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## Clonality and genetic structure of an endangered aquatic plant, *Typha minima*, in the French Alps: consequences for conservation

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1 **Clonality and genetic structure of an endangered aquatic plant, *Typha minima*, in the French**  
2 **Alps. Consequences for conservation.**

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15

16 **Abstract**

17 The dwarf bulrush (*Typha minima* Hoppe) is a perennial aquatic plant that has been rapidly  
18 disappearing in Northern Europe following flood control methods (dikes, dams,  
19 embankments). Floods, by erasing and creating new banks, maintain a metapopulation system  
20 (extinction/recolonization of populations).

21 The largest and most diverse populations are located in France. In order to identify the size of  
22 the metapopulations, we studied clonality, genetic diversity and genetic structure of an  
23 extensive sample of the French populations using AFLP markers.

24 Clonality was high (on average, each genotype was found in three copies) but variable across  
25 sites: some genotypes had a high number of copies (> 20) and were distributed over several  
26 river catchments while 239 genotypes were unique. Genetic diversity was high but did not  
27 accumulate downstream indicating both up- and down-stream long distance gene flow  
28 through pollen and seeds.

29 Genetic diversity is structured in three major clusters. One (cluster N) is restricted to sites  
30 north of 44°4N. The other two (clusters S and E), coexist in river catchments or even in the  
31 same site. However, the highest  $F_{st}$  were found between cluster E and clusters N or S,  
32 indicating a recolonization from different refugia, one possibly located east of the Alps (cluster  
33 E) and one or two on the Western side.

34 Therefore conservation actions should take into account these three major conservation units  
35 (CU) in France. These CU cover large areas. It is thus important to maintain a natural river  
36 dynamics with frequent extinction/recolonization events over whole drainage basins.

37  
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39 restoration  
40

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52

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54 Delphine Rioux and Lucie Fiorèse did the genotyping. Lucie Fiorèse and Irène Till-Bottraud  
55 analyzed the data. Erwan Roussel produced the maps. Irène Till-Bottraud and Erwan Roussel  
56 wrote the original draft. All authors reviewed and commented the article.

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69 Durance, Aygues and Var. Rolf Holderreger provided site location of *T minima* in Csencsics and  
70 Müller 2015.

71

72 **Introduction**

73 The dwarf bulrush (*Typha minima* Hoppe) is a perennial aquatic plant that was widely  
74 distributed along pioneer habitats in the braided reaches of alpine European rivers until the  
75 middle of the 19<sup>th</sup> century (Prunier et al. 2010a; Csencsic and Müller 2015). It has since been  
76 rapidly disappearing from landscapes and is now considered as an endangered species  
77 throughout Europe (IUCN 1982). It is restricted to highly fragmented landscapes of open sand  
78 banks and islands along rivers and their secondary channels or dead arms (Armand et al.  
79 2008). In Europe, the species is now present from Eastern Romania to Italy and South-Eastern  
80 France (Austria, Czech Republic, France, Germany, Switzerland, Hungary, Italia, Yugoslavia and  
81 Romania). Most of the existing populations are in France, in more or less continuous areas  
82 along rivers in the Isère, Durance, Var and Arve drainages, and with some sites in Alsace. Some  
83 large populations are still found in Italy and Austria while Germany and Switzerland harbor  
84 only small and isolated populations (Prunier et al. 2010; Csencsic and Müller 2015).

85 As a pioneer species, the eco-hydrological conditions for regeneration include freshly  
86 disturbed, competitor-free sediments. Prime seedling recruitment habitat in the active alluvial  
87 zone occurs at the immediate margins of the main or secondary channels and is highly  
88 dependent on water table level fluctuations. *T. minima* populations have an effect on  
89 hydrogeomorphological processes, fluvial landforms and related habitat conditions by  
90 trapping fine sediment, nutrients and organic matter. This ecosystem engineering effect  
91 (sensu Jones et al. 1994) creates favorable conditions for the establishment of the next  
92 succession stages with *Alnus* and *Salix* shrubby and woody communities (Prunier et al. 2010b)  
93 and the elimination of the species through competition. On the other hand, the largest floods  
94 create new fluvial bars built with fluvial sediments reworked through banks and bed erosion  
95 and more generally rejuvenates the river landscape, leading to the disappearance of  
96 populations, together with the creation of new colonisation sites. This natural river dynamics  
97 leads to a metapopulation system, with fragmented populations submitted to frequent  
98 extinctions and recolonisations (Hanski et al. 2017). This means that it is crucial for the survival  
99 of populations that floodplains have near-natural sediment and flow dynamics and do not fall  
100 below a minimum size (Csencsic and Müller 2015).

101  
102 During the past two centuries, dikes were constructed along river beds to reduce river  
103 dynamics, control flood and gain cultivable land. This thereby drastically reduced *T. minima*  
104 population sizes and increased population isolation. The lower regression in France can be  
105 attributed to the strategy implemented by 19<sup>th</sup> century hydraulic engineers. Girel (2010)  
106 suggests that, even if the channelization of French alpine braided rivers initially contracted  
107 more drastically the fluvial bandwidth compared to the *golène* embankment practiced in Italy  
108 or Switzeland in which a narrow channel is associated to a larger floodplain, this kind of  
109 hydraulic engineering turned out, in the long term, to be more favourable to biodiversity. The  
110 construction of relatively wide (100 – 150m) channels allowed the centennial flood to run its  
111 course and permitted, within the channel, the lateral development of islands or sandbars on  
112 which typical alluvial taxa such as *Calamagrostis pseudophragmites* or *Myricaria germanica*  
113 could be maintained in addition to *T. minima* (Greulich 2017). Several conservation or  
114 reintroduction programs are underway in Switzerland (Werner 1998, Csencsic et al. 2008,  
115 Lambelet-Haueter et al. 2009), Austria (Csencsic and Müller 2015) and France (Greulich 2017,  
116 Popoff et al. 2021). To produce viable and autonomous conservation units, the major  
117 challenge of these reintroduction programs is to maintain or restore the metapopulation  
118 dynamics. For this, it is necessary to determine the size or range of existing effective

119 metapopulations. This can be answered by studying the genetic diversity and structure of  
120 natural populations in areas where metapopulations are still functional. In these areas, the  
121 genetic structure inferred from exhaustive (or extensive fine-grained) sampling should allow  
122 to identify the number and range of functional metapopulations (Pritchard et al. 2000).

123

124 The genetic diversity of *T. minima* was studied in the remaining Swiss populations using  
125 genetic markers (isozymes). It showed a low level of genetic diversity (Galeuchet et al. 2002),  
126 interpreted as the consequence of the drastic reduction in population size and number in the  
127 last century. Another study was conducted using microsatellite markers on 12 natural and 9  
128 reintroduced populations from Switzerland, Austria, Germany France and Italy (Csencsik and  
129 Müller 2015). They found (i) a higher diversity in the French and Italian populations compared  
130 to Swiss and Austrian ones, confirming the low diversity found by Galeuchet et al. (2002) in  
131 Switzerland and (ii) a strong genetic structure with populations clearly separated into seven  
132 geographic groups that could represent different metapopulations. This study, however, does  
133 not provide information on the actual range of these metapopulations because sampling was  
134 very patchy and, for the French populations, very limited compared to the actual number of  
135 sites where *T. minima* can still be found (only three samples from France). One study  
136 performed on 30 sites collected along a 60 km section of the Isère River using AFLP markers  
137 (Till-Bottraud et al. 2010) showed a much higher diversity compared to the Swiss populations  
138 of Galeuchet et al. (2002), which could be a consequence of using a more variable set of  
139 markers, and no major genetic discontinuity indicating that the whole section belongs to one  
140 single metapopulation. Although this study used a comprehensive sample along a river  
141 segment, sampling was uneven across sites due to very variable population sizes, which may  
142 have affected inferences.

143 All three studies indicated a high level of clonality and long-distance dispersal of genets (over  
144 30 km; Till-Bottraud et al. 2010). In successional species such as *T. minima*, the level of  
145 clonality may provide indications on the regeneration and age of the populations (young  
146 populations with a high regeneration by seeds will have a low level of clonality, while older  
147 populations exhibit mostly clonal reproduction; Galeuchet et al. 2002). Clonality is also  
148 important to identify as it can have a significant impact on spatial genetic structure (SGS)  
149 because some multilocus genotypes can be overrepresented over small distances (Chenault  
150 et al. 2011). Finally, over longer distances (among sites) clonality provide information on  
151 rhizome fragment dispersal.

152

153 In this context, we decided to extend the sampling of Till-Bottraud et al. (2010) to the whole  
154 distribution range of *T. minima* in Southeastern France, using an extensive sampling scheme  
155 and to analyze the whole dataset in order to answer the following questions:

- 156 1-What is the genetic diversity and clonality of the French *T. minima* populations?
- 157 2-How is this diversity structured? Is it possible to identify large groups / metapopulations?
- 158 3-Is it possible to infer the historical sources (glacial refugia) of these groups?
- 159 4-What is the level of gene flow within and between these groups?
- 160 5-What are the consequences for conservation?

