Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology

Official Journal of the Societa Botanica Italiana

ISSN: 1126-3504 (Print) 1724-5575 (Online) Journal homepage: http://www.tandfonline.com/loi/tplb20

Morphometric study of the complex Moehringia sect. Pseudomoehringia McNeill from the western Mediterranean

Juan Lorite, Ana González-Robles, Carlos Salazar-Mendías & Julio Peñas

To cite this article: Juan Lorite, Ana González-Robles, Carlos Salazar-Mendías & Julio Peñas (2018): Morphometric study of the complex Moehringia sect. Pseudomoehringia McNeill from the western Mediterranean, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, DOI: 10.1080/11263504.2017.1418448

To link to this article: https://doi.org/10.1080/11263504.2017.1418448

PLANT BIOSYSTEMS - AN INTERNATIONAL JOURNAL DEALING WITH ALL ASPECTS OF PLANT BIOLOGY, 2018
https://doi.org/10.1080/11263504.2017.1418448

Taylor & Francis
Taylor & Francis Group

Morphometric study of the complex *Moehringia* sect. *Pseudomoehringia* McNeill from the western Mediterranean

Juan Lorite[a,b], Ana González-Robles[c], Carlos Salazar-Mendías[c] and Julio Peñas[a]

[a]Department of Botany, University of Granada, Granada, Spain; [b]Interuniversitary Institute for Earth System Research, Granada, Spain; [c]Department of Animal Biology, Plant Biology and Ecology, University of Jaén, Jaén, Spain

ABSTRACT
Moehringia. sect. *Pseudomoehringia* McNeill is endemic to the western Mediterranean, having its origin and diversification centre in the Iberian Peninsula and mountains of northern Morocco. Both the relationship with the genus *Arenaria* as well as the taxonomy within the section have been largely controversial. To disentangle these issues, we conducted a morphometric study using herbarium material. We measured 12 relevant morphological traits from 148 selected herbarium sheets. Data were analyzed using different statistical methods: general linear models, multiple factorial analysis and linear discriminant analysis. We found significant differences between *Arenaria balearica* and the rest of the genus *Moehringia* taxa. Within this genus, we identified three well-discriminated species: *Moehringia fontqueri*, *M. glochidisperma* and *M. intricata*. Within *M. intricata* complex, we discerned virtually no differences amongst most of the subspecies (*intricata*, *giennensis* and *tejedensis*) with the exception of subsp. *castellana*. We propose: (i) to maintain *A. balearica* separate from *Moehringia* sect. *Pseudomoehringia*; (ii) to consider three species in the section *Pseudomoehringia*: *M. fontqueri*, *M. glochidisperma* and *M. intricata*; (iii) to recognize only two subspecies within *M. intricata* complex: subsp. *intricata* and subsp. *castellana*. To clarify the taxonomy of this threatened group is of great interest because it might help to prioritize conservation measures.

ARTICLE HISTORY
Received 23 June 2017
Accepted 13 December 2017

KEYWORDS
Arenaria; Caryophyllaceae; threatened species; Spain; Tyrrhenian islands; Morocco

Introduction

Caryophyllaceae is a family composed of 85–90 genera and ca. 2200 species, including both annual and perennial herbs (rarely woody plants), living in a wide range of habitats (Simpson 2010). This group is distributed worldwide, mainly in the Holarctic area, with the Mediterranean and Irano-Turanian biogeographic regions being two major origin and diversification centres (Fior et al. 2006).

Traditionally, the *Caryophyllaceae* family has been divided into three subfamilies: *Alsinoideae*, *Caryophylloideae* and *Paronychioideae*. This distinction has been based on morphological characters (Fior et al. 2006). The genus *Moehringia,* belonging to the *Alsinoideae* subfamily, includes 31 species distributed throughout temperate areas of the Northern Hemisphere (Fior et al. 2006). Most of the species have a European distribution. Three areas were considered important speciation centres of the genus, (i) The Balkans, (ii) the mountains of the Iberian Peninsula and (iii) the central European Alpine system (Minuto et al. 2006; Fior and Karis 2007). However, some taxa appear in North Africa, Southeast Asia and North America (Fior and Karis 2007). Most of the species are locally or regionally endemic, due to their habitat specificity, inhabiting rock crevices. Only few species, usually of woodland habitats, have a wide distribution area (Fior and Karis 2007).

Within the family the genus *Moehringia* has been controversial since its first description (Linnaeus 1753) due to the strong resemblance to certain species of the genus *Arenaria* (Fior and Karis 2007). It is believed that these morphological similarities reflect the close relation between these two genera (McNeill 1962; Minuto et al. 2011).

Fior et al. (2006) and Fior and Karis (2007) examined the relationship between the Iberian and North African species of the genera *Moehringia* and *Arenaria*. They concluded *Moehringia* is paraphyletic to *Arenaria*, and Iberian taxa belonging to *Moehringia* sect. *Pseudomoehringia* McNeill are more closely related to *Arenaria* (Fior and Karis 2007). Molecular as well as morphological data (i.e. homology of the strophiole), suggest this close relationship, first presumed by McNeill (1962). Consequently, Fior and Karis (2007) transferred *Moehringia sect. Pseudomoehringia* McNeill to *Arenaria.* Accordingly, they proposed new combinations and nomenclatural changes for these taxa belonging to *Moehringia* sect. *Pseudomoehringia*, such as: *Arenaria glochidisperma* (J.M. Mont.) Fior & P.O. Karis, for *Moehringia glochidisperma* J.M. Mont; *Arenaria tejedensis* (Willk.) Fior & P.O. Karis, for *Moehringia tejedensis* Willk; *Arenaria suffruticosa* Fior & P.O. Karis, for *Moehringia intricata* Willk; and *Arenaria funiculata* Fior & P.O. Karis, for *Moehringia fontqueri* Pau. However, they did not downscale the study to subspecies level.

CONTACT Juan Lorite ✉ jlorite@ugr.es

Recently, Sadeghian et al. (2015), using ITS and *rps16* sequence data, considered this section to be a well-supported monophyletic group within *Arenaria,* and moved the section *Pseudomoehringia* to *Arenaria,* in accordance with Fior and Karis (2007) proposal. Also, we include *Arenaria balearica* L., the closest relative of the *sect. Pseudomoehringia* (Fior and Karis 2007), in order to check its morphological relationship with this section.

Within the Iberian taxa included in the section *Pseudomoehringia* taxonomic treatment of some taxa have been largely controversial as well. While some authors distinguished two taxa within *M. intricata* (=*A. suffruticosa* Fior & P.O. Karis) at subspecies level: *M. intricata* Willk. subsp. *giennensis* Díaz de la Guardia, Mota & Valle, *M. intricata* Willk. subsp. *tejedensis* (Willk.) J.M. Monts. and *M. intricata* Willk. subsp. *castellana* J.M. Monts. (e.g. Díaz de la Guardia et al. 1991, 2011b), based on the morphology and anatomy of its leaves and, the shape of its seed papillae. Others (e.g. Montserrat-Martí 1990), only recognize two: *M. intricata* subsp. *tejedensis* and *M. intricata* Willk. subsp. *castellana* J.M. Monts. In fact, Fior and Karis (2007) stated that

> establishment of a stable taxonomy for the Iberian taxa requires more field work and study of all available herbarium material, and while this is beyond the scope of our study, we clearly indicate the need for reinterpretation of the taxa in question.

