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1 **Genetic diversity and population structure analyses in the Alpine plum**  
2 **(*Prunus brigantina* Vill.) confirm its affiliation to the Armeniaca section**

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25

26 **Abstract**

27 In-depth characterization of the genetic diversity and population structure of wild relatives  
28 of crops is of paramount importance for genetic improvement and biodiversity  
29 conservation, and is particularly crucial when the wild relatives of crops are endangered.  
30 In this study, we therefore sampled the Alpine plum (Briançon apricot) *Prunus brigantina*  
31 Vill. across its natural distribution in the French Alps, where its populations are severely  
32 fragmented and its population size strongly impacted by humans. We analysed 71 wild *P.*  
33 *brigantina* samples with 34 nuclear markers and studied their genetic diversity and  
34 population structure, with the aim to inform *in situ* conservation measures and build a core  
35 collection for long-term ex-situ conservation. We also examined the genetic relationships  
36 of *P. brigantina* with other species in the Prunophora subgenus, encompassing the Prunus  
37 (Eurasian plums), Prunocerasus (North-American plums) and Armeniaca (apricots)  
38 sections, to check its current taxonomy. We detected a moderate genetic diversity in *P.*  
39 *brigantina* and a Bayesian model-based clustering approach revealed the existence of  
40 three genetically differentiated clusters, endemic to three geographical regions in the Alps,  
41 which will be important for *in situ* conservation measures. Based on genetic diversity and  
42 population structure analyses, a subset of 36 accessions were selected for *ex-situ*  
43 conservation in a core collection that encompasses the whole detected *P. brigantina* allelic  
44 diversity. Using a dataset of cultivated apricots and wild cherry plums (*P. cerasifera*)  
45 genotyped with the same markers, we detected gene flow neither with European *P.*  
46 *armeniaca* cultivars nor with diploid plums. In contrast with previous studies, dendrograms  
47 and networks placed *P. brigantina* closer to Armeniaca species than to Prunus species.  
48 Our results thus confirm the classification of *P. brigantina* within the Armeniaca section; it  
49 also illustrates the importance of the sampling size and design in phylogenetic studies.

50

51 **Keywords:** Apricot, Prunus, classification, genetic structure, core collection, taxonomy

52

## 53 Introduction

54 Many wild crop relatives are endangered, because of fragmented and reduced habitats  
55 as well as crop-to-wild gene flow (Cornille et al. 2013b). In order to protect the biodiversity  
56 of wild crop relatives, we need to understand their population subdivision and genetic  
57 diversity distribution (Allendorf et al. 2012; Fahrig 2003). Studying the genetic diversity of  
58 crop-related species is not only important for biodiversity conservation but also for the  
59 sustainable use of valuable genetic resources through the set-up of *ex-situ* germplasm  
60 collections (Li and Pritchard 2009). Developing such collections requires obtaining a  
61 sufficient number of individuals to be representative of the species diversity (Frankel and  
62 Brown 1983; Glaszmann et al. 2010; Govindaraj et al. 2014). Core collections of woody  
63 perennial species have the additional advantages of being propagated vegetatively and  
64 maintained for decades, as clonemates, in field collections (Escribano et al. 2008).

65 Within the genus *Prunus* L. (stone fruit species), the subgenus *Prunus* (also called  
66 *Prunophora* Neck. to avoid confusion with the *Prunus* section and the *Prunus* genus)  
67 includes three sections: the Eurasian and North American plums (sections *Prunus* and  
68 *Prunocerasus* Koehne, respectively) and apricots (section *Armeniaca* (Mill.) K. Koch),  
69 which are all native from the Northern hemisphere (Rehder 1940) (Figure 1). It was shown  
70 that an ancient radiation of the *Prunus* genus through the Old and New Worlds and  
71 independent dispersal events across the North-American and Eurasian continents gave  
72 rise to, on one side, the *Prunocerasus* species, and on the other side, species of the  
73 *Prunus* and *Armeniaca* sections (Chin et al. 2014). Following the Rehder's classification,  
74 within the *Prunophora* subgenus the section *Armeniaca* comprises only diploid species,  
75 in which six species are recognized, based on morphological features: *P. armeniaca* L.  
76 (common apricot), *P. sibirica* L. (wild apricot in Northeastern Asia), *P. mandshurica*  
77 Maxim. (Northeast China and Eastern Russia), *P. mume* (Sieb.) Sieb. & Zucc. (South  
78 China and Japan), *P. holosericeae* Batal (South-West China) and *P. brigantina* Vill.  
79 (Figure 1). The first five species all originate from Asia, ranging from Central to North-East  
80 Asia, while *P. brigantina* (synonym *P. brigantiaca*, <http://www.theplantlist.org>) is native  
81 from Europe (Villars 1786). This species still grows in wild, patchy thickets in the Alps  
82 along the border between France and Italy in the Northern Mediterranean area, where it  
83 is considered either as an apricot or a plum species (Hagen et al. 2002; Pignatti 1982).

84 *Prunus brigantina*, alternatively called the Briançon apricot or the Alpine plum, was  
85 first reported in the French book <*Histoire des Plantes de Dauphiné*> (Villars 1786). It grows  
86 in arid places in shrub and sparse thickets in the Alps, above 1,400 m altitude. Like other  
87 *Prunus* species, *P. brigantina* is hermaphrodite and is pollinated by insects, it flowers in

88 May and its fruits ripen from August to September (Noble et al. 2015; Tison and De  
89 Foucault 2014). In natural stands, *P. brigantina* trees grow 2 to 5 meters high with non-  
90 spiny branches and have heart leaves with double-serrated teeth (Figure 2a). The full-  
91 fledged drupe from *P. brigantina* has a small size and appears glabrous with yellowish  
92 fruit skin (Figure 2a). In the Alps, *P. brigantina* fruits are collected by locals to make jam  
93 (Couplan 2009), and their seeds used to be processed for oil production instead of olive  
94 or almond (Dupouy 1959). It is locally called ‘Marmottier’ or ‘Afatoulier’ and is recognized  
95 as an endemic fruit tree in Europe. Its small and fragmented distribution suggests that it  
96 may be threatened. However, there is currently insufficient information available to  
97 evaluate the current genetic diversity of *P. brigantina* or its population subdivision, which  
98 could contribute to determine the potential threats to this species or its conservation status  
99 (IUCN Red List) (Branca and Donnini 2011), and to develop and inform conservation  
100 programs.

101 Previous phylogenetic studies questioned the Rehder’s classification of *P.*  
102 *brigantina* in the Armeniaca section (Hagen et al. 2002; Reales et al. 2010; Takeda et al.  
103 1998; Zhebentyayeva et al. 2019). However, only one or two *P. brigantina* samples were  
104 analysed, that had not been collected *in situ* but instead obtained from germplasm  
105 repositories such as the Kew Royal Botanical Garden (UK), the Czech national genetic  
106 resources of Lednice, the French Centre of genetic resources at INRAE-Montfavet or the  
107 Japanese Chiyoda experimental station. Because of the ability of *P. brigantina* to be  
108 propagated by grafting and its interfertility with species from both sections Prunus and  
109 Armeniaca, the analyzed trees could be clonemates or hybrids, and their origin was  
110 unknown. Moreover, sampling only one or two individuals per species is known to lower  
111 the accuracy of phylogenetic analyses (Heled and Drummond 2010; Wiens and Servedio  
112 1997). The genetic relationships between *P. brigantina* and other apricot species in the  
113 section Armeniaca therefore remain unclear.

