 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI)  
168 gene corresponding to the region spanning nucleotides 2201 to 2990 of the *Drosophila yakuba*  
169 sequence in each sample (Clary and Wolstenholme 1985). Both strands of the fragment were  
170 sequenced to verify its identity, using the primer pair Jerry (C1-J-2183)/Pat (L2-N-3014)  
171 published by Simon et al. (1994) as follows. The fragments were amplified in 20 µl reaction  
172 mixtures consisting of 4.0 mM MgCl<sub>2</sub>, 300 µM dNTP, 0.5 µM of each primer, 0.5 units of  
173 HotstarTaq DNA polymerase and approximately 10-40 ng DNA in a thermocycler (Eppendorf  
174 AG, Hamburg, Germany). The amplification protocol consisted of 15 min denaturation at 95°C  
175 followed by 36 cycles of 30 s denaturation at 94°C, 30 s annealing at 47°C, 1 min 30 s extension  
176 at 72°C, and a final 10 min extension step at 72°C. The amplified fragments were  
177 electrophoretically separated on a 1 % agarose gel and stained with ethidium bromide to verify  
178 their quality. The target PCR product was purified, using a QIAquick PCR Purification kit  
179 (QIAGEN GmbH, Hilden, Germany) or ExoSAP-IT (USB, Cleveland) following the  
180 manufacturers' instructions, then sequenced at Uppsala Genome Center  
181 ([http://www.igp.uu.se/facilities/genome\\_center/](http://www.igp.uu.se/facilities/genome_center/)) or by Macrogen (Seoul, Korea;  
182 <http://www.macrogen.com>).

183

184 After removing low quality sections at the end of each sequenced fragment, we aligned the  
185 acquired sequences using the ClustalW algorithm (Thompson et al. 1994) as implemented in  
186 BioEdit (Hall 2007), then manually edited the alignment. To ensure that the dataset included no  
187 sequences of nuclear copies of the mtDNA COI gene (so-called NUMTs; Lopez et al. 1994), we  
188 translated the sequences into amino acids and checked that the resulting electropherograms  
189 indicated no unexpected stop codons or double peaks (Song et al. 2008). Three sequences that  
190 yielded ambiguous electropherograms (e.g. double peaks) were removed from the dataset prior to  
191 analysis. In addition, we removed 14 sequences with identical overlaps to two identified NUMT  
192 sequences, JN133882 and JN133883, published by Bertheau et al. (2011).

193

#### 194 *Genealogical relations between haplotypes*

195 To reconstruct the genealogical relations between haplotypes, we constructed a haplotype  
196 network using the Median Joining algorithm implemented in Network 4.611 with epsilon set to  
197 10 (Bandelt et al. 1999, <http://www.fluxus-engineering.com>) and a phylogenetic tree using the  
198 Bayesian method implemented in BEAST v1.7.5 (Drummond and Rambaut 2007) with the HKY  
199 model (Hasegawa et al. 1985) defined by Bertheau et al. (2013). We fixed a Yule speciation  
200 process as prior of tree and default options were used for all other prior and operator settings.  
201 Two independent MCMC analyses were run for 50 million iterations of posterior sampling with  
202 logging to file every 10 000 iterations. Equilibrium was confirmed by Effective Sample Size  
203 (ESS) values larger than 200 as calculated in Tracer v1.5 (Rambaut and Drummond 2009). When  
204 two independent runs converged to the same posterior distribution and same estimates, we  
205 combined tree files and discarded 10% of the sampled trees as burn-in in LogCombiner v1.7.5  
206 (<http://beast.bio.ed.ac.uk/LogCombiner>). Remaining trees were summarized in the form of a  
207 maximum clade credibility tree using TreeAnnotator implemented in BEAST and the resulting  
208 tree was visualized in FigTree v1.4.0. (<http://tree.bio.ed.ac.uk/software/figtree>).

209

#### 210 *Genetic structure*

211 To assess the genetic structure among populations we applied spatial analysis of molecular  
212 variance as implemented in SAMOVA 1.0 (Dupanloup et al. 2002) to identify geographically  
213 homogeneous clusters that are maximally differentiated from others. We applied a simulated  
214 spatial annealing procedure (calculated with geographical distances) for  $K = 2-8$  to identify the

215 optimal number of population groups. We then selected the optimal K-value, defined as the value  
216 that yielded the maximum  $\Phi_{CT}$  value (plateau) while excluding configurations with single-  
217 population groups (which indicate disappearance of group structure; Magri et al. 2006).

218  
219 In addition to SAMOVA, we applied Discriminant Analysis of Principal Component (DAPC)  
220 (Jombart, 2008). DAPC discriminates individuals associated with pre-defined groups according  
221 to a model that maximizes the variance between groups while minimizing the variance within  
222 groups. The groups used for the DAPC were determined *a priori* with the *K*-mean clustering  
223 algorithm. The analyses were run through the *ape* (Paradis et al. 2004) and *adegenet* (Jombart  
224 2008) packages implemented in R software (v 2.14; R Development Core Team 2011) on a  
225 standardized allele frequency table, obtained by scaleGen, with the binomial method. We selected  
226 the optimal number of groups using the minimum Bayesian Information Criterion (BIC) and a  
227 configuration without single-population groups as criteria. As DAPC assigns individuals to  
228 groups and SAMOVA sites to groups, we obtained comparable results from averaging individual  
229 posterior membership probabilities of DAPC at the site level. Results of the optimal  
230 configuration retained for clustering analyses were plotted on a map (Figure 3).

