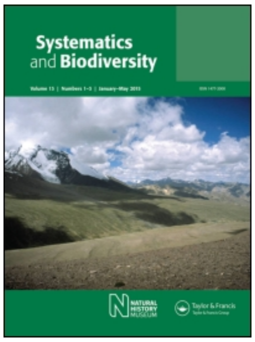

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Research Article

Species limits and morphometric and environmental variation within the South Andean and Patagonian *Mulinum spinosum* species-group (Apiaceae-Azorelloideae)

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Delimiting species is an important, but frequently difficult aspect of systematics that should be addressed using data from multiple sources. Here we combine morphometric analyses and environmental characteristics to delimit species in the South Andean and Patagonian taxonomically difficult species-group composed by *Mulinum spinosum*, *M. echinus* and *M. leptacanthum* (Apiaceae-Azorelloideae). Molecular phylogenies have shown that these three species form part of a polytomy together with other *Mulinum* species, and therefore these data are not useful for their delimitation. We include measurements of 25 morphometric variables from 163 herbarium specimens and perform univariate and multivariate principal component analysis (PCA) and discriminant analysis (DA) to establish the limits amongst the three mostly sympatric, morphologically similar, and phylogenetically unresolved species. We also use 19 bioclimatic and three geographic variables from localities of the specimens to infer environmental characteristics of the taxa and test their relation with morphological variation. Morphological evidence supports the inclusion of *M. echinus* within the morphologically and climatically variable *M. spinosum*, and rejects its recognition as a distinct taxon at any rank. On the contrary, *M. leptacanthum* is considered a morphologically distinct species, generally restricted to high altitude areas of the southern Andes with a cooler and wetter climate. Within the widespread *M. spinosum*, environmental gradients of precipitation and temperature relate to morphological gradients (e.g., in leaf and inflorescence sizes, in leaf acicularity, and in fruit-wing width). These last results showed that the large morphological variation in vegetative and reproductive characteristics of this species that grows in arid and semiarid habitats are related to regional climatic gradients that have probably been important in the evolution of this species' plasticity, diversification, and differentiation.

Key words: climatic gradients, discriminant analysis, fruit morphology, geographic range, leaf acicularity, leaf size, Patagonia, principal component analysis, southern Andes, species delimitation

Introduction

In recent years, species delimitation has re-emerged as a major topic in systematics (e.g., Camargo & Sites, 2013; Sites & Marshall, 2003; Wiens, 2007; Wiens & Penkrot, 2002). Coincidentally, new ideas that support novel types of research for species delimitation have been proposed (Wiens, 2007). First, a general agreement to consider species as lineages that has important implications for species delimitation (e.g., de Queiroz, 2007). Second, the introduction of coalescent simulations that incorporate data from multiple loci to test hypotheses about species limits (Carstens & Dewey, 2010; Fujita, Leache, Burbrink, McGuire, & Moritz, 2012; Knowles & Carstens, 2007). And last, the use of ecological information such as analyses of climatic data, in combination with molecular and morphological data, to help delimit species (Raxworthy, Ingram, Rabibisoa, & Pearson, 2007; Rissler & Apodaca, 2007). Therefore, the importance of using detailed comparative studies applying multiple methods and data sets for species delimitations has become generalized (Sites & Marshall, 2003; Wiens, 2007).

In particular, the use of environmental niche modelling has turned out to be a useful approach to understand how abiotic factors (e.g., temperature, precipitation, and seasonality) impact the geographic limits of lineages and species (Raxworthy et al., 2007; Rissler & Apodaca, 2007; Wiens & Graham, 2005). For example, between two genetically unresolved lineages with unique ecological niches, gene

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flow can be impeded, and this would support the two lineages as distinct even with limited genetic divergence (Raxworthy et al., 2007; Rissler & Apodaca, 2007). Also, closely related species often occupy different ecological niches and can exhibit differences in geographic distributions despite their shared evolutionary history (Grossenbacher, Veloz, & Sexton, 2014). Thus, environmental characteristics have now been used to understand species limits and support species delimitations in many recent works on taxonomically difficult species-complexes and cryptic species-groups, including animals: e.g., *Aneides* (black salamander; Rissler & Apodaca, 2007) and *Phrynosoma* (horned lizard; Piedra-Malagón, Albarrán-Lara, Rull, Piñero, & Sosa, 2016), and plants: e.g. *Crataegus* (Rosaceae; Leaché et al., 2009), *Nassauvia* (Asteraceae; Nicola, Johnson, & Pozner, 2014), *Orinus* (Poaceae; Su, Wu, Li, & Liu, 2015).

Mulinum Pers. (Apiaceae-Azorelloideae) is a taxonomically difficult genus of 10 species of subshrubs and cushion plants endemic to arid and semiarid regions of southwestern South America, from Bolivia to southern Chile and Argentina, that has only recently been revised comprehensively (Fernández, Ezcurra, & Calviño, 2017). Several of its species are ecologically important because they are dominant in open, arid or semiarid plant communities of the southern Andes and Patagonia. Some have been reported as nurse-plants, and as such, key elements in the maintenance of Andean and Patagonian biodiversity (Nuñez, Aizen, & Ezcurra, 1999). A number are also used in traditional medicine (Villagrán, Castro, & Sánchez, 1998b; Muñoz, Montes, & Wilkomirsky, 2001; Villagrán et al., 1998a; Martínez, 2003), or have value as forage (Cavagnaro, Golluscio, Wassner, & Ravetta, 2003; Seoane et al., 2011).

Within *Mulinum* there are groups of species that are difficult to identify because they show great morphological similarity and their ranges of distribution overlap. *Mulinum spinosum* (Cav.) Pers. together with the partially sympatric *M. echinus* DC. and *M. leptacanthum* Phil. of the southern Andes and Patagonia, form a species-group in which species limits are not clear, especially between *M. echinus* and *M. spinosum* (Constance, 1988). The three species have been separated in keys taking mostly into account leaf-size, leaf-shape, and numbers of flowers in the umbels (Constance, 1988; Martínez, 2003), but with clear superposition in the ranges of these characters. Chloroplast DNA phylogenies using sequence data from *rpl16* intron and *trnD-trnT* spacer (Nicolas & Plunkett, 2012) have shown that *M. spinosum*, *M. echinus*, and *M. leptacanthum* fall in a polytomy together with other *Mulinum* species. This polytomy is also recovered based on more rapidly evolving cpDNA markers (i.e., *rps16* intron, *trnQ-rps16*, *rps16-trnK(UUU)* exón 5′, *trnG(GCC)-trnS(GCU)*, and *rpl32-trnL(UAG)*) and/or the nuclear ribosomal ITS region, suggesting a rapid radiation involving these species (Fernández et al., submitted). Therefore, the available molecular phylogenetic information is not useful for species delimitation in this group of closely related species, which enhances the importance of detailed morphometric and environmental studies to delimit them and to make taxonomic decisions.

