



HAL
open science

Morphological polymorphism and rDNA internal transcribed spacer (ITS) sequence variation in *Armeria* (Plumbaginaceae) from south-eastern France

Alex Baumel, P. Auda, F. Torre, F. Médail

► **To cite this version:**

Alex Baumel, P. Auda, F. Torre, F. Médail. Morphological polymorphism and rDNA internal transcribed spacer (ITS) sequence variation in *Armeria* (Plumbaginaceae) from south-eastern France. *Botanical Journal of the Linnean Society*, 2009, 159 (2), pp.255-267. 10.1111/j.1095-8339.2008.00925.x. hal-01451813

HAL Id: hal-01451813

<https://hal.science/hal-01451813>

Submitted on 25 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Morphological polymorphism and rDNA internal transcribed spacer (ITS) sequence variation in *Armeria* (Plumbaginaceae) from south-eastern France

ALEX BAUMEL^{1*}, PASCAL AUDA^{1,2}, FRANCK TORRE¹ and FREDERIC MEDAIL¹

¹Université Paul Cézanne Aix-Marseille III, Institut Méditerranéen d'Ecologie et de Paléoécologie (IMEP, UMR-CNRS 6116), Europole méditerranéenne de l'Arbois, BP 80, 13545 Aix-en-Provence cedex 04, France

²ECO-MED, Tour Méditerranée 13e étage, 65 avenue Jules Cantini, 13298 Marseille cedex 20, France

The *Armeria* populations (Plumbaginaceae) scattered along the south-eastern French coastline are small and often endangered by habitat destruction. Because their taxonomic status remains unclear, we conducted a molecular and phenetic analysis to test for the existence of different taxa and to set conservation priorities. Multivariate analysis of morphometric data demonstrated that the majority of coastal populations are similar to inland populations of *Armeria arenaria* subsp. *bupleuroides*. However, discriminant analysis isolated two distinct populations. One of these has a morphology intermediate between the narrow endemic *Armeria belgenciensis* and *A. arenaria* subsp. *bupleuroides*. However, rDNA internal transcribed spacer (ITS) sequences display no fixed variation, even between highly remote populations. We discuss these results in the light of recurrent hybridization in *Armeria*, and propose the description of two new subspecies of *Armeria arenaria* (**subsp. *pradetensis*** and **subsp. *peirescii***) located in the Var department.

ADDITIONAL KEYWORDS: discriminant analysis – evolutionary conservation – Mediterranean Basin – morphometry – Provence – reticulation.

INTRODUCTION

Hybridization is an important process in plant evolution (Stebbins, 1967; Grant, 1971; Arnold, 1997) and represents a source of genetic variation and speciation as it predisposes colonizing populations to rapid adaptive radiation (Seehausen, 2004). However, hybridization is not randomly distributed among families and genera, but associated with certain characters, such as outcrossing, high phenotypic plasticity or clonality (Ellstrand, Whitkus & Rieseberg, 1996). At low systematic levels, crossing between lineages interrupts divergence and produces a reticulate pattern of evolution (e.g. Fuertes Aguilar, Rosselló &

Nieto Feliner, 1999b). Reticulate evolution promotes gene flow and the exchange of exclusive characters. The resulting decrease in taxonomic resolution for morphological or molecular characters can lead to poor discrimination and identification of species. In this context, insufficient sampling of the polymorphism resulting from such gene flow can lead to the assignment of diverse taxonomic ranks (species, subspecies or varieties) to individual variants. Several methods exist for the empirical delimitation of species (Sites & Marshall, 2003, 2004), and these frequently require the combined use of morphological characters and DNA markers.

The genus *Armeria* L. (Plumbaginaceae) is a model issue of the delimitation of species boundaries. Polymorphism in *Armeria* is high and complicated by

*Corresponding author. E-mail: alex.baumel@univ-cezanne.fr

frequent hybridization and introgression, without a resulting loss of species diversity (Fuertes Aguilar, Rosselló & Nieto Feliner, 1999a). *Armeria* currently includes c. 120 small perennial herbaceous species, 92 of which are located in the Mediterranean Basin (Greuter, Burdet & Long, 1989). This diversity is concentrated in the Iberian Peninsula, where seven (Bernis, 1954, 1955, 1957) or 54 (Nieto Feliner, 1990) species have been described, depending on the species concept used.

Within Plumbaginaceae, the genus *Armeria* is characterized by a rosette vegetative system, capitulate inflorescences and a dimorphic reproductive system (Lledó *et al.*, 1998). Polyploidy is rare in *Armeria*, and almost all chromosome counts in the genus are diploid with $2n = 18$ (Donadille, 1969; Moore, 1982). Most populations are composed of obligate outcrossers as a result of a self-incompatibility system (Baker, 1966; Vekemans *et al.*, 1990), with weak internal reproductive barriers (Nieto Feliner, Gutiérrez Larena & Aguilar, 2004). Crossing experiments (Baker, 1966; Nieto Feliner, 1997) and the structure of rDNA internal transcribed spacer (ITS) sequence variation have demonstrated that extensive introgression has occurred at both coarse (Fuertes Aguilar & Nieto Feliner, 2003) and fine (Fuertes Aguilar *et al.*, 1999a, b; Nieto Feliner, Fuertes Aguilar & Rosselló, 2001a; Nieto Feliner *et al.*, 2004) geographical scales. Thus, the geographical structure of ITS variation is partly independent of and conflicts with the 'classical' taxonomic approach.

An estimated 21 *Armeria* taxa have been described for France, 16 of which are located in the Mediterranean region (Kerguélen, 1993; Médail, *ined.*). In this study, we present an analysis of morphological and rDNA ITS sequence variation focused on taxa belonging to the *A. arenaria* (Pers.) Schult. group from Provence (south-eastern France), and especially populations situated along the coastline of the Var department. In the molecular phylogenetic analyses of Fuertes Aguilar & Nieto Feliner (2003), *A. arenaria* is one of the seven widely distributed species in the genus which share intraspecific morphological variability and between-clade branching patterns in the rDNA ITS phylogenetic trees. However, the sampling of *A. arenaria* in south-east France was limited to a single population in the Bouches-du-Rhône department, and the major part of the region of Provence was not considered.

We initiated this study, in part, because of the inadequately described systematic status of several restricted populations, despite the previous morphological investigations of Donadille (1969). Several of these populations are extremely small, isolated and threatened by human activities, and, beyond systematics, our research aims to set conservation units as

recommended by recent work concerning Evolutionary Significant Units (for a review, see Fraser & Bernatchez, 2001).

MATERIAL AND METHODS

MATERIAL

Most of the populations from south-east France considered here are included in the *A. arenaria* aggregate (= *A. plantaginea* Willd.), which encompasses several subspecies: (1) subsp. *arenaria*, located throughout the species range; (2) subsp. *bupleuroides* (Godron & Gren.) Greuter & Burdet, relatively common on dolomitic and siliceous rocky grasslands in Provence, the southern Maritime Alps and the Drôme department (Albert & Jahandiez, 1908; Molinier, 1980; Charpin & Salanon, 1985; Garraud, 2003); and (3) subsp. *praecox* (Jordan) Kerguélen, mainly located in xeric alpine valleys of the southern Alps (Chas, 1994), but also along the coastline of the Maures and Esterel mountain chains in Provence (Molinier, 1954). Another morphologically distinct taxon, *Armeria belgenciensis* Donadille ex Kerguélen, has been described as a narrow endemic taxon restricted to dolomitic soils near Solliès-Toucas (Var) (Donadille, 1969, 1995). *Armeria belgenciensis* is characterized by thin, linear (filiform) leaves and a flowering season from August to September. The taxa of the *A. arenaria* group differ from *A. belgenciensis* principally by their lanceolate leaves and spring flowering. However, 100 m away from the *A. belgenciensis* population, at a mountain pass called 'Baisse du Pas Etroit', there is a spring flowering population with sublinear leaves, designated as *A. arenaria* subsp. *bupleuroides*. The systematic status of populations on the Var coastline, where our sampling was concentrated, is no less uncertain. These consist of: (1) a copper mine waste population situated on the Cap Garonne (Le Pradet, western Var, near Toulon), which has been treated as *A. bupleuroides* (Albert & Jahandiez, 1908); and (2) populations situated along the coastline of the Maures and Esterel mountain chains (eastern Var), which have been treated as *A. bupleuroides* Gren. & Godr. (Albert & Jahandiez, 1908; Camus & Camus, 1912), *A. praecox* Jord. (Molinier, 1954; Géhu, Biondi & Bournique, 1992) or *A. arenaria* subsp. *arenaria* (Gandioli & Salanon, 2000).