231 Phylogenetic reconstructions suggested the presence of three clades and the genetic structure  
232 analyses identified 2 to 3 clusters. Thus, we tested for the presence of phylogeographic structure  
233 by comparing two estimates of genetic variation: *Gst* (based solely on differences in haplotype  
234 frequencies; Pons and Petit 1995) and *Nst* (based on differences in haplotype frequencies and the  
235 genetic distances between haplotypes; Pons and Petit 1996). According to Pons and Petit (1996),  
236 a significantly higher value of *Nst* indicates that genealogically closely related haplotypes co-  
237 occur within populations more often than random expectations. SAMOVA group assignments  
238 were treated as populations for these analyses. The computed indices and their significance were  
239 assessed with 1000 permutations in PermutCpSSR 2.0 (<http://www.pierroton.inra.fr/genetics/labo/Software/Permut/>).

241  
242 Genetic differentiation among populations was quantified by comparing pairwise fixation indices  
243 ( $F_{ST}$ ), and proportions of genetic variation within and between the identified geographical groups  
244 of samples were estimated by analysis of molecular variance (AMOVA) using Arlequin 3.5  
245 software (Excoffier and Lischer 2010).

246

247 *Summary statistics*

248 For populations at each locality we calculated the following diversity indices using DnaSP 5  
249 (Librado and Rozas 2009): number of haplotypes, haplotype diversity (Hd), nucleotide diversity  
250 (Pi) and the mean number of pairwise differences (MNPd). We also calculated allelic richness (r)  
251 using the rarefaction method proposed by El Mousadik and Petit (1996), as implemented in  
252 Contrib 1.02 (<http://www.pierroton.inra.fr/genetics/labo/Software/Contrib>).

253

254 To explore regional diversity, the sampling sites were classified into seven geographical groups:  
255 northwestern Scandinavia (NWS), northeastern Scandinavia (NES), southwestern Scandinavia  
256 (SWS), southeastern Scandinavia (SES), the Baltic States and Russia (BSR), Denmark (DEN),  
257 and central Europe (CE) (Table 1). We then tested for possible differences in summary statistics  
258 calculated for the geographical groups using non-parametric Kruskal-Wallis analysis of variance  
259 (as we could not be certain that the data were normally distributed).

260

261 *Neutrality departure tests*

262 Finally, to detect signatures of possible demographic expansion events in the sampled  
263 populations' evolutionary history we calculated two statistics: Fu's FS (Fu 1997) and Tajima's D  
264 (Tajima 1989) using simulations in Arlequin and DnaSP 5.0, respectively. These statistics  
265 indicate if populations are in mutation-drift equilibrium (Wright-Fisher model) or if there are  
266 signs of processes that distort the pattern (Ramos-Onsins and Rozas 2002). In the absence of  
267 selection, negative values of these statistics indicate that the number of alleles exceeds random  
268 expectations, potentially due to population expansion. Fu's statistic reportedly detects population  
269 expansion more sensitively than Tajima's (Fu 1997).

270

271 **Results**

272

273 *Genetic diversity*

274 The final alignment consisted of sequences of the targeted 698-bp fragment of the mtDNA COI  
275 gene obtained from 342 individuals. In total, 33 polymorphic nucleotides and 39 haplotypes were

276 identified (Figures 1 and 2). The identified haplotypes are available in GenBank database  
277 (Accession numbers JX845179- JX845217).

278 Three major haplotypes were found, designated *h1*, *h20* and *h11*. These were detected in 160  
279 (47%), 63 (18%) and 39 (11%) of the 342 analyzed individuals, respectively. The other  
280 haplotypes mostly consisted of single mutation variants of these three major haplotypes (Figure  
281 1). As only 448 bp overlapped with sequences published by Bertheau et al. (2011) we could not  
282 fully recover all of the haplotypes. However, the three most abundant haplotypes (*h1*, *h11* and  
283 *h20*) overlapped with the most common haplotype detected by Bertheau et al. (2011), HTI, and  
284 two others (*h13* and *h18*) overlapped with the other two major haplotypes they reported (It1 and  
285 HTII). Not surprisingly, as haplotype HTII was only found in southern European populations in  
286 large-scale studies (Stauffer et al. 1999; Bertheau et al. 2013), we detected it in Central European  
287 populations but not in any of the Scandinavian populations (Figure 1).