161

162

## 163 **Material and Methods**

164

### 165 **Study species**

166 The dwarf bulrush (*Typha minima* Hoppe) is wind pollinated. Seeds are embedded in a hairy  
167 pericarp. They are disseminated both by wind and water and can remain on the water surface  
168 for a long time without sinking (Baur et al 2015). It is rhizomatous with a strong potential for  
169 vegetative growth and colonisation when the habitat is favourable. Rhizome fragments can  
170 be transported along the river to colonise new sites (Csencsics et al. 2008). In natural  
171 conditions, seeds are short-lived (they cannot survive more than a few weeks, Galeuchet et  
172 al. 2002), and germination only occurs immediately after arrival on suitable bare moist alluvial  
173 bars (Baur et al. 2015).

174

### 175 **Sampling**

176 The samples collected in 2008 along the Isère River (Till-Bottraud et al. 2010) were  
177 complemented in the fall of 2010 by 108 sites belonging to five river catchments (Fig.1) in  
178 order to cover the whole distribution range in southeastern France and resulting in a quasi-  
179 exhaustive sampling of this area, except for some sites that were inaccessible. One site  
180 consisted in one patch where *T. minima* was present and separated by at least 20 m from  
181 other individuals. Sampling effort was adjusted to site size, *i.e.* the larger the site, the more  
182 samples were collected, and therefore, some sites were represented by very few samples.  
183 Consequently, for population-level analyses (diversity estimates), we merged sites that were  
184 geographically close to each other (within a radius of ~500m) and had few samples into a  
185 single population, resulting in 23 merged populations and 20 isolated sites (Table 1). The  
186 number of samples per population/site varied from 1 to 127 with an average of 29 samples  
187 and a total 1069 samples. For the genetic structure analyses no merging was performed. One  
188 sample consisted of a 5 cm long fragment of green leaf stored in silicagel.

189

### 190 **Genetic analyses**

#### 191 DNA extraction and AFLP genotyping

192 Total DNA was extracted using the DNeasy 96 plant extraction kit (QIAGEN) according to the  
193 manufacturer instructions. The AFLP procedure followed Vos et al. (1995) with minor  
194 modifications. Digestion of genomic DNA was performed together with ligation of double  
195 stranded adaptors for 2 h in a 11 µL mix using 1 U of *Mse*I, 5 U of *Eco*RI (New England Biolabs)  
196 and 1 U of T4 DNA Ligase (Roche). Products were diluted 1:10 and preselective polymerase  
197 chain reaction (PCR; 120 s at 72°C, 30 cycles 30 s at 94°C, 30 s at 56°C, 120 s at 72°C with a  
198 final elongation of 10 min at 72°C) was carried out in a 25 µL volume containing Tris–HCl and  
199 MgCl<sub>2</sub> both at 1.5 mM, dNTP mix at 0.2 mM each, each primer at 0.2 µM, and 0.5 U of  
200 *Ampli*Taq DNA polymerase (Applied Biosystems). After a 1:20 dilution of pre-selective PCR  
201 products, the selective amplification (10 min at 95°C, 13 cycles 30 s at 94°C, 60 s at 65–55°C,  
202 and 60 s at 72°C, 23 cycles 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C with a final elongation  
203 of 10 min at 72°C) was carried out in a 12.5 µL volume containing Tris–HCl and MgCl<sub>2</sub> both at  
204 2.5 mM, dNTP mix at 0.2 mM each, each primer at 0,2 µM (*Eco*RI + 3 / *Mse*I + 3), and 1 U of  
205 *Ampli*Taq Gold DNA polymerase (Applied Biosystems). Three primer combinations were  
206 chosen which resulted in clear bands of sufficient variability (EAAT– MCAC, EAGC–MCTG and  
207 EAGA–MCTA). PCR products were purified using columns of half to half Sephadex G50 at 5%  
208 and Sephacryl S200. Finally 1.5 µL of the purified products was mixed with 10 µL of HiDi  
209 formamide and 0.1 µL Genescan ROX 500 size standard (Applied Biosystems), and  
210 electrophoresed on an ABI PRISM 3130 XL capillary sequencer (Applied Biosystems). PCR  
211 products from each primer combination were run separately.

212 The results from the 2008 sample were published in Till-Bottraud et al. (2010). In order to  
213 analyze simultaneously both samples (2008 and 2010), 16 DNA extracts from the 2008 sample  
214 were randomly distributed in the 96-well plates with the 2010 samples before the AFLP  
215 procedure and electrophoresis. In addition, 13 samples were duplicated in 2008 and 18 in  
216 2011 to check the reliability of AFLP genotyping. Raw data from the 2008 and 2010 AFLP  
217 genotyping were then collected and sized together using GENEMAPPER 3.7 (Applied  
218 Biosystems). The AFLP profiles were scored using a semi-automated procedure to code  
219 presence (1) or absence (0) of markers. Markers were defined manually with GENEMAPPER  
220 for presence or absence of each marker in each individual. The quality of markers were then  
221 automatically checked with an R script described in Herrmann et al. (2010), similar to that of  
222 Whitlock et al. (2008). This script removes non-reproducible markers and screens phenotype  
223 identification (presence/absence of markers). AFLP fragments shorter than 50 bp and  
224 monomorphic fragments were discarded. Only fully repeatable and polymorphic markers  
225 (lowest allele frequency above 0.01) were kept, resulting in 80 markers and a total genotyping  
226 error of 0. Eight samples did not amplify correctly and were discarded, resulting in 1061  
227 samples.

228

## 229 **Data analysis**

### 230 1- Clonality

231 The analysis of clonality was performed using the Genalex version 6.5 (Peakall and Smouse  
232 2006) software using the “Multilocus match” procedure. From this data, we estimated the  
233 number of genets and the proportion of distinguishable genotypes (G-1/N-1; Arnaud-Haond  
234 et al. 2007) for the whole sample and for each population.

235 To test the discrimination power of the dataset, we computed the probability of occurring  
236 multiple times through random mating (probability of identity,  $p(id)$ ) for each of the replicated  
237 genotypes and for 60 randomly chosen unique genotypes following Till-Bottraud et al. (2012).  
238 To avoid biased estimates due to the high number of ramets of several replicated genotypes,  
239 we used the band frequencies estimated from the genets to compute these probabilities.  
240 Moreover, as our dataset is strongly structured in 3 major clusters (see below), we computed  
241  $p(id)$  on 20 unique genotypes from each cluster.

242

### 243 2- Diversity

244 The subsequent analyses were performed on the genets: within each population, we kept one  
245 ramet for each replicated genotype in order to obtain unbiased diversity estimates (Chenault  
246 et al. 2011). Eight of the 43 populations/sites had less than five genets. In the 35 remaining  
247 populations we estimated general parameters of genetic diversity [percentage of polymorphic  
248 loci (%P), effective number of alleles (Ne) and expected unbiased heterozygosity (uHe)] using  
249 the Genalex software. We tested whether the three diversity parameters were correlated, and  
250 whether diversity was correlated with population size, using sample size (N) as an estimate of  
251 population size, or to the number of genets (G) using a linear regression ( $lm$  function in R). To  
252 see if there was an accumulation of diversity downstream, we tested whether there was a  
253 linear variation of uHe along the two major rivers (Isère and Durance) by computing as above  
254 the linear regression between uHe and the distance from most upstream population (Table  
255 1).

256 We tested isolation by distance by performing Mantel tests between the genetic ( $F_{st}/1-F_{st}$ ) and  
257 geographic distance between populations. We computed two types of geographic distances:  
258 the euclidian distance (Datm) corresponding to a straight line between each pair of

259 populations and a “river” (Dval) distance corresponding to the distance along the river  
260 corridors using classical GIS tools for hydrographic network analysis (*ArcGIS 10.5 Network*  
261 *Analyst Tools*). We computed the genetic distances using Spagedi 1.5a (Hardy and Vekemans  
262 2002) and performed the Mantel tests using Genalex. We performed Mantels for the whole  
263 sample and for the Isère and Durance rivers separately.

264  
265

### 266 3- Structure

267 We first analyzed our data using the R package MEMGENE (Galpern et al. 2014). This software  
268 was designed to detect spatial patterns of genetic structure using Moran’s eigenvector maps  
269 and a regression framework to eliminate the issues related to the Mantel test (mantel tests  
270 suffer from inflated type I error rate; Legendre and Fortin 2010; Guillot and Rousset 2013). It  
271 is moreover supposed to be efficient at detecting weak spatial genetic structure (Galpern et  
272 al. 2014). MEMGENE requires the geographic coordinates of each sample, thus only a subset  
273 of 306 samples could be used.

274 We then used the bayesian structuring method STRUCTURE (v 2.3.4; Pritchard et al. 2000). All  
275 analyses were done on a dataset containing only one ramet for each replicated genotype, with  
276 20 replicate runs for each K value, a Burnin period of 100 000 iterations followed by 200 000  
277 additional MCMC iterations, with an admixture model, uncorrelated allele frequencies and  
278 recessive alleles. The data was visualized using CLUMPAK (Kopelman et al. 2015). In all cases,  
279 all runs provided the same solution for the best K. We used two methods to infer the genetic  
280 structure of our sample. First, we followed the method of Evanno et al. (2005) to detect major  
281 clusters and sub-clusters. The best K was estimated using  $\Delta K$ , visually verifying that the highest  
282 values of LnP were not obtained with K=1. A first run was done on the whole sample with K=1  
283 to 6 (preliminary shorter runs showed that the best number of clusters was small). A second  
284 run was then performed on each cluster identified by the first run with K = 1 to 6. A third run  
285 was performed on the sub-clusters identified by the second run with K = 1 to 6. This method  
286 has, however, been criticized in case of uneven sample sizes (Kalinowski 2011; Puechmaille  
287 2016). We therefore used the “corrected PP” of Puechmaille (2016) to estimate the number  
288 of clusters. The “corrected PP” performs reasonably well even in the case of uneven sample  
289 sizes. Given the very uneven distribution of the species along river corridors, and the structure  
290 of our sample, which was meant to capture this distribution we could not *a priori* delineate  
291 populations or sub-populations, precluding the use of Puechmaille’s other parameters. We  
292 then computed pairwise  $F_{st}$  between the clusters using Genalex.