114 To provide useful guidelines for *P. brigantina* conservation, a critical first task is first  
115 assessing its genetic diversity distribution and population subdivision, in order to assess  
116 whether local specificities need to be conserved. A second important aspect is to clarify  
117 the taxonomic position of the different populations of *P. brigantina*. Indeed, differentiated  
118 populations assigned to a given Latin species may actually represent different species  
119 placed far apart in phylogeny, as found recently for *P. sibirica* (Liu et al. 2019). However,  
120 there is currently no robust data on the genetic diversity or population structure of *P.*  
121 *brigantina*. In the current study, we therefore conducted extensive sampling of *P.*  
122 *brigantina* in its natural habitat and genotyped samples using 34 nuclear markers (Liu et al.  
123 et al. 2019). We assessed the genetic diversity of *P. brigantina* and its population structure,

124 as well as its relationship with Eurasian *Armeniaca* and *Prunus* species. We questioned  
125 the affiliation of *P. brigantina* to either the *Armeniaca* or *Prunus* sections. For this purpose,  
126 we performed a population genetic analysis using datasets with the main members of the  
127 *Armeniaca* and *Prunus* sections and five outgroup species from the North-American  
128 section *Prunocerasus*. Based on our molecular data, we identified a collection of unique  
129 genotypes and selected the best subset for building a *P. brigantina* core collection,  
130 maximizing allelic diversity, which will be very useful for further *P. brigantina*  
131 characterization and for stone fruit crop improvement. In contrast to previous phylogenetic  
132 studies, our population trees and networks further confirmed that *P. brigantina* is closer to  
133 apricot species than to plum species.

134

## 135 **Materials and Methods**

### 136 ***In situ P. brigantina* sampling**

137 A total of 71 wild *P. brigantina* trees were collected in 2017 from three sampling sites, in  
138 southeast France, across the Alps (Figure 2a and Table S1). Young leaves and mature  
139 fruits from each tree were collected for DNA extraction and seedling growth, respectively.  
140 At least one seedling from each sampled tree was kept for possible inclusion in a core  
141 collection.

142 Representatives of other species of the *Armeniaca* section (*P. armeniaca*, *P. mume*, *P.*  
143 *sibirica*, *P. mandshurica*), including two *P. brigantina* accessions maintained by the Centre  
144 of genetic resources at INRA-Montfavet but with unknown origin, were previously  
145 described and genotyped with the same set of molecular markers (Liu et al. 2019).

146

### 147 ***In situ* and *ex situ* sampling of representatives of *Prunus* and *Prunocerasus* species**

148 Part of the plum and plum-related material analysed in this study was kindly provided by  
149 the North-American national repository (ARS-USDA, Davis, California, USA), the  
150 Bourran's collection of *Prunus* (*Prunus* Genetic Resources Centre or *Prunus* GRC,  
151 France) or was collected *in situ*, between 2008 and 2019, in Azerbaijan (Caucasia),  
152 Kazakhstan and Kyrgyzstan (Central Asia) (Table S1b). One *P. cerasifera* sample (X29)  
153 was collected *in situ* in South-West of France, along the Garonne river (Le Tourne-  
154 Langoiran) and another one in the French Alps (FR\_070) (Table S1b). In total, 82 diploid  
155 samples were genotyped in the current study, genotypes for polyploids being difficult to  
156 analyse. The diploid samples included representatives from *P. cerasifera* ( $N=66$ ) (or



157 'cherry plum', alternatively called 'myrobolan' in Europe, *P. divaricata* Ledeb. in Caucasia  
158 and *P. sogdiana* in Central Asia) and other species from the *Prunophora* subgenus: *P.*  
159 *mexicana* ( $N=1$ ), *P. munsoniana* ( $N=1$ , also called *Prunus rivularis*), *P. maritima* ( $N=1$ ), *P.*  
160 *americana* ( $N=1$ ) and *P. subcordata* ( $N=1$ ). *P. salicina* (Japanese plum) samples ( $N=10$ )  
161 were composed of five cultivated accessions which included one plumcot, a hybrid  
162 between *P. salicina* and *P. armeniaca* (called 'Rutland' in the ARS-USDA database,  
163 P0489) and five wild *P. salicina* accessions, sampled in China (Table S1b). *Prunus*  
164 *cerasifera* accessions used in this study originated from Europe ( $N=13$ ), from Caucasia  
165 and Russia ( $N=29$ ) and from Central Asia (more precisely from Kazakhstan and  
166 Kyrgyzstan,  $N=24$ ) (see Table S1b for details).

167

### 168 **DNA extraction, microsatellite markers and polymerase chain reaction (PCR)** 169 **amplification**

170 Genomic DNA was extracted as described previously (Decroocq et al. 2016), either from  
171 lyophilized leaves, bark or fresh flowers. We used 34 microsatellite markers distributed  
172 across the eight *P. armeniaca* chromosomes and showing good amplification success as  
173 well as substantial polymorphism within the different species of the section *Armeniaca*  
174 (Liu et al. 2019). The same set of microsatellite markers were used to amplify PCR  
175 fragments in species of the *Armeniaca* (*P. brigantina* incl.), *Prunus* (*P. cerasifera* incl., see  
176 supplemental information) and *Prunocerasus* sections. Detailed information on these  
177 microsatellite markers, including their repeat motifs, sequences, and amplification  
178 conditions are available in (Liu et al. 2019). PCR amplification and fragment size  
179 genotyping were performed on an ABI PRISM 3730 (Applied Biosystems) as described  
180 previously (Decroocq et al. 2016). Alleles were scored with the GENEMAPPER 4.0  
181 software (Applied Biosystems).

182

### 183 **Analyses of population subdivision and genetic relationships**

184 To assess the probability of observing unrelated individuals with the detected similar  
185 genotypes given the population allelic frequencies, we used GENODIVE and the  
186 corrected *Nei's* diversity estimate with a threshold of 50 (Meirmans and Van Tienderen  
187 2004). We later retained only one individual of each pair detected as clonemates or  
188 siblings for further analyses.

189 We identified population subdivision with the STRUCTURE software v. 2.3.3 (Pritchard et  
190 al. 2000), without the use of *a priori* grouping information and assuming that individuals

191 had mixed ancestry with correlated allele frequencies among populations. The clustering  
192 method implemented in STRUCTURE is based on Monte Carlo Markov Chain (MCMC)  
193 simulations and is used to infer the proportion of ancestry of genotypes in  $K$  distinct  
194 clusters. We simulated  $K$  values ranging from 2 to 10 for the *P. brigantina* population and  
195 three additional datasets (Table 1 and S1c), obtained with the same genetic markers on  
196 Armeniaca and Prunus species originating from Central and Eastern Asia (Liu et al. 2019)  
197 (Supplemental information). For each  $K$ , we ran 10,000 generations of 'burn-in' and  
198 100,000 MCMC. Simulations were repeated 10 times for each  $K$  value; the resulting  
199 matrices of estimated cluster membership coefficients (Q) were permuted with CLUMPP  
200 (Jakobsson and Rosenberg 2007). STRUCTURE barplots were displayed with  
201 DISTRUCT 1.1 (Rosenberg 2004). The strongest level of the genetic subdivision was  
202 determined using  $\Delta K$  (Evanno et al. 2005), as implemented in the online post-processing  
203 software Structure Harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl  
204 and vonHoldt 2012). Principal components analyses (PCA) were performed to investigate  
205 the genetic structure of *P. brigantina* using the scatterplot3d R package (Ligges and  
206 Mächler 2003) or among the five *Prunophora* species, using the DARwin software  
207 package v 6.0.017 (Perrier and Jacquemoud-Collet 2006). Further genetic differentiation  
208 and relationships were also estimated using a weighted neighbour-joining tree as  
209 implemented in the DARwin software package v 6.0.017 (Perrier and Jacquemoud-Collet  
210 2006).

211 We performed a three-step population subdivision analysis, the first one with only  
212 *P. brigantina* samples, and the second one adding previously obtained datasets of  
213 *Armeniaca* species, including *P. armeniaca*, *P. sibirica*, *P. mandshurica* and *P. mume* wild  
214 and cultivated samples (Liu et al. 2019) (dataset 2 in Table 1). In the third step of the  
215 analysis, we added samples of the Prunus (*P. cerasifera* and *P. salicina*) and  
216 Prunocerasus (*P. mexicana*, *P. munsoniana*, *P. maritima*, *P. americana* and *P.*  
217 *subcordata*) sections (dataset 3 in Table 1). The same procedure to investigate population  
218 subdivision and structure analysis was used with the Armeniaca, Prunus and  
219 Prunocerasus diploid samples. In parallel, we also performed a population subdivision  
220 analysis along the native distribution of *P. cerasifera* (Supplemental information '*Prunus*  
221 *cerasifera* diversity and population structure analysis' and dataset 4 in Table 1). In *P.*  
222 *cerasifera*, a Neighbour-Joining tree based on Nei's standard genetic distance was built  
223 with a bootstrap of 30,000 in PopTreeW (Takezaki et al. 2014).

