

Phenotypic strategies for *Jasione* L. or the challenge that taxonomy face to recent floristic responses along western Eurasia

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Abstract: *Jasione* L. (Campanulaceae) is regarded as one of the recent floristic responses in western Eurasia. The characterization of the morpho-environmental fluctuating asymmetries (FA) to describe the phenotypic responses for *Jasione* L. is here exposed. Continuity is pointed out to describe these morpho-environmental responses, as a redundancy effect (RE), in accordance with the significant similarity involved. In this case, 327 individuals and 14 species were investigated (*J. sphaerocephala* Brullo, Marcenò & Pavone and *J. idaea* Stoj. synonymized as *J. montana* L. var. *montana* and *J. supina* Sieber ex Spreng. subsp. *pontica* (Boiss.) Damboldt). *J. montana* L. and *J. heldreichii* Boiss. & Orph. showed the highest comprehensive morphological response, while more important divergence was observed in *J. foliosa* Cav. and *J. mansanetiana* Roselló & Peris. No significant difference was obtained by the morphological approach without taxonomic differentiation. Six morphogroups (Grs) were detected, and only one (Gr6) showed lower morpho-environmental instability (50% RE). Germoplasmic accommodation was observed during the interglacial and glacial periods along the SW distribution range of the genus. The lack of target phenotype must be the consequence of the intense alterations of potential distributions in *Jasione* since its origin. *J. montana* and *J. heldreichii* showed agglomerative phenotypes characteristics.

Key words: Morphogroup, potential habitat distributions, fluctuating asymmetries, agglomerative phenotype, target phenotype, redundancy effect

1. Introduction

Jasione L. (Campanuloideae/Campanulaceae) is a genus of the west Eurasian region. The distribution of *J. montana* L. described by Hultfén (Parnell, 1985) covers its total distribution. At present, 24 taxa have been described in the genus: 14 species, 9 subspecies, and 4 varieties. However, their segregation based on morphology remains controversial since the diagnostic characters traditionally used often overlap (see Appendix A). As suggested for the Iberian taxa (Sales and Hedge, 2001), there are few diagnostic characters in the genus. Hairiness, life form, pedicel and corolla length, as well as the number of bracts around the inflorescence have been used to distinguish taxa. However, the descriptions in Appendix A confirm the variability of these characters and explain both the taxonomic (Parnell, 1987; Leitão and Paiva, 1988; Mota et

al., 1988; Sales et al., 2004; Villegas and Lumbreras, 2008) and anatomical (Bokhari and Sales, 2001) complexity of *Jasione*.

Although *Jasione* is monophyletic, the position of this clade within the Campanuloideae is still unresolved and species are difficult to diagnose (Sales et al., 2004; Pérez-Espona et al., 2005; Haberle et al., 2009). Additionally, *Jasione* showed a considerable genome rearrangement, not uncommon in Campanulaceae, supporting the significant divergence of this group (Haberle et al., 2009; Crowl et al. 2014, 2016). Crowl et al. (2016) hypothesized that the Campanuloideae originated during the Eocene period, and migratory fluxes between Africa and the Palearctic could explain its expansion to its current range. Geographically, *Jasione* is restricted to north and western Eurasia, as well as the Mediterranean basin (Tutin, 1976; Sales and Hedge,

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2001). A land bridge emerged from the collision between the Arabian Peninsula and the Eurasian plate (Dilek, 2006; Mantovani et al., 2006) preventing the *Jasione* genetic flux towards east, because the highlands barrier emerged in this process (Macey, 2000; Garfunkel, 2004; Djamali et al., 2012). Therefore, the Mediterranean region has been suggested as the ancestral biogeographic area of *Jasione*, derived from that original Miocene germplasm and the expansion towards western Eurasia (Roquet et al., 2009; Yoo et al., 2018).

Given the biogeographic evolution proposed for *Jasione*, the present work focuses on its current morpho-environmental characterization throughout its distribution. The morpho-environmental make-up is regarded here as the morphological response to the environmental potential capacity, i.e. the biological accommodation of *Jasione*. In this sense, environmental dynamics of any region would be responsible for the morphological variability detected. Any divergence or discontinuity of the morphological response in any environmental unit would be the consequence of relevant changes upon the climate dynamics in that unit. The “Fluctuating asymmetry” approach (FA; Parsons, 1990) was applied here to describe such morpho-environmental dynamics. FA is traditionally associated with deviations from the development of bilaterally symmetric traits (Klingenberg, 2015; Tucić et al., 2018), and it is even used to compare morphological variations between populations (Sarre, 1996). In our case, FA was used to describe the relationship between the environmental variability (amplitude and correlation) involved (Klingenberg et al., 2019), and the morphological response detected (amplitude and correlation). A similar approach, in this case for group *Dianthus pungens* L., was applied for Castro et al. (2020). Thus, the numerical value of FA will reflect the relationship between morpho-environmental amplitudes (i.e. morpho-environmental possibilities), and the continuity of responses (i.e. morpho-environmental correlations). This continuity effect is discussed here as a redundant effect (RE): the higher correlation, the higher RE. Therefore, RE is regarded as the parameter that represents the similarity of the responses. Based on this hypothesis, the FA approach emerges as a very useful tool to quantify the morphological resilience of the plants analysed, as the morphological response to thermo-pluviometric and altitudinal parameters—the environmental variables analysed in the present work. Despite the difficulties involved in the phenotypic characterization (Valladares et al., 2007), and according to the complexity of the environmental interactions integrated into the morphological response of individuals (De Kroon, 2005), a phenotypic analysis such as this must involve complex morpho-environmental correlations. The results obtained should contribute to a better taxonomic

classification of *Jasione*. The morpho-environmental FA will also contribute to quantifying the phenotypic plasticity in *Jasione* (Fusco and Minelli, 2010). On the basis of the phenotypic distance (Valladares et al., 2006), in our case the RE or the continuity between individuals (analysis of correlation between morpho-environmental responses analysis), it should also be possible to recognise unstable (with low RE) and stable groupings (or those with high RE). Stable groupings would be those with low morpho-environmental FA and would be described here as “target phenotype” (Ancel, 2000). In contrast, unstable groupings would have the higher morpho-environmental FA and no differential phenotype response would be associated. This latter case would explain the taxonomic difficulties in the genus.

2. Materials and methods

2.1. Morpho-taxonomic analysis

The taxonomic assessment was based on the nomenclatural and morphological revisions of the genus (Parnell, 1987; Leitão and Paiva, 1988; Mota et al., 1988; Sales et al., 2004; Villegas and Lumbreras, 2008; Bartók, 2014), or general floras (Tutin, 1976; Damboldt, 1978; Sales and Hedge, 2001; Pignatti, 2018). The taxonomic complexity of *Jasione* results in the numerous nomenclatural combinations published so far, the extensive synonymy, and the succinct morphological description (based on the diagnostic characters used in the genus) in Appendix A. This emphasises the ambiguities in distinguishing species, subspecies, and varieties.

A previous morphological analysis involving 327 individuals (Appendix B) was performed based on the matrix obtained from 21 morphological variables (Appendix C). The variables were selected based on the characters used to differentiate species and infraspecific ranks in previous taxonomic treatments.

Dendrograms for the standardized morphological matrix representing the similarity analysis—Ward distances and Manhattan City-block clusters—were produced. The dendrograms were organized by species and no infraspecific taxonomic rank was considered in the morpho-taxonomic analysis. Conversely, individuals of all species, subspecies, and varieties described in *Jasione* were morpho-environmentally analysed. Also, the average values of variables analysed per species were dendrographically represented to separate similarities. Clusters of species were described, and a forward stepwise Discriminant Canonical Analysis (DCA) was performed. The most discriminant variables involved in the differentiation of the clusters, described in tabular form, involves F to remove, p-level, and tolerance to measure the redundancy with the other variables, as statistical values. This multivariate description has usually applied

for previous morpho-environmental characterizations (Rocha et al., 2012a, 2012b, 2017, 2018). The multivariate analysis here described was carried out using the software STATISTICA v. 9.1 (StatSoft Inc., 2010).

2.2. Morphological analysis

For this analysis the same matrix was used as in the morpho-taxonomic approach. For the morpho-environmental analysis the individuals were not previously segregated by species, with the objective of describing the real morphological variability.

A similarity analysis—Ward distances and Manhattan City-block clusters—was performed again, and a new dendrogram was obtained. This similarity characterization was necessary to describe a new set of clusters, each one representing a morphogroup (Gr). Grs are described here as groups of individuals with similar morphological responses according to their morphological distance obtained from this multivariate analysis. Each Gr was also environmentally analysed. The environmental variables used for this analysis are shown in Appendix E. Twenty-three environmental variables were initially considered as potential predictors for the current habitat distribution of *Jasione*, based on (1) their biological relevance to the species distribution, and (2) on several other habitat modelling studies (Kumar et al., 2006; Murienne et al., 2009; Kumar and Stohlgren, 2009; Rocha et al., 2012a).

