

# Population genetic structure and management perspectives for *Armeria belgenciensis*, a narrow endemic plant from Provence (France)

Alex Baumel<sup>1,\*</sup>, Frédéric Médail<sup>1</sup>, Marianick Juin<sup>1</sup>, Thibault Paquier<sup>2</sup>, Marie Clares<sup>3</sup>,  
Perrine Laffargue<sup>3</sup>, Hélène Lutard<sup>3</sup>, Lara Dixon<sup>2</sup> & Mathias Pires<sup>2</sup>

<sup>1</sup>Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille Univ, Avignon Université, CNRS, IRD, Faculté des Sciences et Techniques St-Jérôme, Marseille, France

<sup>2</sup>Conservatoire botanique national méditerranéen de Porquerolles (CBNMed), 34 avenue Gambetta, 83400 Hyères, France

<sup>3</sup>Conservatoire d'Espaces Naturels (CEN), L'Astragale, 888 chemin des Costettes, 83340 Le Cannet-des-Maures, France

\*Corresponding author: [alex.baumel@imbe.fr](mailto:alex.baumel@imbe.fr)

**Background and aims** – Conserving threatened biodiversity needs a robust knowledge and delineation of the taxonomical entities targeted for *in* or *ex situ* conservation. In the genus *Armeria* (Plumbaginaceae), the frequent correlation between geographical isolation and morphological differentiation led to the delineation of some taxa consisting of few populations. *Armeria belgenciensis* is a narrow endemic represented by one population in southern France, which recently experienced a strong demographic decline caused by repeated disturbances of its habitat and was the object of a demographic rescue. Within two kilometres of *A. belgenciensis*, the presence of *A. arenaria* subsp. *peirescii*, also represented by one small population, poses the question whether the two populations are genetically isolated and should be considered as separated taxa for conservation.

**Material and methods** – The genetic structure of *Armeria belgenciensis* and the geographically close populations of *Armeria arenaria* were analysed based on 328 AFLP markers using model-based and model-free clustering methods. In addition, flowering phenology was estimated to address the possibility of pre-zygotic isolation between *A. belgenciensis* and *A. arenaria* subsp. *peirescii*.

**Key results** – The genetic structure supported a close relationship between *A. belgenciensis* and *A. arenaria* subsp. *peirescii*, compared to other populations of *A. arenaria*. Despite its recent decline, the genetic diversity of *A. belgenciensis* was not particularly low compared to the other studied populations or other rare species in this genus. The existence of gene flow between these two populations is likely given their moderate differentiation and the detection of admixture by one of the clustering methods. The phenological study showed that cross-pollination is possible during a short time period.

**Conclusion** – We propose to place these two populations under the name *A. belgenciensis*, but to keep them as two different management units until there is no evidence of a harmful effect due to small effective sizes.

**Keywords** – Biodiversity; conservation; differentiation; endemism; genetic diversity; clustering; Mediterranean; phylogeography; plant; population; species; systematics.

## INTRODUCTION

Conserving threatened species requires to properly design conservation units. Different strategies can be applied to conserve rare endemic plants (e.g., Van Rossum et al. 2017), but all need to be based on a robust knowledge and delineation of the entities targeted for *in* or *ex situ* conservation (Ryder 1986; Médail & Baumel 2018; Levin 2019). Delineating conservation units is never straightforward, and it often requires the judicious use of available data. However, data are often too restricted to support the exclusiveness or distinctiveness of population sets (Crandall et al. 2000). In the case of incipient species, insufficient isolation and divergence may have precluded the formation of morphologically discrete and reciprocally monophyletic entities for the molecular loci considered. In such cases, combining systematics with population genetic approach is crucial to consider the balance between recognizing ongoing diversification (Moritz 2002; Daïnou et al. 2014) and the risk of over-splitting taxa into populations that may compromise both management and rescue, and ultimately their long-term persistence (Frankham et al. 2012).

The genus *Armeria* (Plumbaginaceae), known as “thrift”, exemplifies this scenario. Integrated by about eighty small perennial polycarpic mostly Mediterranean species, its centre of diversity is in the Iberian Peninsula (Nieto Feliner 1990). Molecular systematic studies in *Armeria* showed the recurrence of hybridization due to the weakness of reproductive barriers and frequent range fluctuation (Fuertes Aguilar et al. 1999; Tauleigne-Gomes & Lefèbvre 2005; Piñeiro et al. 2011; Nieto Feliner et al. 2019). Therefore, the delineation of taxa within this genus is based on a correlation criterion between ecological, geographical and morphological isolation which often results in taxa consisting of few populations (Nieto Feliner 1990). Due to frequent introgressive hybridization (Nieto Feliner et al. 2019) and sometimes also the lack of information on DNA barcode markers, population genetic approaches based on multilocus markers are highly relevant to set conservation units since they are expected to provide a finer reconstruction of the evolutionary history of *Armeria* taxa.

We report here the results of a study on the genetic diversity structure of *Armeria belgenciensis* Donadille ex Kerguelen and geographically closely related populations of *Armeria arenaria* (Pers.) Schult. *Armeria belgenciensis* is a narrow endemic represented by only one population, which recently experienced a strong demographic decline caused by repeated disturbances in its habitat and was the object of a demographic rescue. Within two kilometres of *A. belgenciensis* the presence of *Armeria arenaria* subsp. *peirescii* Baumel, Auda & Médail represented also by one small population, poses the question of whether the two populations are genetically isolated and should be considered as separated taxa for conservation. According to the literature (Tison et al. 2014), *A. arenaria* subsp. *peirescii* flowers one to two months earlier than *A. belgenciensis*. However, their spatial proximity, strong morphological similarity and sharing of the same ITS ribosomal DNA genotype suggested hybridization between them (Baumel et al. 2009).

We applied a population genetic approach to evaluate the isolation between *A. belgenciensis* and *A. arenaria* subsp. *peirescii*. Considering that *Armeria* taxa can exchange genetic material when they meet, our approach is based on the assumptions that genetic differentiation among taxa is expected to be higher than within taxa and their isolation has to be supported by their genetic clustering. Our sampling was therefore designed to estimate (1) genetic admixture between *A. belgenciensis* and *A. arenaria* subsp. *peirescii* and (2) genetic differentiation among them and close neighbour populations belonging to *A. arenaria* (*A. arenaria* subsp. *bupleuroides* Godron & Gren. and *A. arenaria* subsp. *pradetensis* Médail, Baumel & Auda). We also monitored flowering phenology of *A. belgenciensis* and *A. arenaria* subsp. *peirescii* to test for their hypothetical pre-zygotic isolation.

Our genotyping method is based on Amplified Fragment Length Polymorphism (AFLP) because AFLP markers have a higher genome coverage than single locus DNA barcode markers while having a lower cost than genotyping by sequencing methods. Usefulness of AFLP markers for phylogeography or systematics of recently diverged taxa has been proven in several case studies (Despres et al. 2002; Duminil & di Michele 2009; Hardion et al. 2012; De Riek et al. 2013; Medrano et al. 2014; Gutiérrez-Larruscain et al. 2019).