In order to resolve these taxonomic discrepancies, we used rDNA ITS sequences and multivariate analysis of morphometric data to assess population differentiation according to geographical origin. The collection localities spanned the range of *Armeria* in Var, where in-depth studies of nine populations were performed. These populations were also compared with specimens from five populations in the depart-

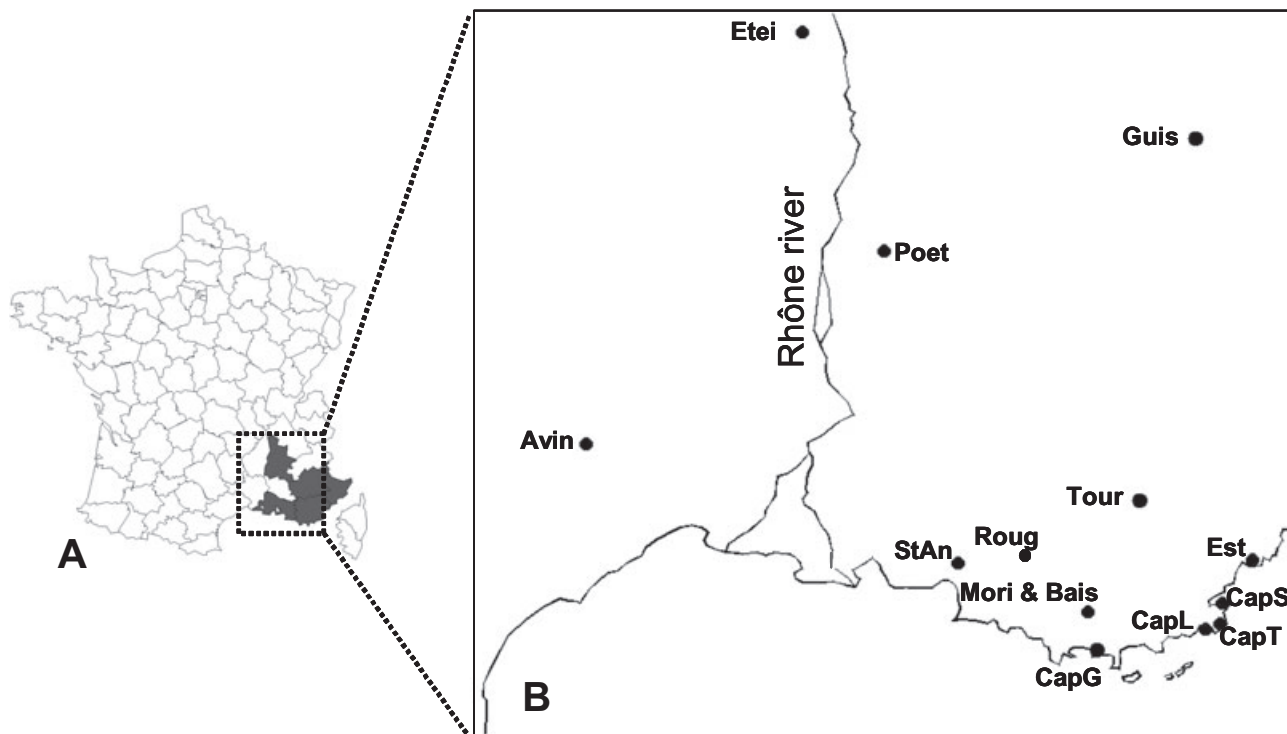


Figure 1. Geographical location of the study. A, Departments in which *Armeria arenaria* subsp. *bupleuroides* occurs in France are shaded in grey; the box corresponds to the area of study. B, Sampling sites for the study. See Table 1 for abbreviations.

ments of Ardèche, Bouches-du-Rhône, Drôme, Hautes-Alpes and Hérault (Fig. 1). The geography and taxonomic treatment of these 14 populations are indicated in Table 1. Almost all *Armeria* populations of the Var coastline have been inspected and sampled. The northern localities of the Var were not exhaustively surveyed, but their taxonomic status has been less debated by botanists. Extensive sampling of *A. arenaria* will be necessary to delimit the distribution of subspp. *arenaria*, *bupleuroides* and *praecox*, which are used here only as standards for comparison.

Ten herbarium specimens were collected in the field for each population. For each one, one capitulum was conserved in 70% alcohol in order to measure the reproductive characters on fresh material. Most of the collected samples were used for morphological measurements and one sample per population was used for DNA extraction and ITS sequencing. Samples from the Morières-la-Tourne, Cap Dramont, Avinière and Eteize populations were used only for molecular analysis because herbarium specimens were too few to allow a robust morphometric analysis. Populations of less than six specimens were excluded, and the final analysis matrix consisted of 106 individuals for morphometric analysis and 12 for rDNA ITS sequencing. Holotypes for new taxa are conserved at the Muséum d'Histoire Naturelle d'Aix-en-Provence

(AIX), and other herbaria vouchers are conserved at the Université Paul Cézanne, Mediterranean Institute of Ecology and Palaeoecology (IMEP, Aix-en-Provence, F. Médail herbarium).

MORPHOMETRY

Vegetative characters were measured on herbarium specimens after at least one week of drying. Vegetative material of *Armeria* is naturally dry and does not change after a few days in a herbarium press. The 21 characters included in the analysis were derived from the measurements of an initial set of 34 selected characters according to previous systematic studies (Bernis, 1954; Donadille, 1969; Nieto Feliner, Fuertes Aguilar & Rosselló *et al.*, 2001b). By selecting only variable and clearly measurable characters, 18 quantitative and three qualitative characters were retained (Table 2). When possible, three replicates of quantitative measures were taken per specimen and averaged for use in statistical analyses.