To better describe the Grs, the results were also discussed on the basis of taxon percentages. Therefore, it was suggested that the correlation between Grs and species should describe the distribution of taxa per Gr and a “corresponding Principal Component Analysis” (cPCA) was carried out. The expected results would recognise taxonomic preferences per Gr. This multivariate description separated the taxa with clear preferences from those without apparent correlation. The latter was here regarded agglomerative taxa, since they were responsible for the similarity between Grs. In other words, depending on the percentage of those agglomerative taxa per Gr, we differentiate the most similar (i.e. with the higher percentages within the agglomerative species) from those most divergent (i.e. with the lower percentages within the agglomerative taxa). The agglomerative properties referred to here were also considered responsible for the continuity between taxa. This continuity effect was confirmed by discriminant analysis (discriminant canonical analysis, DCA).

2.3. Potential habitat distributions and modelling scenarios

Distribution maps of realized distribution of specimens analysed and potential habitats, with potential habitat suitability $\geq 50\%$, were drawn. Different approaches are described in both cases (Jiménez-Valverde et al., 2008):

the geographic location of samples (realized maps), or areas with potential ecological capacities to include the species (potential habitat maps). The potential habitats distribution maps were elaborated based on the environmental information per Gr. Modelling of potential habitat suitability was performed using the Maxent v. 3.3.3 program, based on the environmental constraints (Phillips et al., 2006). It only requires species presence data and environmental variables in GIS layers for the studied area. The potentially suitable habitat per Gr was calculated according to the presence of each individual, which was assessed previously. Twenty-three environmental variables (Appendix E) were considered, initially, as potential predictors for *Jasione* current habitat distribution, based on their biological relevance to plant species distributions and on several other habitat modelling studies (Kumar et al., 2006; Murienne et al., 2009; Kumar and Stohlgren, 2009; Rocha et al., 2012a). All variables were downloaded with a 30 s (ca. 1 km) spatial resolution, from the WorldClim dataset¹ (Hijmans et al., 2005). The current climate data over the period between 1950 and 2000 and Paleoclimate data for the Last Glacial Maximum (LGM; - 22,000 years BP) were derived from CCSM4 (Collins et al., 2006).

To reduce multicollinearity among the environmental variables, and select the environmental variables for modelling the potential habitat distribution, these were subjected to cross-correlations (Pearson correlation coefficient, r), based on the values for each of the individuals occurrence records. The accuracy of the modelling approach and the probability of presence must be ranked through receiver-operating characteristic (ROC) plots (Fielding and Bell, 1997), as well as the area under curve (AUC) (Phillips, et al. 2006). At the same time, locations with random background probability were used as pseudoabsences, for all analysis in Maxent (Phillips et al., 2001, 2006). This analysis was necessary to describe the potential habitat distribution of every Gr in order to describe the continuity hypothesis introduced in the morpho-environmental analysis. In this case, the potential areas per Gr were decisive to establish overlap areas between them. The overlapping boundaries were regarded as a result of the continuity effect, in contrast with the divergent distributions where no overlapping was observed.

Models of past scenarios were also drawn (LGM scenario) with the aim of describing the potential distribution of *Jasione* under glacial circumstances. The climate predictors used here were down-scaled climate data from simulations with Global Climate Models (CCSM4 was chosen), for the Last Glacial Maximum (LGM).

2.4. Fluctuating asymmetry (FA)

A fluctuating asymmetry (FA) analysis was necessary to describe the morphological responses of individuals to

¹ www.worldclim.org

the environmental variability (Castro et al., 2020). The FA value per Gr was obtained by calculating the difference between the environment and the morphological amplitudes and correlations. The amplitudes defined the range of environmental or morphological possibilities, while correlations described the redundant effect (RE). This parameter was used to describe the environmental or morphological continuity, since the correlations or RE would be the consequence of the similarity between individuals (morphological RE), or the places where those individuals were located (environmental RE). Thus, the RE was necessary to establish the morpho-environmental similarity for each Gr. Therefore, the final formula for the FA used here was:

$$FA = \frac{\log[Env] - \log[Morph]}{Env - Morph}$$

where [Env] is the total environmental amplitude, [Morph] the total morphological amplitude, Env the average environmental correlation, and Morph the average morphological correlation per Gr. That is, the FA depends on the effect of the morphological amplitude and the correlation upon the environmental amplitude and correlation, for every Gr. While amplitude describes the total range of environmental ([Env]) or morphological ([Morph]) variability, the correlation represents the redundancy of the environmental (Env) or the morphological (Morph) responses. Higher environmental influences were associated with positive values for $\log[Env] - \log[Morph]$, or for $Env - Morph$. In contrast, negative values are more relevant for the morphological results. The quotient between these differences indicates the influence between the amplitude (total range) and the relative values (RE involved in the environmental or morphological results): FA near 0 means very similar influence of both (environmental and morphological) in terms of amplitude (potential range of responses) and correlation (continuity and similarity -RE- in the responses). Therefore, the stronger the asymmetries of the FA values, the further from 0 these values were. Amplitude and correlation ranges were described by bar diagrams per Grs to better illustrate their amplitude or correlation influence in the FA value. The dispersion, or difference $\log[Env] - \log[Morph]$ or $Env - Morph$, was also represented by a bar diagram. Such representation was very useful to show the differences of FA among Grs. Finally, the FA description was finalised with box diagrams, where means, standard deviations, and amplitudes per Gr were given.

3. Results

3.1. Morpho-taxonomic analysis

The diagnostic characters used to segregate specific and infraspecific taxa in *Jasione* (Appendix A) do overlap,

so species delimitation being obviously difficult. This ambiguity in the taxonomic segregation was the reason for the present morpho-environmental analysis, which was carried out at species level. Even so, all the areas referred to for species and infraspecific taxa were obviously analysed (Appendix B).

Based on our morpho-taxonomic analysis, two taxa were reduced to synonyms. The morphological characterization of *J. sphaerocephala* showed total overlapping with *J. montana*, more so than with *J. laevis*. Brullo et al. (1981) suggested placing this taxon between both species, preferably closer to *J. laevis*. The holotype of *J. sphaerocephala* from the Calabrian region ("Calabria meridionale, fra Monasterace e Stilo, 9/7/1978, Brullo and Marcenò") was investigated, as well as other specimens from southern Italy (and similar habitats). No other material suggests its occurrence in the area and no supplementary or additional information was pointed out by Pignatti (2018), to consolidate the existence of this taxon. Morphological characters such as colour of petals, tap-root diameter, or leaf dimension are not reliable because they overlap with *J. montana*. Based on these descriptions and the analysis of individuals from the region, we decided to place *J. sphaerocephala* under the synonymy of *J. montana* var. *montana*. A similar solution was found for *J. idaea* Stoj. All morphological characters overlapped between *J. idaea* and *J. supina* but for the persistence of hairs all-over the plant in *J. idaea*. Even so, the presence of hairy peduncles was observed in some specimens of *J. supina* (FI, Lucania (Basilicata) tem. Serino; E00913678, Pelister, Baba mts.). Therefore, we decided to consider *J. idaea* a synonym of *J. supina* (Appendix A)

The dendrogram obtained from the average values per species from the morphological matrix is in Figure 1a. Based on the species differentiation and the morphological similarities, five primary clusters of taxa were recognised (C1–C5), grouped into two primary branches: one involving the alpine and subalpine taxa (C5: *J. bulgarica* - *J. orbiculata* - *J. cavanillesii* - *J. supina*), plus the endemic group from the SE mountains of Spain (C4: *J. foliosa* and *J. mansanetiana*); the other branch including *J. heldreichii*, *J. montana*, *J. corymbosa*, *J. penicillata*, *J. laevis*, *J. crispa*, *J. maritima*, and *J. sessiliflora* in clusters C1–C3. PCAs for each morpho-taxonomic cluster are also represented in Figure 1a. The DCA for these five clusters is in Figure 1b, and the results confirm those in the similarity analysis. While the first root of the DCA segregates *J. foliosa* and *J. mansanetiana* from the other species, the second root distinguishes *J. montana* - *J. heldreichii* from the other ones. Nevertheless, according to the low F-values (Table) and the relevant overlap observed between clusters, there was not enough significance to segregate all the taxa. The height of individuals, the length of peduncles (lp), and the