288  
289 Analysis of all aligned sequences yielded haplotype diversity (Hd) and nucleotide diversity (Pi)  
290 values of 0.71 (SD=0.02) and 0.002 (SD=0.000), respectively (Table 1). Both of these variables  
291 differed among geographical regions according to a Kruskal-Wallis ANOVA by ranks test  
292 (H=14.48, p=0.05 and H=13.24, p=0.04, respectively). However, no significant differences were  
293 detected in either of them in pairwise comparisons of the regions' populations (data not shown).

294  
295 As shown in Table 1, allelic richness (Ar) was particularly low in populations of the South  
296 Swedish mainland (ASA, BNS, TON, OMB, GRA, NAS and TIE) and highest in three  
297 populations of the Baltic States (LL, 1.58; JUR, 1.59 and NOR, 1.59), two populations of  
298 southern Finland (LIK, 1.74 and UKK, 1.71), two populations of the Norwegian part of the  
299 Scandes Mountains (NLS, 2.00 and TOK, 1.70), the Gotland island population (GOT, 2.00), one  
300 population of Central Sweden (VIN, 1.63) and the three populations of central Europe (SLO,  
301 2.00; WIE, 1.40; SOP, 1.59).

302  
303 *Genealogical relations*

304 The phylogenetic tree reconstructed by BEAST revealed three clades (designated H<sub>A</sub>, H<sub>B</sub> and H<sub>C</sub>)  
305 corresponding to the three main haplogroups (Figure 2). When plotted on a map, the haplotype

306 frequencies revealed a partition between northern and southern Scandinavia, with H<sub>C</sub> and H<sub>A</sub>  
307 haplogroups mainly present in the southern and northern parts, respectively (Figures 1 and 2).

308

### 309 *Genetic and geographic structures*

310 The spatial analysis of molecular variation (SAMOVA) provided highest support for three  
311 genetic clusters, designated 1, 2 and 3 (Table 1). Cluster 1 included all northern samples, except  
312 those from the Finnish (ROV) and Lithuanian (JUR) locations. Cluster 2 included all samples  
313 from Denmark, southern Norway, Sweden, Finland, the Baltic States, Slovakia and Russia.  
314 Cluster 3 included the remaining two populations from central Europe (WIE and SOP). It also  
315 indicated that 42% of the total variation was distributed among clusters, just 3% among  
316 populations within clusters, and most (55%) within populations (Table 2). For a *K*-value of 3, F<sub>CT</sub>  
317 was 0.42 (p<0.001), F<sub>ST</sub> was 0.45 (p<0.001) and F<sub>SC</sub> was 0.05 (p<0.001). The global F<sub>ST</sub> (with no  
318 grouping of samples) was 0.35 (p<0.001) and F<sub>IS</sub> was 0.10 (p<0.001).

319

320 The DAPC analysis favored a structure with two genetic clusters. The BIC curve decreased  
321 continuously between *K*=2 and *K*=7, but values over *K*=2 led to additional clusters with a single  
322 individual, thus there was no support for a structure with more than two clusters. DAPC with *K*=2  
323 gave very similar partitioning to the SAMOVA with *K*=3 (Figure 3), except that the latter  
324 grouped two central European samples into an additional cluster.

325

326 When considering groups identified by the SAMOVA analysis as populations, phylogeographic  
327 structure was detected with a (slightly) significant difference (p=0.02) between *Gst* (0.215) and  
328 *Nst* (0.290) statistics.

329

330 Pairwise fixation indices between populations are shown in Supplementary Figure S1. Within  
331 geographical regions, differentiations were mostly low to moderate (70% of pairwise  
332 comparisons within a region gave F<sub>ST</sub> values <0.15). Among geographical regions, those from  
333 northern Europe were generally well differentiated from those of Central Europe (CE) (70% of  
334 pairs >0.15), but few pairwise F<sub>ST</sub>-values exceeded 0.5 (indicating <15% differentiation).

335 However, populations from southwestern Scandinavia were clearly differentiated from those of

336 neighboring northern regions, as 90% of the pairwise calculated  $F_{ST}$ -values between SWS and  
337 NWS or NES populations exceeded 0.5.

338

### 339 *Demographic expansion*

340 Fu's test of analysis of departure from neutrality detected signs of expansion in seven  
341 populations, all from geographical groups in the eastern part (Baltic States and Russia, BSR;  
342 south-eastern and north-eastern Scandinavia, SES and NES) of the study region (Table 1).

343 However, Tajima's D statistics were negative for populations in only two of these locations  
344 (KAL and VIN, in northern Sweden).

345

## 346 **Discussion**

347

348 Our fine-scale survey of mitochondrial haplotypes of northern European *Ips typographus*  
349 populations provides new insights into the post-glacial history of this forest pest. We identified  
350 not only genetic structure but also phylogeographic structure between the northern and southern  
351 parts of Scandinavia. We also detected high genetic diversity in northern and eastern populations,  
352 but low genetic diversity in southern populations of Scandinavia.

353

354 These phylogeographic patterns suggest that the current maternal lineages of Scandinavia have at  
355 least two different geographic origins. Neither the phylogeographic structure nor the genetic  
356 diversity distributions are consistent with northward expansion via a single re-colonization route  
357 from an entry-point in southern Scandinavia. Thus, we rejected scenario 1, postulating a single  
358 post-glacial re-colonization route to Scandinavia from the south (Stauffer et al. 1999; Bertheau et  
359 al. 2013).