224 In order to test whether there was a pattern of isolation by distance, we performed  
225 a Mantel test between a matrix of Edwards' genetic distances and a matrix of Euclidean



226 geographic distances in *P. brigantina* using the R adegenet package (Jombart and Ahmed  
227 2011).

228

### 229 **Genetic diversity, differentiation and core collection constitution**

230 We used GENALEX 6.501 (Peakall and Smouse 2012) to estimate the number of alleles  
231 ( $N_a$ ), the effective number of alleles ( $N_e$ ), i.e., the number of equally frequent alleles that  
232 would achieve the same expected heterozygosity as in the sample, the observed  
233 heterozygosity ( $H_o$ ), the unbiased expected heterozygosity ( $H_E$ ) and the Shannon index  
234 ( $H$ ) (Shannon 1948). Genetic differentiation among genetic clusters (Jost'D) was estimated  
235 in GENODIVE (Meirmans and Van Tienderen 2004). The allelic richness ( $A_r$ ) and the  
236 private allelic richness ( $A_p$ ) were calculated after adjustment for sample size differences  
237 among groups through the rarefaction procedure implemented in ADZE Allelic Diversity  
238 Analyzer v1.0 (Szpiech et al. 2008), setting the sample size to five.

239 The maximization (M) strategy (Schoen and Brown 1993) implemented in the  
240 COREFINDER software was used to generate a core *P. brigantina* tree collection  
241 maximizing the number of alleles based on our dataset. The maximization strategy  
242 consisted in detecting the smallest sample that captured 100% of the genetic diversity  
243 present within the entire germplasm collection. We further used the Mann-Whitney U test  
244 to check the genetic diversity difference between the core collection and the entire *P.*  
245 *brigitantina* sample.

246

## 247 **Results**

### 248 **Genetic diversity and population structuration in *P. brigantina***

249 Thirty-four microsatellite markers used in a previous study (Liu et al. 2019) were tested  
250 for our *P. brigantina* population study. Four markers (AMPA109, ssr02iso4G, BPPCT008  
251 and BPPCT038) failed to amplify or generated over 50% of missing data and were  
252 consequently eliminated. Six other markers (BPPCT030, CPPCT022, CPSCT004,  
253 UDP98-412, UDA-002 and PacB26) gave poor amplification in *P. brigantina*, yielding  
254 more than 10% missing data. This may be because of poor marker transferability, as most  
255 of the above microsatellite markers were developed from genomic data on other *Prunus*  
256 species, such as peach, almond, apricot and Japanese plum. The remaining 24  
257 microsatellite markers performed well in *P. brigantina* and were used in this study (Table  
258 S2). In our *P. brigantina* sample, the number of alleles ( $N_A$ ) was 121 (mean of 5.04 per

259 marker) and the number of effective alleles ( $N_E$ ) 59.57 (mean of 2.48 per marker) (Table  
260 S2).

261 The biologically most relevant genetic clustering of *P. brigantina* was found to be  
262  $K=3$ : the DeltaK statistics indicated that it was the strongest population subdivision level  
263 (Figure S1A) and further increasing  $K$  yielded many admixed individuals (Figure S2). The  
264 three inferred genetic clusters (blue, yellow and orange colours in Figure 2b and S2)  
265 corresponded to three French national parks “Queyras”, “Ecrins” and “Mercantour”,  
266 respectively. Weak but significant genetic differentiation (mean Jost’s  $D=0.117$ ) was found  
267 among these three *P. brigantina* populations (Table 2). Both the Josts’  $D$  and the PCA  
268 indicated that the *P. brigantina* “Queyras” cluster was the most differentiated from the two  
269 other ones, the “Ecrins” and “Mercantour” clusters being found genetically closer one to  
270 each other (Table 2, Figure 3).

271 An additional subdivision of the Ecrins cluster was found at  $K=8$ , revealing  
272 differentiation between the dark blue and yellow clusters (Figure S2). The Mantel test on  
273 the three *P. brigantina* clusters indicated no significant relationship between genetic  
274 differentiation and geographic distance ( $P=0.308$ , by Monte Carlo permutation tests,  
275 Figure S3), indicating a lack of isolation by distance.

276

### 277 **Genetic relationships between *P. brigantina* and other Prunophora species**

278 To obtain a better understanding of the genetic relationships between *P. brigantina* and  
279 other Armeniaca species, we combined the current *P. brigantina* data with a former  
280 Armeniaca dataset built with the same 24 microsatellite markers (Liu et al. 2019) (Tables  
281 1 and S1c). We performed a Bayesian clustering analysis on the full Armeniaca dataset,  
282 including wild and cultivated *P. armeniaca*, *P. sibirica*, *P. mandshurica* and *P. mume*  
283 (Table 1). We obtained a similar structure as the one described in (Liu et al. 2019) for the  
284 previous dataset, and *P. brigantina* differentiated in a distinct cluster, from  $K=3$  and above  
285 (yellow colour in Figure S4). No gene flow with other species of the section Armeniaca  
286 was detected (i.e. no individuals who would have admixed ancestry between the yellow  
287 cluster and other clusters), in particular in between wild *P. brigantina* and cultivated  
288 apricots which, yet, partly share habitats over Western Europe (Figure S4).

289 We further questioned the genetic relationship of *P. brigantina* with other members  
290 of the Prunophora subgenus, i.e. species of the Prunus and Prunocerasus sections.  
291 Because *P. cerasifera* (cherry plum), a species of the Prunus section, is partly sharing  
292 habitats with *P. brigantina*, we significantly extended the sampling of *P. cerasifera* species  
293 compared to (Horvath et al. 2008), including accessions from the cherry plum native area,

294 i.e. Caucasia and Central Asia, to obtain a better representation at the species level. We  
295 then explored the genetic differentiation of this species over its Eurasian distribution. We  
296 found genetically differentiated clusters of cherry plums, with contrasted geographical  
297 distributions from Central Asia to Europe (detailed results are presented in the  
298 supplemental information ‘*Prunus cerasifera* diversity and population structure analysis’).  
299 Caucasia appears to be a diversification center of wild cherry plums, with two distinct  
300 genetic clusters that may result from geographical isolation. This dataset was later merged  
301 with representatives of the *Prunus*, *Armeniaca* and *Prunocerasus* sections, to infer the  
302 origin of *P. brigantina* and its genetic relationships with species of the *Prunophora*  
303 subgenus (Tables 1, S1b and S1c). In the following step, we focused on species that  
304 shared, partly, habitats with *P. brigantina*, i.e. *P. cerasifera* and cultivated *P. armeniaca*,  
305 together with other *Armeniaca* (*P. mume*), *Prunus* (*P. salicina*) and *Prunocerasus* species.  
306 For this, we used genotyping data based on 23 microsatellite markers (see the  
307 supplemental information ‘*Prunus cerasifera* diversity and population structure analysis’).

308 The delta  $K$  peaked at  $K=3$ , indicating that this was the strongest level of population  
309 subdivision (Figure S1B). However, further relevant clustering was observed at higher  $K$   
310 values (Figure S5). From  $K=7$  and above, all taxonomic species separated in specific  
311 clusters: green for *P. brigantina*, pink for *P. armeniaca*, blue for *P. cerasifera*, grey for *P.*  
312 *mume*, orange for *P. salicina* and black for *Prunocerasus* (Figure S5). Again, we could not  
313 find any admixture footprints between *P. brigantina* and other *Prunus* species, while there  
314 may be some footprints of introgression from *P. cerasifera* into *P. salicina* (see admixed  
315 individuals indicated by blue stars in Figure S5), although the blue and orange  
316 heterogeneous bars may alternatively result from low assignment power due to the low  
317 number of *P. salicina* individuals.