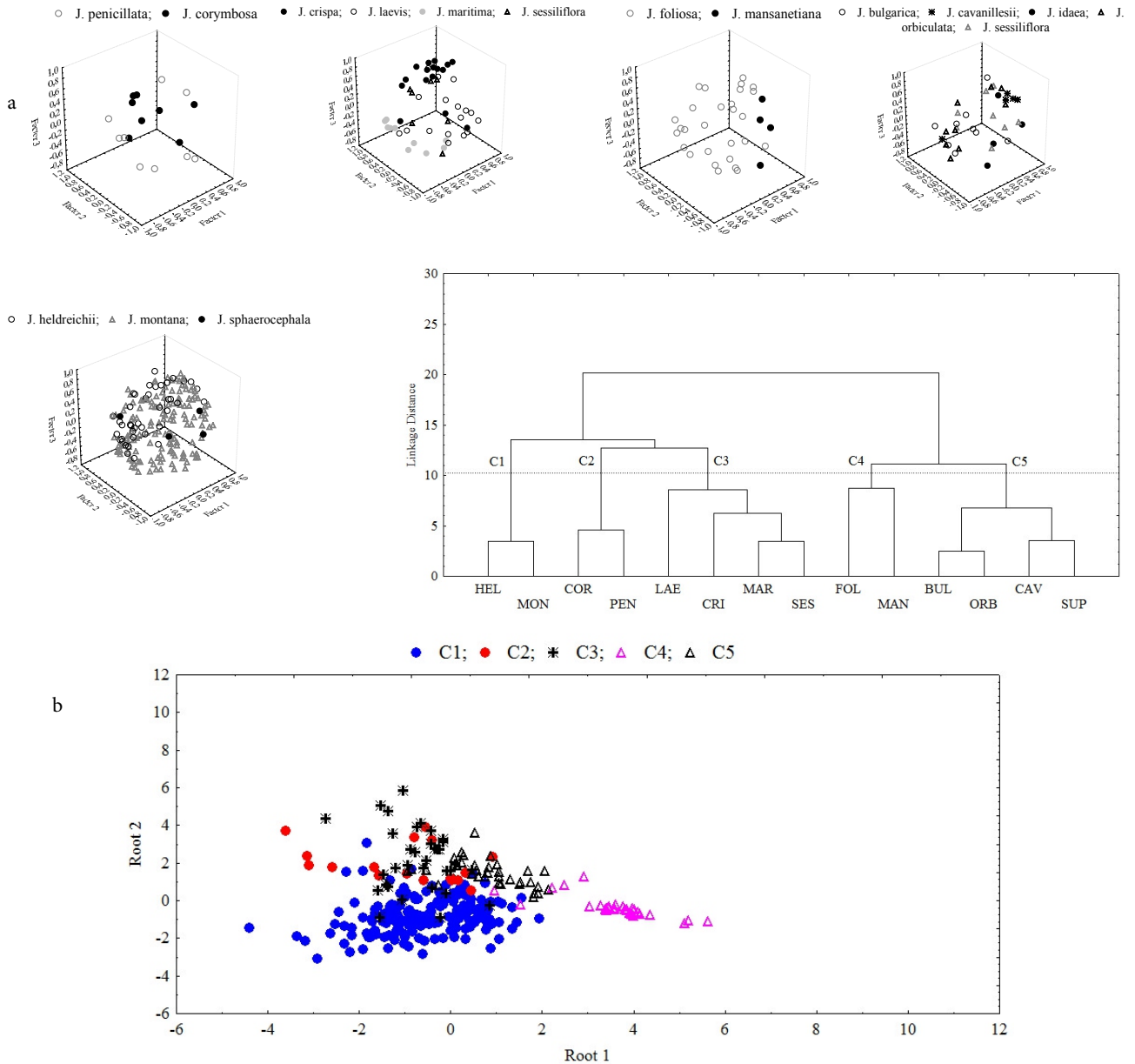


Figure 1. a. The analysis of similarity of the average morphological values per species reveals five clusters (C1 to C5), and the PCAs graphics confirm the correlation between taxa involved (in these graphics *J. sphaerocephala* e *J. idaea* were included to confirm the synonymization with *J. montana* and *J. supina*, respectively); b. Based on the analysis of similarity, the five clusters were discriminated (DCA), and C4 is distanced from the other clusters for Root1, while C1, on one side, and C2, C3 and C5, on the other, show opposite responses for Root2 (BUL, *J. bulgarica*; CAV, *J. cavanillesii*; COR, *J. corymbosa*; CRI, *J. crispa*; FOL, *J. foliosa*; HEL, *J. heldreichii*; LAE, *J. laevis*; MAN, *J. mansanetiana*; MAR, *J. maritima*; MON, *J. montana*; ORB, *J. orbiculata*; PEN, *J. penicillata*; SES, *J. sessiliflora*; SUP, *J. supina*); c the realized map of distribution for the specimens analysed (● C1; ● C2; * C3; △ C4; △ C5).

presence and length of hairs at the margin of calyx teeth (Pcd), proved to be the most discriminant variables.

The statistical values (mean, median, minimum, maximum, percentile 10%, percentile 90%, and standard deviation) per taxon that support the results exposed in Figures 1a–1c are in Appendix D.

Taxa organization along the clusters is revealing differences. While greater differences were detected in the group of clusters C1–C3 (larger dissimilarities were obtained in this case), the same was not observed for the other branch (C4–C5). In this last group of clusters, alpine and subalpine taxa showed high similarity and grouped in

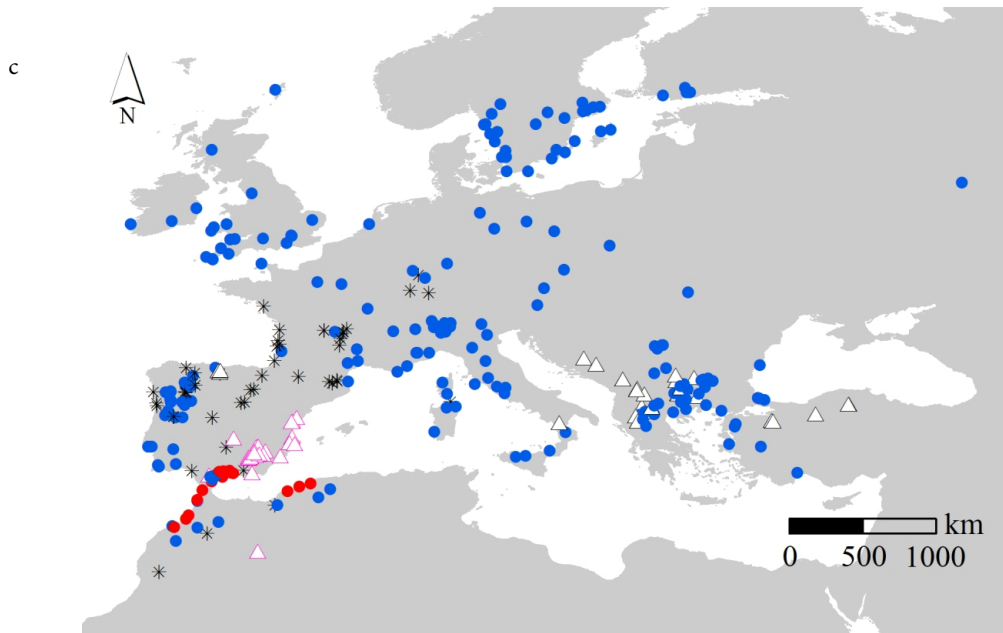


Figure 1. (Continued).

cluster C5, in spite of their disjunct distribution in the west (*J. cavanillesii*) and the east of the Mediterranean region and surrounding mountains (*J. bulgarica* - *J. orbiculata* - *J. supina*). Interestingly, the similarity of taxa included in cluster C1 (*J. montana* and *J. heldreichii*) revealed the widest presence along the CDA, in contrast with the other clusters, especially when compared with C5. The map for the realized distribution of specimens analysed (in this case, by groups of clusters C1-C3) is exposed in Figure 1c.