The taxa studied are mainly rupicolous and the importance of this habitat for conservation is widely acknowledged. Firstly, cliffs and rock crevices habitats take part in the well-preserved habitats worldwide (Torres et al. 2007). They have largely escaped human activities due to their difficult access (Kelly and Larson 1997). Also, these are naturally highly fragmented habitats, favouring population isolation and also constituting a refuge for natural and human-induced changes (Polunin 1980; Thompson 2005). The result is a high number of rare and endemic plant species (e.g. Thompson 2005; Pérez-García et al. 2012). Specifically, the Red List of Spanish Flora (Moreno-Sáiz 2008) includes some species of *Moehringia* under different threat categories (see Table 1). Thus, taxonomical problems constitute a challenge for scientifically sound conservation planning and therefore clarification is vital for biodiversity protection.

With the aim of disentangling the taxonomy of the group, we sought to analyze the relationship between the western Mediterranean taxa (Iberian Peninsula, Tyrrhenian Islands and northern Morocco) of the genus *Moehringia* sect. *Pseudomoehringia*, and the relation with the closely related *Arenaria balearica,* based on morphological characters.

Materials and methods

The present study examines the species of the complex *Moehringia* sect. *Pseudomoehringia* plus the closely related *Arenaria balearica* for western Mediterranean area, covering both continental areas (mountains of Iberian Peninsula and northern Morocco) and islands (Tyrrhenian Islands; Balearic archipelago, Corsica, Sardinia, Tavolara and Montecristo; see Figure 1).

Since Fior and Karis (2007) did not establish the taxonomy of the section at subspecies level, for an operative reason we used the taxa recognized by of *Flora Vascular de Andalucía Oriental* and *Flora iberica* (as belonging to *Moehringia*) as Operational Taxonomic Units (OTUs). We studied seven taxa (see Table 1): *A. balearica* L. (AB), *M. intricata* Willk. subsp. *intricata,* (MII); *M. intricata* Willk. subsp. *castellana* J.M. Monts. (MIC): *M. intricata* Willk. subsp. *tejedensis* (Willk.) J.M. Monts. (MIT); *M. intricata* Willk. subsp. *giennensis* Díaz de la Guardia, Mota & Valle (MIG); *M. fontqueri* Pau (MF) and *M. glochidisperma* J.M. Monts. (MG).

Firstly, we searched for herbarium samples of the selected species at the Global Biodiversity Information Facility database (GBIF; www.gbif.org) and requested samples in herbaria and other botanical collections (Universidad de Almería, HUAL; Instituto Botánico de Barcelona, BC; Universidad del País Vasco, BIO; Universidad de Córdoba, COA; Universidad de Granada, GDA and GDAC; Escuela Técnica Superior de Ingenieros de Montes de Madrid, EMMA; Real Jardín Botánico de Madrid, MA; Universidad de Málaga, MGC; Universidad de Murcia, MUB; Universidad de Salamanca, SALA; Swedish Museum of Natural History, S; Botanische Staatssammlung München, M; Jardín Botánico de la Universidad de Valencia, VAL; and Ludwig Maximilians Universität, MSB).

Secondly, starting with the 540 herbarium specimens received, we selected well-preserved specimens containing the main diagnostic characters (flowers, fruits, etc.), striving to gather 5–7 samples per taxa and geographical unit whenever possible. For *M. fontqueri* and *M. glochidisperma*, due to the low availability

Table 1. Main features of the taxa studied. *N* = number of herbarium specimens used in morphometric analysis. In brackets the number of georeferenced samples included in Figure 1. Threat = IUCN threat categories of the taxa according to Blanca et al. (2011): VU: vulnerable, EN: endangered, CR: critically endangered, NE: not evaluated, DD: data deficient.

Taxon	*N*	Distribution	Substrate	Elev. range (m.asl)	Threat	Chromosome number
Arenaria balearica L. (AB)	30 (15)	Western Mediterranean islands	Calcareous (limestones)	400–1440	LC	2n = 24
M. fontqueri Pau (MF)	7 (7)	Sierra Nevada (South-Eastern Spain)	Siliceous (micaschists)	1800–2500	EN	2n = 24
M. glochidisperma J.M. Monts. (MG)	7 (5)	Western Rif (Morocco)	Calcareous (limestones and dolomites)	2050	DD	?
M. intricata Willk. subsp. *castellana* J.M. Monts. (MIC)	12 (12)	Calcareous mountains of Central-East Spain	Calcareous (limestones and dolomites)	900–1300	NE	2n = 26
M. intricata Willk. subsp. *giennensis* Díaz de la Guardia, Mota & Valle (MIG)	27 (15)	Baetic range	Calcareous (limestones and dolomites)	1400–2200	VU	2n = 26
M. intricata Willk. subsp. *intricata* (MII)	33 (23)	Baetic range	Calcareous (limestones and dolomites)	1400–2200	VU	2n = 26
M. intricata Willk. subsp. *tejedensis* (Willk.) J.M. Monts. (MIT)	32 (4)	Sierras of Almijara and Tejeda (Baetic range)	Calcareous (limestones and dolomites)	1400–2200	CR	2n = 26