360

361 The observed patterns are also inconsistent with scenario 3, which incorporates prolonged  
362 isolation of *I. typographus* populations in one of the cryptic north-western refugia of its host plant  
363 (e.g. Andøya and Trøndelag; Figure 1) (Kullman 2008; Parducci et al. 2012). This is because the  
364 divergence observed between the northern and southern population pools is quite shallow and all  
365 haplotypes differed only by one or two mutational steps. Moreover, we detected no signs of

366 population expansion in populations of the north-western coast as none of the Tajima and Fu's  
367 statistics were significantly negative for this area.

368  
369 Therefore, the observed phylogeographic patterns are more consistent with scenario 2, which  
370 postulates post-glacial re-colonization of inland Scandinavia via a southern and a northern route.  
371 One of these routes is similar to the southern re-colonization route suggested by Stauffer et al.  
372 (1999) for *I. typographus*, which was also apparently used by its host plant *P. abies* (Giesecke  
373 and Bennett 2004; Tollefsrud et al. 2009) and other species, e.g. the bush cricket (Kaňuch et al.  
374 2013). However, in contrast to its host plant, the genetic diversity of *I. typographus* observed in  
375 southern Scandinavia is particularly low, suggesting that few dispersal events across the Baltic  
376 Sea were successful, at least for matrilineal lineages. Considering the geographic distribution of  
377 the H<sub>A</sub> haplotypes and the cluster assignments (both of which identified a second genetic pool  
378 along the Gulf of Finland and in the Scandes Mountains), the second re-colonization route could  
379 correspond to a postulated expansion of its host plant from a north-eastern refugium, probably  
380 located in the Russian plains (Kostroma, Figure 1) (Lagercrantz and Ryman 1990; Tollefsrud et  
381 al. 2008). Moreover, samples from sites near the border between southern Finland and southern  
382 Russian Karelia, which are among the closest to Kostroma, yielded some of the highest diversity  
383 indices and significantly negative Tajima and Fu's statistics, indicative of an expansion event.  
384 Particularly high diversity was also observed at some locations in central Sweden and southern  
385 mid-Norway (e.g. TOK and DEL sites), probably because they were in a zone of secondary  
386 contact between the southern and northern gene pools. Further, we detected relatively weak  
387 differentiation between neighboring populations but relatively high mean numbers of pairwise  
388 differences in this region, which may be indicative of a zone where populations from different  
389 isolated refugia mixed (Petit et al. 2003).

390  
391 The putative northern re-colonization route has not been detected by large-scale molecular  
392 surveys (Stauffer et al. 1999; Sallé et al. 2007; Bertheau et al. 2013), probably because they did  
393 not include high latitude sampling sites in northern Scandinavia. Our use of a relatively large  
394 gene fragment, 140 bp longer than mitochondrial fragments sequenced in the large-scale surveys  
395 (Stauffer et al. 1999; Bertheau et al. 2013), may also have contributed to the fine resolution of  
396 genetic variation we obtained.

397  
398 In conclusion, the findings of this study are consistent with the previous conclusion that fine-  
399 scale analysis of mitochondrial markers is valuable for high-resolution exploration of the  
400 population history of highly-dispersive species such as *I. typographus* (Krascsenitsová et al.  
401 2013). Our application of this approach to Scandinavian populations provided the first indications  
402 that the region was re-colonized after the last glaciation via two pathways: from the northeast to  
403 the northwest through Finland and across the Baltic Sea to southern Scandinavia. The two routes  
404 explain the current partitioning between southern and northern populations of *I. typographus*, and  
405 may be related to observed differences in behavior between them. They also provide potentially  
406 useful information for improving pest management. For instance, removing attacked trees should  
407 be more efficient for managing populations dominated by the southern gene pool since they seem  
408 to hibernate under spruce bark more often than their northern counterparts (Komonen et al.  
409 2011). In addition, future models for predicting effects of climate change on the species'  
410 population dynamics may benefit by applying specific scenarios to the populations from the two  
411 gene pools. However, it has been suggested that the weaker dispersal of females than males of the  
412 species could have caused discrepancies between large-scale mitochondrial and nuclear patterns  
413 (Sallé et al. 2007). Therefore, further investigations should utilize already identified  
414 microsatellite markers (Sallé et al. 2007; Stoeckle and Kuehn 2011) to assess, at fine-scale, if the  
415 phylogeographic patterns observed in this study are representative of the whole species'  
416 population history or are limited to matrilineal lineages.

417

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425

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- 565  
566

567 **Figure 1. a)** Geographical locations of the collection sites and haplotype frequencies of *Ips*  
568 *typographus* in northern Europe. The abbreviations of collection sites are defined in  
569 Supplementary Table S1. Each color indicates a specific haplotype in the network (1b). The total  
570 area of each concentric circle is proportional to the sample size, and the proportions of the colors  
571 in each circle represent the corresponding haplotype frequencies. Arrows indicate locations of  
572 putative northern refugia of the host plant *Picea abies*: A, Andøya; T, Trøndelag; K, Kostroma  
573 (Tollefsrud et al. 2008; Parducci et al. 2012).