318 We further explored the genetic differentiation and relationships among all  
319 *Prunophora* samples using an unrooted weighted neighbour-joining tree (Figure 4). In the  
320 tree, the delimitation of *P. brigantina* as a distinct species from other apricot and plum  
321 taxonomic species was well supported (100% bootstrap support). *Prunus brigantina* trees  
322 appeared genetically closer to the *Armeniaca* species (*P. armeniaca* and *P. mume*) than  
323 to other *Prunus* and *Prunocerasus* species, which is consistent with Rehder’s taxonomy.  
324 The principal component analysis (PCA) supported the differentiation of *P. brigantina* from  
325 other species of the *Armeniaca* section, and from the *Prunus* and *Prunocerasus* sections  
326 (Figure 5). Both the NJ tree and the PCA indicated that plum species (*P. cerasifera* and  
327 *P. salicina*) were partly overlapping, in particular the cultivated Japanese plums and cherry  
328 plums; the wild *P. salicina* trees in contrast appeared well separated from *P. cerasifera*  
329 (Figures 4 and 5). The overlapping may be the result of low power to distinguish the groups

330 based on few individuals or of hybridization between cultivated trees. One particular case  
331 of hybridization is P0489, cv. Rutland plumcot. Breeders' information indicates that it is a  
332 hybrid between plum and apricot. In our structure barplots, NJ tree and PCA (Figures S5,  
333 4 and 5), P0489 in fact appeared admixed between the two plum species, *P. salicina* and  
334 *P. cerasifera*, and not with apricot.

335

### 336 **Construction of a *P. brigantina* core collection**

337 We used the COREFINDER program to identify the smallest core collection that  
338 would be sufficient to capture the whole diversity detected based on our 24 microsatellite  
339 markers. Based on the maximizing strategy implemented in COREFINDER, we propose  
340 the use of a core set of 36 individuals (~49% of the whole *P. brigantina* sample) that  
341 captures 100% of the detected diversity (Figure 6, Table S3). Pairwise comparisons using  
342 Mann-Whitney U tests showed no significant differences in diversity indexes ( $I$ ,  $H_o$ , and  
343  $H_E$ ) between the *P. brigantina* entire Alpine sample ( $N=71$ ) and the core collection ( $N=36$ )  
344 (Tables S2, S4 and S5). This indicates that our core collection can be used as an *ex-situ*  
345 germplasm repository.

346

### 347 **Discussion**

348 The current study showed that *P. brigantina* is still found in a few Alpine valleys,  
349 along the border between France and northwest Italy, where it grows above 1,400 m  
350 altitude as single isolated trees (except for the plateau of Nevache, where they are present  
351 as a denser population), in arid places such as shrub thickets. In France, it is confined to  
352 the three southeastern departments of Alpes-Maritimes, Alpes-de-Haute-Provence and  
353 Hautes-Alpes. The sustainability of *P. brigantina* habitat is threatened by forest  
354 fragmentation. This raises the question of the long-term conservation of this species and  
355 no germplasm accessions of *P. brigantina* are reported by EURISCO to be held in  
356 European *ex-situ* collections. Because large field collections of perennial crops are  
357 expensive to maintain, the identification of a restricted number of representatives of *P.*  
358 *brigantina* population for *ex situ* conservation would be very useful in the perspective of  
359 Alpine ecosystem restoration and future breeding programs. Core collections are  
360 representative subsets of germplasm collections that are developed to improve the  
361 efficiency of germplasm evaluation while increasing the probability of finding genes of  
362 interest (Simon and Hannan 1995). Therefore, our current core collection will serve in the  
363 future for *P. brigantina* conservation as well as for stone fruit breeding programs benefiting  
364 from *P. brigantina* resilience characteristics, especially in a context of Mediterranean

365 climate changes. However, the most efficient strategy for biodiversity conservation  
366 remains the preservation of the natural habitat of endangered species.

367 Thanks to an extensive dataset of *Prunophora* species, we also questioned here  
368 the genetic relationships of *P. brigantina* with other species of the *Prunus* and *Armeniaca*  
369 sections. Species of the *Prunocerasus* section were not integrated in the analysis except  
370 as outgroups because they are naturally distributed on different continents and do not  
371 overlap in their respective natural habitats with *P. brigantina*. Through Bayesian analyses,  
372 *P. brigantina* appears as a *bona fide* species, clearly distinct from other apricot species  
373 and from plum species, with no footprint of admixture. Our results are in accordance with  
374 previous studies that indicate a clear differentiation of *P. brigantina* from other *Armeniaca*  
375 apricot species (i.e. *P. armeniaca* and *P. mume*) but do not support a close relationship  
376 with species of the *Prunus* section (Chin et al. 2014; Reales et al. 2010; Shi et al. 2013;  
377 Zhebentyayeva et al. 2019). This might be due to the fact that our sampling covers a larger  
378 diversity panel than in the former studies, both in *Armeniaca* and *Prunus* sections, *P.*  
379 *brigantina* included. Indeed, sampling only one or two individuals per species is expected  
380 to lower the accuracy of phylogenetic analyses (Wiens and Servedio 1997). In our  
381 analyses, *P. brigantina* was closer to species of the *Armeniaca* section than to the *Prunus*  
382 section. While *P. brigantina* should still be considered as an *Armeniaca* species, it has  
383 diverged from *P. armeniaca* long before *P. mume*, thus representing the most genetically  
384 distant apricot-related species within the *Armeniaca* section (Hagen et al. 2002; Liu et al.  
385 2019).

386 Contradictory results had been obtained from a phylogeny of Eurasian plum  
387 species based on chloroplast DNA sequences (Reales et al. 2010), where *P. brigantina*  
388 grouped together with European *Prunus* species, such as the polyploid *P. spinosa*, *P.*  
389 *insititia* and *P. domestica*, and the diploid *P. ramburii* Boiss. species; it was clearly  
390 separated from *P. armeniaca* (apricot). The proximity in chloroplast genotypes between  
391 *P. brigantina* and the polyploid *Prunus* species might indicate the Alpine plum as a  
392 parental contributor in interspecific hybridization of polyploid *Prunus* species  
393 (Zhebentyayeva et al. 2019). Organelles are however known to introgress much more  
394 often than nuclear DNA and chloroplast genealogies are often discordant from nuclear  
395 phylogenies (Coyne and Orr 2004). The other plum species that grouped with *P. brigantina*  
396 in chloroplast genealogy, *P. ramburii*, is a relict, wild species endemic in the southern  
397 Spanish mountains (Sierra Nevada and Sierra Baz) While its distribution is in Europe, it  
398 does not overlap with that of *P. brigantina*. Hence, its morphological features are distinct  
399 from Alpine plum, forming bushes with tiny, blue/violet drupes and narrow leaves  
400 (<http://www.anthos.es/index.php?lang=en>). Therefore, the incongruence between our



401 results with those obtained earlier based on the chloroplast genome echoes the  
402 conclusions of others that despite the many advantages and widespread use of  
403 chloroplast DNA in phylogenetic studies, caution has to be taken in the use of organellar  
404 variation for inferring phylogeny (Doyle 1992; Lee-Yaw et al. 2019; Soltis and Kuzoff  
405 1995).

406 Nevertheless, by extending the sampling set of both *P. brigantina* and plum  
407 species, our study provides compelling evidence that *P. brigantina* grouped in the  
408 Armeniaca section. It illustrates the importance of the sample size and sampling design  
409 that encompasses here a larger genetic diversity at the species level than in previous  
410 studies (Hagen et al. 2002; Horvath et al. 2008; Reales et al. 2010; Zhebentyayeva et al.  
411 2019). It also questioned the relevance of the classification into sections of the Prunophora  
412 subgenus, at least for the Eurasian sections, i.e. Armeniaca and Prunus. Species of the  
413 two sections are sharing habitats and they are interfertile, in particular between diploid  
414 species, thus resulting in a number of hybrids and probably new species (Cici and Van  
415 Acker 2010; Layne and Sherman 1986). Although the genetic differentiation of the Prunus  
416 and Armeniaca sections from the Prunocerasus section is clear (Krüssmann 1978), the  
417 relationships among taxa of the two Eurasian sections are not well resolved as illustrated  
418 by the role of cross taxa hybridization in Japanese apricot (*P. mume*) adaptive evolution  
419 (Numaguchi et al. 2020). The previous controversial classification of *P. brigantina* either  
420 in the Armeniaca section or in the Prunus section reflects the difficulty of assigning a clear  
421 barrier between species of those two sections; an analysis of the entire subgenus using a  
422 shared set of same nuclear markers could provide greater resolution and would place the  
423 findings presented here into a Prunophora-wide perspective.