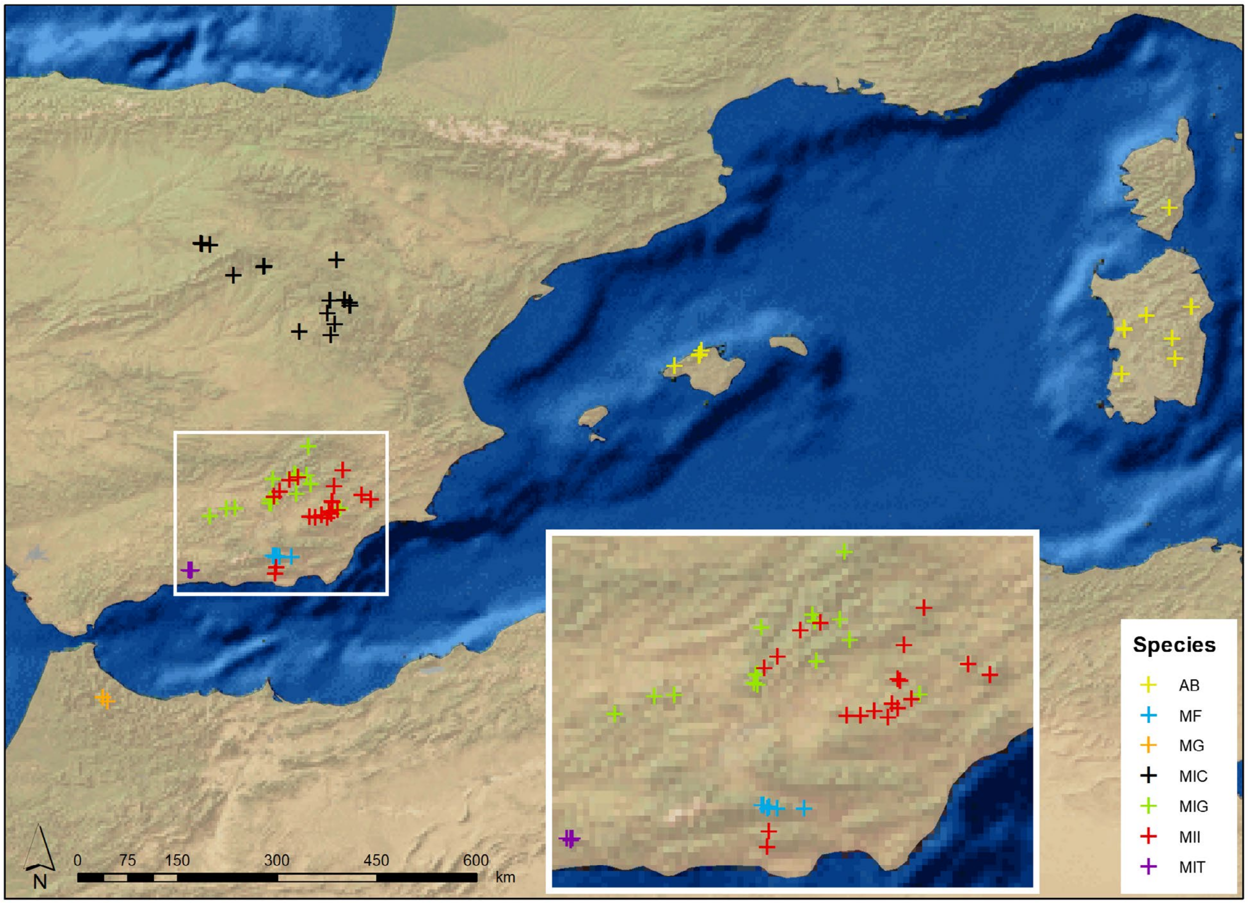

Figure 1. Location of the georreferenced herbarium samples studied.
Abbreviations for taxa: AB = *Arenaria balearica*, MF = *M. fontqueri*, MG = *M. glochidisperma*, MIC = *M. intricata* subsp. *castellana*, MIG = *M. intricata* subsp. *giennensis*, MII = *M. intricata* subsp. *intricata*, MIT = *M. intricata* subsp. *tejedensis*.

of herbarium samples, we collected new material in the habitat. Particularly, for *M. glochidisperma*, only type-specimens from the first Font Quer collection were available (Montserrat-Martí 1985).

Next, we selected 12 diagnosis characters as variables, according to the literature on the selected taxa (López-González 1990; Montserrat-Martí 1990; Díaz de la Guardia 2011a, 2011b; see Table 2). We recorded the selected variables in each herbarium sample by means of a digital calliper (±0.001 mm) and a trinocular microscope (Motic SL47) to examine and measure characters in detail. We took three measurements per specimen and the average per specimen was calculated, except for plant height, which was measured only one time per specimen. These average values per specimen were used hereafter in all the statistical analyses performed. Moreover, we added three supplementary variables: taxon (AB/MF/MG/MIC/MIG/MII/MIT), soil type (calcareous/siliceous) and geographic area (Tyrrhenian/C-Spain/Baetic/Rif).

To analyze the data by means of multivariate analyses, we filtered the raw matrix to remove the missing values (not allowed in these analyses). These missing values usually referred to seeds and petals, either missing or seriously damaged in some herbarium specimens. For variables with less than 25% of the missing values and evenly distributed across the different taxa studied, we made a mean imputation that has no significant effect in the multivariate analyses (Hair et al. 1999). After that, the resulting matrix consisted of 148 samples for the 12 variables (see Tables 1 and 2).

Statistical analysis

We performed all the statistical tests using R v. 3.1.3 (R Core Team 2015). Differences between the morphological quantitative variables were tested fitting Generalized Linear Models (GLMs), continuous variables were modelled by specifying a gaussian error distribution, while for discrete variables (countings) poisson error distribution and logit-link function was selected. The accuracy of the fitted models was tested by graphical exploration (Q–Q plot) of the residuals. Multiple comparisons were made using the R "multcomp" package (Hothorn et al. 2008) in order to explore the differences across taxa.

As for the multivariate relationship of the taxa studied, as well of the weight of each variable in the multivariate space, we performed a Multiple Factorial Analysis (MFA) using FactomineR (Husson et al. 2015). In this analysis, we included the quantitative variables (both continuous and discrete) as active variables and qualitative informative variables as supplementary (i.e. not influencing MFA; see Table 2).

Additionally, we performed a linear discriminant analysis (LDA) using *MASS* package (Venables and Ripley 2002). After this analysis, we made an a priori classification (i.e. hypothesis, which is the name of the taxon assigned in each herbarium sheet) with the result of the analysis, which is an actual classification. Here we show the contingency table with the confusion matrix (showing the discrepancy between the hypothesis and the result of the discriminant analysis; Appendices 1 and 2).

Results

Table 3 lists the results (mean ± SE) for the different morphological variables in each of the seven taxa studied. These values showed significant differences ($p < 0.05$ at *post hoc* Tuckey test) for all the variables in at least one taxa (indicated by different letters in the table), with only one exception for the number of styles that showed no significant differences amongst taxa. For most of the variables, *Arenaria balearica* significantly differed with respect to most of the other taxa, especially with regard to taxa belonging to the *Moehringia intricata* complex (MIC, MIG, MII and MIT). Notably, this is the only taxon that presented a clear leaf petiole (0.79 ± 0.05 mm), which is absent in all the other taxa studied. Also, *Moehringia fontqueri* and *Moehringia glochidisperma* showed significant differences for some characters in comparison with *M. intricata* complex. Within the *M. intricata* group almost all the values of the variables overlapped and consequently did not significantly differ in most cases.

Figures 2–4 present the results from the MFA. We constructed several scatterplots with the first two factorial axes accounting for 59.24% of the variance. Figure 2 shows two scatterplots with the position of each sample in these two first factorial axes. All the samples were categorized with supplementary explanatory variables (see Table 2). Circles show the confidence ellipses around the categories at a level of 0.95. The geographical area, on the left, showed a clear distinction between the Tyrrhenian area (with only *A. balearica*) and the rest of the areas. In the left part, we have represented the taxa studied. A clear distinction emerged for *A. balearica*, while *M. fontqueri* and *M. glochidisperma* appeared well separated. Regarding the *M. intricata* group, the probability ellipsoids appeared to overlap substantially for all the taxa, with none of them forming a clear cluster. Figure 3 shows the correlation for each of the variables, grouping leaf variables (in green), and flower variables (in blue). Leaf-petiole length and flower-pedicel length were positively correlated with *A. balearica*, while many traits such as leaf length, stamen-filament length and sepal-margin length negatively correlated with *A. balearica*. Though plant height showed a strong effect in the MFA, this variable does not offer strong taxonomical value because it is such a plastic character and varied widely within a given taxon, apart from its value depending on the way that material was collected. Figure 4 shows the position of the centroids of each taxa and the coloured lines show the strength of each group of variables. Clear differences arose between *A. balearica* and the rest of the taxa. *M. fontqueri* differed with respect to the *M. intricata* group in leaf characters but was similar to this group in flower characters while *M. glochidisperma* differed in flower characters but proved more similar to the *M. intricata* group in leaf characters. Within the *M. intricata* group, only slight differences appeared in flower and leaf characters, the most determinant character being plant height, which, as stated, is a highly plastic character.