3.2. Morphological analysis

As explained in the methodology, no previous differentiation by species is involved in this morphological approach. Nevertheless, the results obtained are in the line of those showed in the taxonomic characterization. Here, again, there are two major primary clusters in the dendrogram not grouped by species obtained from the morphological matrix (Figure 2a). In this case, and in the same line of the results obtained from the morpho-taxonomic characterization, the first of the two branches (G1-G2) is dominated by *J. heldreichii* and *J. montana* (49% and 48%, respectively; see Appendix D-). The second branch is clearly much more complex (G3-G6). Two circumstances could explain the complexity of this second cluster of individuals: (1) the presence of *J. montana* and *J. heldreichii* is very heterogeneous along this second branch, with very variable frequencies; and (2) the presence of *J. foliosa* - *J. mansanetiana* is clearly divergent and closely grouped. Nevertheless, along these two primary clusters six potential morphogroups, Gr1-Gr6, are clear. The dendrogram from their average values (Figure 2b) does contribute to better

understanding the results. Two primary branches and four sets of Grs are clear: Gr1-Gr2, in the first branch; Gr3, Gr4-Gr5, and Gr6, in the second. In this second branch, Gr4-Gr5 indicate intermediate morphological response between the divergent Gr3 and Gr6. Taxa percentage per Gr is given in Appendix D.

The correlation analysis for the six Grs and each species is represented in the PCAs of Figure 3. While Gr1 and Gr2 are characterized by the clustering of *J. montana* (48% of locations analysed for this species), *J. heldreichii* (49%), and *J. laevis* (56%), Gr4 and Gr5 include *J. bulgarica* (44%), *J. heldreichii* (41%), *J. montana* (43%), and *J. orbiculata* (44%). The strong presence of *J. bulgarica* and *J. orbiculata* in the latter two Grs brings them closer to the divergent morphogroups, Gr3 and Gr6. These have the lowest percentages for *J. heldreichii* and *J. montana*: 10% and 7%, for Gr3, and 0% and 3% for Gr6. The highest percentages in Gr3 were for *J. foliosa* (100%), *J. mansanetiana* (100%), *J. supina* (67%), and *J. cavanillesii* (60%); and, finally, Gr6 was constituted by *J. penicillata* (100%), *J. crispa* (88%), *J. sessiliflora* (71%), and *J. maritima* (56%). Therefore, *J. montana* and *J. heldreichii* showed a very remarkable agglomerative effect since they are present along the six Grs.

Those higher percentages of *J. heldreichii* and *J. montana* along Gr1-Gr 2 and Gr4-Gr5 bring them closer in the PCA. In this 3-factorial approach, the divergent Gr3 is sustained by *J. orbiculata* and *J. supina*; while Gr6 is supported by *J. maritima*, *J. sessiliflora*, *J. crispa*, and *J. penicillata*. The morphological proximity between Gr1-Gr2 and Gr4-Gr5 is confirmed in the DCA (Figure 4). The cloud for Gr6 is clearly wider than the others. Gr3 clusters

Table. The DCA for taxonomical, morphological, and environmental matrices per clusters (C1–C6) and per Grs (Gr1 – Gr6) revealed several differences: F to remove is clearly lower for the environmental characterization of the Grs when compared with the taxonomic and morphological approach; also, the p-values are higher for the environmental variables, in contrast with the taxonomical and morphological values; the morphological characterization shows the most discriminant values when compared with the taxonomic or environmental results. Very significant redundancies are observed for all approaches, which explains the F-values obtained.

Var.	Wilks' lambda	Partial	F-remove	p-value	Toler.
Taxonomical (clusters C1 to C6)					
Ip	0.1058	0.6862	32.2368	0.0000	0.8064
Pcd	0.1004	0.7233	26.9710	0.0000	0.8847
Sn	0.0923	0.7869	19.0941	0.0000	0.9706
Ll	0.0887	0.8182	15.6645	0.0000	0.5702
Ba	0.0870	0.8344	13.9909	0.0000	0.5408
Bnl	0.0835	0.8693	10.6027	0.0000	0.7176
Bn	0.0825	0.8803	9.5877	0.0000	0.9103
PLn	0.0815	0.8913	8.6021	0.0000	0.9307
Pi	0.0795	0.9136	6.6691	0.0000	0.7697
La	0.0741	0.9805	1.4033	0.2330	0.4867
Morphological (Gr1 to Gr6)					
Pcd	0.0901	0.3655	97.8928	0.0000	0.9894
Pi	0.0648	0.5083	54.5606	0.0000	0.8783
Ip	0.0505	0.6526	30.0218	0.0000	0.8902
Ll	0.0370	0.8891	7.0323	0.0000	0.7336
Bnl	0.0378	0.8717	8.3025	0.0000	0.9630
La	0.0360	0.9145	5.2710	0.0001	0.7449
Bn	0.0351	0.9390	3.6628	0.0032	0.9373
Sn	0.0346	0.9528	2.7966	0.0175	0.9346
PLn	0.0337	0.9761	1.3826	0.2308	0.8820
Environmental (Gr1 to mGr6)					
p3	0.4321	0.9062	6.4621	0.0000	0.1747
p8	0.4235	0.9245	5.0962	0.0002	0.2255
tM1	0.4230	0.9257	5.0087	0.0002	0.0235
p4	0.4215	0.9288	4.7817	0.0003	0.1198
tM6	0.4179	0.9369	4.2054	0.0010	0.0200
b8	0.4143	0.9450	3.6308	0.0033	0.3598
b7	0.4103	0.9543	2.9913	0.0118	0.0647
b4	0.4081	0.9595	2.6355	0.0237	0.0559
p10	0.4064	0.9633	2.3765	0.0389	0.2005
alt	0.3946	0.9921	0.4965	0.7788	0.1085

in one extreme of the cloud, while Gr1–Gr 2 and Gr4–Gr5 showed clear grouping along both roots of this analysis. The most discriminant variables are Pci, Pcd, and Ip ($F=97.89$, $F=54.56$, and $F=30.02$ respectively, p -level <0.000), but not enough significance was obtained as a consequence of the

considerable overlapping between Grs (Table). Based on these discriminant morphological variables (Pci, Pcd, and Ip), Gr6 and Gr3 are represented by small plants, average length of the peduncle less than 50 mm, when compared with the other Grs.