The species-group of *M. spinosum*, *M. echinus*, and *M. leptacanthum* is mostly distributed in arid regions of the southern Andes and Patagonia, from northern San Juan to southern Santa Cruz provinces in Argentina and bordering regions of Chile, between 28–54 °S and 74–62 °W (Fig. 1). They grow in sandy soils and rocky areas on flat steppes, mountain slopes and summits, from near sea-level to 4000 m. Within this species-group, *M. spinosum* is the morphologically most variable and geographically most extended of its species (Constance, 1988; Fernández et al., 2017; Martínez, 2003). Morphological variation has been related to geographic and climatic gradients in several widespread taxa of the southern Andes and Patagonia (e.g., *Embothrium*, Chalcoff, Ezcurra, & Aizen, 2008; *idem*, Souto, Premoli, & Reich, 2009; *Nassauvia*, Nicola et al., 2014; *Quinchamalium*, Lopez Laphitz, Ezcurra, & Vidal-Russell, 2015), so it seems important to explore this relationship in *M. spinosum*. Studies that determine the environmental characteristics (geographic and climatic) that are related to morphological variation within species can evidence the drivers of phenotypic differentiation that result in genetic adaptation or express in phenotypic plasticity (Chalcoff et al., 2008; Herrera, 2005; Lopez Laphitz et al., 2015; Paiaro, Oliva, Cocucci, & Sérsic, 2012; Sultan, 2000).

In addition, in its extended distribution *M. spinosum* shows latitudinal genetic structuring, with most haplotypes from Patagonia, Argentina, restricted to either northern or southern regions (Sede, Nicola, Pozner, & Johnson, 2012). This suggests that during the evolution of this species, populations of northern Patagonia may have been isolated from the southern ones by the Chubut and Deseado river basins (Sede et al., 2012). Therefore, it is interesting to analyse the morphological variation of *M. spinosum* in relation to environmental characteristics (geographic and climatic) taking into account these possible barriers to gene flow in the evolutionary history of this species. Genetic isolation between populations can result in morphological differentiation, as has been observed in other temperate woody plants (e.g., Douaihy et al., 2012; Sobierajska et al., 2016).

Southern South America provides a unique opportunity to study morphometric variation in relation to environmental gradients. From north to south it is traversed by the southern Andes, which attain more than 6000 m in their central part (e.g., Mt. Aconcagua, 32.65 °S, 6960 m) and 4000 m in the south (e.g., Mt. San Valentín, 46.59 °S, 4058 m), generating steep altitude gradients with associated temperature variation. From north-west to south-east, it is crossed by the arid diagonal of South America that

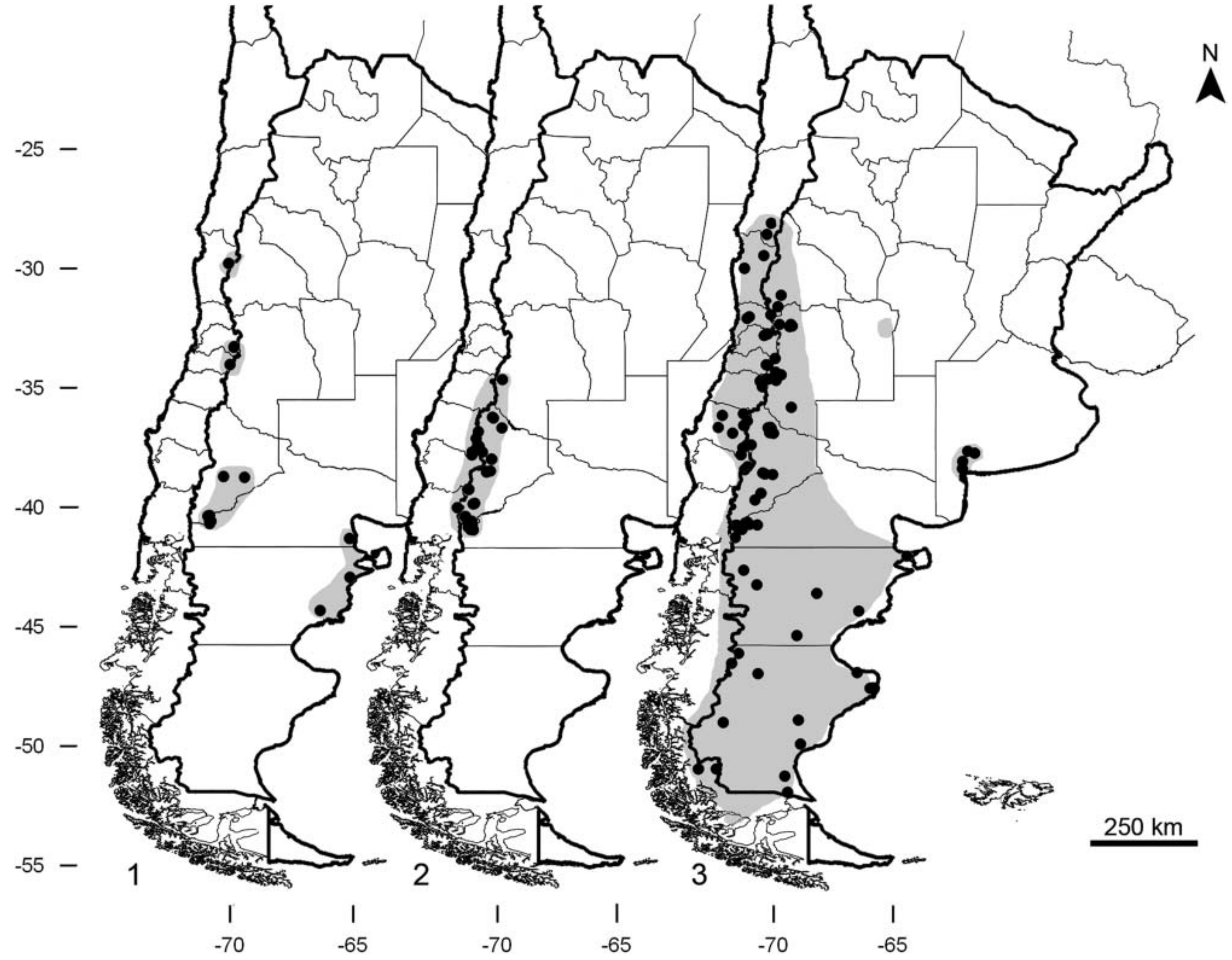

Fig. 1. Geographic distribution of the 163 specimens studied for (1.1) *Mulinum echinus,* (1.2) *M. leptacanthum*, (1.3) *M. spinosum.* Grey shades indicate the complete distribution of each species (Fernández et al., 2017).

comprises the arid and semiarid Pacific coastal Desert, Puna and Prepuna, Monte and Patagonia (nomenclature after Cabrera & Willink, 1980). These deserts and semideserts bordered by the more humid temperate Sub Antarctic forests to the south-west and subtropical Chaco forests to the north-east produce abrupt precipitation gradients. Thus, in southern South America these gradients of altitude, temperature and precipitation combine and produce complex patterns of environmental variation at different scales (e.g., Ferreyra, Cingolani, Ezcurra, & Bran, 1998; Souto et al., 2009).