Table 2. Morphological variables selected and abbreviations used throughout the text.

Variables	Abbreviation
1. Plant height	Height
2. Leaf length	Leaf_length
3. Leaf width	Leaf_width
4. Leaf-petiole length	Leaf_petiol_length
5. Number of flowers per inflorescence	n_flowers_inflor
6. Flower-pedicel length	Flower_pedicel_length
7. Sepal length	Sepal_length
8. Sepal width	Sepal_width
9. Scariose sepal-margin width	Sepal_margin_length
10. Petal length	Petal_length
11. Stamen-filament length	Stamen_filament_length
12. Number of styles	n_style
Qualitative supplementary variables (informative)	
1. Taxon	Taxon (AB, MF, MG, MIC, MIG, MII, MIT)
2. Distribution area	Area (Tyrrhenian, C_Spain, Baetic, Rif)
3. Substrate type	Soil (calcareous vs. siliceous)

Notes: All the variables were measured in mm except variables 5 and 12, which were counted. For supplementary variables the values appear in brackets (see Table 1 for further details).

After the LDA (Tables S1 and S2), we built the contingency matrix (Table 4), showing the confusion between the predicted species (i.e. the taxa identity according to the herbarium sheet) and the label assigned by the model (i.e. actual). We found almost no confusion for *A. balearica*, *M. fontqueri* or *M. glochidisperma*, while within *M. intricata* group (Table 4 in grey) many samples had been misclassified, particularly in *M. intricata* subsp. *giennensis*, where we found a high level of confusion with the rest of the taxa, especially regarding *M. intricata* subsp. *intricata*.

Table 3. Results after the multiple-comparison test (*post hoc* Tukey, variant for GLM tests). Values (mean ± SE) of the measured variables in the taxa studied. AB = *A. balearica*, MF = *M. fontqueri*, MG = *M. glochidisperma*, MIC = *M. intricata* subsp. *castellana*, MIG = *M. intricata* subsp. *giennensis*, MII = *Moehringia intricata* subsp. *intricata*, MIT = *M. intricata* subsp. *tejedensis*. Different letters together with the values indicate significant differences in the *post hoc* Tukey test at $p < 0.05$, performed after the GLMs.

Taxa							
Variables	AB	MF	MG	MIC	MIG	MII	MIT
1. Plant height	10.22 ± 2.06a	63.33 ± 6.49a	96.46 ± 13.89a	151.78 ± 9.36bc	166.46 ± 10.00c	121.65 ± 5.07ab	142.09 ± 13.49ac
2. Leaf length	2.71 ± 0.12a	3.00 ± 0.24ab	6.79 ± 0.69cd	7.43 ± 0.29d	7.92 ± 0.38cd	7.03 ± 0.28cd	5.64 ± 0.41bc
3. Leaf width	1.25 ± 0.05a	1.99 ± 0.14abc	3.56 ± 0.16bc	3.32 ± 0.15c	2.89 ± 0.18c	1.92 ± 0.12b	2.63 ± 0.20bc
4. Leaf-petiole length	0.79 ± 0.05b	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
5. Number of flowers per inflorescence	1 ± 0a	1.39 ± 0.23a	1.83 ± 0.12a	3.91 ± 0.14c	3.31 ± 0.17b	3.65 ± 0.15bc	2.97 ± 0.22b
6. Flower-pedicel length	26.92 ± 1.23d	5.91 ± 0.69a	15.92 ± 1.35ac	15.96 ± 0.57c	14.53 ± 1.00ac	11.04 ± 0.73ab	15.63 ± 1.10bc
7. Sepal length	2.47 ± 0.06a	2.87 ± 1.00abc	3.86 ± 0.12b	3.19 ± 0.07c	3.00 ± 0.07c	3.08 ± 0.07c	3.08 ± 0.13c
8. Sepal width	1.33 ± 0.04ac	1.04 ± 0.09a	1.44 ± 0.09bc	1.52 ± 0.06c	1.17 ± 0.04ab	1.24 ± 0.045ab	1.19 ± 0.05ab
9. Scariose sepal-margin width	0.14 ± 0.02a	0.13 ± 0.01ab	0.31 ± 0.05c	0.26 ± 0.01bc	0.22 ± 0.01bc	0.24 ± 0.01bc	0.24 ± 0.02bc
10. Petal length	3.77 ± 0.10a	6.18 ± 0.72bc	6.39 ± 0.27c	5.78 ± 0.22c	5.67 ± 0.19c	5.00 ± 0.20b	5.84 ± 0.25bc
11. Stamen filament length	2.57 ± 0.07a	4.43 ± 0.45cd	4.95 ± 0.22d	3.38 ± 0.14b	3.96 ± 0.13c	3.41 ± 0.11b	3.86 ± 0.23bc
12. Number of styles	3 ± 0a	3 ± 0a	3 ± 0a	3 ± 0a	2.72 ± 0.08a	3 ± 0a	3 ± 0a