424

## 425 **Conclusion**

426 In this study, we found a low level of genetic diversity in natural *P. brigantina* populations  
427 and identified three genetically differentiated populations, in the Ecrins, Queyras and  
428 Mercantour national parks, respectively. We further successfully established in Bordeaux  
429 a core collection of 36 individuals representing the *P. brigantina* diversity that will be  
430 publicly available through the French Genetic Resources Center. In addition, a population  
431 NJ tree did not support a close relationship between *P. brigantina* and the other Prunus  
432 species, *P. brigantina* being closer to Armeniaca species whilst remaining clearly distinct.  
433 While most of the fruit species originate from Asia or America, many crop wild relatives  
434 still exist both in their center of origin and along their dispersal routes. For example, in pit  
435 and stone fruits, several *Prunus*, *Malus* and *Pyrus* wild species are endemic in Europe



436 and are often threatened by the rapid changes of land use (Cornille et al. 2013a; Welk et  
437 al. 2016). To inform *in situ* and *ex situ* conservation measures and add value to fruit tree  
438 genetic resources, we recommend in-depth characterization of those wild relatives,  
439 similarly to the current study in the Alpine plum.

440

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449 USDA repository (John Preece); we acknowledge the care of the plants at the UMR BFP  
450 (INRAE) by Jean-Philippe Eyquard and Pascal Briard.

451

#### 452 **Statements**

453 Appropriate permissions from responsible authorities for collecting and using *Prunus*  
454 samples from Central Asia and Caucasia were obtained by the local collaborators. The  
455 official authorization for the survey and sampling of *P. brigantina* genetic resources is  
456 registered and accessible through the following link:  
457 <https://absch.cbd.int/database/ABSCH-IRCC-FR-246978>. The rest of the samples were  
458 kindly provided, with due authorizations, by the curators of the French INRAE Genetic  
459 Resources Centre (GRC) and the US ARS-USDA repository, further details are available  
460 on their respective databases.

461

#### 462 **Data availability**

463 The datasets generated by the current study, *i.e.* the SSR genotyping, are available at  
464 the INRAE data portal (<https://data.inrae.fr/>) where they can be freely retrieved.

465

#### 466 ***Captions for the supplementary Figures presented in a separate PDF file***

467 **Figure S1. DeltaK plot as a function of K for the *Prunus brigantina* (A) and**  
468 ***Prunophora* (B) dataset.**

469 **Figure S2. Bayesian clustering on *Prunus brigantina* samples in the French Alps.**

470 *Prunus brigantina* dataset included 71 individuals sampled from the French Alps and two  
471 samples from the French GRC repository. Each individual is represented by a vertical bar,  
472 partitioned into  $K$  segments representing the inferred proportions of ancestry of its  
473 genome.

474 **Figure S3. Isolation by distance (IBD) test in *Prunus brigantina*.**

475 a. Distribution of correlation values between genetic and geographic distances under the  
476 assumption of lack of isolation by distance, drawn from permutations; the observed value  
477 of the correlation between the distance matrices, represented by the black diamond, falls  
478 within the expected distribution which indicates the lack of isolation by distance pattern.

479 b. Pairwise Edwards' distances plotted against Euclidean geographic distances, with local  
480 density of points plotted using a two-dimensional kernel density estimate, displayed in  
481 colour from white to red. The solid line represents the fitted linear regression between  
482 Edwards' genetic and Euclidean geographic distances.

483 **Figure S4. Bayesian analysis on *Armeniaca* and wild *Prunus brigantina***  
484 **accessions.**

485 Genetic subdivision among *Armeniaca* species, *P. brigantina* included, was inferred with  
486 STRUCTURE with 24 microsatellite markers. The 648 samples belong to the six  
487 *Armeniaca* species as follows: *P. brigantina* ( $N=73$ ), *P. armeniaca* (European and Chinese  
488 cultivated  $N=270$  and wild,  $N=204$ ), *P. sibirica* ( $N=84$ ), *P. mume* ( $N=9$ ), *P. mandshurica*  
489 ( $N=8$ ). Each individual is represented by a vertical bar, partitioned into  $K$  segments  
490 representing the inferred proportions of ancestry of its genome. Species and origin of the  
491 accessions are indicated on the top of the figure.

492 **Figure S5. Bayesian analysis on the *Prunus brigantina* dataset together with an**  
493 **extended *Prunophora* dataset.**

494 Genetic subdivision among *Armeniaca*, *Prunus* and *Prunocerasus* species was inferred  
495 with STRUCTURE with 23 microsatellite markers (supplemental information for the list of  
496 markers). The 226 samples belong to three different *Prunophora* species including *P.*  
497 *brigantina* ( $N=73$ ), *P. cerasifera* ( $N=66$ ), *P. armeniaca* ( $N=87$ ), *P. salicina* ( $N=10$ ), *P.*  
498 *mume* ( $N=9$ ), *P. mexicana* ( $N=1$ ), *P. munsoniana* ( $N=1$ ), *P. maritima* ( $N=1$ ), *P. americana*  
499 ( $N=1$ ) and *P. subcordata* ( $N=1$ ). The blue stars (\*), at the bottom of the bar plots,  
500 correspond to Japanese plums (*P. salicina*) admixed with *P. cerasifera*.

501

502 **Legends for the supplementary tables presented in a separate PDF file**

503 **Table S1a. Sampling locations, geographic regions and assigned genetic cluster of**  
504 ***Prunus brigantina* in the French Alps.**

505 FR for an origin from the French Alps. Sampling site is indicated in GPS coordinates, N  
506 for North, E for East.

507 **Table S1b. Sampling locations, geographic regions and/or germplasm repositories**  
508 **of *Prunus cerasifera* samples.**

509 <sup>1</sup> Species affiliation as indicated by the curator of the germplasm collection where the  
510 sample is maintained or as identified *in situ*. <sup>2</sup> Sampling location in decimal degrees. <sup>3</sup>  
511 Origin as indicated in the database of the germplasm repository. n/a, not applicable  
512 because admixed and thus not used in the correlation tests

513 **Table S1c: List of individuals included in the different datasets.**

514 <sup>1</sup> FR refers to France, AZ to Azerbaijan, CH to China, KR to Kyrgyzstan, KZ to Kazakhstan,  
515 OUZ to Uzbekistan, TCH to Czech republic (Lednice repository), TURC to Turkey  
516 (Malatya repository), US to USA (ARS-USDA *Prunus* germplasm repository). For more  
517 details, see Liu et al (2019). Accession numbers starting with A indicate apricot cultivars  
518 and with P, plum cultivars, as displayed in the French GRC database. The sign (-) means  
519 that the sample is maintained in germplasm repository and was not collected *in situ*. The  
520 cross in the last four columns (dataset 1 to 4) means that this sample was used in the  
521 corresponding dataset.

522 **Table S2. Analysis of genetic variability from microsatellite markers for *Prunus***  
523 ***brigantina* population.**

524 *Na*: number of different alleles, and *Ne*: number of effective alleles. *I*: Shannon diversity  
525 index. *He* and *Ho*: expected and observed heterozygosities.

526 **Table S3. The description of individuals retained for the core collection of *Prunus***  
527 ***brigantina***

528 **Table S4. Genetic variability of microsatellite markers for the *Prunus brigantina***  
529 **core collection.**

530 *Na*: number of different alleles, and *Ne*: number of effective alleles. *I*: Shannon diversity  
531 index. *He* and *Ho*: expected and observed heterozygosities.