In this work, we analyze morphological variation of vegetative and reproductive characters and environmental characteristics of *M. spinosum*, *M. echinus*, and *M. leptacanthum* by univariate and multivariate morphometric analyses to establish the limits amongst these species and to infer diagnostic characters for their identification. In addition, we study the effect of environmental gradients within the variable *M. spinosum* by integrating morphological information with climatic, geographic, and genetic data to infer drivers of phenotypic differentiation in this widespread species.

Materials and methods

Sampling

Morphological data of the species were obtained from 163 herbarium specimens from BA, BAB, BCRU, CONC, CORD, HAL, LIL, LP, LY, MA, MERL, NY, PRC, SGO, SI, (abbreviations according to Thiers, 2015) and from specimens collected in the field (SD1, see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2016.1273975). Limits amongst these species were established *a priori* on the basis of observations of all aspects of morphological variation in herbarium material. The morphological characteristics of the type-specimens of the taxon names and their established synonyms were also included in the analyses.

Dried material (e.g., leaves, inflorescences, and fruits) was boiled in water with a drop of detergent to rehydrate, and observations and measurements were made under a stereomicroscope. A total of 25 quantitative morphological characters (15 vegetative and 10 reproductive; Table 1, Fig. 2) were measured in all available specimens of *M. echinus* (N = 16) and in a selection of specimens of *M. leptacanthum* (N = 46) and *M. spinosum* (N = 101) that represent the morphological and geographic variation of each species. To analyse leaf shape, a mature leaf from each specimen was obtained from 3–5 nodes below an inflorescence and was mounted on paper and scanned. The digital images were used to measure perimeter, area, and perimeter/square root area ratio ($P/\sqrt{A}$) using Digimizer (Version 4.6.1; 2005-2016). This ratio is indicative of the degree of lamina incision, since the higher value of the coefficient, the greater the incision (Steinke, Premoli, Souto, & Hedrén, 2008). To assess degree of acicularity of leaf segments, the height/width ratio of the cross-section of the central segment of a mature leaf was calculated. Cross-sections were performed through the widest point between the middle and distal third of the foliar segment, and the height and width of the sections were measured. In some cases it was not possible to evaluate some of the reproductive characters because the material presented only flowers or only fruits, or because the specimens were immature. These missing values represent 17% of the total data of all the species, and for the multivariate analyses were replaced by mean-substitution (i.e., by the mean of the available cases for the variable).

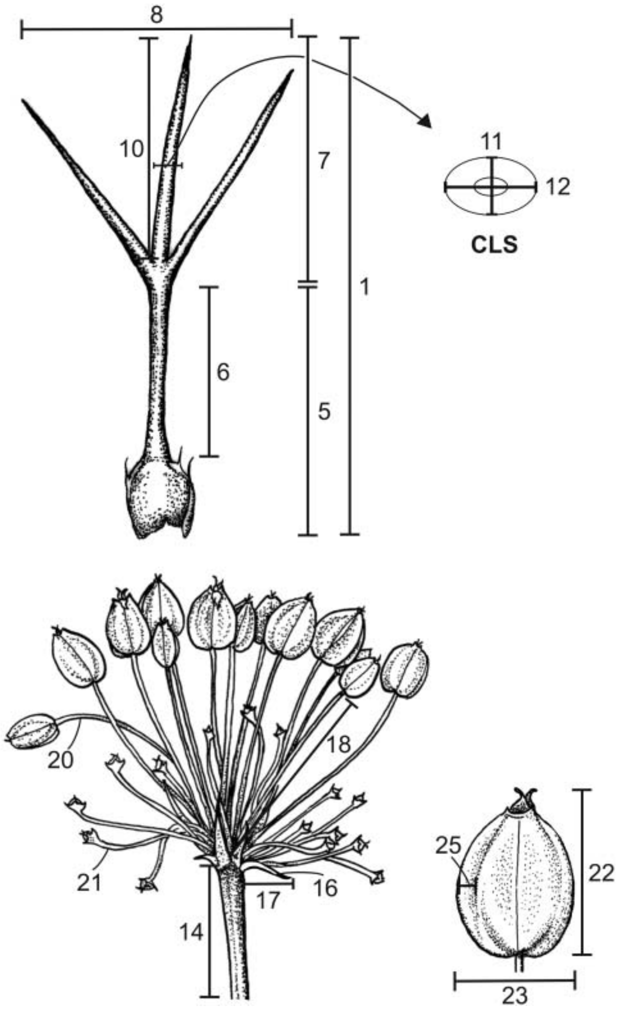

Fig. 2. Leaf, inflorescence, and fruit showing the morphological characters measured for the morphometric analyses, and listed in Table 1. Characters 2–4, 9, 13, 15, 19, and 24 not shown. CLS: transversal section of the central leaf segment.

Climatic and altitude data were obtained from Worldclim 1.4 database, including 19 biologically meaningful 'bioclimatic' variables derived from monthly temperature and rainfall values with a spatial resolution of 2.5 arcminutes (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). From the geographic coordinates of each specimen we extracted values for these bioclimatic and altitude variables using QGIS (Version 2.10 Pisa; 2012). The bioclimatic variables represent annual trends or climatic factors that can be extreme or limiting for plants, such as minimum temperature of the coldest period or precipitation of the driest quarter, etc. Owing to the general overall low density of available climate stations, this database is considered not very accurate in mountainous areas, especially for precipitation (Hijmans et al., 2005). However, in Patagonia the climatic data have been tested against other data bases, and are generally considered reliable (e.g., Waltari, Schroeder, McDonald, Anderson, & Carnaval1, 2014). Nonetheless, to corroborate the reliability of Worldclim altitude data in the southern Andes, we compared their data against altitude of specimens that present this information in labels, and found them highly correlated (Spearman correlation coefficient 0.74).

Data analyses

Species delimitations and diagnostic characters. In order to determine differences amongst species for each morphometric variable, one-way analysis of variance (F-ANOVA) with post-hoc tests for homogeneous groups (Tukey's HSD) were performed for those variables with normal distribution. For variables without normal distribution, non-parametric Kruskal–Wallis (H-KW) was used and multiple comparisons were performed by the method of Dunn. In both, Bonferroni corrections for multiple comparisons were included (Rice, 1989). To graphically explore variation, we constructed boxplots featuring medians, 25th and 75th percentiles, and error bars with 10th and 90th percentiles.

To visualize how specimens belonging to each species are grouped according to their morphological affinities

Table 1. Morphometric and bioclimatic variables and differences amongst *Mulinum spinosum*, *M. echinus*, and *M. leptacanthum*. For each species the mean and standard error of each vegetative (1–13), reproductive (14–25) and bioclimatic (BIO1, BIO12) variable are indicated with ranges in parentheses. Results of ANOVA (F-ANOVA) or Kruskal–Wallis (H–KW) tests with asterisks denote significant differences between species ($P < 0.05$). For each variable, different letters indicate significant differences between means. Morphometric traits are illustrated in Fig. 2.