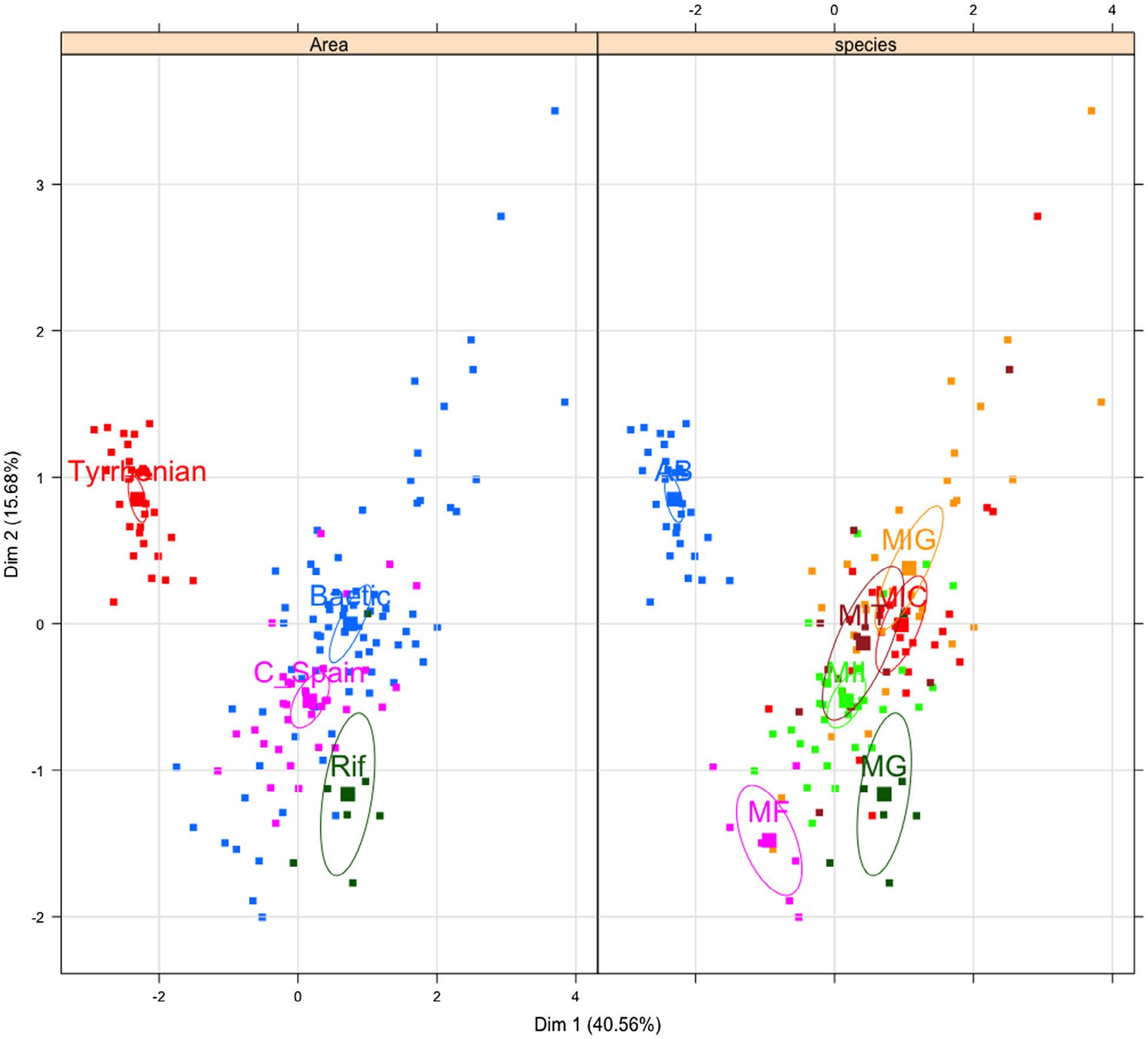

Figure 2. Scatterplot showing the position of each sample in the two first factorial axes.

Notes: Categorical supplementary variables were superimposed. Geographical areas on the left: Tyrrhenian (red), Baetic ranges (blue), Central Spain (pink) and Rif mountains (green), and species on the right: AB = *Arenaria* balearica (blue), MF = *M. fontqueri* (pink), MG = *M. glochidisperma* (dark green), MIC = *M. intricata* subsp. *castellana* (red) MIG = *M. intricata* subsp. *giennensis* (orange), MII = *M. intricata* subsp. *intricata* (light green) and MIT = *M. intricata* subsp. *tejedensis* (brown). Circles show the confidence ellipses around the categories at a level of 0.95.

Discussion

As other authors have concluded (McNeill 1962; Fior et al. 2006; Fior and Karis 2007; Sadeghian et al. 2015), the analyses performed reflected the systematic difficulty of this group, particularly regarding the *Moehringia intricata* complex (Díaz de la Guardia et al. 1991). However, morphometrically, *Arenaria balearica* was clearly distinguished from the rest of the taxa belonging to *Moehringia* sect. *Pseudomoehringia*. This finding fits with the criteria used in *Flora iberica* (López-González 1990; Montserrat-Martí 1990) and *Flora Vascular de Andalucía Oriental* (Díaz de la Guardia 2011a, 2011b). Using a conservative approach, they maintain the taxa in the *Moehringia* genus. Furthermore, other authors agree that *Moehringia* and *Arenaria* also show major differences in the structure and composition of their seeds (Casazza et al. 2008; Minuto et al. 2011).

Within the *Moehringia* taxa, *M. fontqueri* showed a clear distinction, in accordance with molecular data (Fior and Karis 2007). In fact, *M. fontqueri* is the only silicicolous taxon (ecological isolation; Peñas and Lorite 2004), in contrast to the rest of taxa inhabiting calcareous rocks (Montserrat-Martí 1990; Díaz de la Guardia 2011b). In this case, the ecological adaptation could have played a key role in their evolution, as in other taxa of the *Caryophyllaceae* family (Wyatt 1984).

M. glochidisperma also showed differences that can be explained by the ancestral isolation as a consequence of its geographical location (endemic to northern Morocco, Valdés et al. 2002). In fact, during part of the Miocene, these territories were part of the same unit. However, with the movement towards the south-eastern Iberian continental plate and westwards of the Alboran microplate, their floras became separated. This phenomenon formed the Baetic ranges in southern Spain and the Rif mountain range in northern Morocco (Rosenbaum et al. 2002). The closest morphological relationship is with *M. intricata* subsp. *tejedensis*, which is the geographically nearest taxon.