We discuss the implications of our results and propose practical recommendations for the conservation of these two threatened taxa.

## MATERIAL AND METHODS

### Studied taxa

*Armeria belgenciensis* is known from a single location in southern Provence (Solliès-Toucas, Var, France). This population experienced a strong demographic bottleneck from about 1000 individuals in the 1960s (Donadille 1969) to less than 150 individuals in 2004 and 34 in 2007 (Conservatoire botanique national méditerranéen de Porquerolles (CBNMed) unpublished data). A population rescue implemented by CBNMed and Conservatoire d’Espaces Naturels Provence-Alpes-Côte d’Azur (CEN-PACA) since 2009 has helped to restore the census size to nearly 458 individuals in 2019. Within 2 km of the *A. belgenciensis* population, one small population of *A. arenaria* was discovered in 2002 and described in 2009 as *A. arenaria* subsp. *peirescii* (Baumel et al. 2009). In 2019, the census size of *A. arenaria* subsp. *peirescii* was 158 individuals. *Armeria belgenciensis* is located on a dolomitic sandy plateau with an important tree cover mainly consisting of *Pinus pinaster* with *P. halepensis* and *Quercus ilex*. The *P. pinaster* trees are at most 50–60 years old and they regenerate. The area belongs to seven landowners and there is no planned forest management. In 2009 the area with *A. belgenciensis* has been placed under legal protection (“Arrêté préfectoral de protection de biotope”) and is regularly monitored by CBNMed. Small clearings were cut to remove some pines around the main stand of *A. belgenciensis* and their needles were raked. *Armeria belgenciensis* is located in clearings and forest edges, with other psammophilous species such as *Arenaria modesta*, *Helichrysum stoechas*, *Iberis ciliata*, *Iberis linifolia*, *Silene otites* or *Eu-*

*phorbia seguieriana*. In contrast, *A. arenaria* subsp. *pereiscii* grows in an open steep rocky habitat within a vegetation dominated mainly by *Quercus coccifera*, *Rosmarinus officinalis* and *Thymus vulgaris*.

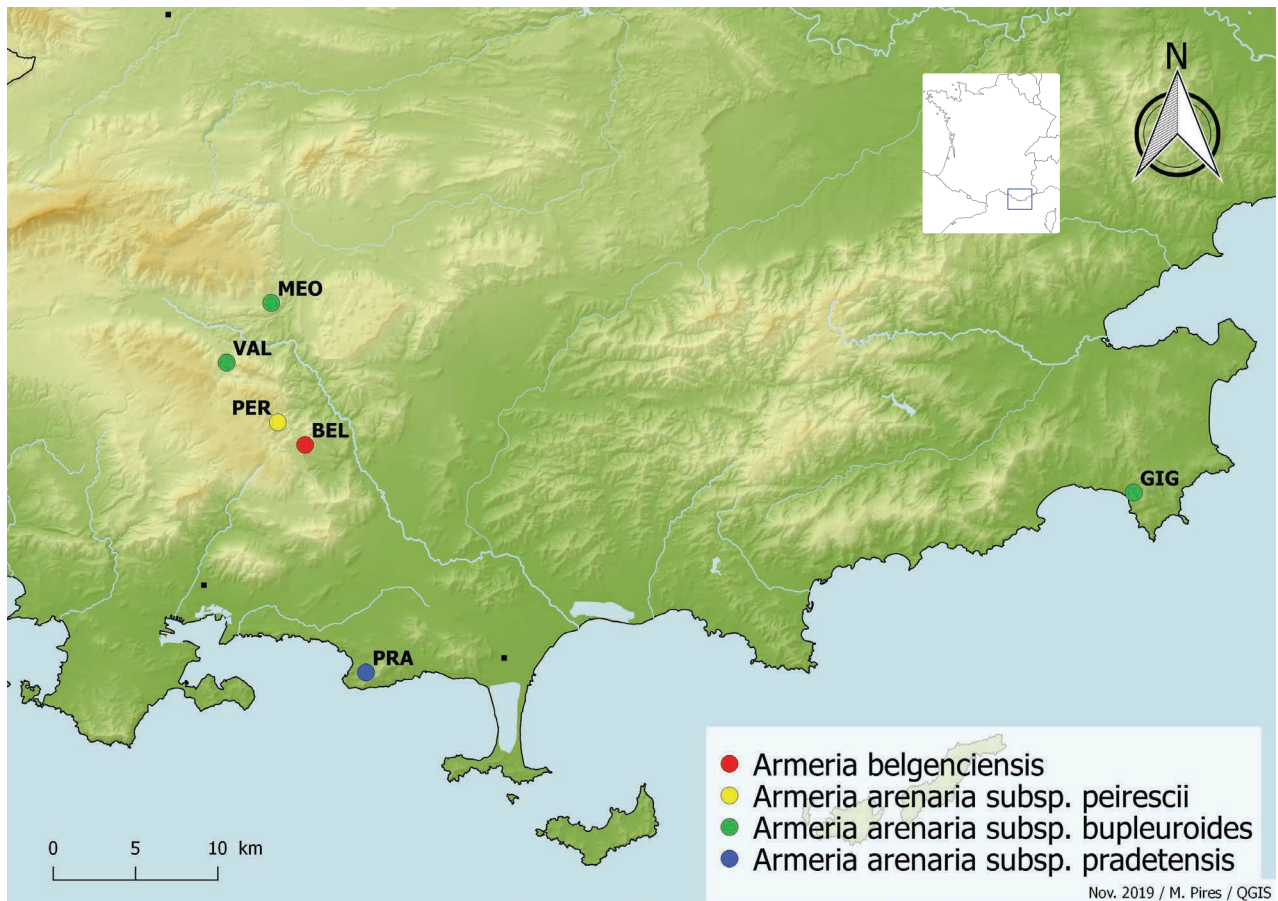
### Sampling

Leaves from two hundred individuals were collected from six populations (fig. 1). According to our first objective described in introduction, the main sampling effort concerned *A. belgenciensis* and *A. arenaria* subsp. *pereiscii* and thus 50 individuals were sampled for each of those two populations (BEL and PER) allowing to comprehensively sample their spatial distribution for detecting admixed individuals (supplementary file 1). According to our second objective the sampling was completed with 50 individuals from two nearby populations of *A. arenaria* subsp. *bupleuroides* (situated at “Aiguilles de Valbelle”, named VAL and “Méounes-les-Montrieux” named MEO), 25 individuals from a population of *A. arenaria* subsp. *pradetensis* situated near the Mediterranean coastline (situated at “Le Pradet” named PRA) and 25 individuals of *A. arenaria* subsp. *bupleuroides* (situated at “Gigaro” named GIG) from an isolated population, 52 km away from *A. belgenciensis*. The sampling coordinates are included in the data sets deposited in Dryad Digital Repository.