293  
294

## 295 Results

### 296 1- Clonality

297 We found clonality in most of the river catchments. We identified 82 replicated and 239  
298 unique genotypes, giving a total of 321 different genets (table 1). Global G-1/N-1 was 0.3, and  
299 ranged from 0.12 to 1 across the 35 populations. The number of copies of the replicated  
300 genotypes ranged from two to 190 (one genotype had 190 ramets, 17 genotypes had 10  
301 ramets or more; Fig.2). Probabilities of identity ( $p(id)$ ) ranged from  $4.8 \cdot 10^{-9}$  to  $5.2 \cdot 10^{-2}$  for the  
302 replicated genotypes and from  $8.1 \cdot 10^{-17}$  to  $1.9 \cdot 10^{-3}$  for the unique genotypes. We found a  
303 significant correlation between  $p(id)$  and the number of copies ( $R^2= 0.611$ ,  $r = 0.78$ ,  $df = 141$ ,  
304  $p < 0.001$ ) and the replicated genotype with the highest number of ramets (190) had the  
305 highest  $p(id)$ . Within six populations, some patches (sampling sites) harbored only one

306 replicated genotype (one site on Arve with 20 samples, four on Isère with 12, 11, 4 and 3  
307 samples and one on Durance with 7 samples). Large replicated genotypes were distributed  
308 over wide areas and often in more than one river catchment (see the distribution of four of  
309 the largest replicated genotypes on the map Fig.3).

310

## 311 2- Diversity

312 Global uHe was 0.144 and ranged between 0.011 (pop AY4) and 0.240 (pop D6) for the 35  
313 populations (table 1). The global number of effective alleles ( $N_e$ ) was 1.21 and ranged from  
314 1.02 to 1.40. The percentage of polymorphic loci (%P) ranged from 2.5 to 85%. All three  
315 estimates were highly correlated with each other (all  $p < 0.001$ ). Diversity (uHe) was correlated  
316 to the number of genets per population (G;  $p = 0.027$ ), but not with sample size (N, a proxy  
317 for population size;  $p = 0.797$ ). In Isère and Durance, diversity did not increase from upstream  
318 to downstream ( $p = 0.927$  and  $0.811$ , respectively).

319 Mantel tests of genetic vs. euclidian (Datm) and river (Dval) distances were significant for the  
320 whole sample ( $p = 0.001$  with Datm,  $p = 0.005$  with  $\log(\text{Datm})$ , and  $p = 0.001$  with Dval), but not  
321 for the Isère and Durance rivers separately (with Datm  $p = 0.382$  and  $0.101$ , respectively).

322

323

## 324 3- Structure

325 MEMGENE results show that almost half of the genetic variation is explained by the spatial  
326 pattern of distribution ( $R^2 = 0.44$ ). The first two eignefunctions represent, respectively, 85  
327 (MEMGENE1) and 13 (MEMGENE2) percent of this explained variation (Fig.4). MEMGENE1  
328 separates the Eastern and Western-most sites together with some sites on the Durance (white  
329 circles) from the rest (black circles). MEMGENE2 separates the Northern and Eastern sites  
330 (white circles) from the Southern sites (black circles).

331 For the first STRUCTURE run,  $\Delta K$  identified two clusters (Red and Blue clusters, Fig.5). Most  
332 genets were assigned to either cluster with membership coefficients ranging from 0.6 to 1 (71  
333 and 242 genets to the Orange and Blue clusters, respectively). This separation corresponds to  
334 the first eigenvector identified by the MEMGENE analysis (Fig.4). The Orange cluster could be  
335 further divided into 2 sub-clusters (Fig.5). One sub-cluster (E, 64 genets) was widely  
336 distributed in the Durance, Aygues and Var catchments, the other sub-cluster (Z, 7 genets)  
337 was restricted to two populations on the Aygues River (AY2 and AY3). The Blue cluster could  
338 be further divided into 3 sub-clusters (Fig.5). One sub-cluster (N, 148 genets) was restricted to  
339 the northern part of the sampling range. The second was restricted to the southern part of  
340 the sampling range (S, 83 genets). The third (X) included only eight genets distributed over the  
341 whole range. This separation corresponds to the second eigenvector identified by the  
342 MEMGENE analysis (Fig.4). Sub-clusters E, N and S did not show further substructure. We also  
343 identified several individuals with mixed ancestry (average membership coefficient over the  
344 20 runs of  $0.5 \pm 0.1$  to two clusters or sub-clusters). Eight genets were of mixed ancestry  
345 between the Orange and Blue clusters and came all from the Durance or Aygues Rivers in  
346 areas where Clusters E and S coexist (see Fig.1). One genet from the Orange cluster on the  
347 Durance River was of mixed ancestry between sub-clusters E and Z. Three genets from the  
348 Blue cluster (two on the Durance River and one on the Isère River) were of mixed ancestry  
349 between sub-clusters N, S or X. Moreover, 11 individuals belonged unequally to two sub-  
350 clusters in the second run (membership coefficient to each sub-cluster ranging from 0.2 to  
351 0.4), and sub-clusters X and Z correspond to genets that showed partial admixture between  
352 the Orange and Blue clusters in the first STRUCTURE run (membership coefficient to each

353 cluster ranging from 0.2 to 0.4). Fig.1 summarizes the distribution of samples assigned to the  
354 different Sub-clusters and mixed ancestry individuals (hybrids) in the different sites.  
355 Using the “corrected PP” parameter on the results from the first STRUCTURE run, we identified  
356 four clusters. Three of them correspond exactly to the N, S and E Sub-clusters using  $\Delta K$  and  
357 the fourth one corresponds to Sub-cluster X and individuals with mixed ancestry between the  
358 Orange and Blue clusters. Genets assigned to Sub-cluster Z were not assigned to a specific  
359 cluster, but showed mixed ancestry between two clusters with a membership coefficient to  
360 one cluster ranging between 0.2 and 0.4 with this method.  
361 The two methods (“ $\Delta K$ ” of Evanno et al. 2005 and “corrected PP” of Puechmaille 2016) thus  
362 converged to identify three major clusters, one or two minor clusters and several genets with  
363 various levels of mixed ancestry. This structure is congruent with the results of the MEMGENE  
364 analysis. The northern cluster (Cluster N) groups all populations north of 44.4°N. South of this  
365 line, Cluster E occurs in all river catchments (Durance, Aygues and Var) while Cluster S is  
366 predominant in the Durance and is present in only two sites in the Aygues River. The minor  
367 clusters X and Z are probably “spurious clusters” as defined by Puechmaille (2016), with no  
368 real biological meaning. Pairwise  $F_{st}$  was 0.85 and 0.86 between Sub-cluster E and Sub-clusters  
369 N or S, respectively, and 0.54 between Sub-clusters N and S.  
370 Clonality was distributed differently in the three major clusters (Fig.2, Table 2). Sub-cluster E  
371 had no replicated genotypes and  $p(id)$  of the unique genotypes from E ranged from  $2 \cdot 10^{-14}$  to  
372  $2 \cdot 10^{-4}$ . Sub-cluster S had the largest replicated genotype (190 ramets) but other replicated  
373 genotypes had no more than 21 ramets. In this cluster,  $p(id)$  ranged from  $4.8 \cdot 10^{-9}$  to  $5.2 \cdot 10^{-2}$   
374 for the replicated genotypes and from  $8.3 \cdot 10^{-10}$  to  $3.2 \cdot 10^{-3}$  for the unique genotypes. Sub-  
375 cluster N had seven highly replicated genotypes (between 20 and 60 ramets) and 6 genets had  
376 between 10 and 20 ramets. In Sub-cluster N,  $p(id)$  ranged from  $4.2 \cdot 10^{-6}$  to  $1.9 \cdot 10^{-2}$  for the  
377 replicated genotypes and from  $8.1 \cdot 10^{-17}$  to  $1.9 \cdot 10^{-3}$  for the unique genotypes. Cluster E had  
378 the highest diversity followed by S and N (uHe estimated on the genets = 0.103, 0.069 and  
379 0.053, respectively; Table 2).

380  
381

## 382 Discussion

### 383 1- Clonality and genetic diversity

384 Clonality was very important in our *Typha minima* populations, with a global level of clonality  
385 (G/N value) of 0.3, meaning that each genotype was found in more than three copies on  
386 average. This value is higher than what was found in 2008 on the Isère sample (G/N value of  
387 0.58, Till-Bottraud et al. 2010). These global values however, hide an extremely large variation  
388 among populations (levels of clonality ranging from 0.12 to 1, with some monogenotypic  
389 patches within six large populations) and among genotypes (one genotype had 190 ramets,  
390 17 genotypes had 10 ramets or more, and 239 genotypes were unique).