DATA ANALYSIS

Multivariate methods were performed according to the principles of phenetic analysis describing morphometric variation. In the first method, principal

component analysis on a correlation matrix was conducted to detect specimen groups on the basis of the 21 morphological characters. To investigate the discriminant power of *a priori* grouping treatments, discriminant analysis was conducted using the ADE4 package of R statistical software (Thioulouse *et al.*, 1996; R Development Core Team, 2006). The objective of discriminant analysis is to find a linear

combination of variables (the discriminant function) that best discriminates among *a priori* defined groups. Two classification factors were tested by discriminant analysis: ‘population’ and ‘taxonomy’. The factor ‘population’ represents the geographical location of the population corresponding to each specimen. The factor ‘taxonomy’ questions the hypothetical existence of well-differentiated morpho-

Table 1. Sampling description

Site	Location and main physiographical characteristics	Taxa studied and correspondence with former taxonomic treatments	Sampling for morphometry, GenBank accession
Cap Lardier	La Croix-Valmer, coast of the Maures massif, Var (83); alt. 10 m; gneiss	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (sub <i>A. praecox</i> : Molinier, 1954)	10 (CapL) EF370060
Cap Taillat	Ramatuelle, coast of the Maures massif, Var (83); alt. 15 m; phyllades	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (sub <i>A. bupleuroides</i> : Camus & Camus, 1912)	10 (CapT) EF370059
Cap de St-Tropez	St-Tropez, coast of the Maures massif, Var (83); alt. 10 m; phyllades	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (sub <i>A. bupleuroides</i> : Albert & Jahandiez, 1908; Camus & Camus, 1912; <i>A. praecox</i> : Molinier, 1954)	10 (CapS)
Cap Dramont	St-Raphaël, coast of the Estérel massif, Var (83); alt. 10 m	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (sub <i>A. arenaria</i> subsp. <i>praecox</i> : Milano, 1960; <i>A. arenaria</i> subsp. <i>arenaria</i> : Gandioli & Salanon, 2000)	(Est) EF370062
St Pierre-de-Tourtour	Tourtour, Var (83); alt. 680 m	<i>A. arenaria</i> subsp. <i>bupleuroides</i>	6 (Tour) EF370067
Rougiers	Rougiers, massif de la Ste-Baume, Var (83); alt. 450 m	<i>A. arenaria</i> subsp. <i>bupleuroides</i>	(Roug) EF370058
Col Ste-Anne	Mimet, massif de l'Etoile, Bouches-du-Rhône (13); alt. 580 m; dolomitic rocks	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (Molinier, 1980)	10 (StAn) EF370061
Montagne du Poët	Poët-Laval, Drôme (26); alt. 600 m, dolomitic rock	<i>A. arenaria</i> subsp. <i>bupleuroides</i> (Garraud, 2003)	10 (Poet)
Cap Garonne	Le Pradet, Colle Noire, Var (83); alt. 90 m; grès-quartzites	<i>A. arenaria</i> subsp. <i>pradetensis</i> (sub <i>A. bupleuroides</i> : Albert & Jahandiez, 1908)	20 (CapG, CapG2) EF370063
Baisse du Pas Etroit	Belgentier, massif de Morières, Var (83); alt. 360 m; dolomitic rocks	<i>A. arenaria</i> subsp. <i>peirescii</i> (sub <i>A. bupleuroides</i> : Albert & Jahandiez, 1908)	10 (Bais) EF370057
Vallée de la Guisane	Serre-Chevalier, Hautes-Alpes (05); alt. 1600 m	<i>A. arenaria</i> subsp. <i>praecox</i> (Chas, 1994)	20 (Guis) EF370064
Avinière	Vallée de la Vis, Hérault (34), alt. 200 m	<i>A. arenaria</i> subsp. <i>arenaria</i>	(Avin) EF370065
Eteize	Suc de Clava, Ardèche (07), alt. 600 m	<i>A. arenaria</i> subsp. <i>arenaria</i>	(Etei) EF370066
Morières la Tourne	Solliès-Toucas, massif de Morières, Var (83); alt. 410 m; dolomitic rocks	<i>A. belgenciensis</i> (Donadille, 1969) (sub <i>A. filicaulis</i> : Albert & Jahandiez, 1908)	(Mori) EF370056

Table 2. Morphological characters

Character	Description
Leaf length (Lof)	Continuous from 26 to 176 (mm)
Leaf width (Laf)	Broadest width of the leaf, continuous from 0.4 to 14.5 (mm)
Leaf shape (FormF)	Four states: 0, linear; 0.33, sublinear; 0.66, sublanceolate; 1, lanceolate
Number of leaf veins (Ner)	Absolute values from 1 to 3
Scape length (Losc)	Continuous from 118 to 472 (mm)
Sheath length (Log)	Continuous from 15 to 54 (mm)
Scape diameter (Dsc)	Measured at the base, continuous from 0.486 to 2.318 (mm)
Outer involucre bract length (Lobre)	Continuous from 2.4 to 11.4 (mm)
Median involucre bract length (Lobrm)	Continuous from 2.7 to 9.5 (mm)
Inner involucre bract length (Lobri)	Continuous from 4.1 to 7.5 (mm)
Number of outer involucre bracts (Nbbre)	Absolute values from 1 to 6
Number of median involucre bracts (Nbbrm)	Absolute values from 1 to 9
Number of inner involucre bracts (Nbbri)	Absolute values from 2 to 8
Length of the calyx spur (LotC)	Continuous from 2.13 to 3.56 (mm)
Width of the calyx spur (LatC)	Continuous from 1.1 to 1.8 (mm)
Calyx length (LoC)	Continuous from 4 to 7 (mm)
Calyx hairiness (PubCa)	Three states: 0, diffuse; 0.5, intermediate between linear and diffuse; 1, linear
Flower bract length (LoPf)	Continuous from 3.9 to 7.4 (mm)
Flower bract width (LaPf)	Continuous from 2.8 to 6.9 (mm)
Number of flowers in spikelet (NbF)	Absolute values, ranging from 1 to 5
Flower colour (Coul)	Two states: 0, white; 1, pink

logical groups that could be allocated to four putative taxa: ‘bup’ (subsp. *bupleuroides*) for the populations of Saint-Anne, Poët Laval, Tourtour, Cap Taillat, Cap de Saint Tropez and Cap Lardier; ‘prae’ (subsp. *praecox*) for the Guisane population; ‘prad’ for the two populations of Cap Garonne (Le Pradet); and ‘peir’ for the population of ‘Baisse du Pas Etroit’ near Belgentier. To perform discriminant analysis, we selected a subset of 14 quantitative characters that were normally distributed (Lof, Laf, Losc, Log, Dsc, Lobre, Nbbre, Nbbrm, LotC, LatC, LoC, LoPf, LaPf, NbF: see Table 2 for details). As for principal component analysis, all variables were standardized before discriminant analysis.

Both principal component analysis and discriminant analysis results were presented as scatter plots using the function ‘s.class’ of the ADE4 package, with ovals drawn around at least 67% of the points belonging to the same class. Each point is connected to its class baricentre (in multidimensional space) by a line. Principal component analysis and discriminant analysis were performed using the ADE4 package of R statistical software. In order to check for cohesiveness of the four putative taxa, according to the taxonomy factor, a Kruskal–Wallis rank sum test of the most discriminant variables in discriminant analysis was performed using R software, considering each taxon versus the rest.