### DNA extraction, AFLP and genetic diversity analysis

Total DNA was extracted using NucleoSpin Plant II Kit (Macherey & Nagel, Germany) from c. 15 mg of silica dried leaves. DNA concentrations were measured using a photometer (Biophotometer, Eppendorf, Germany) and homogenized at 10 ng/μl. The AFLP protocol was conducted on 100 ng of DNA according to Vos et al. (1995) with modifications (see supplementary file 2). Electrophoresis was done using an ABI 3730xl DNA Analyzer.

The marker bands produced by the four primer combinations were entered automatically using the RawGeno application (Arrigo et al. 2009) with stringent criteria to select markers (minimum size peaks greater than 100 UFR, minimum width of 1.2 bp and maximum of 1.75 bp). Despite the stringency of this automatic procedure, it generates an error rate that may be greater than a manual selection. To reduce this error rate, reproducibility of the markers was tested on 12 samples. Markers not reproducible in 100% of the 12 replicates, i.e., having an error rate equal to or greater than 8.33%, were removed. In addition, markers with a frequency lower than 5% or higher than 95% were also removed. Individuals with very few markers, for which the AFLP procedure was not optimal, were removed as well.



**Figure 1** – Map of the populations and taxa collected for the study. Acronyms: BEL: *A. belgenciensis*; PER: *A. arenaria* subsp. *peireiscii*; PRA: *A. arenaria* subsp. *pradetensis*; VAL, MEO, and GIG: three populations of *A. arenaria* subsp. *bupleuroides*. Map created with QGIS version 2.14 (QGIS Development Team 2019).



The genetic diversity structure was investigated using four different methods. First, STRUCTURE (Pritchard et al. 2000) was used to investigate clustering models. From one to 10 genetic groups ( $K = 1-10$ ) were tested allowing admixture at the individual level, with correlated allele frequencies and without prior information on geographical origin. Ten replicate runs per  $K$  were performed, each having a burn-in period of 200 000 generations and a chain length of 1 000 000 generations. The results of the 10 replicates were averaged with CLUMPACK (Kopelman et al. 2015) for each  $K$  model. STRUCTURE HARVESTER (Earl & vonHoldt 2012) was used to obtain likelihood values across the multiple values of  $K$  as well as to apply the delta  $K$  criterion to select the  $K$  model representing the optimal partition of the genetic data set (supplementary file 3).

Second, we used the model-free Discriminant Analysis of Principal Components (DAPC) implemented in the adegenet R package (Jombart et al. 2010). The DAPC uses a k-means algorithm, principal components and discriminant analysis to identify the best clustering of the data set. For DAPC the choice of the  $K$  clustering solution is based on the Bayesian information criterion measure of goodness of fit (R package adegenet, find.clusters and dapc functions; Jombart et al. 2010). Because the challenges for estimating the optimal  $K$  clusters with STRUCTURE (Janes et al. 2017) also apply for DAPC, all the results of STRUCTURE and DAPC from  $K = 1$  to  $K = 6$  are shown in supplementary files 4–6. The retained STRUCTURE and DAPC clusters were depicted using the compoplot function of the adegenet R package. The main pattern of genetic structure revealed by STRUCTURE and DAPC was used to perform a principal coordinate analysis (PCoA) based on inter-individual distances computed with the Dice coefficient (dist.binary function, ade4 R package; Dray & Dufour 2007).

Third, we used the Ward hierarchical clustering method (R package, hclust functions with the correction of Murtagh & Legendre 2014). This last method uses the inter-individual distances computed with the Dice coefficient to generate a dendrogram showing groups that minimize within-group dispersion. Finally, an AMOVA was performed to estimate overall and pairwise differentiations among populations with GeneAlex 6.5 (Peakall & Smouse 2012).

Within population genetic diversity was analysed to estimate the unbiased Nei genetic diversity (HE), the Shannon index (SH) and the standardized index of association (RBARD) with the poppr R package (Kamvar et al. 2014).

### Flowering phenology monitoring

Occasional field observations reported a summer flowering phenology for *A. belgenciensis* and a spring phenology for *A. arenaria* subsp. *peirescii* (Baumel et al. 2009; Tison et al. 2014). To monitor flowering phenology, six permanent plots were established, three for *A. belgenciensis* and three for *A. arenaria* subsp. *peirescii*. The plots were chosen to cover habitat heterogeneity of each taxa according to previous ecological surveys done by CBNMed. The survey was done once per month from April to September in 2017 and twice per week from June to August in 2018, and from May to August in 2019. For *A. belgenciensis* the plots AB1 (30 m<sup>2</sup>),

**Table 1** – Pairwise differentiations (PhiST), obtained by AMOVA on 328 AFLP markers and 187 thrifts. BEL is *A. belgenciensis*, PER is *A. arenaria* subsp. *peirescii*, PRA is *A. arenaria* subsp. *pradetensis*, and VAL, MEO, and GIG are three populations of *A. arenaria* subsp. *bupleuroides*. All values are significant ( $p = 0.01$ , 1000 permutations). The overall PhiST is 0.2 ( $p = 0.01$ , 1000 permutations).

BEL	PER	VAL	MEO	PRA	GIG
0.00					BEL
0.14	0.00				PER
0.22	0.21	0.00			VAL
0.29	0.26	0.13	0.00		MEO
0.31	0.29	0.13	0.15	0.00	PRA
0.42	0.40	0.26	0.30	0.32	0.00 GIG

AB2 (25 m<sup>2</sup>) and AB3 (25 m<sup>2</sup>) included 25, 22 and 23 individuals, respectively. For *A. arenaria* subsp. *peirescii* the demographic density was much lower and finding optimal conditions to monitor individual plants was more difficult. Thus, plots AP1 (100 m<sup>2</sup>), AP2 (5 m<sup>2</sup>) and AP3 (100 m<sup>2</sup>) included 14, 10 and 24 individuals, respectively. For each individual, the number of open flowers and the number of capitula with at least one open flower were counted. The mean and 95% confidence interval for number of flowers and number of capitula per individual were computed and plotted for BEL and PER populations and each census (ggplot2 R package; Wickham 2016).

## RESULTS

### Genetic diversity structure

After the filtering steps of AFLP raw data, 328 markers were selected for a total of 187 individuals. To identify the main pattern of genetic structure we used the models of STRUCTURE assigning the 187 thrift genotypes to genetic clusters. According to the delta  $K$  criterion (supplementary file 3), the optimal solutions were the  $K = 2$  and  $K = 4$  models (fig. 2; but see all in supplementary file 4). For the  $K = 2$  model, BEL and PER populations constituted the first group and the remainder the second group. For the  $K = 4$  model, BEL and PER populations are split to form the two first groups whereas VAL, MEO and PRA constituted the third group and GIG the fourth. The BIC measure of goodness of fit (supplementary file 5) identified six as the optimal number of groups for the DAPC analysis. These six groups corresponded to the six sampled populations albeit with high admixture between VAL and MEO. The STRUCTURE patterns are congruent with the results of DAPC for two and six groups, respectively (fig. 3). The DAPC revealed a higher level of admixture between BEL and PER than STRUCTURE. The Ward algorithm generated a dendrogram with a hierarchical pattern of clustering that is similar to the STRUCTURE and DAPC results (fig. 2). The PCoA of genotypic dissimilarities revealed a pattern of individual grouping that is congruent with STRUCTURE and DAPC admixture plots (fig. 3).