574 **b)** Median-joining haplotype network for 342 sequences of the mtDNA COI gene. The size of  
575 each circle reflects the number of individuals with the corresponding haplotype.

576

577 **Figure 2.** Phylogeny for the 39 Cytochrome *c* oxidase subunit I (COI) haplotypes identified in  
578 the set of *Ips typographus* samples collected from northern and central Europe. The phylogeny is  
579 based on Bayesian analysis implemented in BEAST, assuming a prior Yule speciation process  
580 and applying a HKY+G model (Hasegawa et al. 1985). The colors indicate the three main  
581 haplogroups: H<sub>A</sub> (haplotypes *h20-39*, except *h38*), H<sub>B</sub> (haplotypes *h11-h19* plus *h38*) and H<sub>C</sub>  
582 (haplotypes *h1-10*).

583

584 **Figure 3.** Geographic distribution of clusters for *Ips typographus* populations assigned by  
585 SAMOVA (a) and DAPC (b). In (b), the size of each circle is proportional to the number of  
586 individuals sequenced.

587

**Table 1.** Sampling site information, mtDNA diversity indices and cluster assignments for 47 localities of the spruce bark beetle *Ips typographus* collected from central and northern Europe. Codes indicate localities (see Figure 1 and supporting information Table S1). Number of individuals per site (N); Number of haplotypes (Nb (HT)); Haplotype diversity (Hd); Mean number of pairwise differences (MNPd); Nucleotide diversity (Pi); Allelic Richness with rarefaction to 3 (Ar); Fu's statistic (F<sub>s</sub>); Tajima's statistic (D); SAMOVA cluster (SAM.); DAPC clusters (DAPC, with the number of individuals assigned to one DAPC cluster with P>0.8). Asterisks indicate significance: \*  $p < 0.05$  and \*\*  $p < 0.01$ .

Code	N	Voucher specimen	Region	Nb (HT)	Haplotype ID	Hd (S.D.)	MNPd (S.D.)	Pi (S.D.)	Ar [3]	F <sub>s</sub>	D	SAM.	DAPC
NLS	4	INLS1-4	NWS	4	8, 16, 21, 28	1.00 (0.18)	2.50 (1.69)	0.0036 (0.0007)	2.00	-1.51	-0.80	1	1 (3); 2 (1)
NLV	8	INLV1-8	NWS	3	13, 17, 20	0.46 (0.20)	1.11 (0.80)	0.0016 (0.0007)	0.75	0.39	-0.18	1	1 (6); 2 (2)
OVE	7	IOVE1-7	NWS	4	11, 13, 20, 24	0.86 (0.10)	1.33 (0.94)	0.0019 (0.0004)	1.40	-0.91	0.40	1	1 (3); 2 (4)
VER	9	IVER1-9	NWS	4	9, 11, 20, 25	0.78 (0.11)	1.22 (0.85)	0.0017 (0.0005)	1.50	-0.63	-0.69	1	1 (6); 2 (3)
ORK	7	IORK1-7	NWS	3	8, 20, 23	0.52 (0.21)	0.57 (0.52)	0.0008 (0.0004)	0.86	-0.92	-1.24	1	1 (6); 2 (1)
ROV	7	IROV1-7	NES	4	1, 11, 20, 30	0.81 (0.13)	1.33 (0.94)	0.0019 (0.0005)	1.46	-0.91	0.40	2	1 (2); 2 (5)
KAL	10	IKAL1-10	NES	6	8, 11, 20, 22, 29, 36	0.78 (0.14)	1.00 (0.73)	0.0014 (0.0004)	1.42	-3.88**	-1.74*	1	1 (8); 2 (2)
VIN	10	IVIN1-10	NES	7	11, 13, 20, 26, 27, 31, 34	0.87 (0.11)	1.76 (1.11)	0.0025 (0.0006)	1.63	-3.71**	-1.64*	1	1 (8); 2 (2)
SOL	9	ISOL1-9	NES	4	1, 11, 19, 20	0.69 (0.15)	1.00 (0.74)	0.0014 (0.0004)	1.20	-1.04	-0.36	1	1 (5); 2 (4)
LIK	10	ILIK1-10	SES	7	1, 11, 16, 20, 23, 25, 35	0.91 (0.08)	1.49 (0.98)	0.0021 (0.0004)	1.74	-4.29**	-0.63	1	1 (7); 2 (3)
UKK	7	IUKK1-7	SES	5	1, 11, 20, 23, 32	0.91 (0.10)	1.43 (0.98)	0.0020 (0.0020)	1.71	-2.31*	-0.60	1	1 (4); 2 (3)
POR	5	IPOR1-5	SES	2	1, 27	0.40 (0.24)	1.20 (0.91)	0.0017 (0.0010)	0.60	1.69	-1.05	2	1 (1); 2 (4)
LAP	5	ILAP1-5	SES	3	1, 3, 20	0.70 (0.22)	1.20 (0.91)	0.0017 (0.0007)	1.20	-0.19	-1.05	2	1 (1); 2 (4)
HAM	8	IHAM1-8	SES	2	1, 27	0.54 (0.02)	1.61 (1.06)	0.0023 (0.0005)	0.80	2.99	1.60	2	1 (3); 2 (5)
TUU	7	ITUU1-7	SES	2	1, 15	0.29 (0.20)	0.57 (0.52)	0.0008 (0.0006)	0.43	0.86	-1.24	2	1 (0); 2 (7)
ALA	10	IALA1-10	SWS	4	1, 4, 20, 39	0.53 (0.18)	1.11 (0.79)	0.0016 (0.0006)	0.89	-0.65	-0.82	2	1 (2); 2 (8)
LIL	11	ILIL1-11	SWS	3	8, 11, 20	0.64 (0.09)	0.73 (0.58)	0.0010 (0.0002)	1.03	-0.02	0.20	2	1 (5); 2 (6)
TOK	5	ITO1-5	SWS	4	1, 7, 11, 20	0.90 (0.16)	1.40 (1.02)	0.0023 (0.0006)	1.70	-1.65	-0.18	2	1 (2); 2 (3)
AAS	7	IAAS1-7	SWS	2	1, 6	0.48 (0.17)	0.48 (0.46)	0.0007 (0.0002)	0.71	0.59	0.56	2	1 (0); 2 (7)
ARS	7	IARS1-7	SWS	2	1, 21	0.29 (0.20)	0.57 (0.52)	0.0012 (0.0008)	0.43	0.86	-1.24	2	1 (1); 2 (6)
DEL	7	IDEL1-7	SWS	4	1, 11, 20, 23	0.71 (0.18)	1.33 (0.94)	0.0019 (0.0006)	1.26	-0.91	0.40	1	1 (2); 2 (5)
SIL	11	ISIL1-11	SWS	3	1, 11, 20	0.56 (0.13)	0.95 (0.70)	0.0014 (0.0003)	0.91	0.48	1.18	2	1 (3); 2 (8)
NAS	10	INAS1-10	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (10)