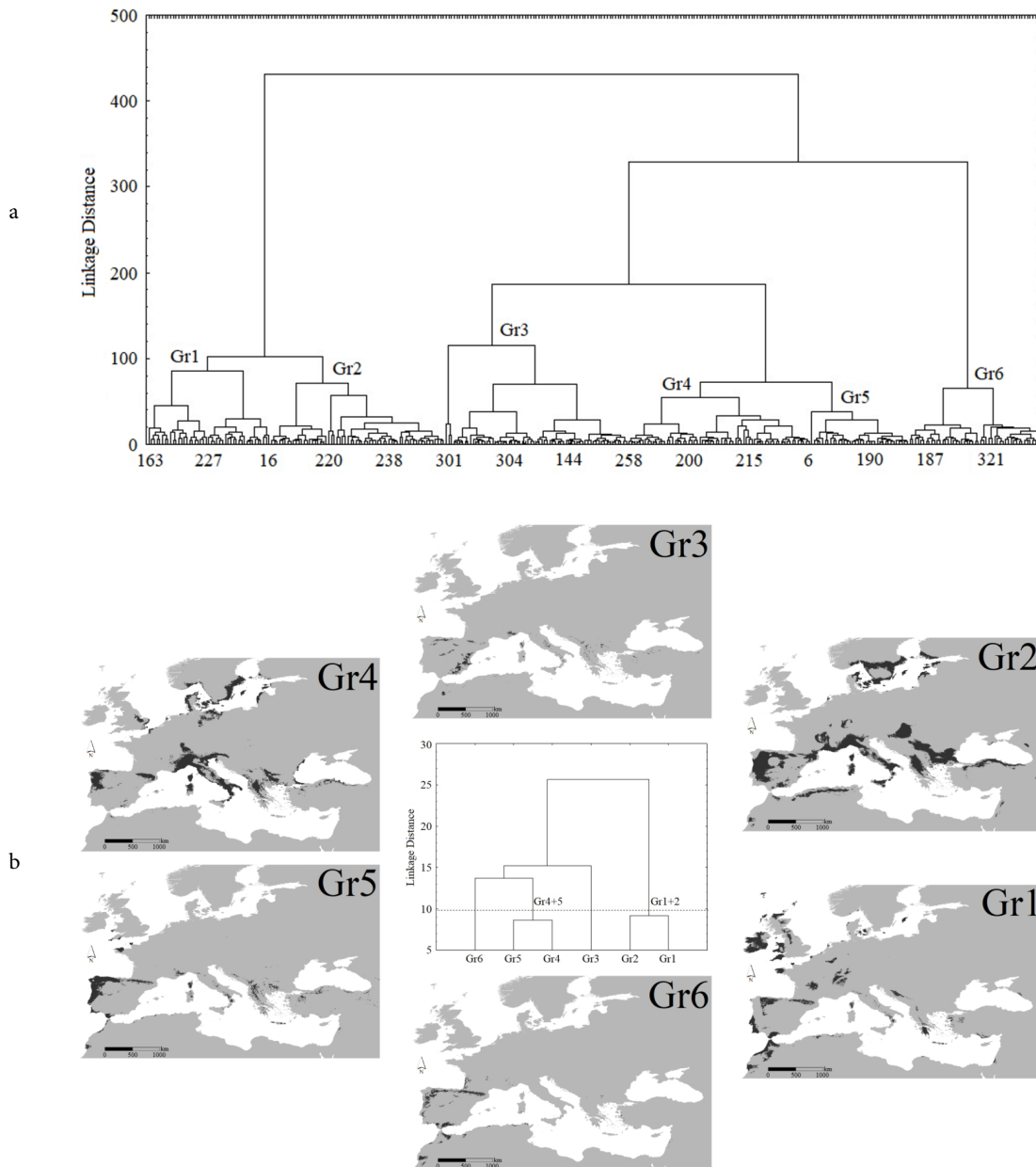


Figure 2. a. There are six Grs (Gr1–Gr6) in the dendrogram obtained from the similarity analysis performed from the morphological matrix; b. A similarity approach for the average values per Gr segregate Gr1 and Gr2 was elaborated, and Gr4 and Gr5 have intermediate morphological variability between Gr6 and Gr3. These results agree with the potential distribution maps for these Grs (Gr3 and Gr6 show the most restricted distribution, in contrast with Gr1–Gr2 and Gr4–Gr5).

This morphometric analysis reveals the presence and length of hairs towards the peduncle apex or calyx teeth margin as the most discriminant characters. Nevertheless, and as referred to above for Gr6 and Gr3, the height of plants (lp) is also important in this Grs differentiation. In fact, the F-values obtained in this analysis are clearly higher than the same values for the taxonomic approach (Table).

3.3. Potential habitat distributions and modelling scenarios

The maps of the potential habitat suitability (probability of occurrence $\geq 50\%$) per Gr (Figure 2b) were elaborated. The narrowest distributions are those of Gr6 in the western part of the Mediterranean basin, and Gr3 with a distribution extending to the eastern Mediterranean (the disjunct distribution between *J. cavanillesii* and *J. bulgarica* - *J.*

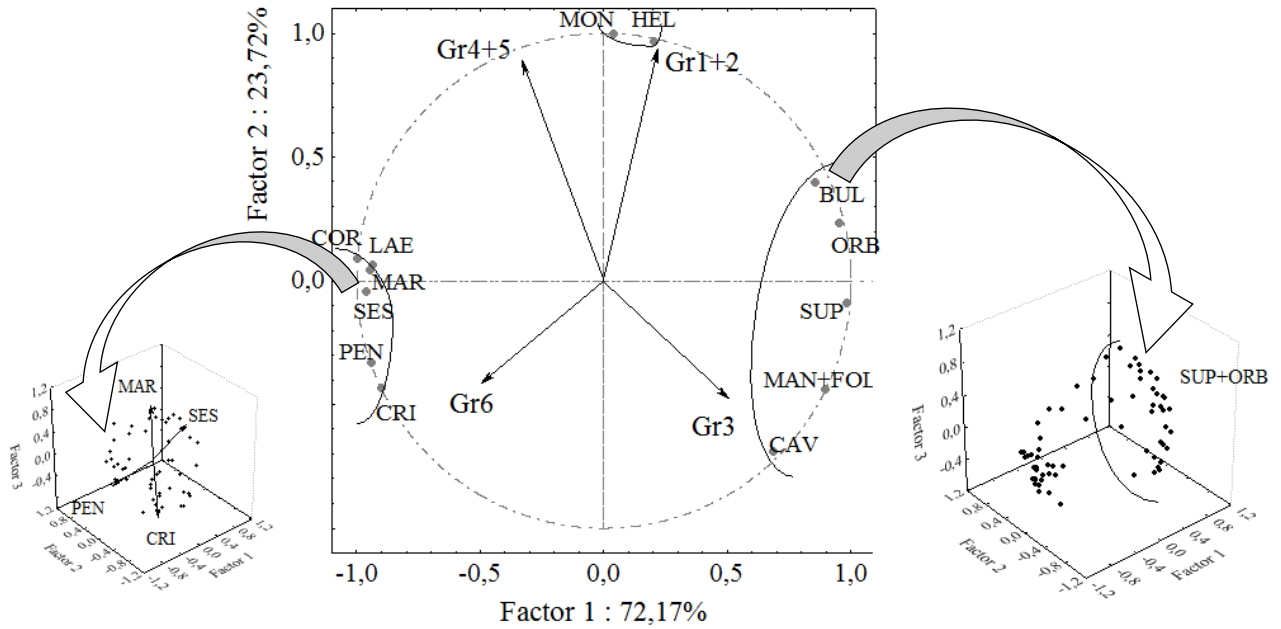


Figure 3. The PCA between average variations per morphogroup and the percentages of each species explain the potential distributions for Gr1-Gr2-Gr4-Gr5, characterized by higher percentages of *J. montana* (MON) and *J. heldreichii* (HEL), in contrast with Gr3 [where most of *J. bulgarica* (BUL) – *J. cavanillesii* (CAV) – *J. foliosa* (FOL) – *J. mansanetiana* (MAN) – *J. orbiculata* (ORB) – *J. supina* (SUP) are concentrated] and Gr6 [with the predominance of *J. corymbosa* (COR) – *J. crispa* (CRI) – *J. laevis* (LAE) – *J. maritima* (MAR) – *J. penicillata* (PEN) – *J. sessiliflora* (SES)]. In the case of Gr3, *J. orbiculata* and *J. supina* show higher correlation values, while for G6 divergences in correlation are exhibited by *J. crispa*, *J. maritima*, *J. penicillata* and *J. sessiliflora*.

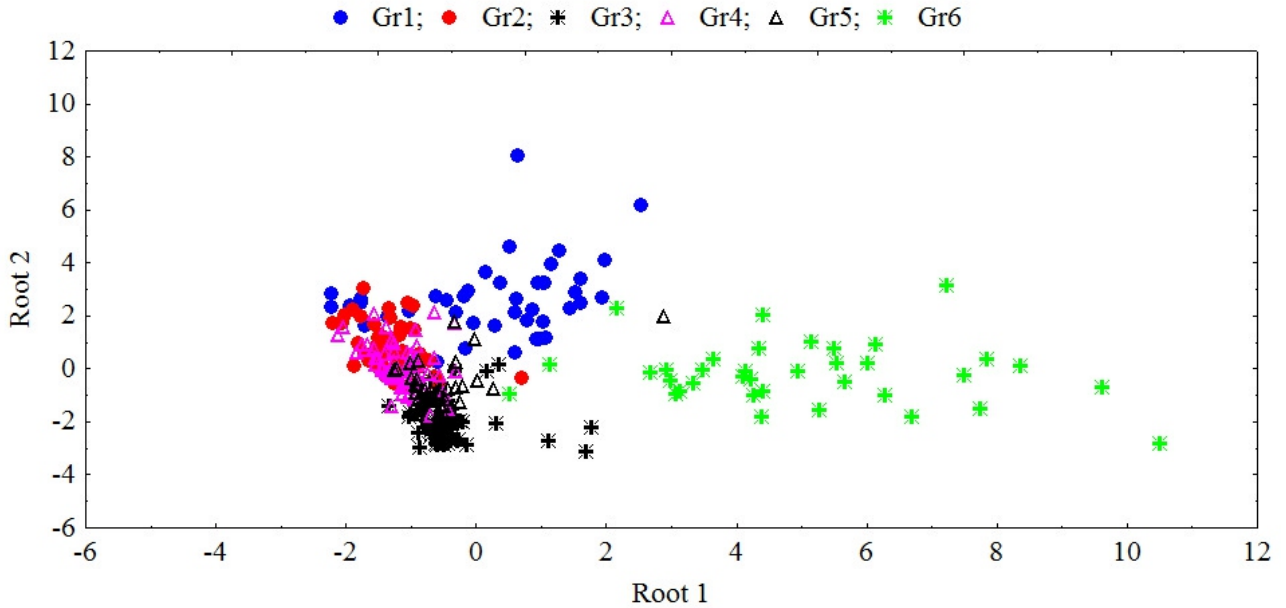


Figure 4. The DCA for the morphological variability per Gr reveals the significant differences between Gr6 and the other Grs along Root 1. Root 2 separates Gr1 from the others, more so from Gr 3.

orbiculata - *J. supina*, referred above). A distribution ranging from the Mediterranean basin, to north-western France and south of England is given for Gr5. This potential distribution is intermediate between that given for Gr6 and Gr3. The

widest distributions are those of Gr1, Gr2, and Gr4, with occurrences along western Eurasia and NW Africa.