The mean PhiST was 0.2 and the minimum pairwise PhiST was 0.13 between VAL and MEO, followed by the PhiST between BEL and PER, whereas the two highest values, 0.4 and 0.42, were obtained between BEL and GIG (table 1).

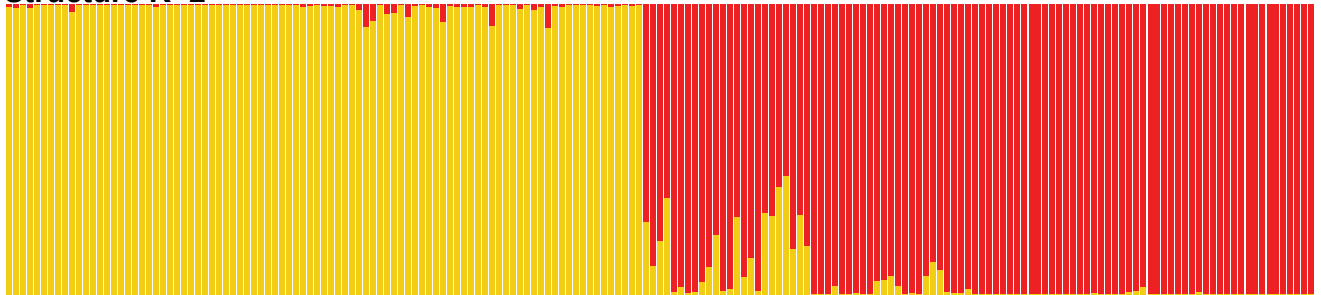
Overall patterns of genetic structure indicate a close relationship between the BEL and PER populations, which form a distinct genetic group from other populations. Moderate differentiation ( $\Phi_{ST} = 0.14$ ), as well as multivariate methods DAPC and PCoA, supported the existence of genetic exchanges between BEL and PER although STRUCTURE

identified a lesser extent of admixture (fig. 2). According to the multivariate methods, the gene flow is symmetrical with the same proportion of introgressed individuals in both populations whereas STRUCTURE supports an asymmetrical introgression of BEL into PER.

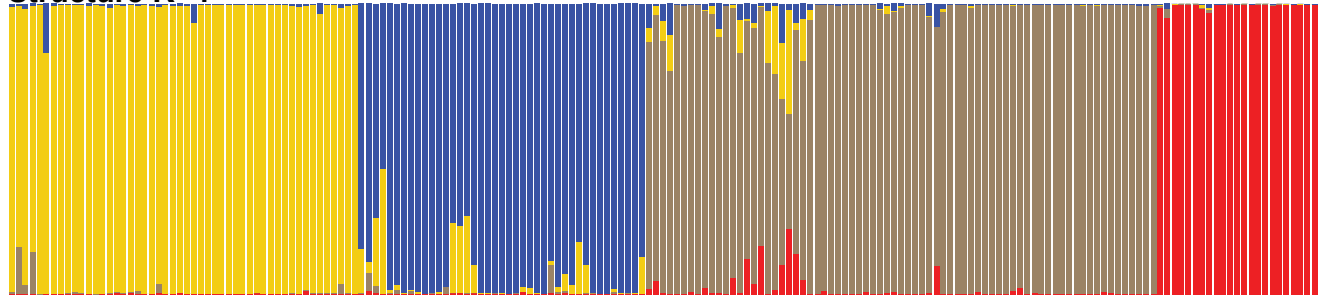
### Genetic diversity

Within-population genetic diversity exhibited similar values in the six populations (table 2). HE ranged between 0.2 and 0.26 and SH between 3.14 and 3.91. Private markers to sin-

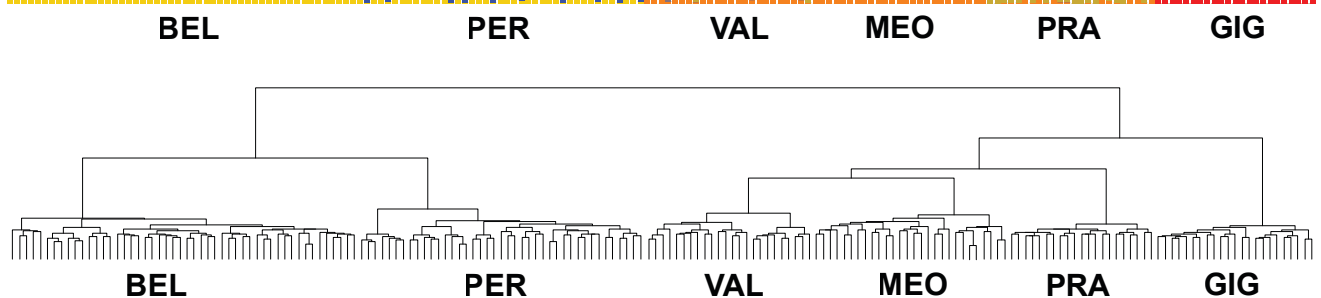
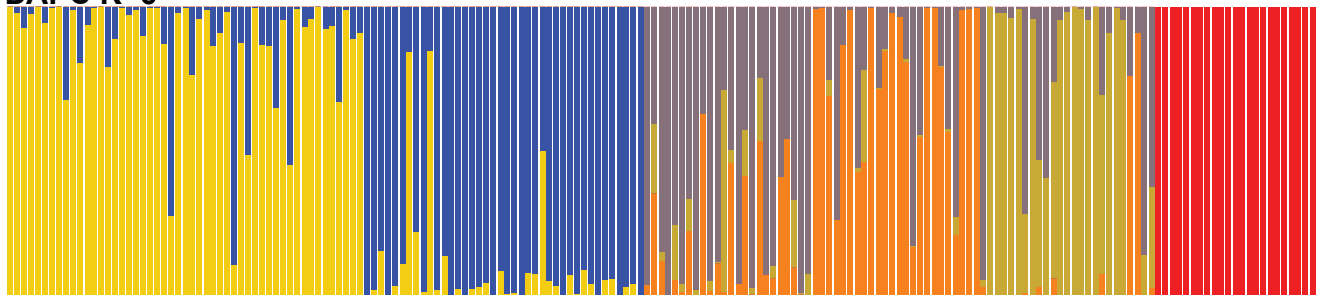
#### Structure K=2



#### Structure K=4



#### DAPC K=6



**Figure 2** – Genetic groups inferred for 187 thrifts using 328 AFLP markers according to three methods of clustering. The admixture proportions are indicated for K = 2 and K = 4 solutions of STRUCTURE and for K = 6 solution of DAPC. The dendrogram was obtained with the Ward algorithm performed on Dice intergenotype distances. Acronyms (BEL, PER...) detailed in caption of fig. 1.