532 **Table S5. Mann-Whitney U tests (two-tailed) between the whole *Prunus brigantina***  
533 **dataset and its core collection.**

534  
535 **Supplemental information presented in a separate PDF file**

536 **Supplemental information ‘*Prunus cerasifera* diversity and population structure**  
537 **analysis’**

538

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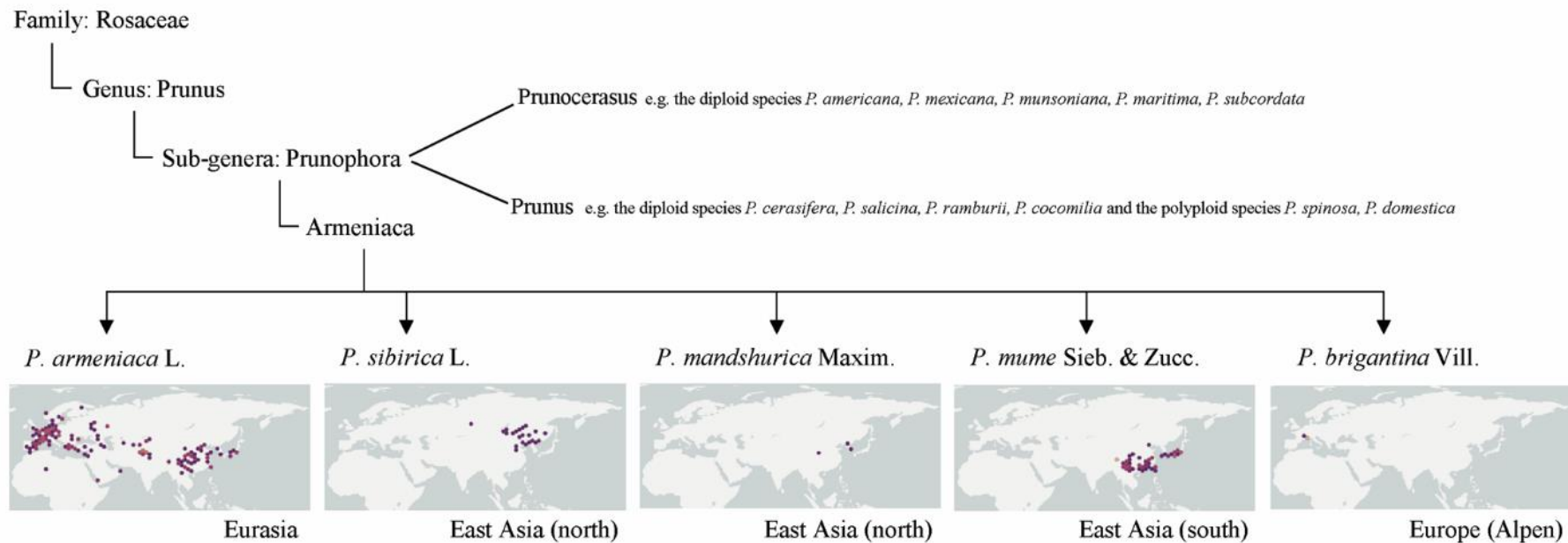


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672



673 **Figures**

674 **Figure 1. Taxonomy and geographic distribution of the different species in the**  
675 **Armeniaca section.** Species classification is based on reports by Rehder (1940). Data  
676 on species distribution were retrieved from the global biodiversity information facility  
677 (GBIF) (<https://doi.org/10.15468/39omei>). Dots represent georeferenced species records  
678 from 1910 to 2017.



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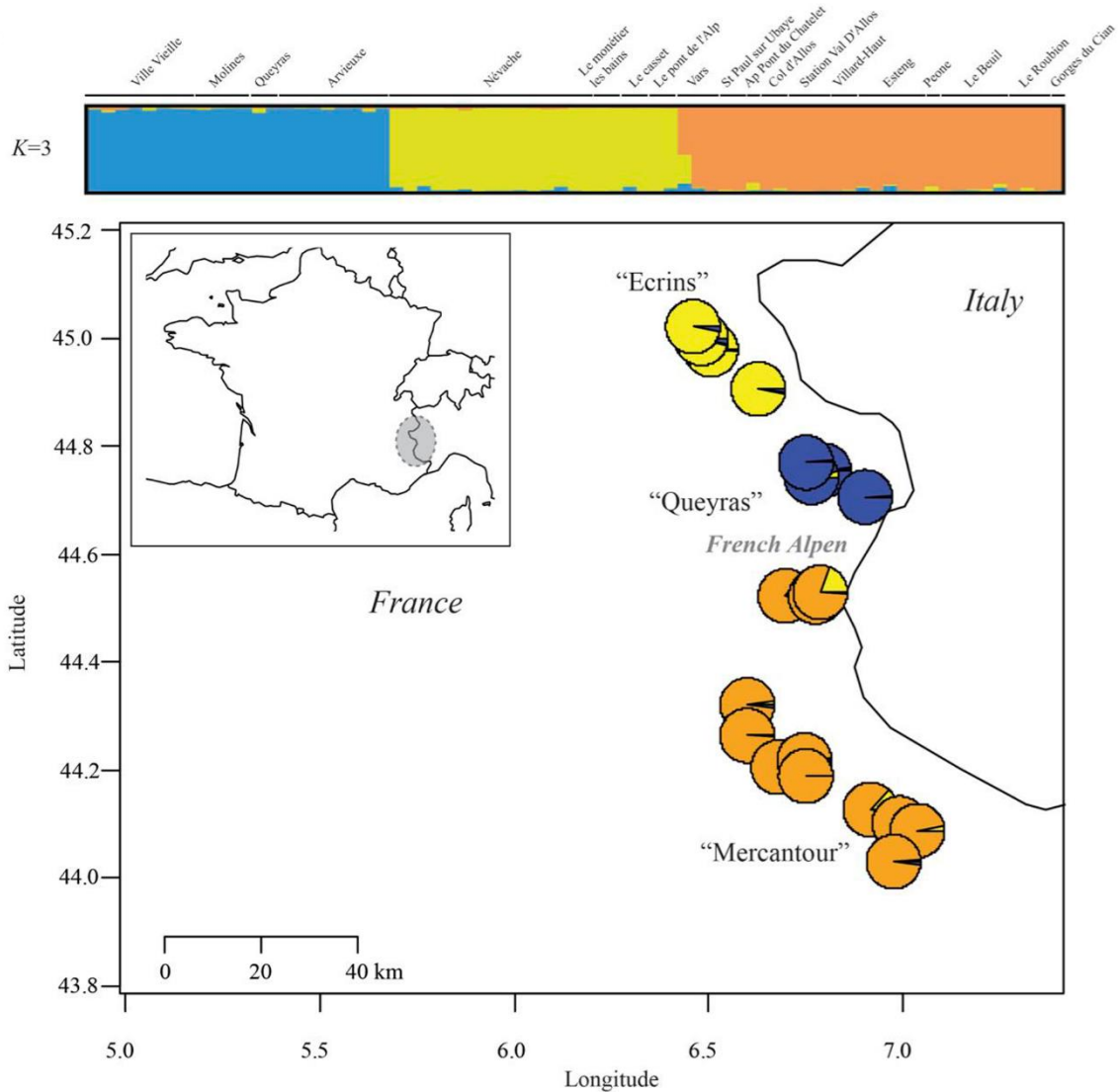
680

681 **Figure 2. *Prunus brigantina* morphological features, genetic clustering and spatial**  
682 **distribution in the French Alps.** a. A *P. brigantina* small tree in its natural habitat  
683 (Arvieux) (left), summery leaves (middle) and ripening fruits (right). b. The three genetic  
684 clusters of *P. brigantina* inferred from the STRUCTURE analysis (Figure S2 at  $K=3$ ) and  
685 their spatial distribution in the French Alps. “Ecrins”, “Queyras” and “Mercantour” refer to  
686 the three national parks in the southeast of France.

a.