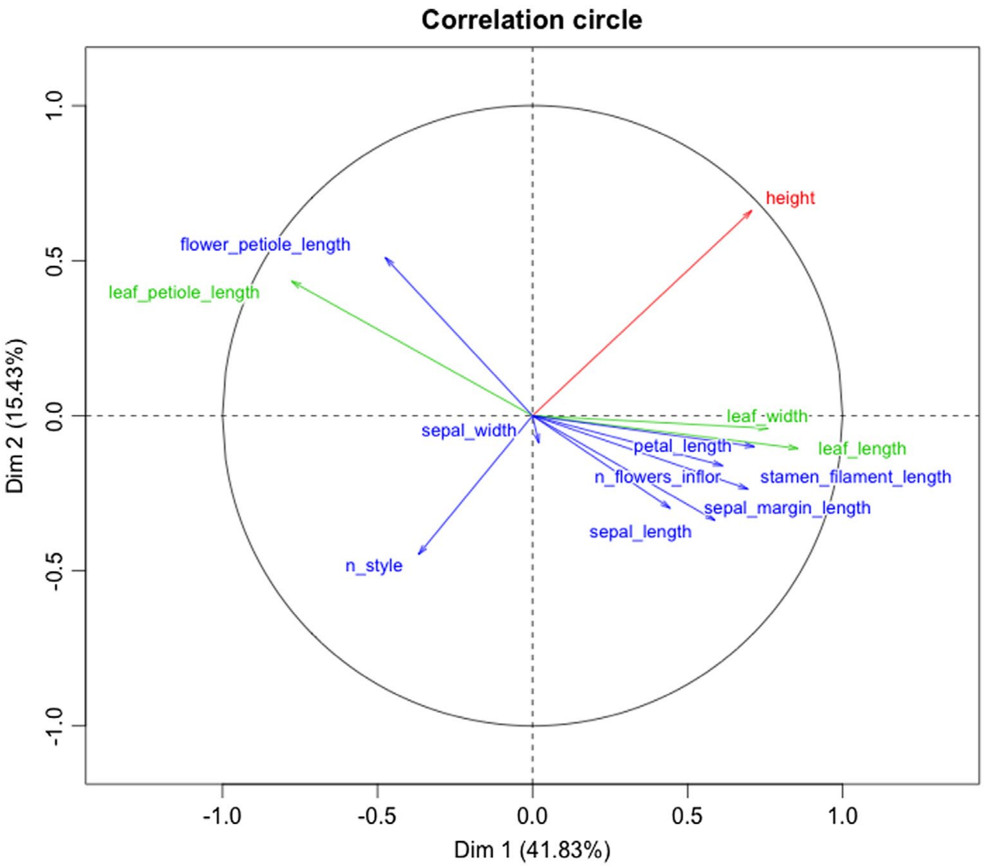

Figure 3. Correlation circle with variables.

Notes: In red "height", in blue "flower variables" and in green "leaf variables". See the abbreviations for variables in Table 2.

Furthermore, our results showed no significant morphometric differences between the *M. intricata* subspecies (*M. intricata* subsp. *intricata, M. intricata* subsp. *giennensis*, and M. *intricata* subsp. *tejedensis*) recognized in the Baetic mountains by reference floras (Montserrat-Martí 1990; Díaz de la Guardia 2011b). The results did not support the maintenance of these subspecies as previously stated by McNeill (1962). *M. intricata* subsp. *intricata* seems to show greater similarities with *M. intricata* subsp. *giennensis*, together with an entangled geographic connections between their populations, lacking a geographical pattern nor morphological differences to support their position as different subspecies. *M. intricata* subsp. *tejedensis* also showed narrow morphometric similarities with these former two. In fact, these similarities prompted some authors to assign this taxon to samples coming from other areas within the Baetic ranges (e.g. Montserrat-Martí 1990).

In the *M. intricata* complex, only *M. intricata* subsp. *castellana* showed quite marked morphological differences in addition to a well-defined geographical entity (Central System and Serrania de Cuenca, Spain), supporting its position as subspecies.

The results found and conclusions drawn are useful not only for taxonomical purposes but also for conservation. In fact, within the Mediterranean hotspot, rupicolous habitats (where the species studied live) have great floristic singularity, acting as refuge areas for many endemic species (Domínguez-Lozano et al. 1996; Casazza et al. 2005; Médail and Diadema 2009). Also, these rocky walls act as biological islands, with rupicolous species being restricted to these conditions. These habitats are small and patchy, being surrounded by environments which are completely hostile for rupicolous species (Youssef et al. 2010). It is important to emphasize that most of the species are under great extinction risk, specifically: *M. intricata* subsp. *intricata* and *M. intricata* subsp. *giennensis* as Vulnerable (VU), *M. fontqueri* as Endangered (EN) and *M. intricata* subsp. *tejedensis* as critically endangered (CR) (Peñas and Lorite 2004; Díaz de la Guardia 2011b).

Based on the results, we recommend the following: i) to maintain *A. balearica* as separate from *Moehringia* sect. *Pseudomoehringia* taxa, ii) to consider three species in the section *Pseudomoehringia: M. fontqueri*, *M. glochidisperma* and *M. intricata*; and iii) to recognize only two subspecies within *M. intricata* complex: *M. intricata* subsp. *intricata* (=*M. intricata* subsp. *giennensis*, =*M. intricata* subsp. *tejedensis*) and *M. intricata* subsp. *castellana*.

Consequently, for conservation purposes, we propose to prioritize *M. fontqueri* as a well-separated species, being the only taxon living on siliceous substrate. Also, the special situation of *M. glochidisperma* should be emphasized. At present, only one population is known (Montserrat-Martí 1985), despite that no threat category has been assigned to this species due to the lack of threat evaluation in most Moroccan flora. Therefore, it is a poorly known taxon needing an exhaustive study of its territory (Western Rif), in order to determine its situation and to find new populations. *M. intricata* subsp. *intricata* and *M. intricata* subsp. *castellana* should be reevaluated in order to determine