Morphometric variables	*M. echinus*	*M. leptacanthum*	*M. spinosum*	F- ANOVA	H-KW
1. Leaf length (mm)	20.2 ± 1.61 a (13–40)	13.7 ± 0.67 a (6.1–30)	35.36 ± 1.13 b (17–73)		102.00*
2. Leaf perimeter (cm)	8.92 ± 1.64 a (1.26–29.6)	5.43 ± 0.25 a (2.54–10.01)	14.66 ± 0.57 b (5.42–29.6)		92.11*
3. Leaf area (cm^2)	0.57 ± 0.13 a (0.18–2.24)	0.30 ± 0.01 a (0.15–0.57)	1.05 ± 0.06 b (0.2–3.11)		71.08*
4. Leaf perimeter/square root of area	12.47 ± 1.24 a (1.4–22.8)	9.80 ± 0.26 b (6.5–14)	15.18 ± 0.42 a (8.6–28.1)		58.01*
5. Petiole and sheath length (mm)	9.8 ± 0.89 a (6–20)	7.34 ± 0.41 a (3–17)	19.12 ± 0.68 b (8–37)		95.30*
6. Petiole length (mm)	5.3 ± 0.79 a (3–14.6)	3.97 ± 0.34 a (1–10)	12.19 ± 0.52 b (3–29)		85.12*
7. Lamina length (mm)	10.53 ± 0.88 a (6–20)	7.15 ± 0.33 a (3–13)	16.53 ± 0.58 b (8.4–36)		91.57*
8. Lamina width (mm)	14.26 ± 1.49 a (9–30)	8.87 ± 0.4 b (3–16)	22.25 ± 0.8 c (7.5–42)		89.67*
9. Lamina length/width	0.76 ± 0.03 (0.55–1.11)	0.83 ± 0.02 (0.54–1.28)	0.76 ± 0.01 (0.3–2)		5.45
10. Central leaf-segment length (mm)	9.8 ± 0.9 a (6–20)	6.1 ± 0.29 b (3–10)	15.23 ± 0.57 c (7–35)		92.37*
11. Central leaf-segment thickness (mm)	0.88 ± 0.03 a (0.6–1.2)	0.67 ± 0.02 b (0.4–1)	0.96 ± 0.02 a (0.2–1.6)		43.70*
12. Central leaf-segment width (mm)	1.03 ± 0.07 a (0.6–1.8)	1.73 ± 0.07 b (1–2.8)	1.6 ± 0.05 b (0.5–3.6)		22.90*
13. Acicularity of central leaf-segment	0.85 ± 0.04 a (0.6–1)	0.39 ± 0.02 b (0.15–0.76)	0.62 ± 0.02 c (0.2–1)		48.81*
14. Peduncle length (mm)	17.14 ± 1.55 a (10–25)	5.48 ± 0.51 b (1–16)	29.4 ± 1.60 a (3–50)		80.48*
15. Leaf length/peduncle length	1.30 ± 0.12 a (0.66–2.1)	3.54 ± 0.42 b (0.75–14)	1.54 ± 0.10 a (0.54–8.66)		36.00*
16. Involucre bract number	5.41 ± 0.43 a (3–9)	5 ± 0.11 a (3–7)	6.47 ± 0.16 b (4–10)		30.27*
17. Bract length (mm)	3.08 ± 0.18 a (2–4)	2.93 ± 0.14 a (1–4.5)	5.09 ± 0.22 b (2–15)		47.36*
18. Pedicel length (mm)	4.54 ± 0.40 a (3–6)	3.4 ± 0.24 a (1–6.5)	7.96 ± 0.37 b (3–22)		60.96*
19. Bract length/pedicel length	0.72 ± 0.05 ab (0.5–1)	0.98 ± 0.07 a (0.33–2)	0.6 ± 0.04 b (0.22–1.5)		20.04*
20. Number of perfect flowers per umbel	8.22 ± 1.75 ab (2–15)	4.45 ± 0.43 a (2–10)	10.38 ± 0.62 b (3–22)		29.15*
21. Number of staminate flowers per umbel	15 ± 3 ab (12–18)	10.52 ± 0.65 a (6–18)	20.36 ± 1.33 b (9–44)		23.93*
22. Fruit length (mm)	5.44 ± 0.34 (4–7)	5.81 ± 0.17 (4.8–7.5)	6.02 ± 0.16 (3–9)		2.56
23. Fruit width (mm)	4.38 ± 0.33 a (3–6)	6.3 ± 0.31 b (3.7–10)	5.71 ± 0.19 b (3–9)	5.90*	
24. Fruit length/fruit width	1.27 ± 0.09 a	0.95 ± 0.03 b	1.08 ± 0.02 c	8.70*	

(*continued*)

Table 1. (*Continued*)

Morphometric variables	*M. echinus*	*M. leptacanthum*	*M. spinosum*	F- ANOVA	H-KW
	(1–1.66)	(0.7–1.28)	(0.66–1.66)		
25. Fruit-wing width (mm)	1.41 ± 0.19 a	2.17 ± 0.14 b	2.08 ± 0.10 b		8.05^{*}
	(1–2.5)	(1–4)	(1–4)		
Bioclimatic variables					
BIO1. Annual mean temperature (°C)	8.22 ± 1.00 a (−0.9–13.7)	6.43 ± 0.36 b (3.2–12.0)	7.51 ± 0.38 a (−2.9–15.8)		6.63^{*}
BIO12. Annual precipitation (mm)	511.1 ± 74.30 a (159–835)	898.1 ± 49.6 b (413–1787)	572.1 ± 34.7 a (79–1505)		28.41^{*}

and to identify the characters that best define the groups, principal component analysis (PCA) was performed with all specimens and considering all morphological characters. Contributions of the variables (correlation values) to each principal component (PC) were interpreted as significant when ≥ |0.6|. Graphs were constructed with axes corresponding to the most informative PCs.

In addition, discriminant analysis (DA) of quantitative morphological variables was used to discriminate amongst the three groups (species) defined *a priori*. Quantitative characters that were not normally distributed were $\log_{10}$ transformed before the analysis. Traits that strongly correlated with other traits as determined by a Pearson's correlation coefficient $r > |0.9|$ were identified, and one of the traits of each correlating pair was excluded from the DA analysis. Mahalanobis distances between the centroids of the three groups were calculated to establish a measure of morphological affinity. The specimens treated as *M. spinosum*, *M. echinus*, and *M. leptacanthum* were re-classified *a posteriori* with DA.

To visualize how specimens of each species are grouped according to their climatic characteristics and to infer if the species have different climatic niches, PCA was performed with the 19 bioclimatic variables obtained from Worldclim database. Contributions of the bioclimatic variables to the PCA axes were interpreted as significant when ≥ |0.6|. Correlations between climate and geographic latitude, longitude and altitude were also explored by treating these latter variables as illustrative (i.e., not participating in the ordination) in the climatic PCA. We then used multivariate analyses of variance (MANOVA) with PCA axes scores as dependent variables and species as the fixed factors to determinate whether species separation in the climatic niches was statistically significant, and Tukey's HSD post-hoc tests were performed to identify homogeneous groups. We also determined climatic differences amongst species for the two bioclimatic variables that represent annual trends (annual mean temperature, BIO1, and annual precipitation, BIO12) using Kruskal–Wallis (H-KW) and multiple comparisons by the method of Dunn (Table 1). These two annual variables were selected for these comparisons, because they resulted in contributing highly to the most informative PCs of the climatic PCA, and correlated to the monthly, seasonal, or daily bioclimatic variables with highest contributions.