DNA ISOLATION, POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SEQUENCING OF rDNA ITS

Total DNA was isolated from leaves stored in silica gel following a slightly modified 2 × CTAB method (Doyle & Doyle, 1987). For all sequences, each amplification reaction (100 mL final volume) contained 50 ng of template DNA, 1 × incubation buffer (Q-biogen), 7.5 mM MgCl₂, 10 mM of each primer and 5 units of Taq polymerase (Q-biogen). Amplification of the ITS region was performed using primers ITS-4 and ITS-7, as described by Fuertes Aguilar *et al.* (1999a). The PCR cycling regime involved 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 48 °C and 2 min extension at 72 °C. PCR products were checked on 1.5% agarose gels before sequencing. As band heterogeneity was never observed on agarose, PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and shipped for direct sequencing (MWG Biotech Company). All fragments were sequenced with ITS-4 and ITS-7 in order to check the quality of the sequences by alignment of the two strands. Sequences were manually aligned with MEGA 3.1 software (Kumar, Tamura & Nei, 2004). The method of Fuertes Aguilar & Nieto Feliner (2003) was used to search for polymorphic sites in the electropherograms which could display an

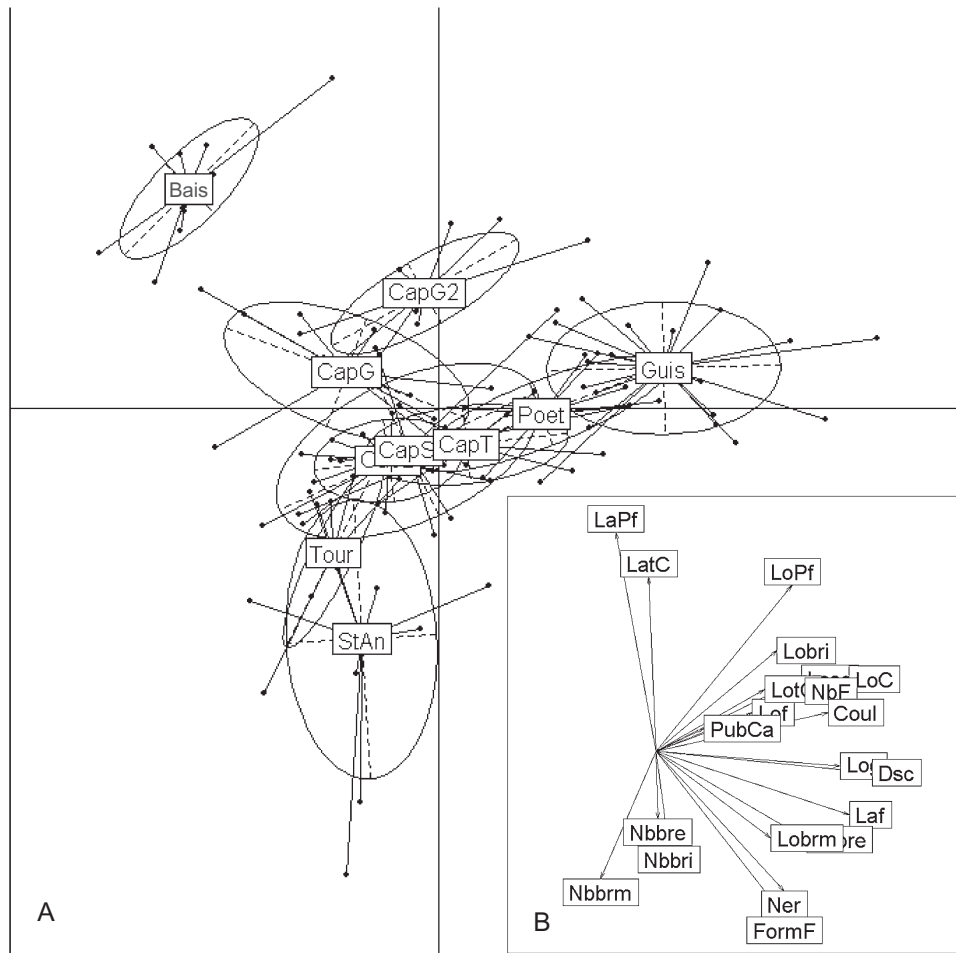


Figure 2. Principal component 1 versus 2, explaining 26% and 17% of the variance, respectively. A, Ovals are labelled according to the population origin of the specimens and encompass at least 67% of the samples. B, Correlations and variable weights.

additive pattern with respect to *Armeria* ITS diversity found in GenBank. Additive polymorphic sites (APSs) were determined by two conditions: (1) if a polymorphic site has two peaks at one nucleotide position, the weakest signal must represent at least 25% of the strongest signal; and (2) two nucleotides involved in a polymorphic site must be found separately in the data set or in previous records in GenBank. APSs can be the mark of past reticulation events.

RESULTS

MORPHOLOGICAL ANALYSIS

The main results of the principal component analysis based on 18 quantitative and three qualitative characters are presented in Figure 2. The first three components explain 26, 17 and 10% of the variance, respectively. A scatter diagram of the first vs. second

components is shown in Figure 2; the other contributions decrease slowly and are not considered here. Principal component 1 (PC1) is strongly positively correlated with scape diameter (Dsc, $r^2 = 71.5\%$), leaf width (Laf, $r^2 = 57.1\%$), calyx length (LoC, $r^2 = 57\%$) and flower colour (Coul, $r^2 = 45\%$). Other correlations between variables and PC1 are under 40%. Principal component 2 (PC2) is positively correlated with the width and length of the flower bracts (LaPf, $r^2 = 73.1\%$; LoPf, $r^2 = 42\%$; respectively) and the calyx spur width (LatC, $r^2 = 46.6\%$), and negatively correlated with the leaf shape (FormF, $r^2 = -42\%$). All other variables are less than 40% correlated with PC2. Only one character, calyx hairiness (PubCa), had a relatively high correlation with PC3 ($r^2 = 49.5\%$). Plants with high values on PC1 possess wide leaves, pink flowers, a wide scape diameter and a long calyx (Fig. 2). Plants with high values on PC2 have linear or sublinear leaves, wide and long flower bracts, and

Table 3. Mean and standard deviation for the most discriminant morphological metric characters (see Table 2 and section on ‘Data analysis’ for abbreviations)

	Lof	Laf	Losc	Dsc	Lobre	LaPf	LoC	LotC
prae	108.5 ± 27.5ns	7.5 ± 2.6***	430.8 ± 82.2***	1.76 ± 0.24***	6.94 ± 1.69ns	5 ± 0.4ns	6.3 ± 0.4***	2.74 ± 0.17***
bup	82.7 ± 33.5***	5.1 ± 1.7**	241.9 ± 76.8***	1.19 ± 0.24ns	6.92 ± 1.75*	4.5 ± 0.6***	5.4 ± 0.6ns	2.75 ± 0.29ns
prad	125.6 ± 28.6***	2.4 ± 0.8***	335.8 ± 84.6*	1.03 ± 0.24**	6.11 ± 1.27ns	5.6 ± 0.7***	5.4 ± 0.5ns	2.9 ± 0.35*
peir	83.6 ± 19ns	1.2 ± 0.4***	311.3 ± 102.3ns	0.86 ± 0.1***	3.48 ± 0.51***	6.2 ± 0.3***	4.9 ± 0.2**	2.48 ± 0.14***

Results of the Kruskal–Wallis rank test are indicated as follows: * $P < 5\%$; ** $P < 1\%$; *** $P < 0.1\%$; ns, $P > 5\%$. All measurements in millimetres.

wide calyx spurs. The third component is, to a large extent, determined by one character, calyx hairiness, which is diffuse in the Cap Garonne populations (CapG), and linearized to diffuse in the other populations. All populations overlap, except for the ‘Baisse du Pas Etroit’ (Bais) population.

Discriminant analysis was based on 14 quantitative characters that were visually checked for normality. Two grouping criteria were tested: population origin and taxonomic hypothesis.

Population origin as a grouping criterion

Discriminant components (DCs) 1, 2 and 3 explain 22, 20 and 16% of the variance in the matrix, respectively. Three characters have a high canonical weight on DC1: LoC (−0.46), LaPf (0.37) and LaF (0.30). DC2 is mainly explained by Losc (−0.56), LotC (0.45) and LoC (−0.44). A DC1 vs. DC2 scattergram (Fig. 3) indicates that discriminant analysis fails to distinguish populations, but rather organizes them along a morphological gradient, ranging from the Guisane population in the Hautes-Alpes (Guis) to the population of Baisse du Pas Etroit (Bais).