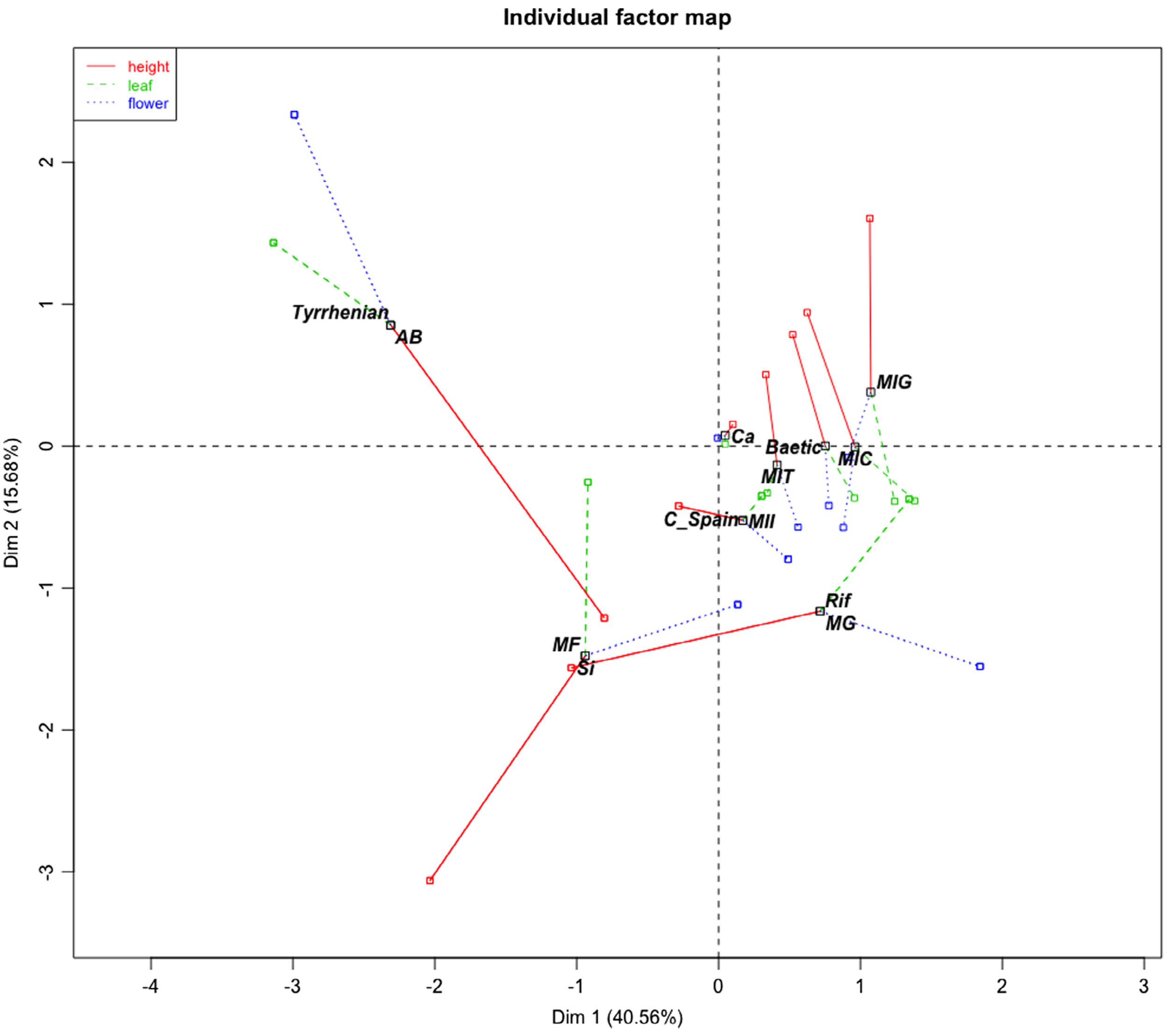

Figure 4. Individual-factor map showing the relative position of the centroids of each taxa, as well as the categorical supplementary variables: area (Tyrrhenian/Central Spain/Baetic Ranges/Rif) and soil (siliceous = Si/calcareous = Ca). Lines show the strength of variable group for each taxon in the MFA performed (red = plant height, blue = flower variables, green = leaf variables).

Abbreviations for taxa: AB = *Arenaria balearica*, MF = *M. fontqueri*, MG = *M. glochidisperma*, MIC = *M. intricata* subsp. *castellana*, MIG = *M. intricata* subsp. *giennensis*, MII = *M. intricata* subsp. *intricata*, MIT = *M. intricata* subsp. *tejedensis*.

Table 4. Confusion matrix. In columns a priori assigned species. In columns the labels assigned by the models after the LDA. In grey, the high level of confusion within the *Moehringia intricata* complex. Abbreviations for taxa: AB = *Arenaria balearica,* MF = *M. fontqueri,* MG = *M. glochidisperma,* MIC = *M. intricata* subsp. *castellana,* MIG = *M. intricata* subsp. *giennensis,* MII = *M. intricata* subsp. *intricata,* MIT = *M. intricata* subsp. *tejedensis*.

Predicted (hypothesis)							
Actual	AB	MF	MG	MIC	MIG	MII	MIT
AB	29	0	0	0	0	0	0
MF	0	2	0	0	0	0	0
MG	0	0	6	0	1	0	1
MIC	0	0	0	19	4	1	2
MIG	0	0	0	2	11	6	2
MII	0	0	0	1	11	25	0
MIT	1	0	1	5	5	1	7

their status of thereat after our taxonomical proposal. For *M. tejedensis*, with well-separated populations with regard to *M. intricata s.l.*, we can consider the existence of Evolutionary Significant Units (ESU) deserving conservation efforts (Waples 1995; Allendorf and Luikart 2007). However, the conservation status of *M. intricata s.l.* should be updated by applying the IUCN guidelines for the regional level (i.e. local in this case, IUCN 2012).

In summary, clarifying the taxonomy of these taxa is a key point for species conservation. This will contribute more information concerning the real conservation status of these taxa, and could help to put into practice better conservation plans to avoid the recession and disappearance of endemic flora. In this way, the main goals to be achieved in terms of biodiversity are to establish effective conservation strategies in minority, fragmented and fragile Mediterranean cliff environments.

Acknowledgements

We are grateful to Dr. M. Martínez-Ortega for helpful comments and D. Nesbitt for the language revision. We also thank Dr. Eva Cañadas for her help the making one of the figures.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Spanish Ministerio de Ciencia e Innovación through the projects [project number CGL2010-16357/BOS] (Phylogeography and conservation of endemic flora of "island" habitats: Ibero-North African species of *Moehringia* sect. *Pseudomoehringia*).

References

Allendorf FW, Luikart G. 2007. Conservation and the genetics of populations. Malden (MA): Blackwell.

Blanca G, Cabezudo B, Cueto M, Salazar C, Morales-Torres C, editors. 2011. Flora vascular de andalucía, Granada, orientalServicio de Publicaciones de las Universidades de Almeria, Jaen y Málaga.

Casazza G, Barberis G, Minuto L. 2005. Ecological characteristics and rarity of endemic plants from Italian Maritime Alps. Biol Conserv. 123: 361–371.

Casazza G, Borghesi B, Roccotiello E, Minuto L. 2008. Dispersal mechanisms in some representatives of the genus *Moehringia* L. (Caryophyllaceae). Acta Oecol. 33: 246–252.

Díaz de la Guardia C. 2011a. *Arenaria* L. In: Blanca G, Cabezudo B, Cueto M, Salazar C, Morales-Torres C, editors. Flora Vascular de Andalucía Oriental. Granada: Servicio de Publicaciones de las Universidades de Almeria, Jaen y Málaga; p. 501–511.

Díaz de la Guardia C. 2011b. *Moehringia* L. In: Blanca G, Cabezudo B, Cueto M, Salazar C, Morales-Torres C, editors. Flora Vascular de Andalucía Oriental, Granada, Servicio de Publicaciones de las Universidades de Almeria, Jaen y Málaga; p. 501-511.

Díaz de la Guardia C, Mota JF, Valle F. 1991. A new taxon in the genus *Moehringia*. Plant Syst Evol. 177: 27–38.

Domínguez-Lozano F, Galicia-Herbada D, Moreno-Rivero L, Moreno-Saiz JC, Sainz-Ollero H. 1996. Threatened plants in peninsular and balearic Spain: a report based on the EU habitats directive. Biol Conserv. 76: 123–133.

Fior S, Karis PO. 2007. Phylogeny, evolution and systematics of *Moehringia* (Caryophyllaceae) as inferred from molecular and morphological data: a case of homology reassessment. Cladistics. 23: 362–372.

Fior S, Karis PO, Casazza G, Minuto L, Sala F. 2006. Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast matK and nuclear ITS rDNA sequences. Am J Bot. 93: 399–411.

Hair JF, Anderson RE, Tatham RL, Black WC. 1999. Análisis multivariante. 5ª ed. Madrid: Prentice Hall Iberia.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. Biom J. 50(3): 346–363.

Husson F, Josse J, Le S, Mazet J. 2015. FactoMineR: multivariate exploratory data analysis and data mining. R packag vers. 1: 29. http://CRAN.R-project.org/package=FactoMineR.

IUCN. 2012. Guidelines for application of IUCN red list criteria at regional and national levels: version 4.0. Gland: IUCN.

Kelly PE, Larson DW. 1997. Effects of rock climbing on populations of presettlement eastern white cedar (*Thuja occidentalis*) on cliffs of the Niagara Escarpment, Canada. Conserv Biol. 11(5): 1125–1132.

Linnaeus C. 1753. Species plantarum. Stockholm: Lars Salvius.