In all cases before conducting the analyses, all measurements were standardized. All statistical analyses were performed using the software SPAD (Version 5.5; 2012) and/or STATISTICA (Version 7.0; 2014).

Morphometric and environmental variation within the widespread *Mulinum spinosum*. The following analyses that combine morphometric, bioclimatic, and geographic variables were performed to relate morphology with environmental characteristics within *M. spinosum*. Morphological and climatic PCAs using the same 25 morphological characters and the 19 bioclimatic variables examined for the previous section were conducted on all specimens of *M. spinosum* (species delimitation based on the results of the analyses performed in the previous section; N = 117). These analyses were performed to select the morphometric and bioclimatic variables with highest contributions to the most informative PCs of the morphological and climatic PCA ordinations. In turn, these selected variables were later used to relate the morphological variation of *M. spinosum* to climatic characteristics by fitting linear regressions between the selected morphometric and the bioclimatic variables. Correlations between morphology and climatic and geographic variables were also explored by treating the environmental variables as illustrative (i.e., not participating in the ordination) in the morphological PCA of *M. spinosum*, and the geographic variables as illustrative in the climatic PCA. In addition, means, standard deviations, and ranges of annual climatic variables (BIO1 and BIO12) were calculated for *M. spinosum s.l.* to characterize its climatic niche.

To evaluate if morphology and/or climate are related with the latitudinal differences in genetic (phylogeographic) structure estimated by Sede *et al.* (2012) for *M. spinosum* between Argentinean populations of northern vs. southern Patagonia, the specimens were separated into two groups following Sede *et al.* (2012): those present in northern Patagonia (i.e., north of the Chubut river basin; N = 60) and those of southern Patagonia (i.e., south of the Deseado river basin; N = 7). These two groups were used as the fixed factors with climatic PCA axes scores as dependent variables in a MANOVA to determinate whether separation in the climatic niches was statistically significant between northern and southern Patagonian groups. Finally, non-parametric Mann–Whitney tests were performed between the northern and southern groups for all morphometric variables, and for the bioclimatic variables BIO1 and BIO12.

Results

Species delimitations and diagnostic characters

The ANOVA and Kruskal–Wallis morphological analyses resulted in statistically significant differences amongst the three species for 23 of the 25 characters (Table 1). Of these, nine characters (1–3, 5–7, 16–18) separated *M. spinosum* from *M. echinus* and *M. leptacanthum*, four (4, 11, 14, 15) separated *M. leptacanthum* from *M. echinus* and *M. spinosum*, three (12, 23, 25) separated *M. echinus* from *M. leptacanthum* and *M. spinosum*, four (8, 10, 13, 24) separated the three species, and three (characters 19–21) separated *M. spinosum* from *M. leptacanthum* but *M. echinus* was not separated from any of these species. The results also show that the ranges of these characters generally overlap amongst the three species. Moreover, except for character 14, these overlaps are important as shown by the boxplots of the morphometric variables where at least 25% of the specimens of the different entities show superposition (SD2, see supplemental material online).

The principal component analysis of morphology (morphological PCA) for the three species showed that the first two components account for 48% of the total variation of the data, with PC1 representing 36%, PC2, 12%, and PC3, 8%. The representation of the first two components shows that on the first axis, from left to right, *M. leptacanthum* separates with little overlap from *M. echinus* and *M. spinosum*, however *M. echinus* overlaps completely with *M. spinosum* (Fig. 3). Axis 2 (PC2) does not separate any of the three species as there is substantial overlap, although *M. echinus* is generally restricted to the bottom of the axis (Fig. 3). The characters that contributed most to PC1 are mainly related to leaf size and inflorescence size (characters 1–2, 5–8, 10, 14, 17–18; Table 2), and the characters that contributed most to PC2 are mainly related to fruit size (characters 22–23, 25; Table 2). Therefore, *M. leptacanthum* specimens generally differ from *M. echinus* and *M. spinosum* in their smaller leaves and umbels (given by shorter peduncles and pedicels), whereas *M. leptacanthum* and *M. spinosum* specimens differ somewhat from *M. echinus* in their larger fruits, although these two species also show fruits as small as those of *M. echinus*.

For the discriminant analysis (DA) only morphological variables 23 and 24 showed normal distribution, so to comply with the assumptions of DA, we applied $\log_{10}$ to each of the rest of the characters. After this transformation, the following characters showed normality: 1, 4–5, 7–8, 10, 12, 19–20. Pearson's correlation coefficients between all pairs of characters showed a significant ($P < 0.05$) and strong ($r \geq |0.9|$) association between characters 5 and 1 and between 10 and 7, hence we eliminated characters 5 and 10 from the analysis. The DA showed statistically significant differences amongst the three species for five of the nine characters analysed (Wilks' $\lambda = 0.27$; F = 28.4; $P < 0.001$), with Mahalanobis distances (MD) between centroids of each species highest between *M. spinosum* and *M. leptacanthum* (MD = 9.60; $P < 0.001$), lowest between *M. spinosum* and *M. echinus* (MD = 3.95; $P < 0.001$), and

Table 2. Correlations of each morphometric variable to the first two axes of the morphological PCA for *M. spinosum*, *M. echinus*, and *M. leptacanthum*. In bold correlations ≥ |0.6|, considered significant.

Morphometric variables	PC1: 36%	PC2: 12%
1. Leaf length	**0.95**	0.14
2. Leaf perimeter	**0.84**	0.06
3. Leaf area	0.32	0.19
4. Leaf perimeter/square root of area	0.58	−0.05
5. Petiole and sheath length	**0.89**	0.11
6. Petiole length	**0.86**	0.14
7. Lamina length	**0.90**	0.17
8. Lamina width	**0.88**	0.15
9. Lamina length/width	−0.12	0.03
10. Central leaf-segment length	**0.89**	0.17
11. Central leaf-segment thickness	−0.02	0.10
12. Central leaf-segment width	−0.35	0.49
13. Acicularity of central leaf-segment	0.48	−0.49
14. Peduncle length	**0.79**	−0.01
15. Leaf length/peduncle length	−0.36	0.23
16. Involucre bract number	0.49	0.03
17. Bract length	**0.63**	0.18
18. Pedicel length	**0.72**	−0.03
19. Bract length/pedicel length	−0.37	0.27
20. Number of perfect flowers per umbel	0.57	−0.20
21. Number of staminate flowers per umbel	0.41	0.14
22. Fruit length	0.10	**0.62**
23. Fruit width	−0.17	**0.83**
24. Fruit length/fruit width	0.26	−0.53
25. Fruit-wing width	−0.12	**0.78**