391 The discrimination power of our dataset was globally high, except for some replicated  
392 genotypes. The genotypes with the highest number of ramets also had the highest probability  
393 of occurring multiple times through random mating indicating that some individuals that we  
394 identified as ramets of a clone could be in fact different genets. However, several clones had  
395 a significantly low probability of occurring multiple times. Moreover, probabilities in the range  
396 of  $10^{-2}$  to  $10^{-4}$ , although arguing for a possible origin of identical genotypes through sexual  
397 reproduction, cannot explain the very high number of copies of some genets. Thus, although  
398 it might be slightly overestimated, clonality is very important in most sites.

399 The global variability of our extensive sample of French populations was similar to the values  
400 obtained on the Isère sample alone in 2008 (Till-Bottraud et al. 2010). Genetic diversity,  
401 estimated on the genets was of 0.144 compared to 0.139 in 2008. However diversity per  
402 population had a broader range than in 2008 (uHe ranged from 0.011 to 0.240 compared to  
403 0.033 to 0.141 in 2008). Galeuchet et al. (2002) studied the clonality and the genetic diversity  
404 of six Swiss natural populations using isozymes. They found a high clonality level and a low  
405 genetic diversity (1 to 17 genotypes per population with a sample size of 30; number of alleles  
406 per locus ranging from 1.3 to 1.8), which could be due to a low discrimination power of  
407 isozyme markers (Till-Bottraud et al. 2010). Csencsics and Müller (2015) studied populations  
408 from France, Italy, Switzerland, Germany and Austria using microsatellite markers and found  
409 a higher diversity in the French and Italian populations compared to Swiss and Austrian ones,  
410 confirming the low diversity found by Galeuchet et al. (2002) in Switzerland.  
411 The genetic diversity values we obtained fall in the medium range of diversity obtained with  
412 AFLPs on other species (Gaudeul et al. 2000; Jump et al. 2006; Tsumura et al. 2007) and is  
413 quite similar that of other clonal species (Widen et al. 1994). Genetic diversity was very  
414 variable across other *Typha* species. Almost no variability was found for *T. latifolia* with  
415 isozymes (Mashburn et al. 1978; Sharitz et al. 1980). Using AFLPs, Lamote et al. (2005) found  
416 a high diversity in Flanders, and greater in *T. angustifolia* compared to *T. latifolia* (%  
417 polymorphic bands = 81 vs. 46%). The same result was found using microsatellite markers in  
418 Ukraine (He= 0.66 and 0.37, respectively; Tsyusko et al. 2005), China (He = 0.205 and 0.101,  
419 respectively; Zhou et al. 2016) and northeastern United States (He = 0.339, and 0.271,  
420 respectively; Pieper et al. 2020). Clonal diversity is similarly quite variable, ranging from 0.32  
421 to 0.86 with AFLPs (Lamote et al. 2005) and microsatellites (Tsyusko et al. 2005; Pieper et al.  
422 2020). As with *T. minima*, clones were observed within populations as well as among  
423 populations (Lamote et al. 2005). A lower clonality was found in *T. angustifolia* than in *T.*  
424 *latifolia* (Lamote et al. 2005; Tsyusko et al. 2005; Pieper et al. 2020).

425

## 426 2- Genetic structure

427 The genetic diversity of the French *Typha minima* populations shows a very strong structure  
428 (three major groups) over large distances, but not among drainage basins. This was  
429 unexpected given the strong geographical discontinuity of the distribution as shown on the  
430 map in Fig.1 (we sampled *T. minima* from almost all know sites). Significant IBD was identified  
431 over the whole range but not along the two major rivers harboring *T. minima* populations  
432 (Durance and Isère) and diversity did not accumulate downstream along these river systems.  
433 The strongest genetic differentiation does not correspond to a geographic structuration:  
434 although the North/South divide is spatially very clear and individuals from Clusters E and S  
435 occur in the same sites or in adjacent sites, the predominant genetic structure was between  
436 Cluster E and Clusters S or N. The genetic differentiation follows this pattern (largest  $F_{st}$  -  
437 greatest genetic differentiation- between Cluster E and Clusters N and S). An additional  
438 difference was that we found no replicated genotypes in Cluster E.

439

440 In a study of populations from France, Italy, Switzerland, Germany and Austria Csencsics and  
441 Müller (2015) identified seven different clusters. One of their French population was collected  
442 on the same site as our Giffre population (Arve catchment, cluster N), another one on the  
443 same site as our D34 population (Durance catchment, Cluster S). Cluster N and S are thus  
444 probably equivalent to the “Giffre/Rhône” and “Durance” clusters, respectively, of Csencsics  
445 and Müller (2015). The third population was collected in the “Haute Durance” reach but we

446 did not find plants in this site in 2010. The Italian populations that were sampled on the Dora  
447 Riparia are geographically close to our southeastern populations of Cluster E (Var River) and  
448 could belong to the same cluster.

449 Over more restricted distances and using isozymes, Galeuchet et al. (2002) found low genetic  
450 differentiation among populations (mean genetic distance of 0.03) and no isolation by  
451 distance. Using microsatellite markers, isolation by distance was found in *T. latifolia*, but not  
452 in *T. angustifolia* in Ukraine (Tsyusko et al. 2005) and China (Zhou et al 2016), while the reverse  
453 was found in the eastern United States (Pieper et al 2020). Genetic structure was also different  
454 between the two species across continents, with 3 distinct groups in *T. latifolia* and one in *T.*  
455 *angustifolia* in Flanders (Lamote et al. 2005), 3 groups in *T. angustifolia* in the eastern United  
456 States with genotypes of two of them scattered across the sampling area (similarly to our E  
457 and S clusters) and one in *T. latifolia* (Pieper et al 2020) and no apparent structure for either  
458 species in Ukraine (Tsyusko et al. 2005). In general aquatic macrophytes tend to show little  
459 geographic structure (*Sisymbrium austriacum* in Belgium, Jacquemyn et al. 2006; *Sparganium*  
460 *emersum* in Flanders, Pollux et al. 2007; *Nuphar lutea* in the Czech Republic; Fer & Hroudova  
461 2008; *Phragmites australis* in the northeastern USA, Kirk et al 2011; *Phragmites australis* and  
462 *Triglochin procerum* in Australia, James et al. 2013; *Ranunculus subrigidus* in the Qinghai-Tibet  
463 plateau, Wu et al. 2019) although Garcia-Giron et al. (2019) identified a strong structure in  
464 *Myriophyllum alterniflorum* over short distances (~30 km) in Spain. The results we obtained  
465 on *T. minima* also show a striking similarity to what was found for the Black Poplar, *Populus*  
466 *nigra*, a protected species that requires a similar habitat for its natural regeneration and which  
467 shows little natural regeneration in northern Europe: Faivre-Rampant et al (2016) found a  
468 large genetic diversity and only a restricted number genetic clusters over Europe, indicating  
469 large scale dispersal and populations or metapopulations distributed over large areas.

470

### 471 3- Historical sources (refugia)

472 Although Kalinowski (2011) shows that the clusters created by STRUCTURE are not  
473 systematically consistent with the evolutionary history of the populations, the convergent  
474 results provided by our different analyses and the  $F_{st}$  values suggest that Cluster E was  
475 separated from Clusters N and S for a longer time, or by stronger barriers.

476 During the late glacial maximum, many European species survived in isolated southern refugia  
477 (Iberian Peninsula, Italy, Balkans; e.g. *Sedum album*, Listl et al. 2017) or at the southern edge  
478 of the cold and dry steppe-tundra area in eastern, central, and southwestern Europe, leading  
479 to strong divergence among different lineages (Petit et al. 2003). Recolonisation from these  
480 refugia was constrained by geographical barriers such as the Alps (Taberlet et al. 1998). On  
481 the other hand, several Alpine species were shown to have survived in local ice-free peripheral  
482 areas or on nunatacks (Schönschwetter et al. 2005). In their review of the phylogeographic  
483 patterns of Alpine species, Schönschwetter et al. (2005) identified a well-supported  
484 southwestern-Alpine peripheral refugium between Nice and the Aoste valley and several  
485 potential peripheral refugia on the western border of the French Alps, the latter confirmed by  
486 a study on *Arabis alpina* with a clear divide between southern and northern populations  
487 (Rogivue et al. 2018). *T. minima*, while not a strictly alpine species, is restricted to the braided  
488 reaches of alpine European rivers and has probably a similar history than some alpine species.  
489 Indeed, the distribution of native North American hydrophytes can be related to historical  
490 processes such as the retreat of the Wisconsin glacier while climatic factors seem to have  
491 limited importance (Santamaria 2002). Moreover, the biogeography of *Ranunculus subrigidus*,  
492 an aquatic macrophyte of the Qinghai-Tibet plateau, is similarly suspected of being influenced

493 by mid-to-late Pleistocene events (Wu et al. 2019). Cluster E could thus originate from an  
494 eastern (Italian) refugium or from the “southwestern-Alpine peripheral refugium” of  
495 Schönswetter et al. (2005), while clusters N and S originate from one or several western  
496 refugia.