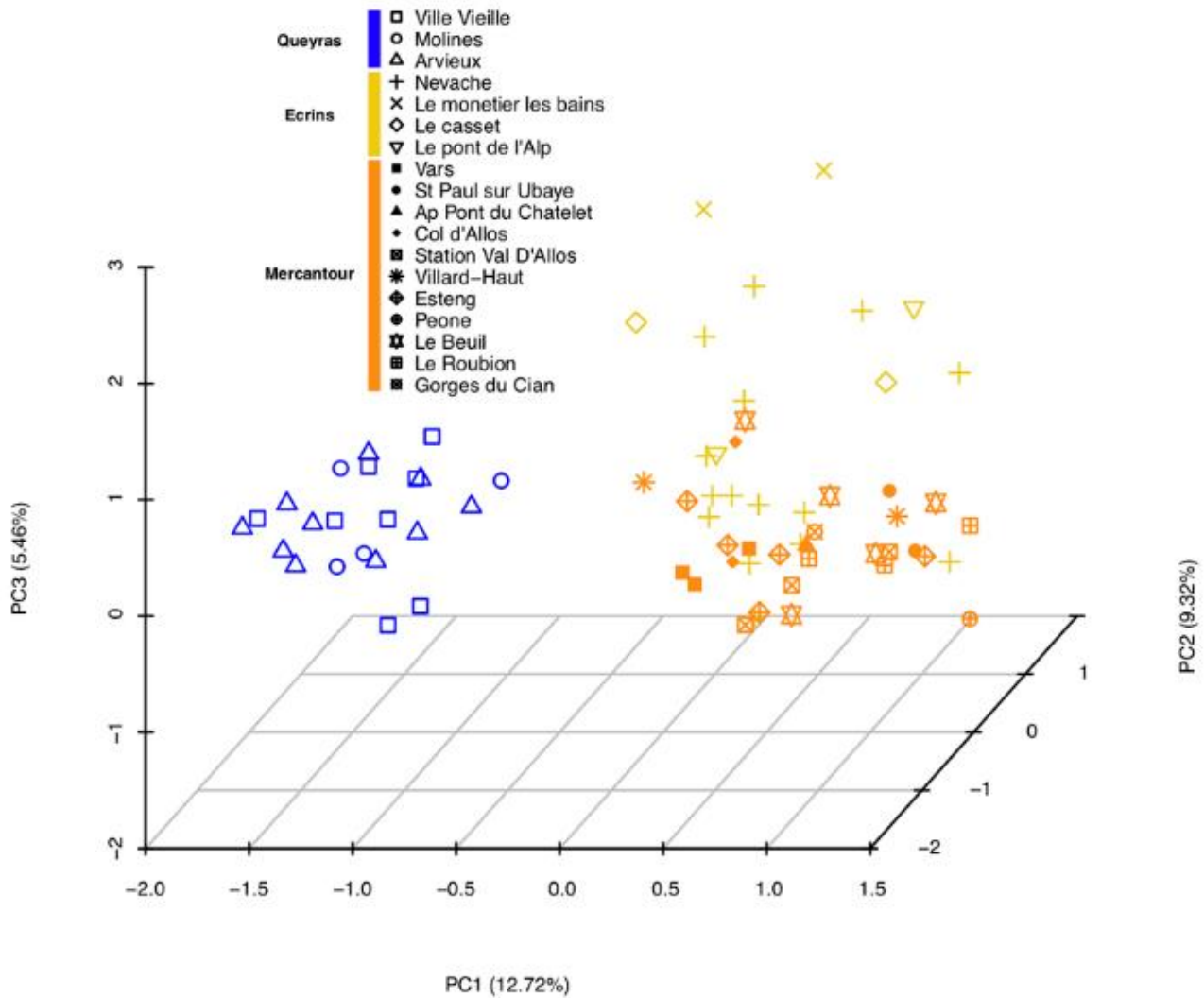
b.



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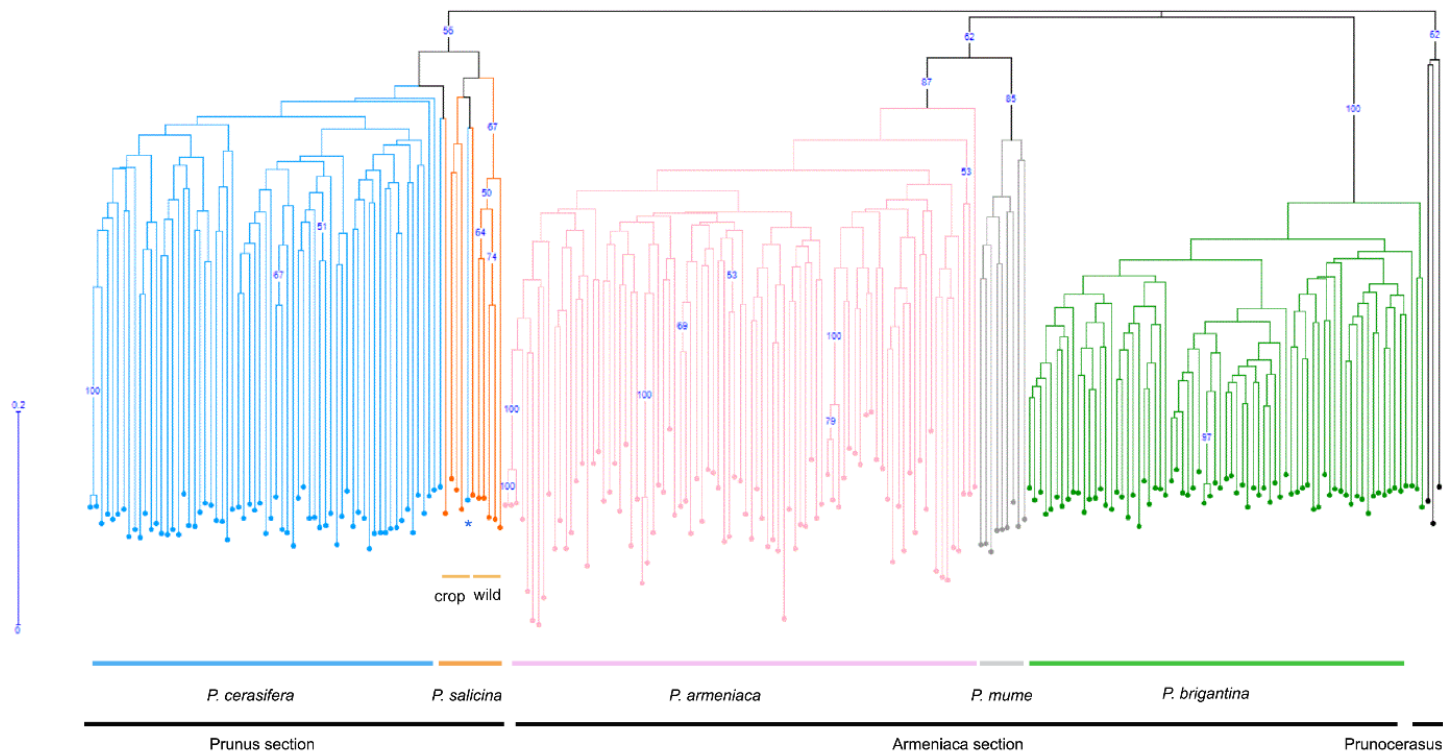
688

689 **Figure 3. Principal components analysis on *Prunus brigantina*.** Colours refer to the  
690 genetic clusters inferred from the STRUCTURE analysis, according to the barplots at  $K=3$   
691 in Figure S2.