Taxonomic hypothesis as a grouping criterion

DCs 1, 2 and 3 explain 45, 38 and 17% of the variance, respectively. The highest variable weights on DC1 are those for LaPf (0.42), Dsc (−0.31) and Laf (−0.30). For DC2, Losc (−0.62), LoC (−0.43) and LotC (0.35) are the principal characters. The taxonomic criterion leads to a better discriminant function as shown by the DC1 vs. DC2 scattergram (Fig. 3). The groups ‘prae’ and ‘bup’ are well individualized, the groups ‘prad’ and ‘peir’ are partly overlapping, and ‘prae’ and ‘bup’ or ‘prad’ and ‘bup’ are close and overlapping for some individuals. Thus, as for the population criterion, no group is completely discrete in the multivariate analysis. However, the Kruskal–Wallis rank test gives evidence for cohesiveness of the four taxa when the most discriminant variables are considered alone (Table 3).

ITS DIVERSITY

Sequencing of the rDNA ITS genes was conducted on one individual from 12 populations: Sainte-Anne

(StAn), Rougiers (Roug), Guisane valley (Guis), Estérel (Est), Cap Lardier (CapL), Cap Garonne (CapG), Morières (*A. belgenciensis*), Baisse du Pas Etroit (Bais), Eteire (Etei), Avinière (Avin), Cap Taillat (CapT), and Tourtour (Tour). The complete sequences containing the ITS1, 5.8S gene and ITS2 are 620 bp long. Fixed nucleotide or indel variations are absent. Therefore, the 12 samples analysed share the same ITS sequence. Nevertheless, following the method of Fuertes Aguilar & Nieto Feliner (2003), we found five APSs in all but two sequences (from Avinière and col Ste-Anne); these APSs are summarized and compared with a set of GenBank sequences representative of *Armeria* ITS diversity (Table 4).

DISCUSSION

In order to delimit morphological groups among *Armeria* populations in Var, we developed a phenetic analysis and rDNA ITS sequencing. As morphological data are mostly continuous and highly variable, we did not apply species delimitation methods, such as population aggregation analysis (Davis & Nixon, 1992; Sites & Marshall, 2003). We preferred to use non-specialized methods more suitable for different kinds and quality of data.

Principal component analysis results reveal a pattern of differentiation between populations, but, in parallel, the variation remains high within populations and overlaps between them, forming a continuous morphological gradient. As an illustration, the *A. arenaria* subsp. *praecox* population (Guis), sampled near Briançon (Hautes-Alpes), characterized by large and lanceolate leaves, a long scape, a long calyx and pink flowers, encompasses some individuals that are morphologically close to some plants from the Var coastline (Fig. 2). This similar leaf morphology could explain why the coastal and halophilous populations of the Maures and Esterel massifs have often been considered as subsp. *praecox* (Molinier, 1954; Géhu *et al.*, 1992). Nevertheless, other characters distinguish these alpine and maritime populations, which are also well dif-

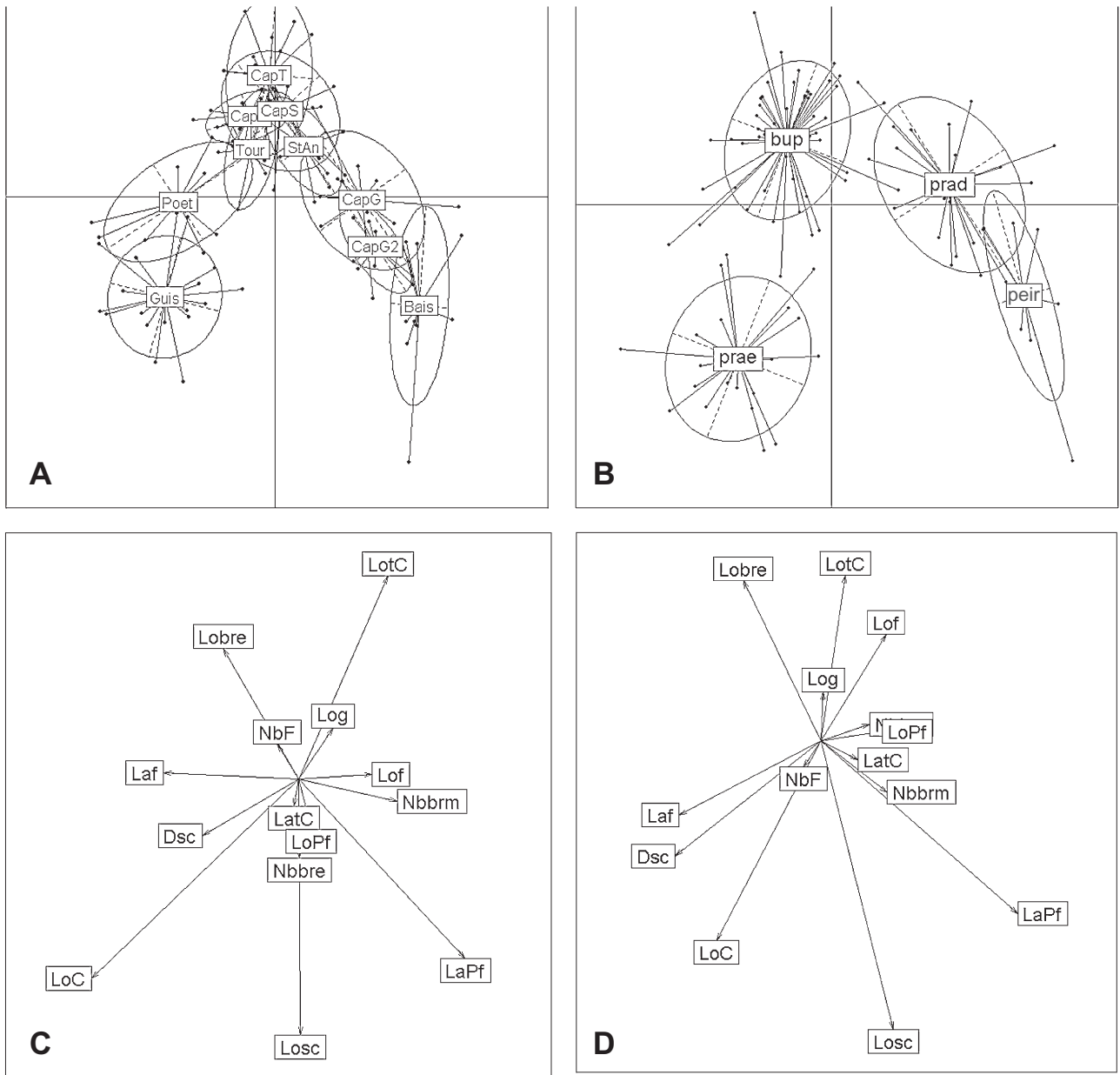


Figure 3. Discriminant analysis results. Scatter plots of canonical discriminant function 1 versus canonical discriminant function 2. Ovals are labelled according to: A, population origin; B, taxonomic hypothesis. C, D, Correlations and variable weights for the population and taxonomic hypothesis criteria, respectively.

ferentiated from an ecological point of view. The maritime populations of *Armeria* in Var should thus be included in subsp. *bupleuroides* (see below), as proposed initially by local botanists (Albert & Jahandiez, 1908; Camus & Camus, 1912). These results show that the delimitation of groups of specimens in *Armeria* is not easy and must be based on statistical sampling.