497 The North/South divide in the French external Alps is interesting: in the Durance catchment,  
498 the most upstream population of the Durance River (HD) is mostly assigned to Cluster N.  
499 Symmetrically, the southernmost population of the Isère catchment, the Drac population, is  
500 assigned to Cluster S. These two populations lie, respectively, north and south of 44.4°N,  
501 suggesting a phylogeographic or a climatic limit at this latitude. Ozenda (1985) describes a  
502 climatic limit in the French Alps at 45°N separating the “Alpes nord-occidentales” from the  
503 “Alpes sud-occidentales”. This limit also corresponds to the divide between southern and  
504 northern populations of *A. alpina* (Rogivue et al. 2018). This suggests that Clusters N and S  
505 either originated from two separate refugia in the French external Alps, or that local climatic  
506 conditions have recently separated these two groups, or as a combination of both causes, *i.e.*  
507 that recent climatic conditions have maintained or even reinforced a divergence due to post-  
508 glacial recolonisation.

509 An alternative explanation for cluster E is that, similarly to what was suggested by Pieper et al  
510 (2020) for *T. angustifolia* in the United States, it is an escaped cultivated lineage. Garden  
511 centers sell *T. minima* by internet over all of Europe. One garden center calls it the “dwarf  
512 Japanese bull rush” indicating a possible foreign origin. However, no plants belonging to  
513 cluster E were found in the northern part of our study area, [which](#) makes this a less plausible  
514 explanation than for *T. angustifolia* in the US.

515

516

#### 517 4- Gene flow within and between groups

518 Although the global large-scale structure is very robust, evidence of current gene flow is  
519 pervasive in our data. First, several clones are distributed along river reaches and even across  
520 river catchments, indicating long-distance transport of rhizome fragments. The distribution of  
521 clones over long river reaches can be explained by downstream transport of rhizome  
522 fragments by water, most probably during bankfull flood events for which sediment transport,  
523 erosion and deposition are the most efficient in the long term (Wolman and Miller, 1960). The  
524 occurrence of the same clone in different river catchments (e.g. clone II in Durance, Aygues  
525 and Drac) suggests other means of transport, such as human accidental transport with river  
526 sediments or active transplantation, or possibly aquatic birds (Santamaria 2002; James et al.  
527 2014). Alternatively, different genets that could not be distinguished due to the medium  
528 discrimination power of our dataset could occupy the different catchments.

529 Second, diversity did not accumulate downstream along these river systems. This indicates  
530 that gene flow is bidirectional (upstream and downstream). The upstream movement is  
531 probably due to wind transport of pollen and seeds, while the downstream movement could  
532 be due to both water and wind transport of seeds, and water transport of rhizome fragments.

533 Third, several genets showed various levels of admixture between clusters or sub clusters,  
534 suggesting gene flow and hybridization over a large range of distances (see Fig.1). Eleven  
535 individuals that were found to have roughly equal probability of belonging to two clusters are  
536 probably F1 hybrids. Some occur in sites where individuals from two different clusters coexist,  
537 but three were found in a river catchment where only one parent cluster is present.  
538 Additionally, 26 individuals belonging unequally to two clusters are possibly the result of  
539 various levels of backcrosses. Some could thus be the result of local hybridization with no

540 further dispersal, but others suggest long distance pollination events across river catchments  
541 or long distance seed dispersal.

542 On the other hand, we identified a strong genetic discontinuity in the upper reaches of the  
543 Drac and Durance Rivers. Important dam systems occur downstream of both the Haute  
544 Durance and the Drac sites (see Fig.1), suggesting that they could represent a barrier to gene  
545 flow. However, as these are the only river reaches inhabited by *T. minima* where dams are  
546 present, generalization is not possible.

547 High levels of gene flow are usually estimated in aquatic plants. In his review, Santamaria  
548 (2002) describes compelling evidence of high dispersal rates due to potential long distance  
549 dispersal by waterfowl and describes a dispersal function that peaks locally but has a thick  
550 tail. This has been confirmed by more recent studies (e.g. Jacquemyn et al. 2006; Pollux et al.  
551 2007; Fer & Hroudova 2008; Kirk et al 2011; James et al. 2013; Wu et al. 2019; Pieper et al.  
552 2020; but see also Garcia-Giron et al. 2019 for an example of strong IBD and gene flow  
553 restricted mostly between neighboring populations).

554

#### 555 5- Consequences for conservation

556 France is the European country with the largest number of natural populations of *T. minima*  
557 (Prunier et al. 2010a). It also harbors a very high genetic diversity, together with Italy  
558 (Csencsics and Müller 2015). Our exhaustive sampling showed that French populations belong  
559 to three different genetic clusters. One of these clusters (N) corresponds to the Western  
560 Switzerland cluster identified by (Csencsics and Müller 2015), another (E) is probably the same  
561 as the one found in Italy, and the third one (S) is unique to France. All this highlights the  
562 responsibility of France for the protection of this species. Consequently, a regional action plan  
563 (“plan régional d’action Auvergne-Rhône-Alpes et Provence-Alpes-Côte d’Azur”) is being  
564 developed by a network of stakeholders (réseau Flore sentinelle <https://floresentinelle.fr/>).

565

566 In most species, existing populations inhabit easily delimited areas that can be set aside for  
567 protection. For pioneer species inhabiting very dynamic habitats and functioning as  
568 metapopulations, as is the case for *T. minima*, protecting sites where the species is present at  
569 a certain point in time is not sufficient. We need to identify the range at which a  
570 metapopulation can be viable, *i.e.* what is the size of a functional conservation unit? For *T.*  
571 *minima*, we identified three major genetic groups that are distributed over very large areas  
572 (each approx.  $2.10^4$  km<sup>2</sup>). We moreover found significant gene flow between sites and even  
573 river catchments, through transport of rhizome fragments, but also pollen and seeds that are  
574 transported both down- and up-stream. This gene flow confirms the large geographical reach  
575 and the strong dynamics of *T. minima* metapopulations.

576 The structure could, however, be the signature of an ancient favorable situation (before the  
577 major flood control actions started in European Alpine rivers ~100-200 years ago) in which the  
578 time elapsed since fragmentation was not sufficient to develop a local genetic structure and  
579 reduce genetic diversity. Such a situation was suspected in two wetland macrophytes in  
580 Australia (*Phragmites australis* and *Triglochin procera*; James et al. 2013), the long-lived Alpine  
581 perennial *Eryngium alpinum* L. (Gaudeul and Till-Bottraud 2008), and in the short-lived  
582 perennial *Laserpitium prutenicum* L., where it was called “silent goodbye” (Reichel et al. 2016).

583 To detect effective actual gene flow would require testing seeds or very young seedlings.

584 In this context, it is important to maintain natural river processes and dynamics with frequent  
585 extinction/recolonization events to maintain functional connectivity along whole drainage  
586 basins or whole river systems. Climate change impacts on Alpine rivers may also drive,

587 through contrasted effects on the upstream and the downstream parts, the development of  
588 a much more contrasted, or even fragmented, riverine landscape restricting the connectivity  
589 between populations (Bard et al., 2015; Giuntoli et al., 2013). On the other hand, there is a  
590 strong incitation in France and the EU to restore fluvial longitudinal and lateral connectivity of  
591 sediment and water in order to thwart the general alpine rivers incision resulting from both  
592 natural and anthropic controlling factors (Peiry et al, 1994). This should result in a much more  
593 natural fluvial dynamics promoting transient fluvial landforms such as braiding islands, which  
594 are a favourable pioneer habitat for *T. minima*.

595  
596 Reinforcement/Reintroduction programs require using genotypes originating from the same  
597 gene pool to avoid maladaptation (Derry et al. 2019) or outbreeding depression (Frankham et  
598 al. 2011). We suspect a climatic limit at Lat 44.4°N between individuals from Cluster N and  
599 Cluster S. This local adaptation should therefore be tested using e.g. reciprocal transplant  
600 experiments. However, as a precautionary approach we suggest the reintroduction of  
601 individuals from Cluster N in the northern part of the range. In the southern part, Clusters E  
602 and W, although exhibiting the strongest genetic differentiation, coexist, indicating similar  
603 climatic requirements, and thus a reduced risk of maladaptation. The risk of outbreeding  
604 depression might however be greater as the low level of clonality of the inter-cluster hybrids  
605 (only three of the 37 admixed genotypes were replicated and had only two copies) indicate a  
606 low vegetative propagation capacity or a reduced lifespan. This interpretation must however  
607 be put into perspective because we found no clonality in Cluster E where we have no  
608 indication of reduced fitness. In the South, we thus suggest to reinforce populations with  
609 individuals from the same cluster or to reintroduce individuals from only one cluster to  
610 prevent inter-cluster hybridization.

## 611 **Conclusions**

613 In this study, we showed that *Typha minima*, an endangered pioneer species, is structured in  
614 three genetic clusters over its distribution range in southeastern France. Similarly to what was  
615 found in several aquatic macrophytes, these clusters span very large areas (2. 10<sup>4</sup> km<sup>2</sup>), exhibit  
616 high levels of diversity and high levels of gene flow via both rhizome fragments and  
617 reproductive propagules (pollen and seeds). This indicates that in this region, three  
618 metapopulations are present and are still potentially viable if the river dynamics is maintained.  
619 The absence of fine-scale genetic structure despite the large geographic discontinuities of the  
620 distribution was unexpected and could be due to a faster rate of habitat loss compared to the  
621 loss of genetic diversity. Evidence of actual (present) gene flow should be investigated by  
622 studying e.g. in situ germinations. The origin of the strong structure is, on the other hand, still  
623 unclear and could be the result of post-glacial recolonization or local adaptation. The latter  
624 could be tested using e.g. reciprocal transplant experiments.