López-González G. 1990. *Arenaria* L. In: Castroviejo S, Aedo C, Laínz M, Muñoz-Garmendia F, Nieto-Feliner G, Paiva J, Benedí C, editors. Flora iberica 2. Madrid: Real Jardín Botánico, CSIC; p. 172-224.

McNeill J. 1962. Taxonomic studies in the Alsinoideae: I. Generic and infrageneric groups. Notes Roy Bot Gard Edinburgh. 24: 79–155.

Médail F, Diadema K. 2009. Glacial refugia influence plant diversity patterns in the Mediterranean basin. J Biogeogr. 36: 1333–1345.

Minuto L, Fior S, Roccotiello E, Casazza G. 2006. Seed morphology in *Moehringia* L. and its taxonomic significance in comparative studies within the Caryophyllaceae. Plant Syst Evol. 262: 189–208.

Minuto L, Roccotiello E, Casazza G. 2011. New seed morphological features in *Moehringia* L. (Caryophyllaceae) and their taxonomic and ecological significance. Plant Biosyst. 145(1): 60–67.

Montserrat-Martí JM. 1985. *Moehringia* glochidisperma (Caryophyllaceae) nova espècie del Rif (Marroc). Collect Bot. 16(1): 113–116.

Montserrat-Martí JM. 1990. *Moehringia* L. In: Castroviejo S, Aedo C, Laínz M, Muñoz-Garmendia F, Nieto-Feliner G, Paiva J, Benedí C, editors. Flora iberica 2. Madrid: Real Jardín Botánico, CSIC; p.172-224.

Moreno-Sáiz JC. 2008. Lista Roja 2008 de la flora vascular española. Madrid: Direcc. General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente y Medio Rural y Marino y Sociedad Española de Biología de la Conservación de Plantas).

Peñas J, Lorite J. 2004. *Moehringia* fontqueri Pau. In: Bañares Á, Blanca G, Güemes J, Moreno-Sáiz JC, Ortiz S, editors. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Madrid: Dirección General de Conservación de la Naturaleza; p.788–798.

Pérez-García FJ, Medina-Cazorla JM, Martínez-Hernández F, Garrido-Becerra JA, Mendoza-Fernández AJ, Salmerón-Sánchez E, Mota JF. 2012. Iberian baetic endemic flora and the implications for a conservation policy. Ann Bot Fennici. 49: 43–54.

Polunin O. 1980. Flowers of Greece and the Balkans: a field guide. Oxford: Oxford University Press.

R Core Team. 2015. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/.

Rosenbaum G, Lister GS, Duboz C. 2002. Reconstruction of the tectonic evolution of the western Mediterranean since the oligocene. J Virt Expl. 8: 107–130.

Sadeghian S, Zarre S, Rabeler RK, Heubl G. 2015. Molecular phylogenetic analysis of *Arenaria* (Caryophyllaceae: Tribe Arenarieae) and its allies inferred from nuclear DNA internal transcribed spacer and plastid DNArps16 sequences. Bot J Linn Soc. 178: 648–669.

Simpson MG. 2010. Plant systematics. 2nd ed. San Diego, CA: Academic Press.

Thompson JD. 2005. Plant evolution in the Mediterranean. Oxford: Oxford University Press.

Torres K, Opazo BM, Rubio F. 2007. Species composition and biogeographic relations of the high plateau of Itatiaia. SE-Brazil. Rev Bras Biol. 30(4): 623–639.

Valdés B, Rejdali M, Achhal El Kadmiri A, Jury SL, Montserrat-Martí JM, editors. 2002. Checklist of vascular plants of N Morocco with identification keys. Madrid: Consejo Superior de Investigaciones Científicas.

Venables WN, Ripley BD. 2002. Modern applied statistics with S. 4th ed. New York (NY): Springer.

Waples RS. 1995. Evolutionarily significant units and the conservation of biological diversity under the endangered species act. In: Nielsen, JL, editor. Evolution and the aquatic ecosystem: defining units in population conservation; Bethesda (MD): American Fisheries Society, Symposium 17.

Wyatt R. 1984. Intraspecific variation in seed morphology of *Arenaria* uniflora (Caryophyllaceae). Syst Bot. 9: 423–431.

Youssef S, Vela E, Baumel A, Tatoni T. 2010. Distribution, habitat and population size variation of *Genista lobelii* (Fabaceae) from the calcareous mountains of Basse Provence (S-E France). Ecol Mediterr. 36(1): 63–76.

Appendix 1. Mean for each variable grouping by the taxa, as obtained after the linear discriminant analysis (in mm)

	AB	MF	MG	MIC	MIG	MII	MIT
1. Plant height	104.22	59.00	96.46	151.78	166.46	121.65	142.09
2. Leaf length	2.71	2.97	6.79	7.43	7.92	7.03	5.64
3. Leaf width	1.25	2.00	3.56	3.32	2.89	1.92	2.63
4. Leaf-petiole length	0.79	0.00	0.00	0.00	0.00	0.00	0.00
5. Number of flowers per inflorescence	1.00	1.00	1.83	3.91	3.31	3.65	2.97
6. Flower-pedicel length	26.92	4.66	15.92	15.96	14.53	11.04	15.63
7. Sepal length	2.47	2.99	3.86	3.19	2.99	3.08	3.08
8. Sepal width	1.33	0.84	1.44	1.52	1.17	1.24	1.19
9. Scariose sepal-margin width	0.14	0.10	0.30	0.26	0.22	0.24	0.24
10. Petal length	3.77	6.63	6.39	5.77	5.67	4.96	5.84
11. Stamen filament length	2.57	5.17	4.95	3.38	3.96	3.40	3.86
12. Number of styles	3.00	3.00	3.00	3.00	2.72	3.00	3.00

Abbreviations for taxa: AB = *Arenaria balearica,* MF = *M. fontqueri,* MG = *M. glochidisperma,* MIC = *M. Intricata* subsp. *castellana,* MIG = *M. intricata* subsp. *giennensis,* MII = *M. intricata* subsp. *intricata,* MIT = *M. intricata* subsp. *tejedensis.*

Appendix 2. Scores for the variables in the first three discriminant axes (LD1–LD3)

	LD1	LD2	LD3
1. Plant height	−0.0060	0.0067	0.0003
2. Leaf length	−0.0254	0.4323	0.1149
3. Leaf width	0.5170	−1.0104	−0.9248
4. Leaf-petiole length	−5.1765	−1.7039	0.2735
5. Number of flowers per inflorescence	0.2126	1.0018	0.3824
6. Flower-pedicel length	−0.0654	0.0363	−0.0903
7. Sepal length	1.0410	−0.7250	−0.5833
8. Sepal width	−0.9573	1.6866	−0.6170
9. Scariose sepal-margin width	3.1490	2.1264	−5.0033
10. Petal length	0.1049	−0.0142	0.0599
11. Stamen filament length	0.5075	−0.5990	0.1493
12. Number of styles	0.3236	−0.8997	−1.0229