**Table 2** – Genetic diversity sampled in 6 thrift populations using 328 AFLP markers. BEL is *A. belgenciensis*. PER is *A. arenaria* subsp. *peirescii*, PRA is *A. arenaria* subsp. *pradetensis*. and VAL, MEO, and GIG are three populations of *A. arenaria* subsp. *bupleuroides*. N is the sample size. HE is the Nei genetic diversity index. SH is the Shanon entropy index. Private is the number of markers unique to a population and RBARD is the association index.

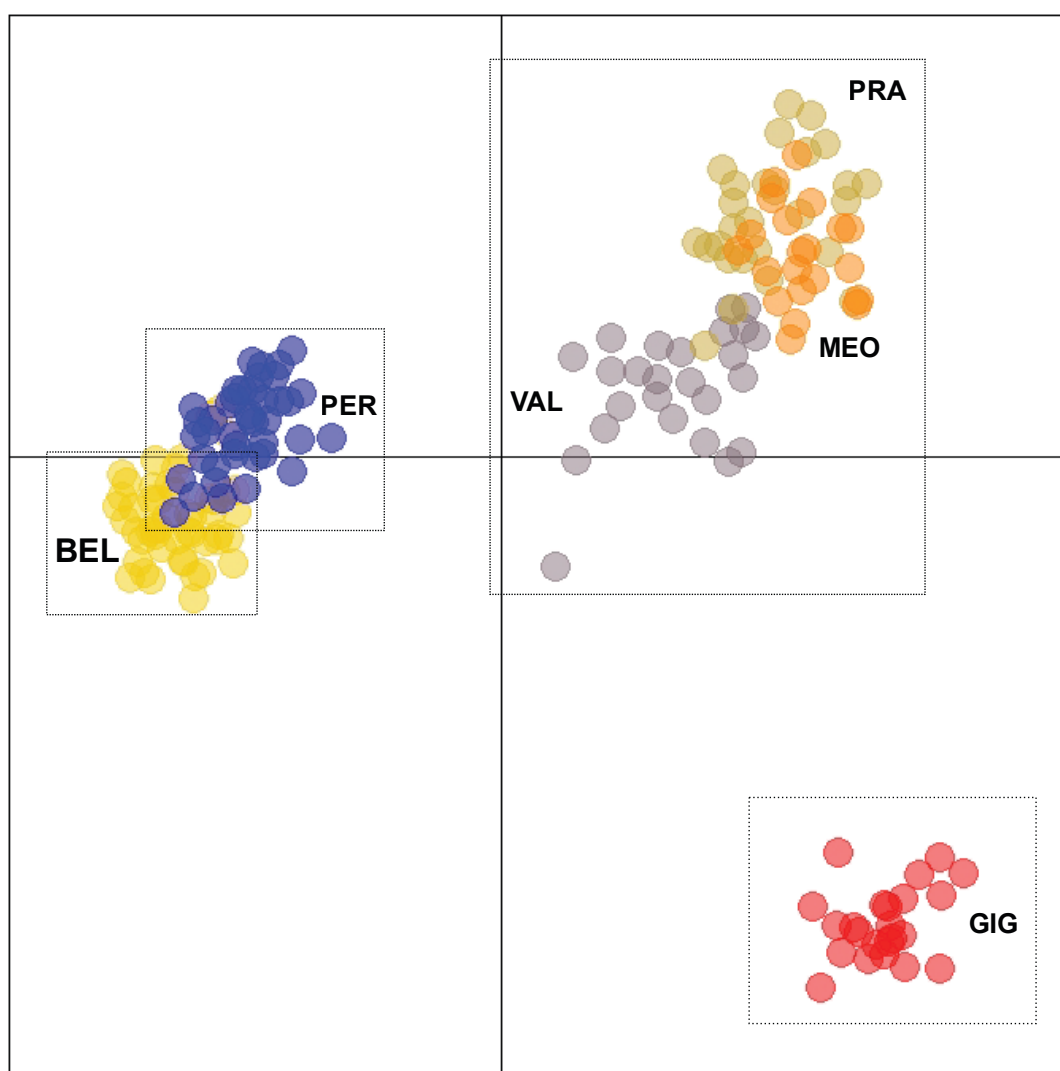
Populations	N	HE	SH	PRIVATE	RBARD
BEL	50	0.23	3.91	1	0.00375*
PER	41	0.23	3.71	1	0.00593*
VAL	24	0.26	3.18	0	0.00642*
MEO	25	0.25	3.22	0	0.0037*
PRA	24	0.24	3.18	0	0.0167*
GIG	23	0.2	3.14	3	0.0037*

gle populations were relatively few. The normalized index of association (RBARD) was significant and positive but small in all populations.