Discriminant analysis was used to confirm the relevance of the *a priori* treatment of specimen groups. Discriminant analysis using the population criterion produces results similar to those of principal component analysis, and most populations in Var are weakly differentiated, except for the Bais population. Leaf (LaF) and scape (Dsc) narrowness and flower bract width (LaPf) are similar for the Bais and CapG

Table 4. Summary of additive polymorphic sites found in the internal transcribed spacer (ITS) region (see Table 1 for abbreviations), compared with a set of GenBank sequences representative of ITS diversity in the genus *Armeria*

		377	386	387	388	556
	This study					
CapL	EF370060	Y	Y	Y	Y	Y
CapT	EF370059	C	Y	Y	Y	Y
Est	EF370062	Y	Y	Y	Y	Y
Tour	EF370067	Y	Y	Y	Y	Y
Roug	EF370058	C	C	T	Y	Y
StAn	EF370061	C	C	T	T	T
CapG	EF370063	Y	Y	Y	Y	T
Bais	EF370057	C	C	T	Y	T
Guis	EF370064	C	Y	T	Y	Y
Avin	EF370065	C	C	T	T	T
Etei	EF370066	Y	Y	Y	Y	Y
Mori (<i>A. belgenciensis</i>)	EF370056	C	C	T	Y	T
	GENBANK					
<i>A. alliacea</i>	AJ225578	C	C	T	T	T
<i>A. beirana</i>	AY179762	C	C	T	T	C
<i>A. hirta</i>	AJ225568	C	C	T	T	C
<i>A. pungens</i>	AY179810	C	C	T	T	C
<i>A. arenaria confusa</i>	AY179757	C	C	C	C	C
<i>A. arenaria arenaria</i>	AY179756	C	C	T	T	T
<i>A. villosa bernisii</i>	AY444134	C	C	C	C	C
<i>A. filicaulis filicaulis</i>	AY179756	C	C	T	T	C
<i>A. leucocephala</i>	AY179788	C	C	C	C	C
<i>A. fontqueri</i>	AY179783	C	C	C	C	C
<i>A. trachyphylla</i>	AY179818	C	C	C	C	C
<i>A. maderensis</i>	AY179796	C	C	C	C	C
<i>A. alpina</i>	AJ225576	T	T	C	C	C
<i>A. maritima maritima</i>	AY179801	T	T	C	C	C
<i>A. nebrodensis</i>	AY179806	C	C	C	C	C

populations. The Bais population is situated less than 1 km away from the rare and highly vulnerable *A. belgenciensis*, which is characterized by thin, linear leaves and a late flowering season (end of summer), whereas all the other taxa analysed flower during the spring. We did not sample this endemic species for the morphological analysis because of its highly endangered status. Although the flowering times of *A. belgenciensis* and the Bais population do not overlap, the shared ITS sequence and their morphological similarities suggest the potential existence of gene flow between them in the past. To a lesser extent, the vegetative and floral similarities between Bais and CapG populations, as well as their geographical closeness (16 km between them), suggest that their distributions overlapped during the past, promoting gene flow.

Discriminant analysis using the taxonomic criterion groups populations from the Maures and Esterel coastline with inland populations. The latter form a group, called 'bup': 'bup' is characterized by lanceolate

leaves of intermediate width, long outer involucral bracts, long corolla tubes and thin flower bracts. This result confirms that the coastal *Armeria* spp. in Var fit the delimitation of subsp. *bupleuroides*. As expected, the population from the Guisane valley near Briançon is well differentiated and corresponds to *A. arenaria* subsp. *praecox* ('prae'). It has wide, lanceolate leaves, a long scape with the largest diameter and a long flower calyx. Finally, the *a priori* treatment did not clearly delimit the hypothetical taxa 'prad' and 'peir', as they both include individuals characterized by sublinear leaves. However, the diffuse hairiness of the calyx appeared to be specific to the 'prad' group. In spite of the important variation found within each taxon, the polymorphism appears to be rather well structured by different characters. The robustness of these characters should be tested in the future by common garden experiments, but we have confidence in our data, which are in accordance with similar results in *Armeria* (Lefebvre & Veke-mans. 1995; Nieto Feliner *et al.*. 2001b).

Despite the observation of phenotypic polymorphism, the 12 ITS sequences sampled across a wide range of plants did not display fixed variation. We propose two non-exclusive hypotheses to explain this absence of fixed variation at a coarse geographical scale. First, the range of *Armeria* in south-eastern France could be the result of a recent expansion of populations from the Iberian Peninsula, in parallel with rapid adaptive and morphological diversification, but, by contrast, a slow drift within neutral DNA sequences. To support this hypothesis, the phylogenetic tree based on ITS sequences of 71 *Armeria* taxa performed by Fuertes Aguilar & Nieto Feliner (2003) indicates that the *A. arenaria* group is included in the Western Mediterranean Clade, together with several Iberian *Armeria*. According to the second hypothesis, the lack of ITS variation could be a result of recent breeding events occurring between formerly differentiated genetic entities, followed by ribotype homogenization during concerted evolution, a not unusual process in the genus (Fuertes Aguilar *et al.*, 1999a). The second hypothesis supports the compilospecies concept formulated by Harlan & de Wet (1963), and adapted for *Armeria* by Fuertes Aguilar *et al.* (1999b) when they discovered the strong geographical structure of rDNA ITS sequence variation in southern Spain; here, different species from the same location have the same ribotype. Compilospecies are widely distributed, polyphyletic taxa which breed with well-adapted endemics (Fuertes Aguilar *et al.*, 1999b); they form a system in which free gene exchange is possible and, at the same time, confuses the phylogenetic signal of molecular markers. In the present study, we looked for APSs which could support the second hypothesis. We found that 10 of the 12 ITS sequences included five APSs. Two APSs (positions 377 and 386) support a reticulation event between the *A. maritima/alpina* and *A. arenaria* clades. Three samples (CapL, Est, Tour) from the coastline of Var have the Y state, pointing to a potential and ancient link between the alpine and Mediterranean populations. The three other APSs (387, 388 and 556) also indicate the possibility of between-clade gene flow, but are less informative because the two states (C and T) have a wider distribution in the genus; therefore, the APS 'Y' may indicate different reticulation events. Another pattern supporting the 'reticulation' hypothesis is that *A. belgenciensis* has the *A. arenaria* ribotype, whereas it is phonologically and morphologically the most differentiated taxon, with a different flowering time and filiform leaves. Moreover, the morphological intermediacy and APS of the Bais population support the possibility of former gene flow, as this population is located less than 1 km distant from *A. belgenciensis*.

In the light of the frequent hybridization in *Armeria*, Nieto Feliner (1987, 1990) proposed that,

instead of the biological species concept, a combination of morphological characters, geographical range and ecological preferences should be used as criteria to delimit species-level taxa. In fact, morphologically and ecologically differentiated groups exist in this genus, despite extensive reticulation (Fuertes Aguilar & Nieto Feliner, 2003). What seems important is that the delimitation of taxa is not arbitrary. Thus, the 'eco-morpho-geo' criterion offers the possibility to label well-separated and localized groups of populations for conservation purposes. This is in agreement with the concept of Evolutionary Significant Units, in which populations with distinct adaptations to different habitats take on importance from a conservation genetics perspective (Moritz, 1994; Crandall *et al.*, 2000).