625  
626 *T. minima* is restricted to the braided reaches of alpine European rivers. These reaches were  
627 severely impacted by the flood control methods implemented during the 19<sup>th</sup> century,  
628 strongly restricting the natural flow dynamics to reduce flood impacts and increase available  
629 farming areas. The consequence was globally detrimental to rare habitats now protected by  
630 the EU Habitats Directive such as water courses of plain to montane levels, alluvial forests,  
631 calcareous fens but also floodplain grasslands and was more globally detrimental to  
632 biodiversity. However, the impact of the channeling system chosen for the Isère River was not  
633 as dramatic as that of the Italian “golena system” used in other Alpine rivers. The awareness

634 that these measures, together with climate change, also impact water availability and  
635 sediment transport along the river continuum, opens avenues for restauration of better river  
636 continuity and of the habitats and species that depend on dynamic riverine systems.

637  
638 The regeneration habitats created by dynamic rivers are also important for other endangered  
639 plant species such as *Populus nigra* or *Centaureum favargerii*, *Corispermum gallicum*, *Polygala*  
640 *exilis* which are notably difficult to detect, but also protected animal species such as the  
641 Eurasian beaver (*Castor fiber*) or the Eurasian otter (*Lutra lutra*). For all these species, natural  
642 / unhampered river dynamics is a crucial factor for their maintenance. *Typha minima*, one of  
643 the finest indicator species for the health or naturalness of a river (Prunier et al 2010a), is a true  
644 umbrella species for these habitats. The rapid metapopulation dynamics of the species  
645 requires now to move away from a classical approach of site protection and to take into  
646 account river dynamics, functionality and the presence of endangered species over large river  
647 sections in all future river development projects such as dams, gravel extraction etc. both  
648 upstream and downstream of the sites where the species is present. Although *T. minima* still  
649 occupies large and continuous river sections, France has a major stake in the protection of the  
650 species in Europe.

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**Table 1:** Characteristics, diversity estimates and cluster assignment of the sites and merged populations studied.

Pop	Isolated/ Merged	River	Year	N	G	D(km)	G-1 /N-1	%P	Ne	SE(Ne )	uHe	SE(uHe)	Main cluster	2nd clusters
Arve	M	Arve	2010	38	12		0,30	37,50	1,149	0,032	0,095	0,019	N	X
Giffre	I	Arve	2010	20	3								N	
Isère1	I	Isere	2010	1	1								N	
Isère3	M	Isere	2010	35	11	0,00	0,29	12,50	1,048	0,019	0,031	0,011	N	
Isère11	I	Isere	2008	22	11	3,23	0,48	12,50	1,060	0,022	0,038	0,013	N	
Isère12	M	Isere	2010	59	24	4,26	0,40	17,50	1,048	0,016	0,034	0,011	N	
Isère15	M	Isere	2010	70	32	14,37	0,45	28,75	1,062	0,020	0,042	0,012	N	
Arc1	I	Isere	2010	7	3								N	
Arc2	I	Isere	2010	22	9		0,38	13,75	1,058	0,022	0,037	0,013	N	
Arc3	M	Isere	2010	29	9	18,67	0,29	12,50	1,041	0,016	0,030	0,010	N	
Isère37	I	Isere	2010	10	7	19,77	0,67	10,00	1,036	0,014	0,027	0,010	N	
Isère42	I	Isere	2008	23	16	35,31	0,68	12,50	1,052	0,020	0,033	0,012	N	
Isère43	M	Isere	2008	19	15	35,69	0,78	21,25	1,083	0,025	0,053	0,015	N	
Isère45	I	Isere	2008	5	4								N	
Isère47	M	Isere	2008	16	7	40,69	0,40	6,25	1,039	0,019	0,024	0,011	N	
Isère49	M	Isere	2008	21	12	42,57	0,55	28,75	1,113	0,027	0,075	0,017	N	X
Isère55	M	Isere	2008	33	18	44,15	0,53	22,50	1,073	0,021	0,050	0,013	N	H
Isère59	I	Isere	2008	23	13	48,68	0,55	10,00	1,048	0,021	0,030	0,012	N	
Isère61	I	Isere	2008	28	9	52,25	0,30	7,50	1,038	0,019	0,024	0,011	N	
Isère62	I	Isere	2008	16	8	55,89	0,47	8,75	1,043	0,019	0,028	0,011	N	
Isère64	M	Isere	2008	25	17	59,37	0,67	17,50	1,050	0,017	0,035	0,011	N	
G72	I	Isere	2010	21	11	60,32	0,50	7,50	1,061	0,024	0,035	0,014	N	
Isère67	I	Isere	2008	10	4								N	
Isère69	M	Isere	2008	16	5	71,77	0,27	7,50	1,044	0,020	0,029	0,012	N	
Drac	I	Isere	2010	3	2								S	
DuranceH	M	Durance	2010	13	10	0,00	0,75	17,50	1,069	0,023	0,045	0,014	N	S

Durance34	M	Durance	2010	51	23	53,82	0,44	45,00	1,117	0,026	0,079	0,016	S	N/H/X
Durance6	M	Durance	2010	29	28	75,69	0,96	81,25	1,401	0,043	0,240	0,022	E	S/H
Durance9	M	Durance	2010	17	12	102,52	0,69	13,75	1,082	0,026	0,050	0,015	S	
Durance43	M	Durance	2010	20	7	125,64	0,32	56,25	1,312	0,037	0,206	0,023	S	H/Z
Asse	M	Durance	2010	77	10	145,64	0,12	18,75	1,071	0,023	0,047	0,014	S	
Durance12	M	Durance	2010	46	25	190,67	0,53	85,00	1,215	0,035	0,140	0,018	S	E/H/X
Durance23	M	Durance	2010	127	27	191,22	0,21	56,25	1,104	0,026	0,069	0,015	S	X/H
Durance15	M	Durance	2010	11	8	193,39	0,70	28,75	1,134	0,031	0,086	0,018	S	X
Durance3	M	Durance	2010	10	8	194,51	0,78	10,00	1,040	0,017	0,028	0,010	S	
Durance2	I	Durance	2010	2	2								E	X
Durance1	I	Durance	2010	6	3								S	
Aygues3	I	Aygues	2010	5	5		1,00	20,00	1,143	0,036	0,088	0,021	Z	E
Aygues4	I	Aygues	2010	15	5		0,29	2,50	1,018	0,013	0,011	0,008	S	
Aygues5	M	Aygues	2010	12	12		1,00	23,75	1,120	0,031	0,073	0,018	E	
Aygues1	I	Aygues	2010	15	6		0,36	6,25	1,030	0,017	0,020	0,010	S	
Aygues2	I	Aygues	2010	20	20		1,00	61,25	1,262	0,036	0,167	0,021	E	Z/H
Var	M	Var	2010	12	12		1,00	20,00	1,108	0,031	0,065	0,017	E	

Grand Mean and SE over Loci and Pops  
(for pops with more than 5 Genets)

Global 1061 321 0,30 100 1,211 0,030 0,144 0,016

825 Pop: site or population name (listed from North to South). Isolated/ Merged; I: sites with a high number of samples or isolated; M: the populations result  
826 from merged sites that were geographically close to each other and had few samples. Year: sampling year. N: sample size. G: number of genets. D: distance  
827 from the uppermost populations (for Isère and Durance Rivers only). G-1/N-1: level of clonality (proportion of distinguishable genotypes). %P: percentage of  
828 polymorphic loci. Ne: effective number of alleles. uHe: unbiased heterozygosity. SE(Ne) and SE(uHe) are the respective standard errors. Main cluster:  
829 cluster to which most of the genets were assigned. 2nd clusters: clusters to which the remaining genets were assigned.

830 **Table 2:** Genetic diversity of the different clusters identified by the STRUCTURE analysis.  
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Cluster	N	G	G-1 /N-1	%P	Ne	SE(Ne)	uHe	SE(uHe)
E	63	63	1,00	41,25	1,171	0,035	0,103	0,019
N	582	141	0,24	60,00	1,075	0,020	0,053	0,012
S	388	82	0,21	45,00	1,101	0,023	0,069	0,014
X	8	8	1,00	72,50	1,248	0,029	0,180	0,018
Z	7	7	1,00	15,00	1,110	0,033	0,064	0,018

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 833 N: sample size. G: number of genets. D: distance from the uppermost populations (for Isère and Durance only).  
 834 G-1/N-1: level of clonality (proportion of distinguishable genotypes). P: percentage of polymorphic loci. Ne:  
 835 effective number of alleles. uHe: unbiased heterozygosity. SE(Ne) and SE(uHe) are the respective standard  
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**Fig.legends**

Fig.1: Map of all investigated sites of *T. minima*. For each site, the size of the circle is proportional to the number of individuals sampled, and the colors show the proportion of individuals assigned to each of the five clusters (N, X, S, E and Z; see fig. 5 and text) or to admixed individuals (H), as determined by the STRUCTURE analysis. To prevent overlapping of symbols, close populations were slightly moved apart.

Fig.2: Distribution of copy number per replicated genotype; number of genotypes with 1, 2, .. 190 copies from Clusters E(blue), S (orange) and N (green), as determined by the STRUCTURE analysis. "15-": 15 to 19; "20-": 20 to 29; "30-": 30 to 39; "40-": 40 to 49; "50-": 50 to 189".