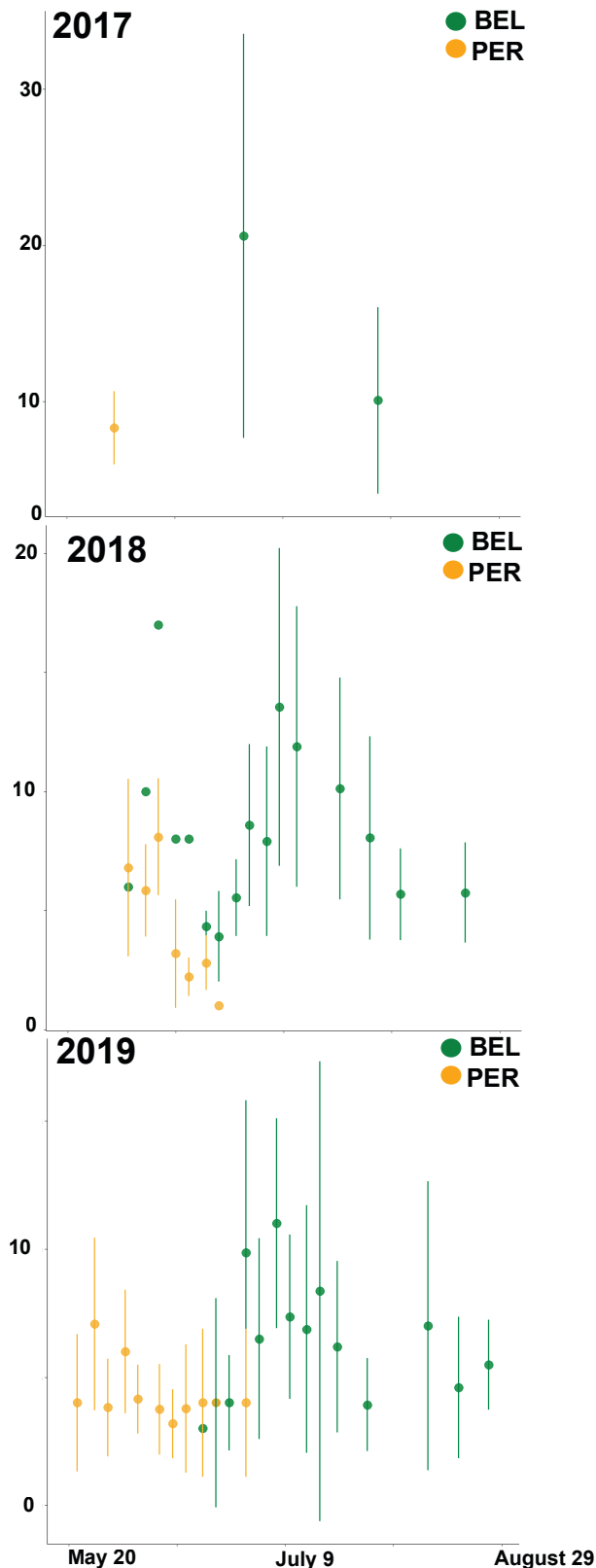
### Flowering phenology

BEL showed a higher number of capitula and flowers per blooming individual. The mean number of capitula per plant was 2.5 (SE 0.13) for BEL versus 2 (SE 0.09) for PER. The mean number of flowers by plant was 8.5 (SE 0.65) for BEL versus 4.9 (SE 0.34) for PER. BEL has also larger confidence intervals for these variables (fig. 4).

The flowering period of PER started earlier than BEL and had a shorter duration (fig. 4). Over the three years of flowering censuses, we observed that PER started blooming in May whereas BEL started in June. The blooming peak was in the beginning of June for PER and in the beginning of July for BEL. In 2017 the frequency of visits was not sufficient



**Figure 3** – Principal coordinate analysis (PCoA) of genetic distances (Dice coefficient) between 187 thrift individuals genotyped by 328 markers. The colours are corresponding to the 6 clusters found by the DAPC method and the boxes are the cluster of the K = 4 model of STRUCTURE. Acronyms (BEL, PER...) detailed in caption of fig. 1.



**Figure 4** – Flowering census of *Armeria belgenciensis* (BEL: 70 plants censused) and *A. arenaria* subsp. *peirescii* (PER: 48 plants censused) populations. The points and lines respectively show the mean and 95% confidence interval of the number of flowers per individual. The x-axis is the same for the three plots.

to observe flowering overlap between the two populations. However, in 2018 and 2019 an overlap was clearly observed between the end of the PER blooming period and the beginning of the BEL blooming period. This overlap, occurring at the end of June/beginning of July, lasted 8 days in 2019 (from June 24 to July 1) and 15 days in 2018 (from June 8 to June 22).

## DISCUSSION

Since the Mediterranean Basin hotspot includes many rare and vulnerable narrow endemic plant species, the number of available molecular systematic studies is not sufficient to understand the complexity of the evolution of many groups and thus to properly design conservation units (Médail & Baumel 2018). Our multilocus molecular approach was able to distinguish a hierarchical genetic differentiation structure with taxonomic and conservation implications.

### Genetic structure and taxonomic implications

The main result of this study based on 328 AFLP markers is that different methods of clustering revealed a close relationship between *Armeria belgenciensis* (BEL) and *A. arenaria* subsp. *peirescii* (PER) as suggested by a previous morphometric study (Baumel et al. 2009). The fact that GIG, the geographically most distant population, was grouped with VAL, MEO and PRA in all analyses stresses the distinctiveness of BEL and PER, even though the latter was described as a subspecies of *A. arenaria*. BEL and PER must therefore be conserved independently of *A. arenaria* and we propose to gather these two populations under *A. belgenciensis*. This proposal has the advantage of establishing a taxonomical scheme based on a robust analysis of genetic differentiation and it also implies the legal protection of a second population of *A. belgenciensis*.

VAL, MEO and PRA also form an original genetic group despite the distance that separates these populations. Within this group, the PRA population (*A. arenaria* subsp. *pradentensis*) is moderately differentiated according to PhiST and forms a genetic group according to the clustering methods. Its genetic isolation is not complete (fig. 2) but it makes sense to keep it as a separate conservation unit, and possibly maintaining its subspecies status based on three arguments: its geographical isolation, its specific rocky habitat located on the waste of an old copper mine and its different morphology (Baumel et al. 2009). Previous works emphasized the evolution of heavy-metal tolerance in *Armeria* (e.g., Veke-mans & Lefèbvre 1997).

Our results also raise the question of the status of the most remote *A. arenaria* population (GIG), which appears to be an isolated and independent genetic group in all analyses, and occurs on a distinct semi-halophilous habitat, near the coastline. On the basis of these results we are facing a systematic dilemma. On the one hand, maintaining *A. arenaria* subsp. *pradentensis* would require describing the GIG populations as an independent subspecies. On the other hand, if we followed the main split in the Ward dendrogram, or the STRUCTURE solution for  $K = 2$ , only two taxa should be recognized: *A. belgenciensis* and *A. arenaria* subsp. *bupleuroides*. Deciding between these two treatments will re-

quire an *ad hoc* study, based on a comprehensive sampling of *A. arenaria* over its entire range.

### Genetic diversity of *Armeria belgenciensis*

The only known population of *Armeria belgenciensis* (BEL) has recently experienced a very strong decline (c. 95% of the census size), which has urged a reinforcement procedure to save the population. It was expected that the genetic diversity of this population would be small relative to other *Armeria* populations. For example, intra-population diversity in *Armeria caespitosa* (Ortega) Boiss., which is endemic to mountainous areas in central Spain encompassing small populations with often less than 500 individuals, was found to be generally low with mean values of 0.18 for HE and 0.24 for SH (García-Fernández et al. 2013). For *Armeria pungens* (Link) Hoffmanns. & Link, a coastal species located in Spain, Portugal and Corsica-Sardinia, the diversity was also quite low with mean values of 0.11 for HE and 0.12 for SH (Piñeiro et al. 2007). These values were even lower for *Armeria maderensis* Lowe (HE = 0.06, SH = 0.11), a rare endemic of the mountains of Madeira (Piñeiro et al. 2011). By comparison, the values reported here for *A. belgenciensis* (BEL) and *A. arenaria* subsp. *peirescii* (PER) populations are much higher (table 2). These values are close to a moderate genetic diversity level, generally reported for Mediterranean endemic plants analysed by AFLP (Fernández-Mazuecos et al. 2014; Jimenez-Mejías et al. 2015; Médail & Baumel 2018). This result indicates that some factors may have limited the impact of the strong recent bottleneck. We suggest the role of the soil seed bank, the rescue performed by the CBNMed and the gene flow from the PER population (see above) as hypothetical mitigating processes.

The RBARD values were significant and positive but small in all populations, rejecting the null hypothesis of no linkage among markers and indicating little deviation from panmictic mixing (Kamvar et al. 2014). Because clonality or selfing are very unlikely in these plants (Baker 1966), this positive and significant values for RBARD are probably caused by a small Wahlund effect. An interesting point is that PER has a lower demographic size, lower genetic diversity (SH), and higher RBARD index. Although the PER population seems stable since its discovery (CBNMed unpublished records), our analyses support a low effective demographic size compared to the greater genetic diversity and higher rate of genetic mixing that characterizes the BEL population.