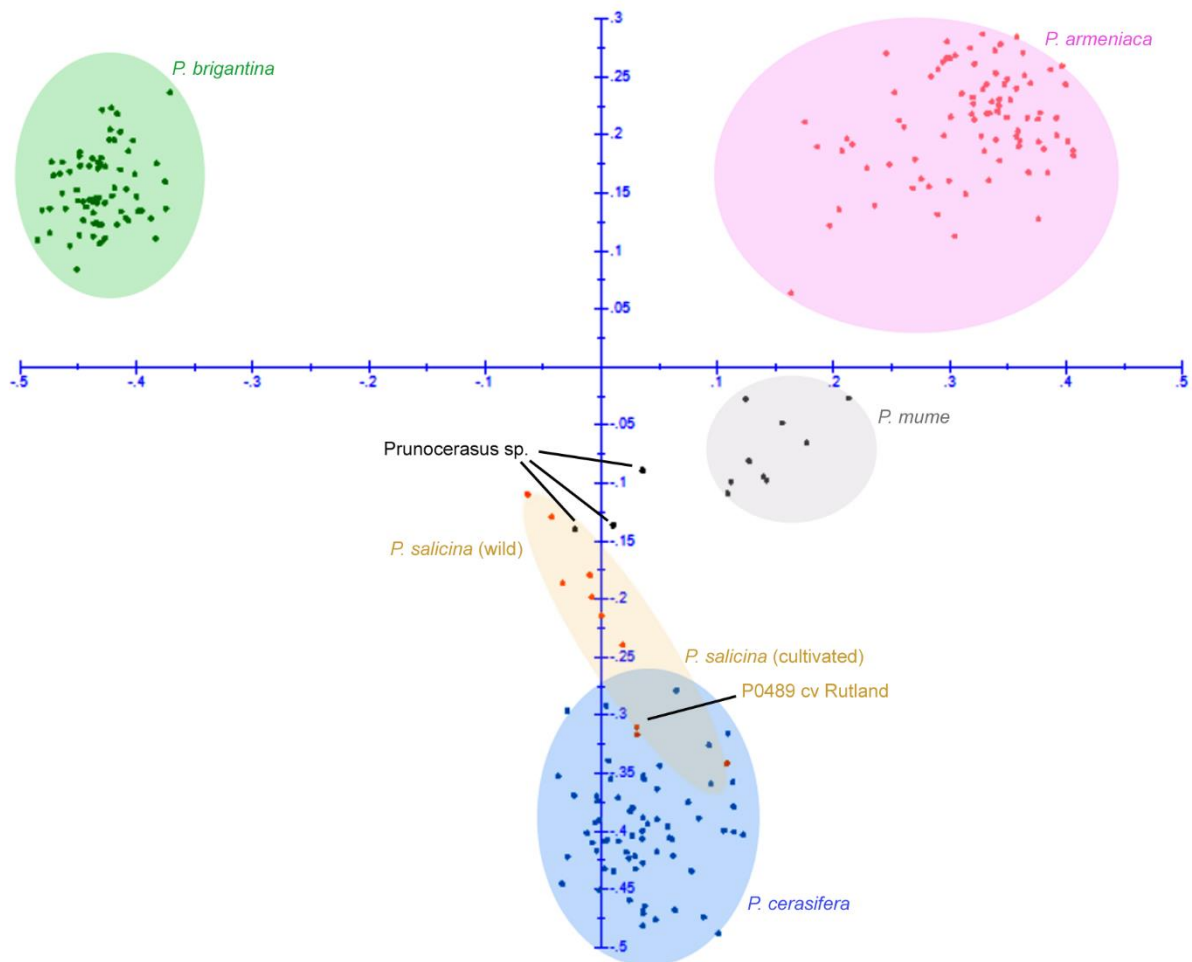
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694 **Figure 4. Unrooted weighted neighbour-joining (NJ) tree of *Prunus brigantina* and other**  
 695 **Prunophora species.** The species are represented by the same colour as the ones used in  
 696 STRUCTURE barplots ( $K=8$ , Figure S5). The NJ tree was built with DARwin, bootstrap support  
 697 values were obtained from 30,000 repetitions. Bootstrap values when greater than 50% are shown  
 698 above the branches. (\*) corresponds to the P0489 plumcot sample. Classification into sections  
 699 was made according to Krüssmann (1978) and Reales et al (2010).



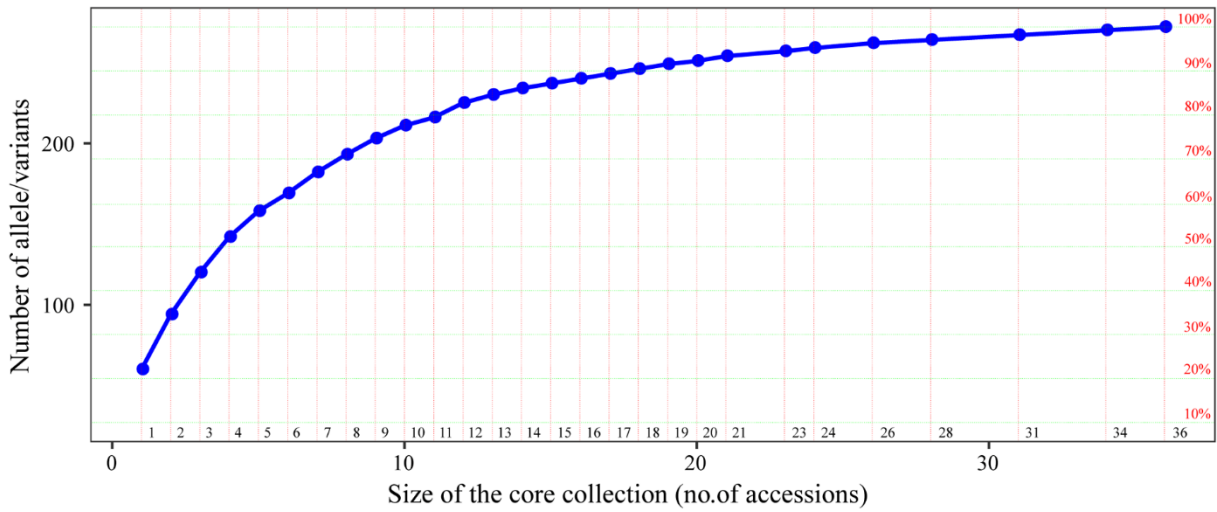
700

701 **Figure 5. Principal components analysis (PCA) on five Prunophora species**  
702 performed with DARwin.  
703 The sampling for this analysis included *P. cerasifera* (N=66) in blue, *P. armeniaca* (N=87)  
704 in pink, *P. brigantina* (N=73) in green, the Chinese apricot tree *P. mume* (N=9) in grey and  
705 Japanese plum, *P. salicina* (N=10) in orange. Black dots correspond to *Prunocerasus*  
706 species (*P. mexicana*, *P. munsoniana* and *P. maritima*). Colours refer to the genetic  
707 clusters inferred from the STRUCTURE analysis, according to the barplots at  $K=8$  in  
708 Figure S5.



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711 **Figure 6. Identification of the core collection of *Prunus brigantina* population based**  
712 **on the strategy maximizing allelic diversity.** The genetic diversity in terms of number  
713 of alleles (left) or percentage of variation compared to the whole dataset (right) is plotted  
714 for different core collection sizes. Details on the accessions retained for each percentage  
715 rate are presented in Table S3.



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717



718 **Tables**719 **Table 1. Different datasets including *Prunus brigantina* and other apricot species in this**  
720 **study.**

721 \* indicate a *P. brigantina* dataset that includes 71 individuals sampled from the French Alps and 2  
722 samples from the French GRC repository. <sup>Δ</sup> Prunocerasus species are represented by *P. mexicana*  
723 (*N*=1), *P. munsoniana* (*N*=1), *P. maritima* (*N*=1), *P. americana* (*N*=1), *P. subcordata* (*N*=1).

Datasets	Description	Number of accessions	Number of accessions for each <i>Prunus</i> species under study ( <i>N</i> )							
			<i>P. brigantina</i>	<i>P. armeniaca</i> (wild)	<i>P. armeniaca</i> (cultivated)	<i>P. sibirica</i>	<i>P. mume</i>	<i>P. mandshurica</i>	<i>P. salicina</i>	<i>P. cerasifera</i>
1	<i>P. brigantina</i>	73*	73*	-	-	-	-	-	-	-
2	<i>P. brigantina</i> and accessions of other <i>Armeniaca</i> species	648	73*	204	270	84	9	8	-	-
3	<i>P. brigantina</i> and accessions of the <i>Armeniaca</i> , <i>Prunus</i> and <i>Prunocerasus</i> sections <sup>Δ</sup>	250	73*	-	87	-	9	-	10	66
4	<i>P. cerasifera</i>	66	-	-	-	-	-	-	-	66

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726 **Table 2. Pairwise population Jost' *D* of *P. brigantina***

Population	Queyras	Ecrins	Mercantour
Queyras	-	0.116	0.14
Ecrins		-	0.097
Mercantour			-

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728