Fig.3: Distribution of four of the largest replicated genotypes. Clone D and Clone E belong to Cluster N; Clone HH and Clone II belong to Cluster S. Clusters determined by STRUCURE and clones determined by Genalex.

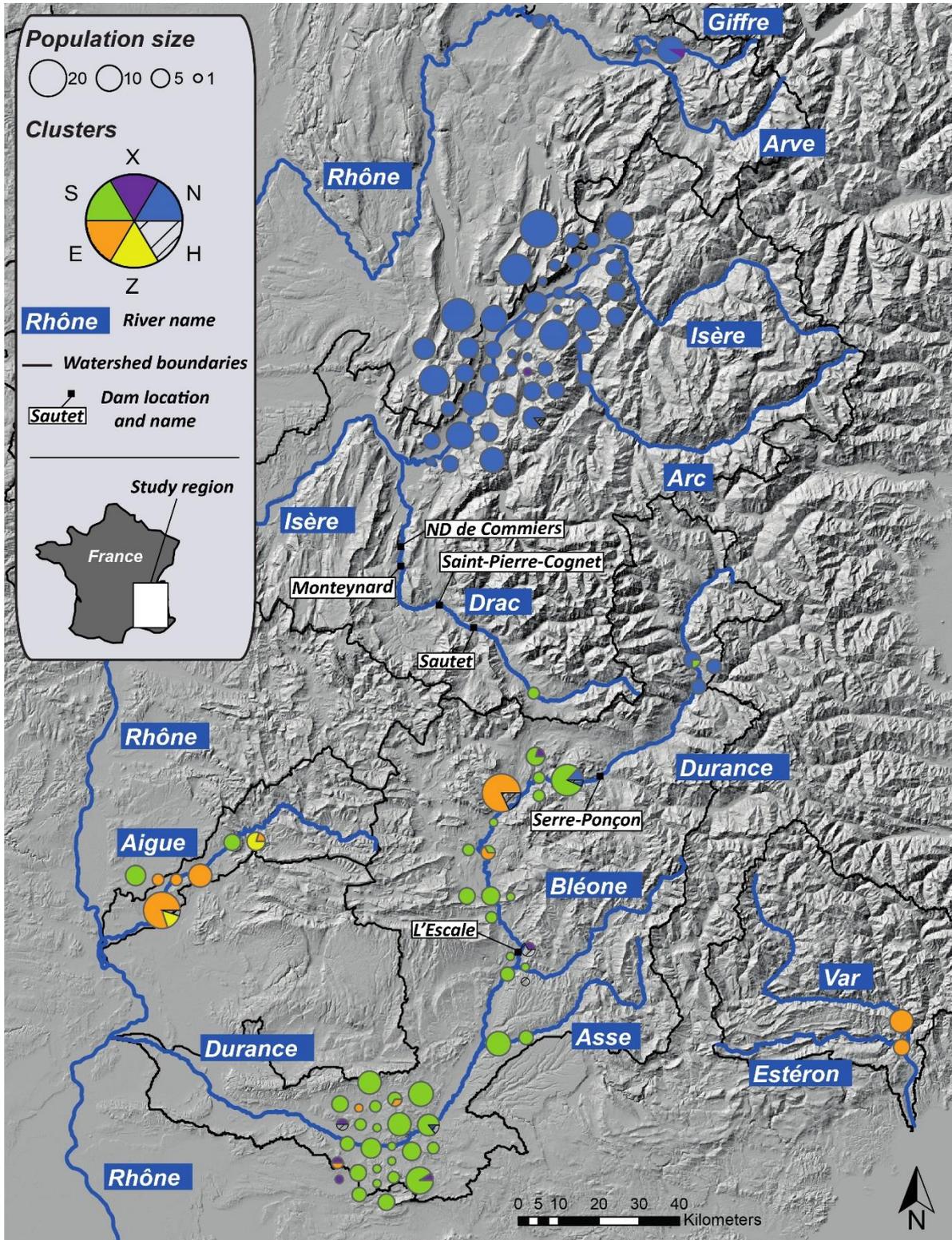
Fig.4: MEMGENE results for eigenvector 1 (left; 85% of the variance explained by the model) and eigenvector 2 (right; 13% of the variance explained by the model). Dashed lines represent watershed boundaries (as in Fig.1). Values next to the circles in the legend box represent the scores: large black and white circles describe opposite extremes on the MEMGENE axis (eigenvector) and the size of the circle diminishes towards the center of the axis. In the graph, circles of a similar size and color indicate individuals with similar scores and groups of circles with similar scores close to each other indicate a spatial pattern.

Fig.5: Inference of population structure obtained from Bayesian analysis in STRUCTURE. The first run (First analysis) indicated an optimum of two clusters (Orange and Blue clusters). Each cluster was further subdivided into two or three sub-clusters (Second analysis). For each analysis, the population structure is shown on the left (sites arranged from north to south) and the  $\Delta K$  values are on the right.

**Supplementary material:**

Fig.S1: Map of all investigated sites of *T. minima* showing the grouping of sites into populations (each population is represented by a different color).

878 **Fig.1:** Map of all investigated sites of *T. minima*. For each site, the size of the circle is  
 879 proportional to the number of individuals sampled, and the colors show the proportion of  
 880 individuals assigned to each of the five clusters (N, X, S, E and Z; see fig. 5 and text) or to  
 881 admixed individuals (H), as determined by the STRUCTURE analysis. To prevent overlapping  
 882 of symbols, close populations were slightly moved apart.  
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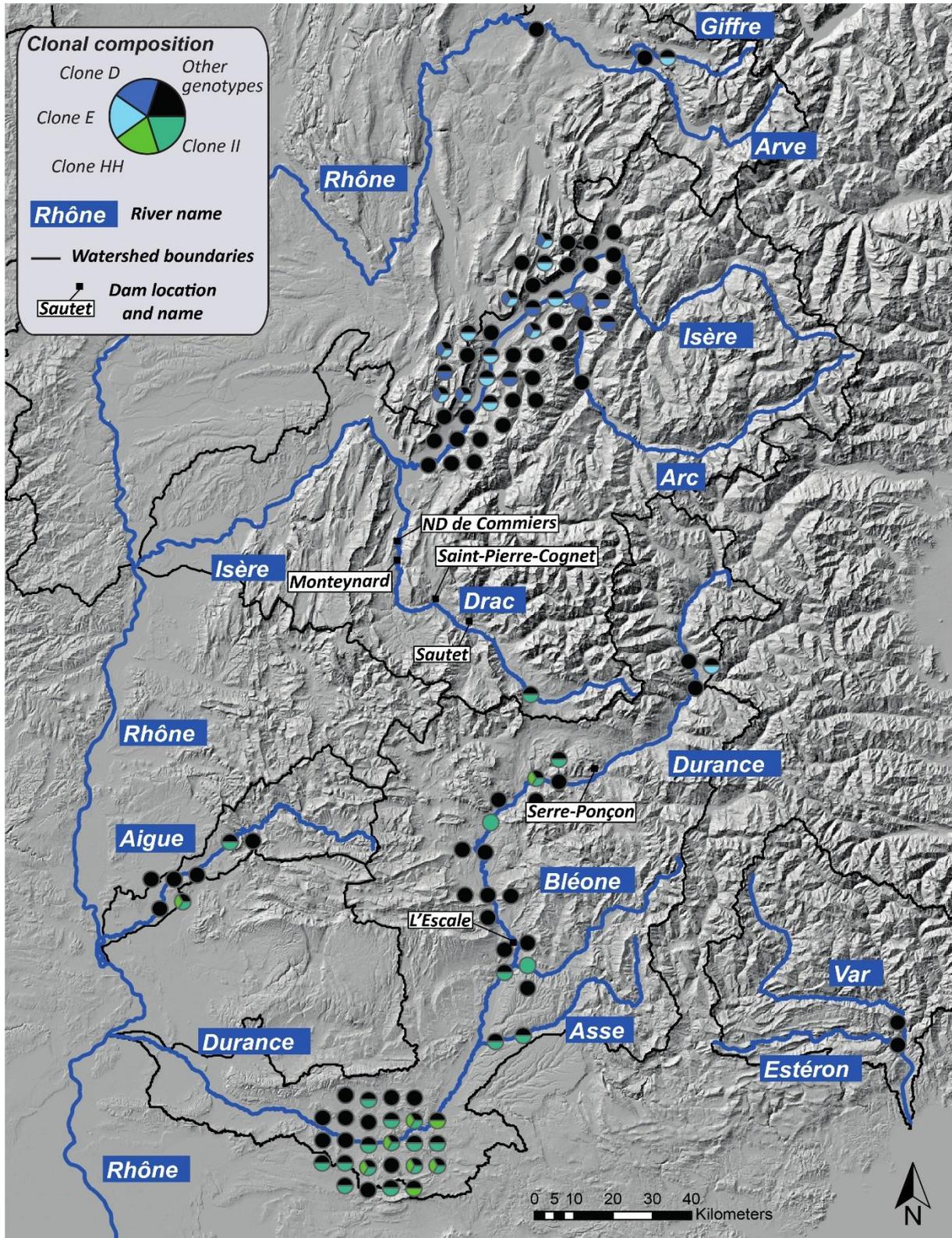
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**Fig.2:** Distribution of copy number per replicated genotype; number of genotypes with 1, 2, .. 190 copies from Clusters E(blue), S (orange) and N (green), as determined by the STRUCTURE analysis. "15-": 15 to 19; "20-": 20 to 29; "30-": 30 to 39; "40-": 40 to 49; "50-": 50 to 189".  
Excel histogram