In conclusion, we propose the following taxonomy for the *Armeria* populations living along or near the Var coastline. First, *A. belgenciensis* is undoubtedly well differentiated and must conserve its species rank. Only one population of less than 200 individuals exists according to a census performed in 2003 (A. Baumel, pers. observ.), and it is one of the most threatened plants in France (Donadille, 1995). In addition to its small population size, *A. belgenciensis* is also endangered by its proximity to a road; furthermore, a great deal of sheep grazing, with a large impact on the local vegetation, was observed in 2004, together with destruction of an important part of the population in 2005 as a result of road alteration work. At present, the future of this narrow endemic is seriously jeopardized, with the persistence of less than 50 individuals in 2005 (A. Aboucaya and A. Baumel, pers. observ.). Secondly, *Armeria* populations from the Maures and Esterel mountain chains should be included in *A. arenaria* subsp. *bupleuroides*, as they are similar to the inland populations sampled in Var, Bouches-du-Rhône and Drôme. The subspecies *bupleuroides* is therefore a widely distributed endemic taxon of south-eastern France, present from the coastline to the southern parts of the Alps, as populations from the northern part of Var also belong to this taxon (A. Baumel, unpubl. data). A more in-depth sampling should be performed in the Alpes-de-Haute-Provence and Hautes-Alpes departments to explore the relationship between subsp. *bupleuroides* and subsp. *praecox*, as we have sampled only one locality of the latter. Finally, considering that two populations of *Armeria* in the Var department need taxonomic recognition, we decided to apply the operational criterion developed by Nieto Feliner *et al.* (2001b). We propose the recognition of the uniqueness of the 'Bais' and 'Prad' populations, despite some similarities shared with both *A. arenaria* subsp. *bupleuroides* and *A. belgenciensis*. This could be another mark of reticulate evolution that should be

recognised by botanists, and we propose to describe them as two new subspecies.

DESCRIPTION OF TWO NEW SUBSPECIES OF *ARMERIA* FROM THE VAR DEPARTMENT

The Cap Garonne population, growing on copper mine wastes, should be distinguished as a different subspecies with respect to its sublanceolate leaves, unique calyx hairiness and particular ecology on a metalliferous (copper) substrate. We propose that it should be named as follows.

ARMERIA ARENARIA (PERS.) SCHULT. SUBSP. *PRADETENSIS* MÉDAIL, BAUMEL & AUDA, SUBSP. NOV.

Diagnosis: Ab subsp. *bupleuroide* (Godron & Gren.) Greuter & Burdet differt: foliis longioribus (125.6 ± 28.6 mm non 82.7 ± 33.5 mm), angustioribusque (2.4 ± 0.8 mm non 5.1 ± 1.7 mm) latis; ab subsp. *bupleuroide* quoque differt: calyce pilis sparse diffusis vestito et non distincte lineatis.

Holotype: France, Var department (83), Le Pradet, Colle Noire, Cap Garonne, alt. 90 m, gres-quartzites. F. Médail, P14., 9.vi.2002, AIX. Isotype: F. Médail, P14., 9.vi.2002, Herbarium F. Médail, (IMEP, Université Paul Cézanne, Aix-Marseille III, Aix-en-Provence).

Description: Scapes 17–47 cm. Leaves 50–165 × 1.5–5.2 mm, monomorphic, sublinear to sublanceolate, three-veined, the point mostly acute but sometimes obtuse. Outer involucre bracts 2–6, length 4.2–8.3 mm with an awn easily visible. Middle involucre bracts 1–4, length 3.4–5.5 mm. Inner involucre bracts 3–6, length 4.8–6.6 mm. Spikelet bracts 5.3–7.2 mm long and 4.1–6.9 mm wide. Flower number by spikelet 1–4. Calyx 4.7–6.2 mm long with diffuse pubescence, tube 2.2–3.3 mm long and 1.2–1.6 mm wide. Corolla white.

Derivation: The name *A. arenaria* subsp. *pradetensis* (Pradet thrift) is given in reference to the village of Le Pradet, the municipality in which it grows.

The population of Baisse du Pas Etroit, south of Belgentier (Bais), must be differentiated from *A. arenaria* subsp. *bupleuroides*, notably by its linear to sublinear leaves. We propose that this population should be described as follows.

ARMERIA ARENARIA (PERS.) SCHULT. SUBSP. *PEIRESCII* BAUMEL, AUDA & MÉDAIL SUBSP. NOV.

Diagnosis: Ab subsp. *bupleuroide* (Godron & Gren.) Greuter & Burdet, differt: foliis linearibus vel sublinearibus (1.2 ± 0.4 mm non 5.1 ± 1.7 mm), involucri phyllis brevioribus (3.48 ± 0.51 mm non 6.92 ± 1.75 mm).

Holotype: France, Var department (83), Belgentier, massif de Morières, Baisse du Pas Etroit, alt. 360 m; dolomitic rocks. A. Baumel & P. Auda, Bais5, 27.v.2004, AIX. Isotype: A. Baumel & P. Auda, Bais5, 27.v.2004, Herbarium F. Médail, s.n. (IMEP, Université Paul Cézanne, Aix-Marseille III, Aix-en-Provence).

Description: Scapes 23–59 cm. Leaves 56–103 × 0.4–1.6 mm, linear to sublinear, one-veined, the apex acute. Three outer involucre bracts, length 2.4–4.1 mm with a short awn. Three to four middle involucre bracts, length 2.7–4 mm. Five inner involucre bracts, length 5.2–7.3 mm. Spikelet bracts 5.2–7.1 mm long and 5.6–6.7 mm wide. Flower number by spikelet 2–3. Calyx 4.6–5.3 mm long with linear pubescence, tube 2.3–2.7 mm long and 1.4–1.8 mm wide. Corolla white. This population occurs at the location called ‘Baisse du Pas Etroit’, near the village of Sollies-Toucas, but in the Belgentier municipality, and contains no more than a few hundred individuals. We consider its linear leaves as potential proof of gene flow with *A. belgenciensis*.

Derivation: The name *A. arenaria* subsp. *peirescii* (Peiresc thrift) is given in reference to Nicolas-Claude Fabri de Peiresc (1580–1637), a famous humanist, jurist, philosopher, astronomer and botanist, who lived for several years in his country house at Belgentier.

CONCLUSION

This work suggests that an increased number of diverse molecular markers must be used in *Armeria* to refine the taxonomic definition. It provides valuable data to understand how phenotypic diversity evolved despite weak reproductive barriers between the taxa. For the time being, future conservation units should be considered only on the basis of multivariate morphometric analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the following: Laurence Affre, Roger Cruon, INFLOVAR, Pierre Donadille, Marianick Juin, Claude Lefèbvre, Pierre Quézel and Jean-Marc Tison for their help during different

steps of this work, Carey Suehs for checking of the English, and Gonzalo Nieto Feliner and an anonymous reviewer for their helpful comments on the manuscript.