The environmental variables used were: alt (m), b4, b7, b8, P (mm), Tmax (°C), Tmin (°C). The modelling for the

past scenario confirmed the SW Mediterranean basin as the biological reservoir or genetic pool for the individuals analysed (Figure 5). SW of the Atlantic Eurasian region (S Great Britain and SW France), the Iberian Peninsula and the Balearic Islands, and NW Africa were confirmed in all projections for every Gr.

3.4. Fluctuating asymmetry (FA)

The discriminant values obtained for the morphological analysis are clearly higher than those of the environmental approach (average precipitation for March (p3), $F=6.46$, and average precipitation for August (p8), $F=5.10$, $-p\text{-level}<0.000$ in both cases-) (Table). The reason for this significant decrease must be the most remarkable overlap between the environmental variables per Gr (Figure 6). In other words, the environmental characterization for the Grs, previously carried out in the morphological analysis, is also not statistically significant.

Observing the graphic for the DCA (Figure 6), the environmental overlap between Grs is evident. In this sense, the first root shows some differences between Gr6 and Gr4, in contrast with root 2, where no significant differences are represented. Only Gr3 and Gr6 had more concentrated results along the years, since their average

precipitation in August is lower (36.24 mm for Gr3, and 37.23 mm for Gr6) than the other Grs. The higher average precipitation in March (84.23 mm) of Gr6 also separated it from the other.

The morpho-environmental FA diagrams in Figures 7a–7d reveal wider amplitudes for all the Grs, and, at the same time, confirm the DCA representation of Figure 6. Two values for FA per Gr are positive (Gr6, $FA=0.20$; Gr1, $FA=0.28$). The FA values for the other Grs are negative (Gr3, $FA = -0.26$; Gr2, $FA = -0.29$; Gr5, $FA = -0.40$; Gr4, $FA = -3.03$). Some morpho-environmental restrictions were observed for Gr5 only, but not very significant (values very close to 50% of the potential amplitude). Significant asymmetries were detected between correlations and amplitudes. Only Gr6 has high morphological correlation, while higher environmental amplitudes are detected for Gr4. Morphological and environmental REs are clearly low for Gr1, Gr2, Gr3, and Gr5. Disparity of morphological combinations characterizes these Grs. The very low FA value for Gr6 is explained by the very similar values obtained for $\log[\text{Morph}]$ and Morph (morphological continuity or high RE along the morphological range), while the same parameters for Env were clearly different

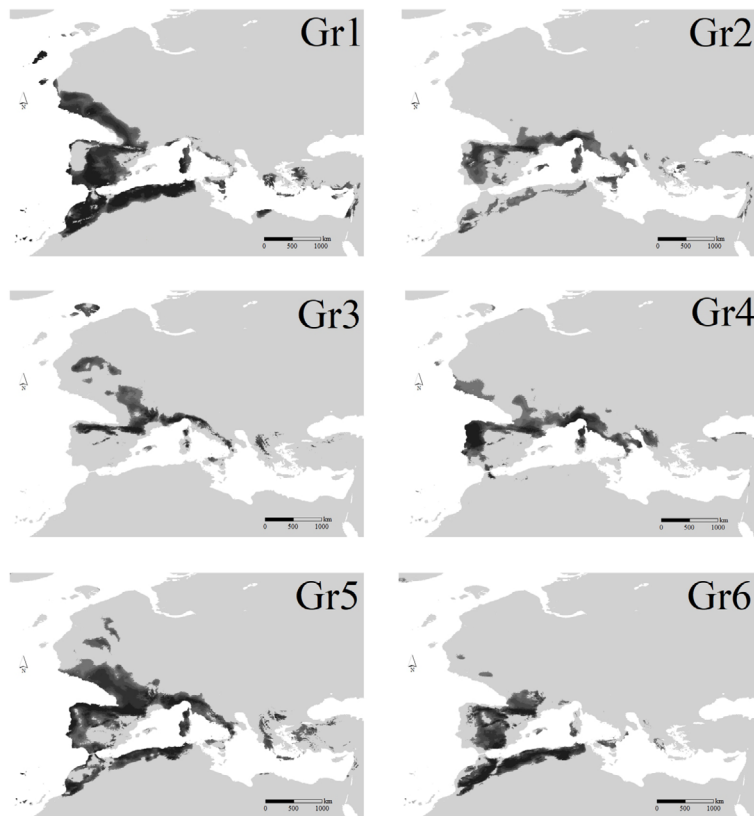


Figure 5. Potential habitat areas for Last Glacial Maximum scenario. Under this climatic scenario, the persistence of *Jasione* along the SW of the current potential distribution is confirmed.

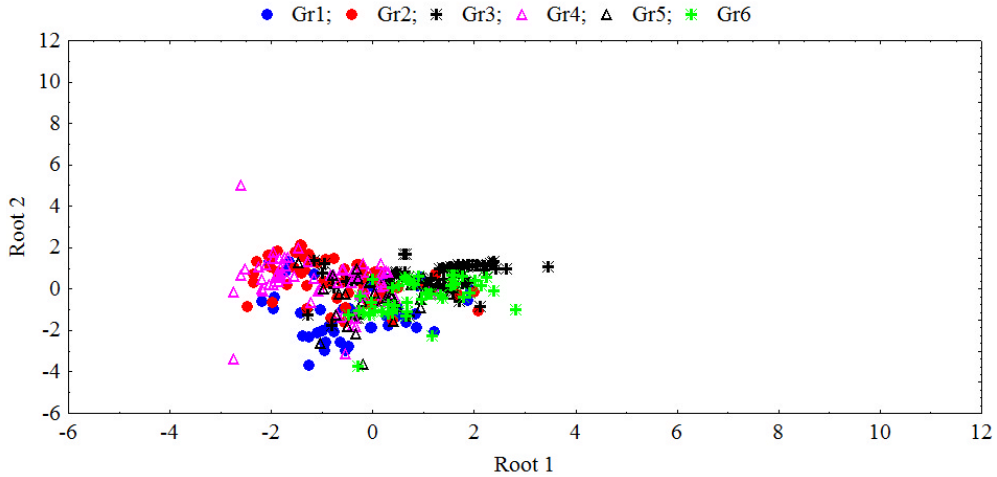


Figure 6. Wider variability is observed along the Root 1 for the morpho-environmental DCA, but the clear overlapping between Grs for both Roots is very significant.

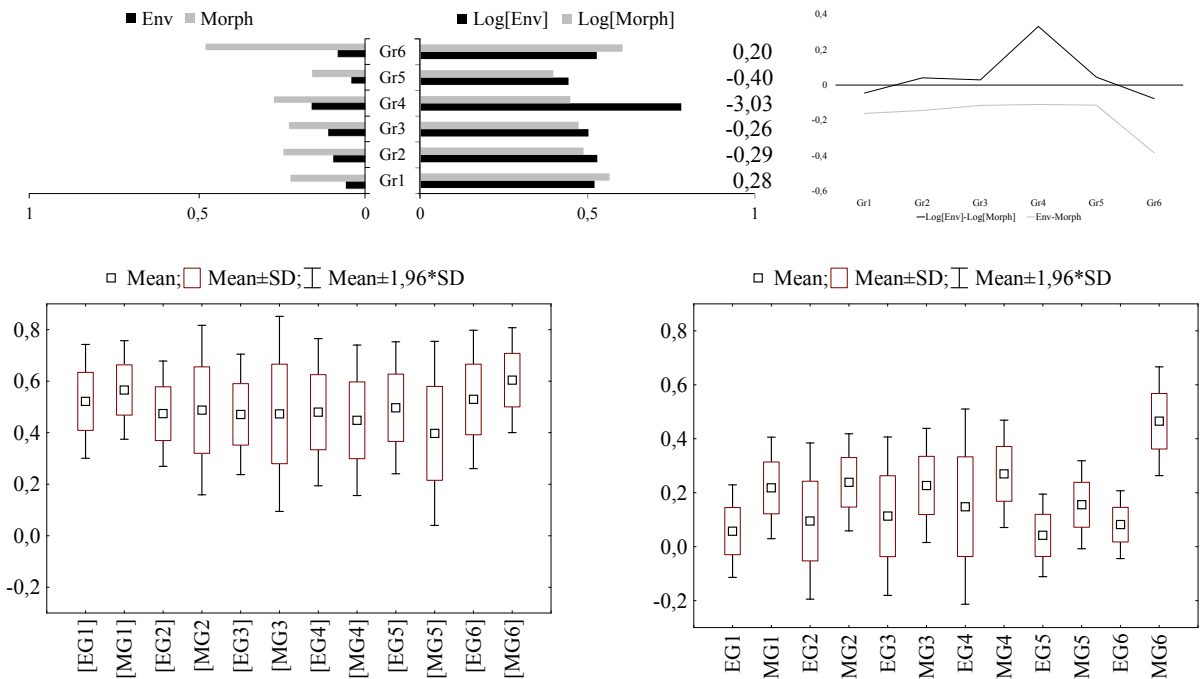


Figure 7. a. Bars diagram for amplitude (Log[Env] and Log[Morph]) and average correlation (Env and Morph) values per Gr (Gr1-Gr6), and their respective FA on the right side of this graphic; b. Dispersion graphic for Log[Env] - Log[Morph] and Env - Morph, per Gr; c boxes plots for environmental ([EG1]-[EG6]) and morphologic amplitude ([MG1]-[MG6]); d. Environmental (EG1-EG6) and morphological correlation (MG1-MG6) (E, environmental; M, morphological; G, morphogroups -Gr-).