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TIE	5	ITIE1-5	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (5)
GRA	5	IGRA1-5	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (5)
CHA	8	ICHA1-8	SWS	3	1, 11, 20	0.61 (0.16)	0.96 (0.73)	0.0014 (0.0004)	1.00	0.14	0.93	2	1 (2); 2 (6)
SKI	7	ISKI1-7	SWS	2	1, 38	0.29 (0.20)	0.57 (0.52)	0.0008 (0.0006)	0.43	0.86	-1.24	2	1 (0); 2 (7)
VAT	10	IVAT1-10	SWS	2	1, 13	0.20 (0.15)	0.40 (0.40)	0.0006 (0.0004)	0.30	0.59	-1.40	2	1 (0); 2 (10)
ORS	9	IORS1-9	SWS	3	1, 13, 20	0.42 (0.19)	0.83 (0.65)	0.0012 (0.0006)	0.60	0.02	-0.94	2	1 (1); 2 (8)
TIB	9	ITIB1-9	SWS	2	1, 20	0.22 (0.17)	0.44 (0.43)	0.0006 (0.0005)	0.33	0.67	-1.36	2	1 (1); 2 (8)
OMB	7	IOMB1-7	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (7)
GOT	4	IGOT1-4	SWS	4	1, 5, 11, 27	1.00 (0.18)	2.17 (1.50)	0.0031 (0.0009)	2.00	-1.74	-0.07	2	1 (1); 2 (3)
SAV	4	ISAV1-4	SWS	2	1, 11	0.50 (0.26)	0.50 (0.52)	0.0007 (0.0004)	0.75	0.17	-0.61	2	1 (0); 2 (4)
MAR	5	IMAR1-5	SWS	2	1, 2	0.60 (0.17)	0.60 (0.56)	0.0009 (0.0002)	0.90	0.63	1.23	2	1 (0); 2 (5)
ASA	7	IASA1-7	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (7)
BNS	9	IBNS1-9	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (9)
TON	5	ITON1-5	SWS	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (5)
NOR	8	INOR1-8	BSR	5	1, 5, 11, 20, 36	0.86 (0.11)	1.46 (0.99)	0.0021 (0.0005)	1.59	-1.86*	-0.22	2	1 (0); 2 (8)
RUG	7	IRUG1-7	BSR	2	1, 11	0.57 (0.12)	0.57 (0.52)	0.0008 (0.0002)	0.86	0.86	1.34	2	1 (0); 2 (7)
E2B	3	IE2B1-3	BSR	1	1	0.00 (0.00)	0.00 (0.00)	0.0000 (0.0000)	0.00	0.00	0.00	2	1 (0); 2 (3)
LL	10	ILL1-10	BSR	6	1, 11, 20, 27, 33, 36	0.84 (0.10)	1.84 (1.15)	0.0026 (0.0004)	1.58	-2.05*	0.18	2	1 (5); 2 (5)
JUR	8	IJUR1-8	BSR	5	1, 11, 20, 36, 37	0.86 (0.11)	1.29 (0.90)	0.0018 (0.0004)	1.59	-2.17*	-0.73	1	1 (5); 2 (3)
FEL	7	IFEL1-7	DEN	2	1, 10	0.29 (0.20)	0.29 (0.34)	0.0004 (0.0003)	0.43	-0.10	-1.01	2	1 (0); 2 (7)
RUD	7	IRUD1-7	DEN	4	1, 10, 14, 20	0.71 (0.18)	1.33 (0.94)	0.0019 (0.0006)	1.26	-0.91	-0.88	2	1 (1); 2 (6)
SLO	3	ISLO1-3	CE	3	1, 11, 13	1.00 (0.27)	1.33 (1.10)	0.0019 (0.0006)	2.00	-1.22	0.00	2	1 (0); 2 (3)
WIE	5	IWIE1-5	CE	3	11, 12, 18	0.80 (0.16)	1.00 (0.80)	0.0014 (0.0004)	1.40	-1.69	0.99	3	1 (0); 2 (5)
SOP	9	ISOP1-9	CE	5	1, 8, 11, 18, 20	0.86 (0.09)	1.39 (0.94)	0.0020 (0.0003)	1.59	-0.48	0.24	3	1 (2); 2 (7)
MEAN	7			39		0.71 (0.02)	1.75 (1.11)	0.0019 (0.0001)		-0.51	-0.26		