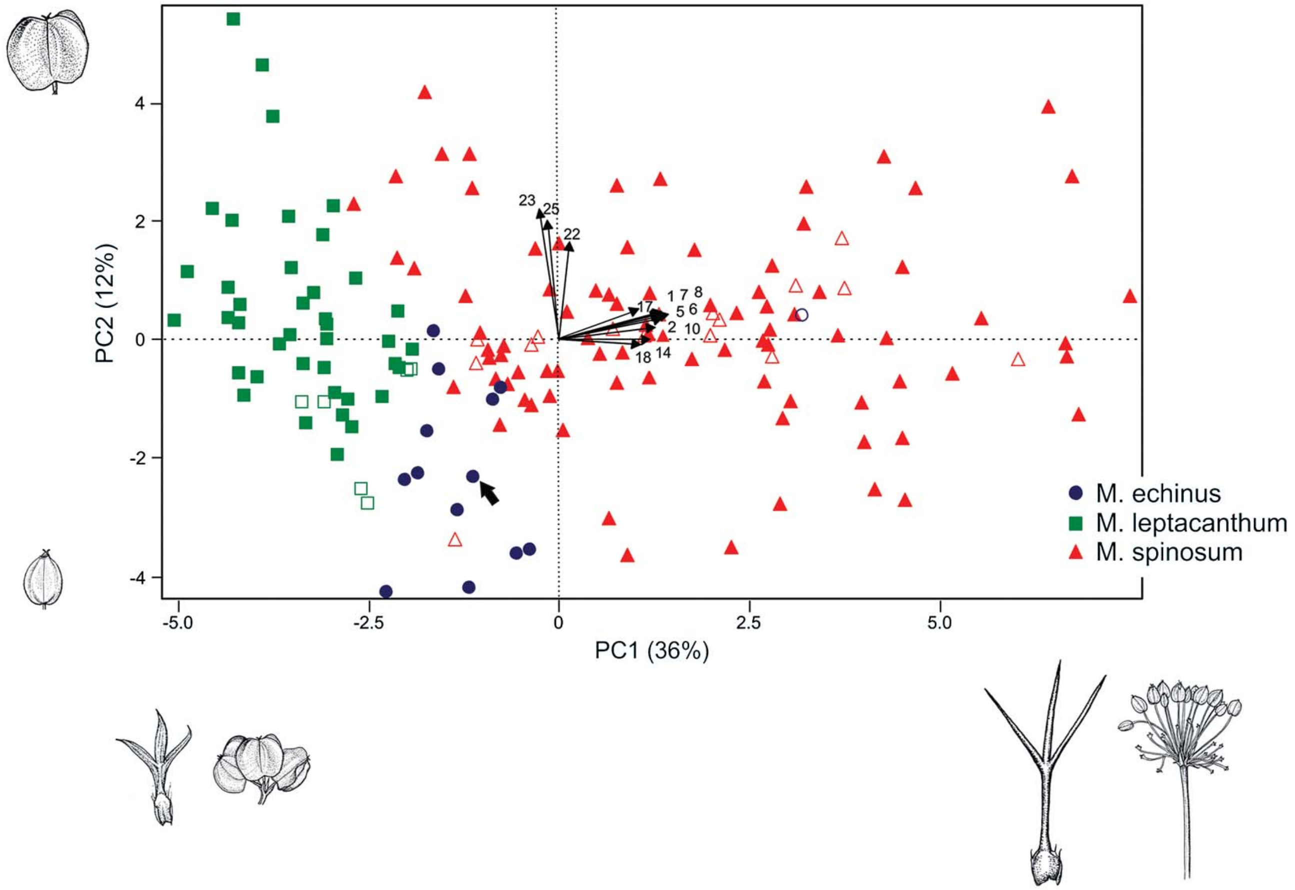

Fig. 3. Morphological principal component analysis (PCA) for species delimitation: scatterplot of the first two axes (PC1 and PC2) of 163 specimens of *Mulinum spinosum*, *M. echinus*, and *M. leptacanthum* based on 25 morphometric variables. Percentage of total variance associated to each PC is provided in parentheses. Unfilled circles, squares and triangles represent type-specimens of each species. Thick arrow indicates specimen collected by Haenke in Chile (HAL 26839), discussed in the text. Thin arrows indicate the contribution of significant morphological characters to the first two axes. Numbers correspond to the morphological characters listed in Table 1.

intermediate between *M. leptacanthum* and *M. echinus* (MD = 4.83; $P < 0.001$). The first discriminant function explained 90% of variance of the data (Wilks' $\lambda = 0.27$; $\chi^2 = 204.50$; $P < 0.001$) and separated mainly *M. leptacanthum* from *M. spinosum* and *M. echinus* with little overlap (figure not shown). The second function accounted for the remaining 10% of the total variance (Wilks' $\lambda = 0.81$; $\chi^2 = 32.02$; $P < 0.001$) and separated *M. echinus* from *M. leptacanthum* and *M. spinosum*, although with substantial overlap (figure not shown). The characters that most contributed to these functions are related to leaf size, inflorescence characteristics and fruit shape (Function 1: 1, 8, 19, 24; Function 2: 12).

In addition, results of the DA showed that out of a total of 15 individuals of *M. echinus*, one was classified as *M. leptacanthum* and five (including two type-specimens of the species) were classified as *M. spinosum*. Also, of a total of 46 individuals of *M. leptacanthum*, three were classified as *M. spinosum* and none as *M. echinus.* Lastly, of a total of 102 individuals of *M. spinosum*, five were classified as *M. leptacanthum* and one as *M. echinus*. Therefore, the percentage of individuals (including types) which were classified correctly by DA was 60% for *M. echinus*, 95% for *M. leptacanthum*, and 94% for *M. spinosum*. Consequently, the high percentage of misclassifications (40%) in *M. echinus* together with the lowest Mahalanobis distance of this species with *M. spinosum* does not support a clear delimitation between the two.

The principal component analysis of climatic data (climatic PCA) for the three species showed that the first two components account for 65% of the total variation of the data, with PC1 representing 36%, PC2, 29%, and PC3, 17%. On axis 1, from left to right, *M. echinus* and *M. leptacanthum* completely overlap with *M. spinosum*, although *M. echinus* appears somewhat more to the left and *M. leptacanthum* appears less dispersed and more to the right