(low RE, or very low environmental continuity along the environmental range). In other words, the morphological response in Gr6 is much more continuous (50% of the morphological responses are continuous along the potential morpho-environmental range) than the morphological response for the other Grs. However, very diverse environmental responses are observed throughout the six Grs. Based on these results, Gr6 is clearly the most

morpho-environmentally stable Gr: under very different environmental combinations, morphological responses are also diverse but without significant divergences.

4. Discussion

The morpho-environmental results obtained here could be one more example of those taxa circumscribed in the new Miocene lineages (in this case included in Wahlebergieae

group -Roquet et al., 2009-) exposed to the Pliocene-Pleistocene environmental variations (Benítez-Benítez et al., 2018, Vargas et al., 2018). The Pliocenic maritime transgression along the Mediterranean basin, and the subsequent Pleistocene glacial-interglacial dynamic (Hellwig, 2004) may be responsible for recent floristic responses in western Eurasia (Feliner, 2014). The onset of the Mediterranean climate in the Miocene (Cavender-Bares et al., 2016; Vargas et al., 2018), more specifically in the Serravallian-Tortonian periods (Rundel et al., 2018), correlates with the recent and rapid origin of *Jasione* in the Mio-Pliocene (Sales et al., 2004; Pérez-Espona et al., 2005), and therefore, difficulties should be expected in segregating species and infraspecific taxa (Grundt et al., 2006; Hoffmann et al., 2017). In fact, the taxonomic accounts published so far (Tutin, 1976; Damboldt, 1978; Sales and Hedge, 2001; Pignatti, 2018) showed great morphological overlaps for all taxa (see Table and Appendix A).

In this morpho-environmental characterization, two species, *J. montana* and *J. heldreichii*, had unique responses (Figures 1a, 2a, and 3), and the percentages of them per morphogroup confirmed their morpho-environmental agglomerative responses. At the morphological level, such divergence between *J. montana*-*J. heldreichii* and the other taxa were supported by the length of peduncle, and length of hairs of sepal's teeth (Table and Figure 4). Nevertheless, the discriminant values obtained were not particularly relevant to distinguish morphological differences. This lack of significant morphological differences explains, to some extent, the congregative effect of *J. montana* and *J. heldreichii* in relation to the other species.

The discrimination values obtained were higher in the case of the morphological analysis in relation to the taxonomical results (Table). The presence and length of hairs and the height of plants were the most discriminant variables for this characterization. These morphological variables were decisive to distinguish the divergences (Gr3 and Gr6), as well as differences between Gr5 and the other Grs (Gr1, Gr2 and Gr4). Short plants and the presence of hairs on the plant is a very common combination for Gr3, Gr6, and Gr5, in contrast to the other Grs, where these characters were largely variable. Similarity and agglomerative patterns for *J. montana* and *J. heldreichii* were morpho-environmentally confirmed.

FA proved very useful in the morpho-environmental description of the individuals analysed. Based on the differences between amplitudes (Log[Env]-Log[Morph]) and correlations (Env-Morph), three FA tendencies were observed: Gr1 and Gr6, where differences for amplitudes and correlations were negative; Gr2, Gr3, and Gr5, with positive differences for amplitude, and negative differences for correlation; and, finally, the case of Gr4, where differences for amplitude is also positive and remarkably

higher, when compared with Gr2, Gr3, and Gr5. Wider morphological amplitude or correlation displayed negative values for FA, in contrast with positive ones. In these last cases, environmental amplitude and correlation must be dominant in the morpho-environmental response of individuals. The morphological responses for Gr6 and Gr1 are very relevant. For Gr6, the morphological RE was c. 50%, in contrast with Gr1, where RE was close to 10%. In other words, while Gr6 showed more continuity in the morphological responses, Gr1 was associated with great dispersal of morphological combinations. This higher or lower continuity of the morphological responses resulted from interindividual variation and its developmental instability (Møller and Shykoff, 1999). In our case, neither morphological nor environmental divergences should be drawn from stable morphological or environmental responses per Gr. Such a result is possible only for high RE, since the continuity of response is the only way to avoid any morphological or environmental divergence. The percentages of taxa could explain better this hypothesis. In fact, *J. penicillata* (100%), *J. crispa* (88.24%), *J. sessiliflora* (71.43%), and *J. maritima* (55.56%) were included in Gr6 where morphological FA is very close to 0. The morpho-taxonomic characterization distributed these taxa in clusters C2 and C3. *J. montana* (48%), *J. heldreichii* (49%), and *J. laevis* (56,25%) were the most characteristic species for Gr1, included in C1 and C3. Gr6 was clearly more restrictive in terms of taxa, in contrast with Gr1 where more divergence should be expected at morphological level. In other words, both morphological and environmental similarities were decisive to describe a high RE and, consequently, FA values close to 0.

Very diverse morpho-environmental responses were observed for the other Grs (Figure 2b). For example, Gr1 showed very low RE (high morphological amplitudes and very low morphological correlations). The presence of taxa with distinct distributions for this Gr, such as *J. corymbosa* (C2), *J. laevis* (C3), *J. montana* (C1), and *J. heldreichii* (C1) could explain its low RE. Gr3 and Gr5 showed similar results, even more spread along the morpho-taxonomic clusters (C1-C6). On the other hand, Gr4 was remarkable for its widest environmental amplitude, which is confirmed by its potential range of distribution. On the contrary, narrow realized potential areas were observed for Gr3 and Gr5, with, totally or almost so along the Mediterranean basin. However, the standard deviation detected for these Grs (see box plots) for the morphological amplitude was clearly higher, as well as their average values. In Gr3 was concentrated the whole presence of *J. foliosa* and *J. mansanetiana* (endemic Iberian taxa), as well as relevant percentages of more two geographically restrict taxa, *J. cavanillesii* (also an Iberian endemic) and *J. supina* (eastern Mediterranean basin). These results described

major morphological divergences for Gr3. Something similar was observed for Gr5, where eastern (*J. bulgarica*, *J. heldreichii*, and *J. orbiculata*), and western Mediterranean basin species were included (*J. corymbosa*, *J. maritima*, and *J. sessiliflora*). In these cases, heterogeneity in the response is the main conclusion, i.e. remarkable asymmetries were detected between morphological and environmental responses. Only Gr6 seems to have low morphological asymmetric response for wide environmental diversity. Therefore, FA values were excellent parameters to quantify these differences.

Models on LGM scenario (Figure 5) show the morphological stability detected for Gr6. Under the LGM scenario, the potential area of distribution worked as apparent refuge for *Jasione*. Once again, morphological stability is confirmed, and this area could be described as an incipient regional target phenotype (Fusco and Minelli, 2010). In fact, no significant divergence was observed in the morphological analysis, when comparing Gr6 with the other Grs. On the other hand, the concentration of taxa in Gr6 also described differences about the other: relevant percentage of *J. penicillata*, *J. crispa*, *J. sessiliflora*, and *J. maritima* are represented in this Gr. Therefore, morphological stability is apparently canalizing morphological variability, revealing potential future target phenotypes. However, *Jasione* constitutes an example of a young lineage, and such morphological response would also be very recent.