Phylogeography of northern populations of *Ips typographus*

**Table 2.** Analysis of molecular variance (AMOVA) for three genetic clusters identified by spatial analysis of molecular variance (SAMOVA) based on the sequence variation in 47 samples of *Ips typographus* collected in central and northern Europe.

Source of variation	d.f.	Sum of squares	Variance components	% of variation	F-stat
Among clusters	2	58.77	0.36 Va	42.23	Fct=0.42
Among populations within clusters	44	28.78	0.03 Vb	3.09	Fsc=0.05
Within populations	295	136.90	0.46 Vc	54.67	Fst=0.45
Total	341	224.45	0.85		

Figure 1

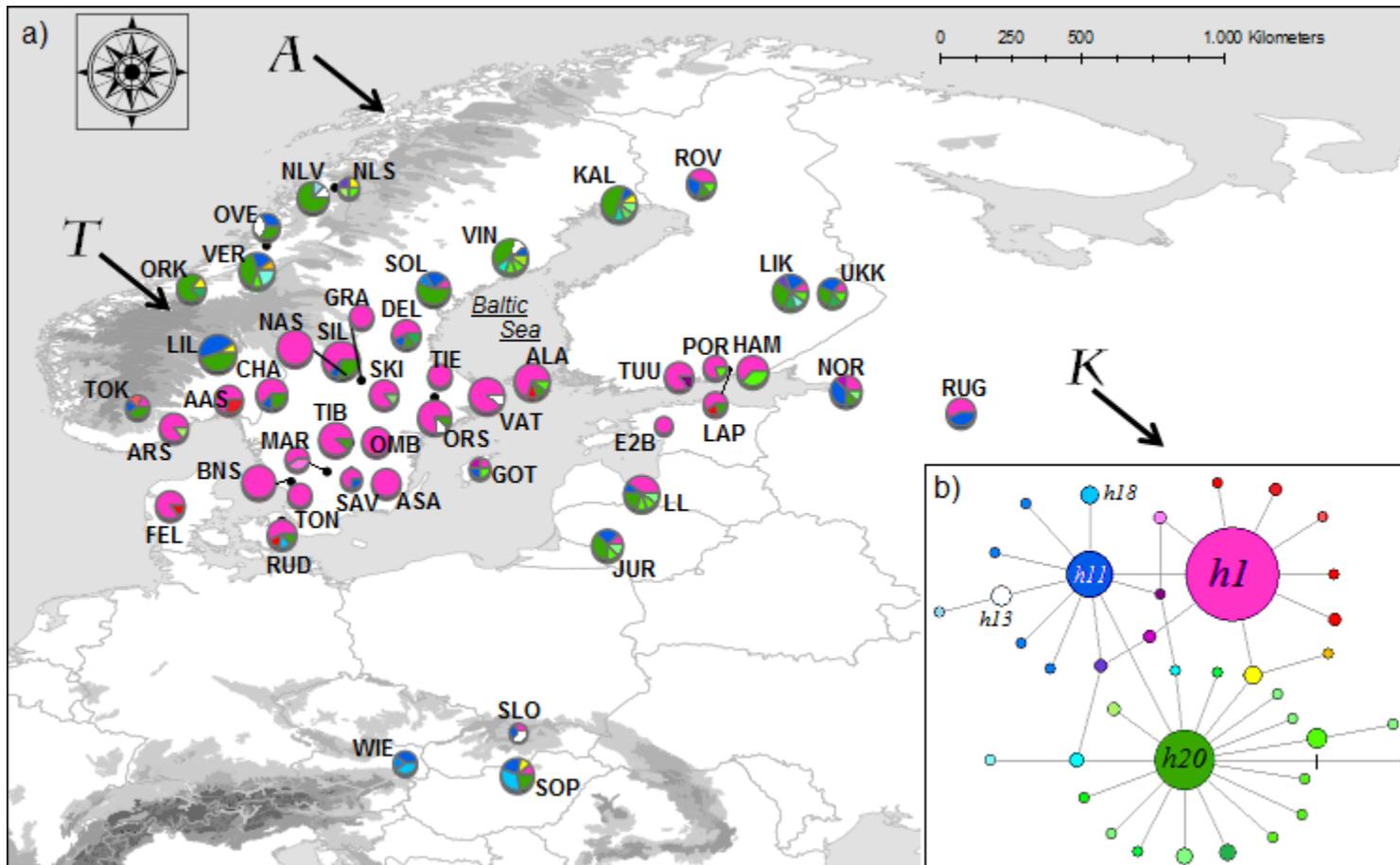


Figure 2

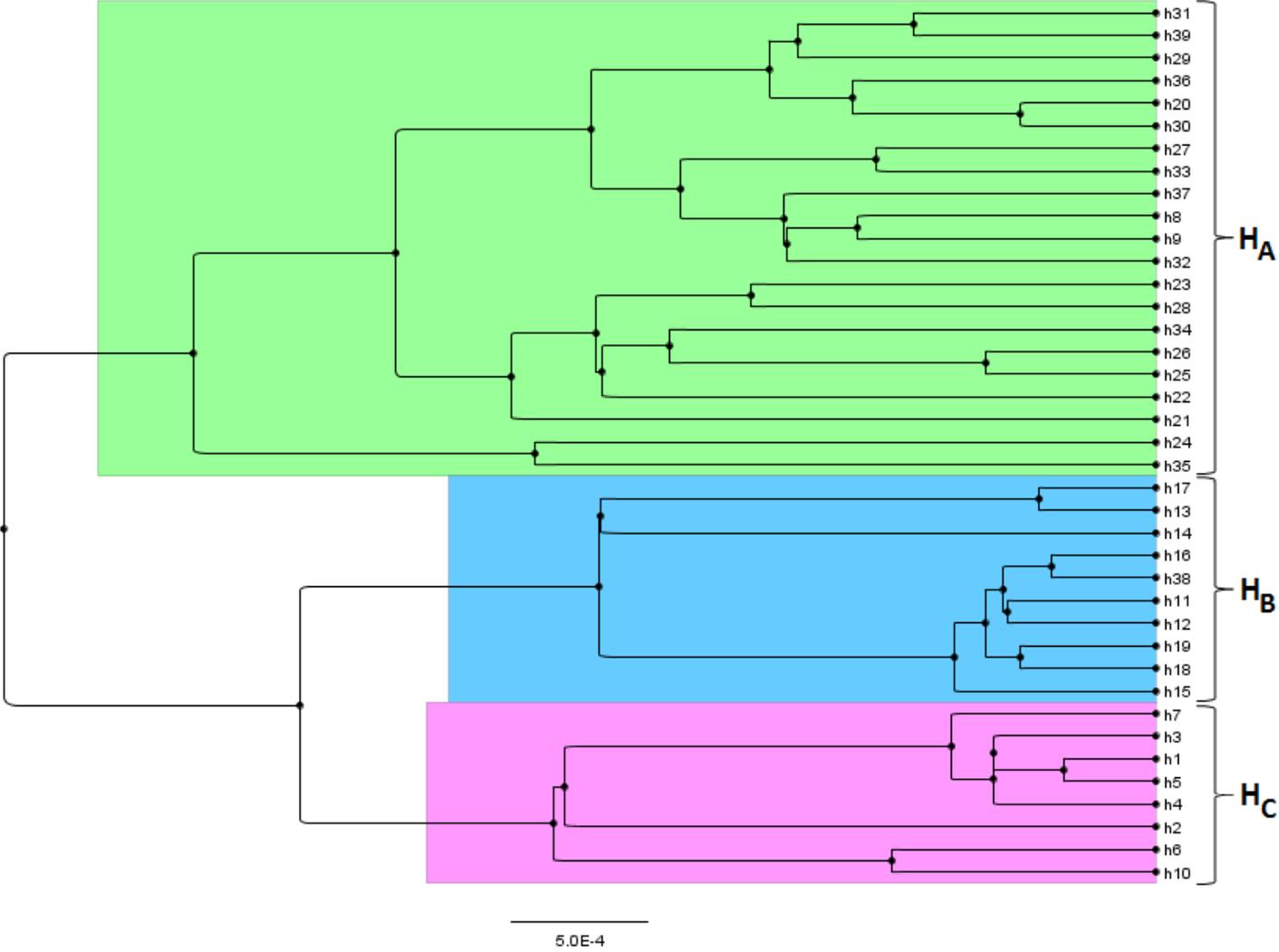


Figure 3