### Implications for the conservation of *Armeria belgenciensis*

To our knowledge no forest fires occurred in the area during the last 50 years, meaning that the woody vegetation (forest and matorral) could have acted as a physical barrier between BEL and PER for several generations. Incongruences among the clustering methods limit our discussion about the extent and the direction of gene flow. Progress in this direction will require more data and estimating parameters of an isolation by migration model. However, the BEL and PER populations are not strongly differentiated and are probably connected by low gene flow. Their flowering overlap (fig. 4) is narrow but sufficient to allow cross pollination in early summer by

the numerous generalist pollinators regularly observed visiting *Armeria* (García-Camacho & Escudero 2009; Nieto Feliner et al. 2019). Moreover, a hiking trail passing close to both populations may facilitate ectozoochorous dispersal of the fruits (enclosed in their calyx) between the populations (Tauleigne-Gomes & Lefèbvre 2005). Gene flow, even low, may contribute to their persistence in the long term because the small size of these two populations (458 individuals for *A. belgenciensis* and 158 individuals for *A. arenaria* subsp. *peirescii*) are well under the effective number of 1000 individuals recommended to maintain evolutionary potential (Frankham et al. 2014). A growing body of studies highlights the pivotal role of biological connectivity for the genetic rescue of small fragmented populations as well as to minimize the extinction risk under future environmental change (Frankham 2015; Ralls et al. 2018). Our recommendation is, therefore, to keep these two populations separated as two different management units for seed sourcing or for any further population recovery plan, but to not act against their connectivity, for example by installing fences or removing trails passing nearby. Increasing connectivity between the BEL and PER populations is not pertinent right now but could be considered in the future to prevent the harmful effects of small demographic sizes, i.e., inbreeding, allele effect or limited adaptive potential (Hamilton & Miller 2016).

### SUPPLEMENTARY FILES

Six supplementary files are associated with this paper:

Supplementary file 1: Map of the sampling of *A. belgenciensis* and *A. arenaria* subsp. *peirescii*.

<https://doi.org/10.5091/plecevo.2020.1702.2165>

Supplementary file 2: AFLP genotyping method.

<https://doi.org/10.5091/plecevo.2020.1702.2167>

Supplementary file 3: Result of STRUCTURE HARVESTER.

<https://doi.org/10.5091/plecevo.2020.1702.2169>

Supplementary file 4: Admixture proportions inferred by STRUCTURE.

<https://doi.org/10.5091/plecevo.2020.1702.2171>

Supplementary file 5: DAPC optimal K number of genetic clusters.

<https://doi.org/10.5091/plecevo.2020.1702.2173>

Supplementary file 6: Admixture proportions inferred by DAPC.

<https://doi.org/10.5091/plecevo.2020.1702.2175>

### DATA SETS

Data sets are available on the DRYAD database:

<https://doi.org/10.5061/dryad.cvdncjt17>

- AFLP data 187 *Armeria* x 328 markers, with individual coordinates (Lambert II), excel file.
- Flowering census data, excel file.
- Scripts with data formatted for analyses in R.



## ACKNOWLEDGEMENTS

The authors would like to gracefully thank the staff who performed the field monitoring survey for their precious help: Rémi Obtiz, Simon Beuro, Mallauray Hamon, and Antoine Carroué. This study benefited from equipment and services from the molecular biology facility (SCBM) at IMBE and was supported by Fondation Itancia (France) and Fondation du Patrimoine (France).

## REFERENCES

- Arrigo N., Tuszyński J.W., Ehrich D., Gerdes T., Alvarez N. (2009) Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics* 10: 33. <https://doi.org/10.1186/1471-2105-10-33>
- Baumel A., Auda P., Torre F., Médail F. (2009) Morphological polymorphism and rDNA internal transcribed spacer (ITS) sequence variation in *Armeria* (Plumbaginaceae) from south-eastern France. *Botanical Journal of the Linnean Society* 159(2): 255–267. <https://doi.org/10.1111/j.1095-8339.2008.00925.x>
- Baker H.G. (1966) The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. *Evolution* 20(3): 349–368. <https://doi.org/10.1111/j.1558-5646.1966.tb03371.x>
- Crandall K.A., Bininda-Emonds O.R., Mace G.M., Wayne R.K. (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution* 15(7): 290–295. [https://doi.org/10.1016/S0169-5347\(00\)01876-0](https://doi.org/10.1016/S0169-5347(00)01876-0)
- Daïnou K., Mahy G., Duminił J., Dick C.W., Doucet J.-L., Donk-pégan A.S.L., Pluijgers M., Sinsin B., Lejeune P., Hardy O.J. (2014) Speciation slowing down in widespread and long-living tree taxa: insights from the tropical timber tree genus *Milicia* (Moraceae). *Heredity* 113: 74–85. <https://doi.org/10.1038/hdy.2014.5>
- Despres L., Lorient S., Gaudeul M. (2002) Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 11(11): 2337–2347. <https://doi.org/10.1046/j.1365-294X.2002.01618.x>
- De Riek J., De Cock K., Smulders M.J.M., Nybom H. (2013) AFLP-based population structure analysis as a means to validate the complex taxonomy of dogroses (*Rosa* section *Cinaneae*). *Molecular Phylogenetics and Evolution* 67(3): 547–559. <https://doi.org/10.1016/j.ympev.2013.02.024>
- Donadille P. (1969) Contribution à l'étude du genre *Armeria* Willd. (Plumbaginaceae). III. Clé générale des taxons français. *Bulletin de la Société botanique de France* 116(9): 511–521. <https://doi.org/10.1080/00378941.1969.10838693>
- Dray S., Dufour A.B. (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22(4): 1–20. <https://doi.org/10.18637/jss.v022.i04>
- Duminił J., Di Michele M. (2009) Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143(3): 528–542. <https://doi.org/10.1080/11263500902722964>
- Earl D.A., vonHoldt B.M. (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Fernández-Mazuecos M., Jiménez-Mejías P., Rotllan-Puig X., Vargas P. (2014) Narrow endemics to Mediterranean islands: moderate genetic diversity but narrow climatic niche of the ancient, critically endangered *Naufraga* (Apiaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 16(4): 190–202. <https://doi.org/10.1016/j.ppees.2014.05.003>
- Frankham R., Ballou J.D., Dudash M.R., Eldridge M.D., Fenster C.B., Lacy R.C., Mendelson J.R., Porton I.J., Ralls K., Ryder O.A. (2012) Implications of different species concepts for conserving biodiversity. *Biological Conservation* 153: 25–31. <https://doi.org/10.1016/j.biocon.2012.04.034>
- Frankham R., Bradshaw C.J.A., Brook B.W. (2014) Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* 170: 56–63. <https://doi.org/10.1016/j.biocon.2013.12.036>
- Frankham R. (2015) Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology* 24(11): 2610–2618. <https://doi.org/10.1111/mec.13139>
- Fuertes Aguilar J., Rossello J.A., Nieto Feliner G. (1999) Molecular evidence for the compilospecies model of reticulate evolution in *Armeria* (Plumbaginaceae). *Systematic Biology* 48(4): 735–754. <https://doi.org/10.1080/106351599259997>
- García-Camacho R., Escudero A. (2009) Reproduction of an early-flowering Mediterranean mountain narrow endemic (*Armeria caespitosa*) in a contracting mountain island. *Plant Biology* 11(4): 515–524. <https://doi.org/10.1111/j.1438-8677.2008.00151.x>
- García-Fernández A., Iriondo J.M., Escudero A., Aguilar J.F., Nieto Feliner G. (2013) Genetic patterns of habitat fragmentation and past climate-change effects in the Mediterranean high-mountain plant *Armeria caespitosa* (Plumbaginaceae). *American Journal of Botany* 100(8): 1641–1650. <https://doi.org/10.3732/ajb.1200653>
- Gutiérrez-Larruscain D., Andrés-Sánchez S., Rico E., Martínez-Ortega M.M. (2019) Advances in the systematics and evolution of western Mediterranean representative species of the *Pentanema conyzae* clade through genetic fingerprinting. *Journal of Systematics and Evolution* 57(1): 42–54. <https://doi.org/10.1111/jse.12431>
- Hamilton J.A., Miller J.M. (2016) Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology* 30(1): 33–41. <https://doi.org/10.1111/cobi.12574>
- Hardion L., Verlaque R., Baumel A., Juin M., Vila B. (2012) Revised systematics of Mediterranean *Arundo* (Poaceae) based on AFLP fingerprints and morphology. *Taxon* 61(6): 1217–1226. <https://doi.org/10.1002/tax.616004>
- Janes J.K., Miller J.M., Dupuis J.R., Malenfant R.M., Gorrell J.C., Cullingham C.I., Andrew R.L. (2017) The K = 2 conundrum. *Molecular Ecology* 26(14): 3594–3602. <https://doi.org/10.1111/mec.14187>
- Jombart T., Devillard S., Balloux F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11: 94. <https://doi.org/10.1186/1471-2156-11-94>
- Jiménez-Mejías P., Fernández-Mazuecos M., Amat M.E., Vargas P. (2015) Narrow endemics in European mountains: high genetic diversity within the monospecific genus *Pseudomisopates* (Plantaginaceae) despite isolation since the late Pleistocene. *Journal of Biogeography* 42(8): 1455–1468. <https://doi.org/10.1111/jbi.12507>