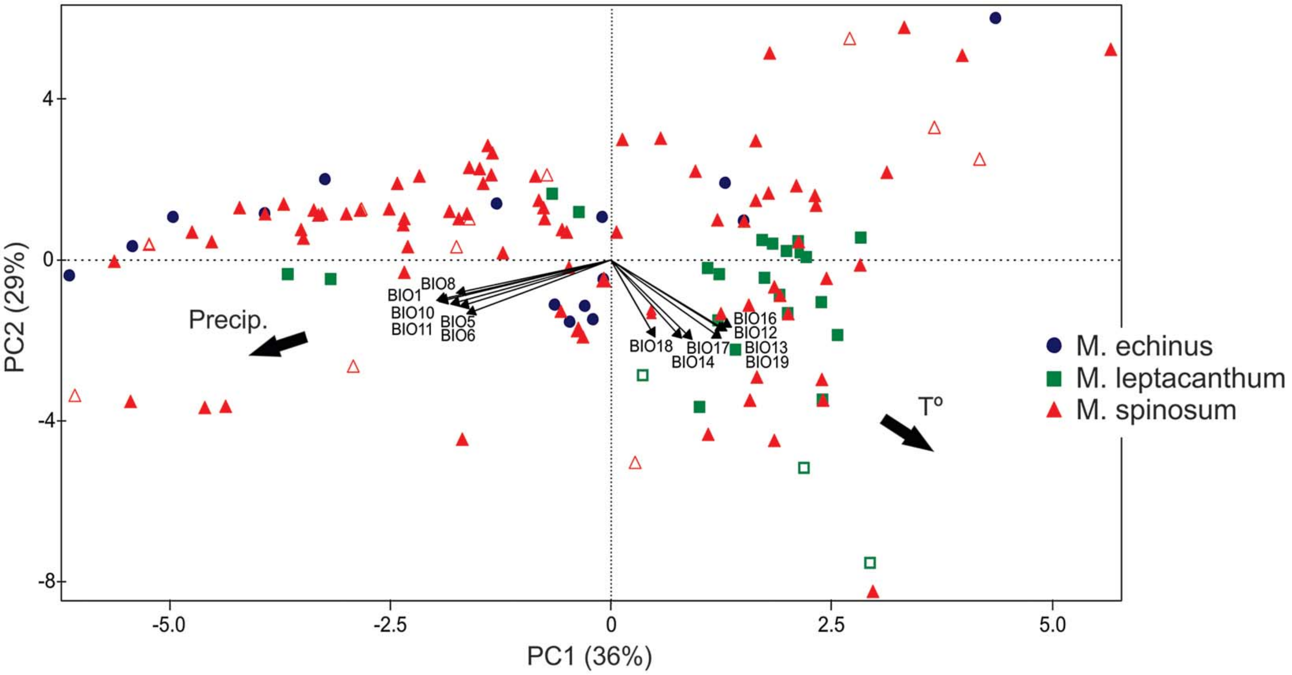

Fig. 4. Climatic principal component analysis (PCA) for species delimitation: scatterplot of the first two axes (PC1 and PC2) of 163 specimens of *Mulinum spinosum*, *M. echinus*, and *M. leptacanthum* studied based on 19 bioclimatic variables. Percentage of total variance associated to each PC is provided in parentheses. Unfilled circles, squares, and triangles represent type-specimens of each species. Thin arrows indicate the contribution of significant bioclimatic variables to the first two axes. Numbers correspond to the bioclimatic variables listed in Table 3. Thick arrows indicate the direction to which precipitation or temperature increase.

(Fig. 4). On axis 2, *M. echinus* appears more to the top and *M. leptacanthum* to the bottom, both superposed with *M. spinosum*. The bioclimatic variables that contributed most to PC1 were mainly related (inversely) to annual mean temperature and other temperature related bioclimatic variables (BIO 1, 5–6, 8, 10–11; Table 3). The ones that contributed most to PC2 were related (inversely) to annual precipitation and other precipitation related bioclimatic variables (BIO 12–14, 16–19; Table 3). Of the illustrative geographic variables, only altitude correlated significantly with PC1 (Table 3). Therefore, *M. leptacanthum* appears generally associated to lower temperatures and higher precipitations, at high altitudes, and *M. echinus* and *M. spinosum* show the widest climatic niches. Overlaps in the climatic variables between *M. spinosum* and *M. echinus* showed that climatic characteristics do not separate these two species (Table 1, Fig. 4). Moreover, the MANOVA analysis using PCA scores of climatic data (climatic PCA) resulted in statistically significant differences amongst species (Wilks' $\lambda = 0.50$; $F_{36,280} = 3.19$; $P < 0.001$). Post-hoc tests revealed that seven of the 18 factors were significant and that *M. leptacanthum* differed in its ecological niche from *M. echinus* and *M. spinosum.* Both BIO1 and BIO12 bioclimatic variables (annual mean temperature and annual precipitation) separated *M. leptacanthum* from *M. echinus* and *M. spinosum* (Kruskal–Wallis; Table 1).

Morphometric and environmental variation within the widespread *Mulinum spinosum*

Taking into account the previous results that show maximum morphological and climatic overlap between *M. spinosum* and *M. echinus*, these two species were considered a single, variable and geographically extended species (hereafter called *M. spinosum s.l.*) in which intra-species variation in relation to environmental characteristics (climatic and geographic) was explored. The morphological PCA of *M. spinosum s.l.* showed that the first two components accounted for 43.6% of the total variation of the data, with PC1 representing 31.6%, PC2, 12%, and PC3, 7.8% (SD3, see supplemental material online). The following variables most contributed to each PC (SD4, see supplemental material online): PC1, leaf size (inversely; variables 1–3, 5–8, 10) and length of the inflorescence (inversely; variables 14, 18), and PC2, leaf acicularity (inversely; variable 13) and fruit size (positively, variables 22–23, 25). Bioclimatic and geographic variables that were treated as illustrative over the morphological PCA to visualize the relationship of the morphological ordination with environmental characteristics presented low correlations with PC1 and PC2 ($< |0.35|$; results not shown). In the climatic PCA of *M. spinosum s.l.* the first two components accounted for 64.3% of the total

Table 3. Correlations of each bioclimatic and illustrative geographic variable to the first two axes of the climatic PCA for *M. spinosum*, *M. echinus*, and *M. leptacanthum.* In bold correlations ≥ |0.6|, considered significant.

Bioclimatic variables	PC1: 36%	PC2: 29%
BIO1. Annual mean temperature	**−0.89**	−0.42
BIO2. Mean diurnal temperature range	0.06	0.11
BIO3. Isothermality	0.46	0.24
BIO4. Temperature seasonality	−0.52	−0.05
BIO5. Maximum temperature of warmest period	**−0.78**	−0.48
BIO6. Minimum temperature of coldest period	**−0.74**	−0.56
BIO7. Temperature annual range	−0.26	−0.02
BIO8. Mean temperature of wettest quarter	**−0.80**	−0.34
BIO9. Mean temperature of driest quarter	−0.58	−0.40
BIO10. Mean temperature of warmest quarter	**−0.90**	−0.42
BIO11. Mean temperature of coldest quarter	**−0.83**	−0.45
BIO12. Annual precipitation	0.57	**−0.80**
BIO13. Precipitation of wettest month	**0.60**	**−0.73**
BIO14. Precipitation of driest month	0.36	**−0.80**
BIO15. Precipitation seasonality	0.31	0.03
BIO16. Precipitation of wettest quarter	0.59	**−0.73**
BIO17. Precipitation of driest quarter	0.42	**−0.82**
BIO18. Precipitation of warmest quarter	0.23	**−0.78**
BIO19. Precipitation of coldest quarter	**0.62**	**−0.69**
Illustrative geographic variables		
Latitude	0.17	0.23
Longitude	−0.57	0.11
Altitude	**0.66**	0.53

variation of the data, with PC1 representing 36%, PC2, 28.3%, and PC3, 16.8% (SD5, see supplemental material online). The following variables most contributed to each PC (SD6, see supplemental material online): PC1, variables related to temperature (inversely, variables BIO1, BIO3, BIO5-BIO6, BIO8, BIO10-BIO11; SD6, see supplemental material online), and PC2, variables related to precipitation (variables BIO12-BIO14, BIO16-BIO19;

Table 4. Relationships between morphometric variables of *Mulinum spinosum s.l.* and annual mean temperature and annual precipitation, indicating the direction of the relationships. n.s. = non-significant.