REFERENCES

- Albert A, Jahandiez E. 1908.** *Catalogue des plantes qui croissent naturellement dans le département du Var*. Paris: P. Klincksieck (Mus. Hist. Nat. Toulon, reprints 1985).
- Arnold ML. 1997.** *Natural hybridization and evolution*. New York: Oxford University Press.
- Baker HG. 1966.** The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. *Evolution* **20**: 349–368.
- Bernis F. 1954.** Revisión del género *Armeria* Willd. con especial referencia a los grupos ibéricos. *Anales del Instituto Botanica A.J. Cavanilles* **11**: 5–288.
- Bernis F. 1955.** Revisión del género *Armeria* Willd. con especial referencia a los grupos ibéricos. Parte segunda (descriptiva de los grupos ibéricos). *Anales del Instituto Botanica A.J. Cavanilles* **12**: 77–252.
- Bernis F. 1957.** Revisión del género *Armeria* Willd. con especial referencia a los grupos ibéricos. Parte segunda (conclusión). *Anales del Instituto Botanica A.J. Cavanilles* **14**: 259–432.
- Camus EG, Camus A. 1912.** *Florule de Saint-Tropez et de ses environs immédiats*. Paris: Lechevalier.
- Charpin A, Salanon R. 1985.** Matériaux pour la Flore des Alpes Maritimes. Catalogue de l'herbier d'Emile Burnat déposé au Conservatoire Botanique de la Ville de Genève. I. Lycopodiaceae-Lentibulariaceae. *Boissiera* **36**: 1–258.
- Chas E. 1994.** *Atlas de la flore des Hautes-Alpes*. Gap: Conservatoire Botanique National Alpin de Gap-Charance, Conservatoire des Espaces Naturels de Provence et des Alpes du Sud, Parc National des Ecrins.
- Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000.** Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* **15**: 290–295.
- Davis JI, Nixon KC. 1992.** Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Botany* **41**: 421–435.
- 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**: 511–521.
- Donadille P. 1995.** *Armeria belgenciensis* Donadille ex Kerguélen. In: Olivier L, Galland JP, Maurin H, Roux JP, eds. *Livre rouge de la flore menacée de France. Tome 1: espèces prioritaires. Collection Patrimoines naturels*, vol. 20. Paris: Muséum National d'Histoire Naturelle, Conservatoire Botanique National de Porquerolles, Ministère de l'Environnement, 37.
- Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19**: 11–15.
- Ellstrand NC, Whitkus R, Rieseberg LH. 1996.** Distribution of spontaneous plant hybrids. *Proceedings of the National Academy of Sciences of the United States of America* **93**: 5090–5093.
- Fraser D, Bernatchez L. 2001.** Adaptive evolutionary conservation: towards a unified concept or defining conservation units. *Molecular Ecology* **10**: 2741–2752.
- Fuertes Aguilar J, Nieto Feliner G. 2003.** Additive polymorphism and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). *Molecular Phylogenetics and Evolution* **28**: 430–447.
- Fuertes Aguilar J, Rosselló JA, Nieto Feliner G. 1999a.** Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology* **8**: 1341–1346.
- Fuertes Aguilar J, Rosselló JA, Nieto Feliner G. 1999b.** Molecular evidence for the compilospecies model of reticulate evolution in *Armeria* (Plumbaginaceae). *Systematic Biology* **48**: 735–754.
- Gandioli JF, Salanon R. 2000.** Données floristiques sur les Alpes-Maritimes franco-italiennes: l'herbier de Joseph Rodié (Institut de Botanique de Montpellier, MPU). *Biocosme Méditerranéen* **171**: 1–195.
- Garraud L. 2003.** *Flore de la Drôme. Atlas écologique et floristique*. Gap: Conservatoire Botanique National Alpin de Gap-Charance.
- Géhu JM, Biondi E, Bournique C. 1992.** Glanures phytosociologiques sur les côtes de Provence. In: Géhu JM, ed. *Colloques phytosociologiques, XIX, Végétation et qualité de l'environnement côtier Méditerranée. Cagliari 1989*. Stuttgart: Borntraeger Verlagsbuchhandlung, Science Publishers, 147–157.
- Grant V. 1971.** *Plant speciation*. New York: Columbia University Press.
- Greuter W, Burdet HM, Long G. 1989.** *Med-checklist: a critical inventory of vascular plants of the circum-Mediterranean countries. Vol. 4, Dicotyledons (Lauraceae-Rhamnaceae)*. Berlin-Dahlem: Conservatoire et Jardin Botanique de la Ville de Geneve, Switzerland, and the Botanical Garden and Museum.
- Harlan JR, de Wet JMJ. 1963.** The compilospecies concept. *Evolution* **17**: 497–501.
- Kerguélen M. 1993.** Index synonymique de la flore de France. *Collection patrimoines naturels*, vol. 8. Paris: Muséum National d'Histoire Naturelle.
- Kumar S, Tamura K, Nei M. 2004.** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**: 150–163.
- Lefèbvre C, Vekemans X. 1995.** A numerical taxonomic study of *Armeria maritima* (Plumbaginaceae) in North America and Greenland. *Canadian Journal of Botany* **73**: 1583–1595.
- Lledó MD, Crespo MB, Cameron KM, Fay MF, Chase MW. 1998.** Systematics of Plumbaginaceae based upon cladistic analysis of *rbcL* sequence data. *Systematic Botany* **23**: 21–29.
- Milano J. 1960.** *Etude phytosociologique des groupements végétaux du littoral de l'Estérel entre Saint-Raphaël et le cap Roux*. Marseille: Rapport DES Géobotanique, Faculté des Sciences de Marseille I.

- Molinier RE. 1954.** Observations sur la végétation de la zone littorale en Provence. *Vegetatio* **5**: 257–267.
- Molinier RE. 1980.** Catalogue des plantes vasculaires des Bouches-du-Rhône. *Bulletin du Musée d'Histoire Naturelle de Marseille*, **40**, num. sp., I-LVI + 375p.
- Moore DM. 1982.** *Flora Europea checklist and chromosome index*. Cambridge: Cambridge University Press.
- Moritz C. 1994.** Defining evolutionary significant units for conservation. *Trends in Ecology and Evolution* **9**: 373–375.
- Nieto Feliner G. 1987.** El género *Armeria* (Plumbaginaceae) en la Península Ibérica: aclaraciones y novedades para una síntesis. *Anales del Jardín Botánico de Madrid* **44**: 319–348.
- Nieto Feliner G. 1990.** *Armeria*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L, eds. *Flora Iberica. Plantas vasculares de la Península Iberica e Islas Baleares. Vol. II. Platanaceae-Plumbaginaceae*. Madrid: Real Jardín Botánico, CSIC, 642–721.
- Nieto Feliner G. 1997.** Natural and experimental hybridization in *Armeria* (Plumbaginaceae): *A. salmantica*. *International Journal of Plant Science* **158**: 585–592.
- Nieto Feliner G, Fuertes Aguilar J, Rosselló JA. 2001a.** Can extensive reticulation and concerted evolution result in a cladistically structured molecular data set? *Cladistics* **17**: 301–312.
- Nieto Feliner G, Fuertes Aguilar J, Rosselló JA. 2001b.** A new species of *Armeria* (Plumbaginaceae) from southern Spain with molecular and morphometric evidence on its origin. *Botanical Journal of the Linnean Society* **135**: 71–84.
- Nieto Feliner G, Gutiérrez Larena B, Fuertes Aguilar J. 2004.** Fine-scale geographical structure, intra-individual polymorphism and recombination in nuclear ribosomal internal transcribed spacers in *Armeria* (Plumbaginaceae). *Annals of Botany* **93**: 189–200.
- R Development Core Team. 2006.** *R: a language and environment for statistical computing*. R foundation for statistical computing, ISBN 3-900051-07-0, <http://www.R-project.org>, 2008.
- Seehausen O. 2004.** Hybridization and adaptative radiation. *Trends in Ecology and Evolution* **19**: 198–207.
- Sites JW, Marshall JC. 2003.** Delimiting species: a renaissance issue in systematic biology. *Trends in Ecology and Evolution* **18**: 462–470.
- Sites JW, Marshall JC. 2004.** Operational criteria for delimiting species. *Annual Review of Ecology and Systematics* **35**: 199–227.
- Stebbins GL. 1967.** *Variation and evolution in plants*. New York: Columbia University Press.
- Thioulouse J, Chessel D, Dolédec S, Olivier JM. 1996.** ADE-4: a multivariate analysis and graphical display software. *Statistics and Computing* **7**: 75–83.
- Vekemans X, Lefèbvre C, Belalia L, Meerts P. 1990.** The evolution and breakdown of the heteromorphic incompatibility system of *Armeria maritima* revisited. *Evolutionary Trends in Plants* **4**: 15–23.