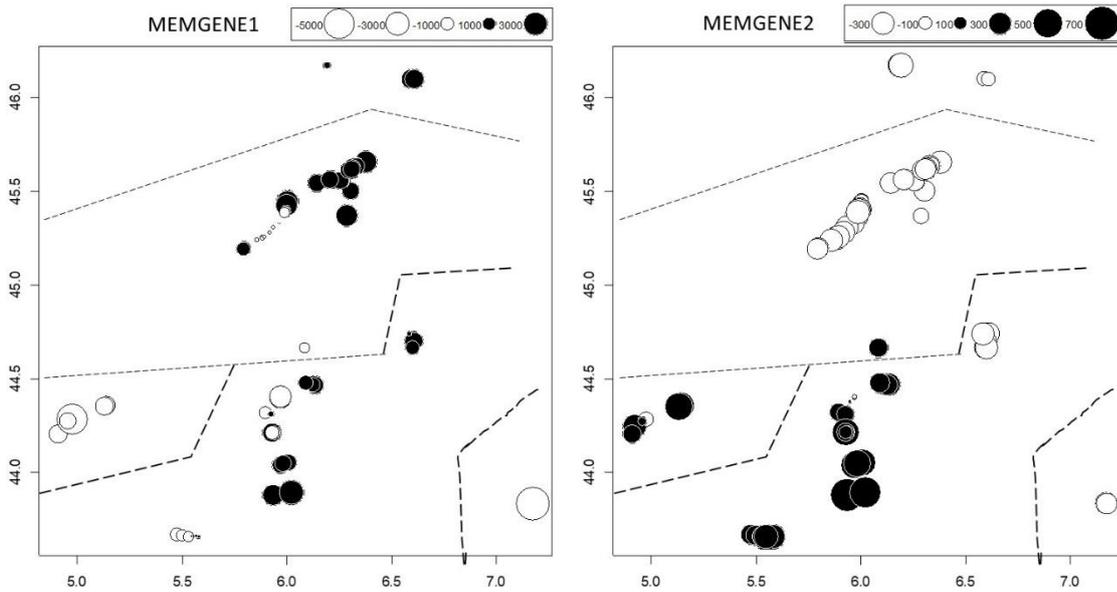
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896 **Fig.3:** Distribution of four of the largest replicated genotypes. Clone D and Clone E belong to  
 897 Cluster N; Clone HH and Clone II belong to Cluster S. Clusters determined by STRUCTURE and  
 898 clones determined by Genalex.  
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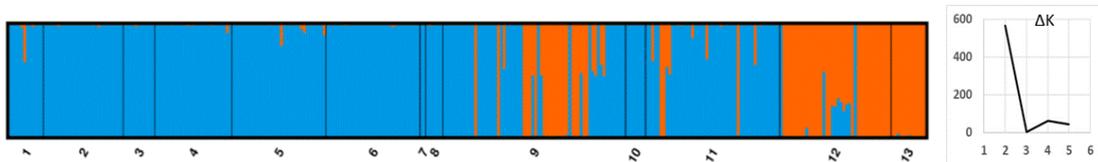
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901  
 902 **Fig.4:** MEMGENE results for eigenvector 1 (left; 85% of the variance explained by the model)  
 903 and eigenvector 2 (right; 13% of the variance explained by the model). Dashed lines  
 904 represent watershed boundaries (as in Figure 1). Values next to the circles in the legend box  
 905 represent the scores: large black and white circles describe opposite extremes on the  
 906 MEMGENE axis (eigenvector) and the size of the circle diminishes towards the center of the  
 907 axis. In the graph, circles of a similar size and color indicate individuals with similar scores  
 908 and groups of circles with similar scores indicate a spatial pattern.  
 909 MEMGENE output  
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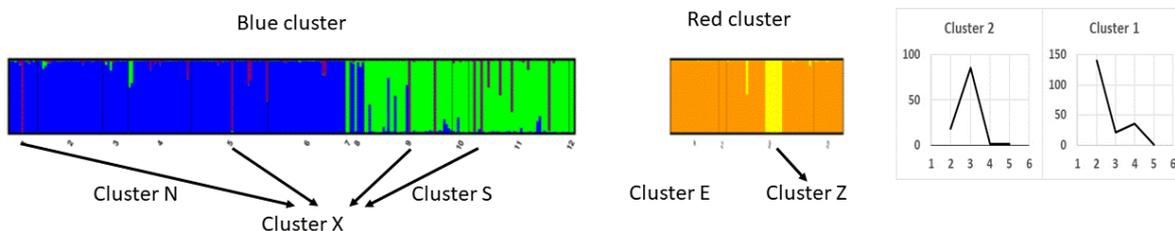


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 912  
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 914 **Fig.5:** Inference of population structure obtained from Bayesian analysis in STRUCTURE. The  
 915 first run (First analysis) indicated an optimum of two clusters. Each cluster was further  
 916 subdivided into two or three sub-clusters (Second analysis). For each analysis, the population  
 917 structure is shown on the left (sites arranged from north to south) and the  $\Delta K$  values are on  
 918 the right.  
 919 CLUMPAK outputs assembled using Powerpoint

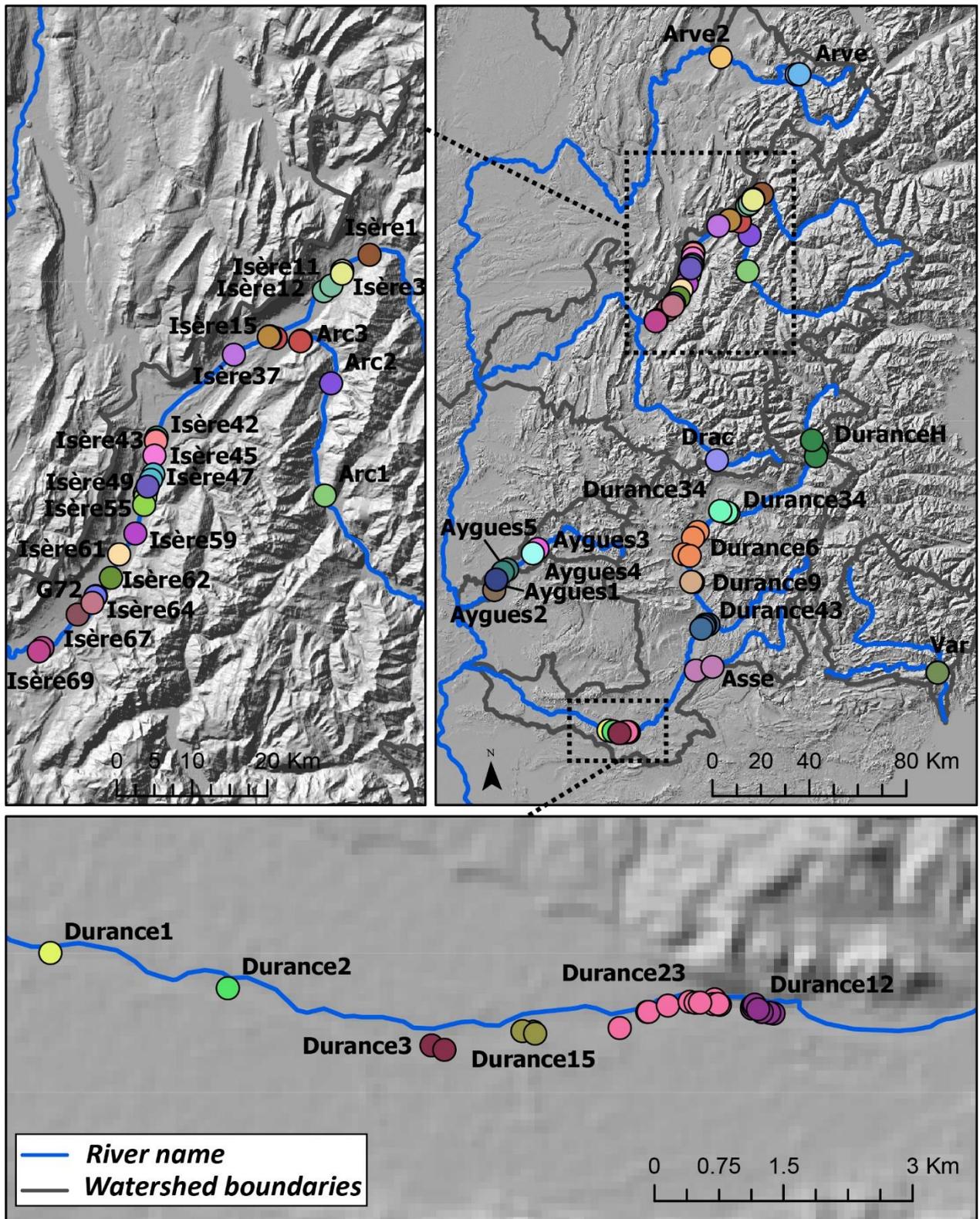
First analysis: 2 clusters



Second analysis: 3 + 2 clusters



920 **Supplementary material:**  
 921 Fig.S1: Map of all investigated sites of *T. minima* showing the grouping of sites into  
 922 populations (each population is represented by a different color).



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