The LGM modelling scenario provided remarkable information on the potential refugia areas (Médail and Diadema, 2009). More irregular geomorphology involves lower climatic impacts along interglacial periods (Bravo et al., 2008). According to the results obtained by this approach, the western Mediterranean basin showed available environmental conditions to accommodate germplasm of *Jasione*. Therefore, the probability of adding more morphogenetic information, especially under those glacial circumstances, could be responsible for positive FA, not only for Gr6 but also for Gr1, as accumulation of germplasm during such critical periods was also detected. These results also agreed with the distribution of cytotypes observed for *J. montana* (Castro et al., 2019): polyploid populations were detected for the western Iberian Peninsula, in contrast with the diploids in the remaining distribution area of the species. Traditionally, polyploidy and apomitic processes are associated with germoplasmatic colonization and diversification (Hojsgaard and Hörandl, 2015). Here, Gr6 was characterized by higher morphological continuity, the highest morphological variability, and major environmental discontinuity. Apparently, the frequency of polyploid taxa could be the consequence of hybridization and introgression by periodic colonization processes (Guo et al., 2012; Crowl et al., 2017). This hypothesis confirms

the refuge scenario for this area (western Mediterranean basin and the south of the European Atlantic region), as well as the incipient target phenotype.

These biogeographic results were also reflected in the FA values. Only Gr6 showed some stability ($\log[\text{Morph}] = 60\%$, and $\text{Morph} = 46\%$), but this is not enough to establish any consolidated target phenotype. Morpho-environmental stability involves the necessary morphological continuity along environmental variability, and that was not proved here. The environmental ranges detected along the occurrence area and the Grs observed were still far from any morpho-environmental stability. Western Mediterranean basin and SW Atlantic region (represented by Gr6, in current scenarios, and Gr6 and Gr1 for LGM environmental conditions) allowed accommodation for this germplasm during the Pliocene-Holocene period. The remaining Grs were exposed to temporary environmental restrictions and the important restriction or even the lack of this germplasm.

These results are in line with those obtained for *Baldellia*, *Dianthus*, *Scilla*, or *Ononis*, in the same area (Rocha et al., 2012b; Almeida da Silva et al., 2014; Rocha et al., 2018; Castro et al., 2019, 2020). In these cases, morphological fluctuations also overlapped, and no significance was detected for their environmental characterization either. Microevolutionary processes (Mayr, 1982; Fernández-Mazuecos and Glover, 2017) could explain these behaviours. In these cases, nongenetic reasons must be involved, in order to establish potential future genetic strategies (Larsen, 2005). Therefore, these morpho-environmental variations must be interpreted as a response to intrinsic and extrinsic signals. Both in these examples and in the present work, the morpho-environmental FA would also be far from any stability and target phenotype. In fact, in those cases it was difficult to identify the infrageneric taxa and there was accumulation of germplasm along the Mediterranean basin—both observed in *Jasione*. Thus, this confirms the trend to higher stability (FA values close to 0) in this area.

Our morpho-environmental results could be regarded as a response of morphology to the environmental dynamics during the Plio-Holocene, according to the hypothesis of the Miocene potential origin explained above. *Jasione* is a genus associated with nanophanerophytic shrubs, grey grasses, rupicolous, or sandy plant communities (Sales and Hedge, 2001; Sopotlieva et al., 2016; Cerabolini et al., 2017; Gajewski et al., 2017; Mahdavi et al., 2017). Such community diversity would be decisive to guarantee its migrations under climatic changes: richness of herbaceous life forms (Tomiolo and Ward 2018), ability for propagule fast migrations (Matlack, 1994), or because of their biological capacities to colonize alpine environments (Alexander et al., 2015). In this sense,

the genetic flux involved along such glacial-interglacial processes (García-Gallardo et al., 2018; Suc et al., 2018) caused remarkable changes in the vegetation. Taiga/mountain woodlands and pockets of temperate forests in most western Eurasia (Willis and Andel, 2004) alternated with steppes and thermophile formations (Zeeberg, 1988). Such a vegetation dynamics must trigger relevant effects in migrations along southern Europe and NW Africa, for chamaephyte and geophyte plants like *Jasione*. Nevertheless, further discussion should be involved in the migration responses for this genus. In fact, alterations in the structure and organization of vegetation should also be decisive for the capacity to accommodate germplasm (Silveira et al., 2016). Under these critical environmental variations, new genetic combinations among different haplotypes, as well as introgressions and coalescence processes merged frequently (Schaal and Olsen, 2000; Widmer and Lexer, 2001; Prentice et al., 2008; Heuertz et al., 2010; Mattioni et al., 2013; Feliner, 2014; Jordan et al., 2018). In this sense, a complex and recent microevolutionary perspective (Mayr, 1982; Bell, 2016) could explain the difficulties in clearly segregating taxa.

Based on our results, we suggest the following germoplasmic trend: periodic advances and setbacks during the Plio-Holocene. In these dynamics, two species were apparently decisive: *J. montana* and *J. heldreichii*. Both were established as generalist responses, based on their wide distribution throughout the area of *Jasione*. The other taxa showed more, or much more, restricted distribution and two tendencies were apparent for these taxa: one concentrated in the west (*J. cavanillesii*, *J. corymbosa*, *J. crispa*, *J. foliosa*, *J. laevis*, *J. mansanetiana*, *J. maritima*, *J. penicillata*), and the other in the east (*J. bulgarica*, *J. orbiculata*, *J. supina*) of the range of the genus. A similar response was observed in the generalist responses since *J. montana* is much concentrated along the centre and the west of that area, while *J. heldreichii* occurs in the east. Also, the higher species diversification observed in the west agrees both with the results projected for FA and the distribution of germplasm under glacial scenarios.

The relevant Plio-Holocene glacial-interglacial processes explain previous results discussed for the morpho-taxonomic variability in *Jasione* (Bokhari and Sales, 2001; Sales et al., 2004; Rubido-Bará et al., 2010; Ferrer-Gallego et al., 2015). According to the results obtained, Plio-Holocene migrations of *Jasione* from the Mediterranean basin, especially from its most western area towards remaining western Eurasia, as a response to those glacial-interglacial rhythms could be suggested. From the phenotypic point of view (future molecular approaches must test this hypothesis), *J. montana* and *J. heldreichii* are the main morphological pool for this migration. Their distribution along the morpho-

taxonomic clusters confirmed their amalgamation effect. Also, biogeographical and ecological differences were clear when comparing them with the other species, both those occurring in alpine-subalpine regions (*J. bulgarica*, *J. cavanillesii*, *J. crispa*, *J. orbiculata*, *J. supina*), and those with more or less restricted distributions (*J. maritima*, *J. corymbosa*, *J. penicillata*, *J. foliosa*, *J. mansanetiana*). Such responses could be explained by the conservative hypothesis in high territories (Engler et al., 2011), or the existence of microrefugia (Gavin et al., 2014; García-Aloy et al., 2017; Coals et al., 2018). Finally, *J. laevis* has intermediate distribution concentrated in the west of *Jasione* range. Recent epigenetic processes subject to intense climatic changes must be associated with the morpho-environmental responses in this species, i.e. either environmental canalizations as response to the climate changes (Flatt, 2005), in order to trigger morphological responses to environmental dynamics (Hall et al., 2007; Palacio-López et al., 2015); or, incipient epigenetic divergence (Bossdorf et al., 2008), without apparent canalizations (Jablonka and Lamb, 2002). The results obtained also describe these dynamics, sustained by high morpho-environmental amplitudes and clearly more restricted morphological correlations, the only exception being Gr6.

In conclusion, *Jasione* seems to be associated with the evolution of the most recent Mediterranean flora, i.e. it is an example of Pliocene new lineages (Rundel et al., 2016). Remarkable instability was revealed by the morpho-environmental FA analysis, with morphological discontinuities and great environmental variability widely associated. Low RE and FA values distant from 0 described this discontinuity in the morphological variability. The recent origin of *Jasione*, and the abrupt and continuous climatic changes during the Plio-Holocene were the suggested explanation for these results. Both facts caused a complex process of possible epigenetic responses, here characterized by very significant morpho-environmental instability, and the lack of target phenotype. Two species revealed decisive, not only to maintain this morpho-environmental instability, but simultaneously to guarantee the response of *Jasione* to those permanent environmental changes. No overlapping in the geographic occurrences was observed in both cases, since *J. montana* spread along the centre and the west of the area, while *J. heldreichii* was confined to the east. The remaining taxa showed restricted distributions, as well as two tendencies in their occurrences, mostly totally or partially included in the Mediterranean basin: one, along the west, and the other in the east. This region was confirmed as the accommodation area for the genetic pool in the glacial periods, especially so the west Mediterranean basin. In fact, the Gr that represented this area (Gr6) was the one closer to zero for the FA values.

These migratory dynamics revealed decisive to guarantee the morphological variability along the range of *Jasione*. However, such a recent lineage has not yet established stable target phenotypes. Because of these circumstances, species or infraspecific taxa identification has turned out to be an extremely difficult task.

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Conflict of interest

The authors declare not having either conflict of interests or competing interests.

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