- Kamvar Z.N., Tabima J.F., Grünwald N.J. (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2: e281. <https://doi.org/10.7717/peerj.281>
- Kopelman N.M., Mayzel J., Jakobsson M., Rosenberg N.A., Mayrose I. (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15: 1179–1191. <https://doi.org/10.1111/1755-0998.12387>
- Levin D.A. (2019) Intraspecific lineages as focal points in the extinction and persistence of species. *Plant Systematics and Evolution* 305: 719–726. <https://doi.org/10.1007/s00606-019-01616-z>
- Médail F., Baumel A. (2018) Using phylogeography to define conservation priorities: The case of narrow endemic plants in the Mediterranean Basin hotspot. *Biological Conservation* 224: 258–266. <https://doi.org/10.1016/j.biocon.2018.05.028>
- Medrano M., López-Perea E., Herrera C.M. (2014) Population genetics methods applied to a species delimitation problem: Endemic trumpet daffodils (*Narcissus* section *Pseudonarcissi*) from the Southern Iberian Peninsula. *International Journal of Plant Sciences* 175(5): 501–517. <https://doi.org/10.1086/675977>
- Moritz C. (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* 51(2): 238–254. <https://doi.org/10.1080/10635150252899752>
- Murtagh F., Legendre P. (2014) Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? *Journal of Classification* 31: 274–295. <https://doi.org/10.1007/s00357-014-9161-z>
- Nieto Feliner G. (1990) *Armeria*. In: Castroviejo S., Lainz M., Lopez G., Montserrat P., Muñoz G.F., Paiva J., Villar L. (eds) *Flora Iberica* vol 2: 642–721. Madrid, Consejo Superior de Investigaciones Científicas.
- Nieto Feliner G., Rosato M., Alegre G., San Segundo P., Rosselló J.A., Garnatje T. Garcia S. (2019) Dissimilar molecular and morphological patterns in an introgressed peripheral population of a sand dune species (*Armeria pungens*, Plumbaginaceae). *Plant Biology* 21(6): 1072–1082. <https://doi.org/10.1111/plb.13035>
- Peakall R., Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537e2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Piñeiro R., Widmer A., Aguilar J.F., Nieto Feliner G. (2011) Introgression in peripheral populations and colonization shape the genetic structure of the coastal shrub *Armeria pungens*. *Heredity* 106: 228–240. <https://doi.org/10.1038/hdy.2010.46>
- Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- QGIS Development Team (2019) QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at <https://qgis.org> [accessed 28 Apr. 2020].
- Ralls K., Ballou J.D., Dudash M.R., Eldridge M.D., Fenster C.B., Lacy, R.C., Sunnucks P., Frankham R. (2018) Call for a paradigm shift in the genetic management of fragmented populations. *Conservation Letters* 11(2): e12412. <https://doi.org/10.1111/conl.12412>
- Ryder O.A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology & Evolution* 1(1): 9–10. [https://doi.org/10.1016/0169-5347\(86\)90059-5](https://doi.org/10.1016/0169-5347(86)90059-5)
- Tauleigne-Gomes C., Lefèbvre C. (2005) Natural hybridisation between two coastal endemic species of *Armeria* (Plumbaginaceae) from Portugal. 1. Populational in situ investigations. *Plant Systematics and Evolution* 250: 215–230. <https://doi.org/10.1007/s00606-004-0247-7>
- Tison J.M., Jauzein P., Michaud H. (2014) Flore de la France méditerranéenne continentale. Turriers, Naturalia publications.
- Van Rossum F., Michez D., Van der Beeten I., Van de Vyver A., Robb L., Raspé O. (2017) Preserving the only endemic vascular plant taxon in Belgium in a highly anthropogenic landscape. *Plant Ecology and Evolution* 150(1): 4–12. <https://doi.org/10.5091/plecevo.2017.1238>
- Vekemans X., Lefèbvre C. (1997) On the evolution of heavy-metal tolerant populations in *Armeria maritima*: evidence from allozyme variation and reproductive barriers. *Journal of Evolutionary Biology* 10(2): 175–191. <https://doi.org/10.1046/j.1420-9101.1997.10020175.x>
- Vos P., Hogers R., Bleeker M., Reijans M., Lee T.V.D., Hornes M., Friters A., Paleman J., Kuiper M., Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23(21): 4407–4414. <https://doi.org/10.1093/nar/23.21.4407>
- Wickham H. (2016) ggplot2: Elegant Graphics for Data Analysis. New York, Springer-Verlag. <https://doi.org/10.1007/978-3-319-24277-4>

Communicating Editor: Katharina Budde.

Submission date: 25 Nov. 2019

Acceptance date: 28 Apr. 2020

Publication date: 8 Jul. 2020