Morphometric variables	BIO1. Annual mean temperature			BIO12. Annual precipitation		
	r^2	*P*	Direction	r^2	*P*	Direction
1. Leaf length	0.05	0.016	+		n.s.	
2. Leaf perimeter		n.s.			n.s.	
3. Leaf area		n.s.			n.s.	
5. Petiole and sheath length		n.s.			n.s.	
6. Petiole length		n.s.			n.s.	
7. Lamina length	0.06	0.010	+		n.s.	
8. Lamina width	0.03	0.046	+		n.s.	
10. Central leaf-segment length	0.05	0.014	+		n.s.	
13. Acicularity of central leaf-segment	0.08	0.006	+	0.08	0.005	−
14. Peduncle length		n.s.		0.08	0.002	+
18. Pedicel length		n.s.		0.04	0.045	+
22. Fruit length		n.s.			n.s.	
23. Fruit width	0.11	0.007	−		n.s	
25. Fruit-wing width	0.14	0.004	−	0.10	0.0	+

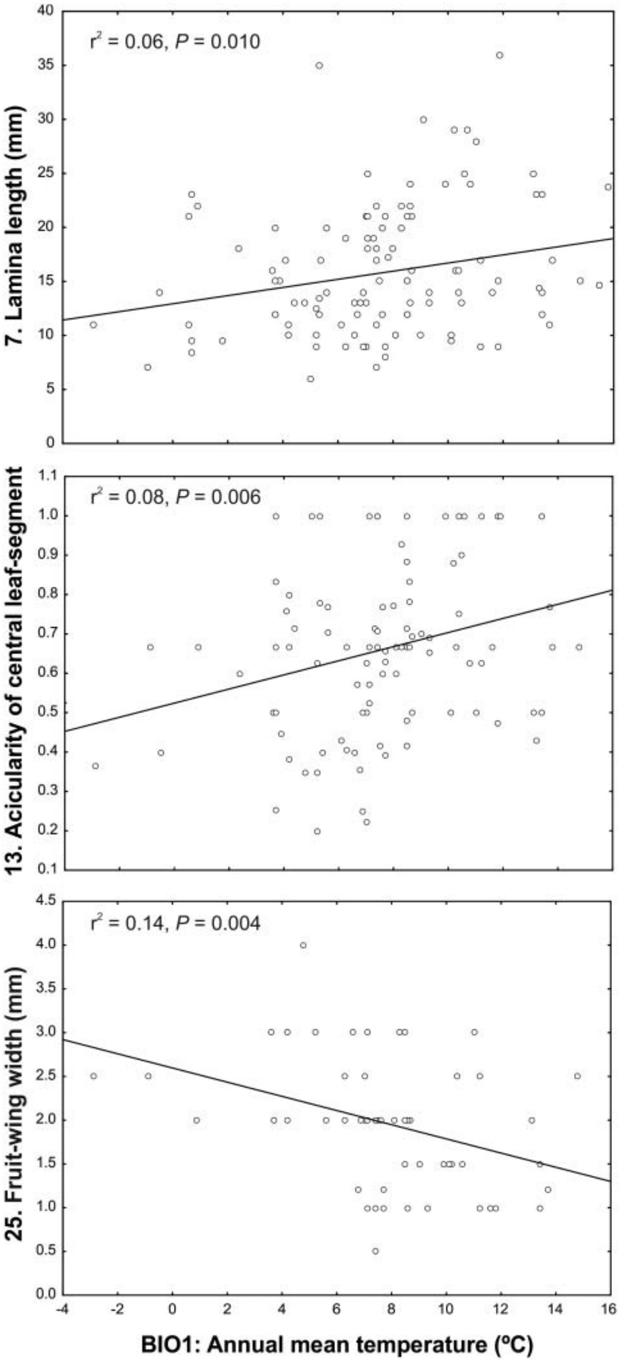

Fig. 5. Relationships between annual mean temperature and morphometric variables (lamina length, leaf acicularity, fruit-wing width) for specimens of *Mulinum spinosum s.l.*, with linear regression fits and associated r^2 and *P* values.

SD6, see supplemental material online). Geographic variables treated as illustrative over the climatic PCA, only showed a significant correlation ($\geq |0.6|$) between altitude and PC1 (SD6, see supplemental material online). Annual climatic characteristics of *M. spinosum s.l.* resulted in annual mean temperature (BIO1) = 7.6 °C ± 0.35 (range: −3 °C to 16 °C), and annual precipitation (BIO12) = 565 mm ± 31.7 (range: 79 mm to 1505 mm).

Linear regressions between morphometric variables with significant contributions to the first two PCs of the morphological PCA of *M. spinosum s.l.* (related to leaf length, peduncle length, leaf acicularity and fruit width; SD4, see supplemental material online) and annual bioclimatic variables with highest contributions to the first two PCs of the climatic PCA (BIO1 and BIO12; SD6, see supplemental material online) showed statistical significance for nine of the 14 morphometric variables ($P < 0.05$, albeit with $r^2 < 0.15$; Table 4). The following morphometric variables were significantly related to annual mean temperature (BIO1; Table 4): leaf size (positive, variables 1, 7–8, 10; Fig. 5), leaf acicularity (positive, variable 13; Fig. 5), and fruit-wing (negative, variables 23, 25; Fig. 5); while the following, to annual precipitation (BIO12; Table 4): leaf acicularity (negative, variable 13; Fig. 6), inflorescence length (positive, variables 14, 18; Fig. 6), and fruit-wing (positive, variable 25; Fig. 6). These results suggest that in *M. spinosum s.l.*, leaf size increases with temperature, and inflorescence size with precipitation; leaf acicularity increases with aridity (i.e., an increase in temperature and decrease in precipitation), and fruit-wing increases with higher precipitation and lower temperature.

The Mann–Whitney tests between the specimens of *M. spinosum s.l.* of northern vs. southern Patagonia for morphology and climate showed statistically significant differences for only four (variables 14–16, 21) of the 25 morphometric variables, and for BIO12 of the bioclimatic variables (SD7, see supplemental material online). Northern Patagonia populations showed larger inflorescences than the southern ones. Therefore, except for some inflorescence characteristics, no other morphological characters showed differences associated with the northern vs. southern Patagonian populations genetically fragmented and isolated, according to the phylogeographic study of *M. spinosum* (Sede et al., 2012). Regarding bioclimatic variables, the MANOVA analysis using PCA scores of climatic data (climatic PCA) resulted in statistically significant differences between the northern and southern groups (Wilks' $\lambda = 0.10$; $F_{18,48} = 22.43$; $P < 0.001$). The phylogeographic north vs. south structuring presented differences in annual precipitation, with northern Patagonia specimens presenting higher mean precipitation than the southern ones (SD7, see supplemental material online).

Discussion

Species delimitations and diagnostic characters

Mulinum spinosum, *M. echinus*, and *M. leptacanthum* have been one of the most difficult groups of species to identify within *Mulinum*. Morphologically, they show greatest similarity, and in practice, the available

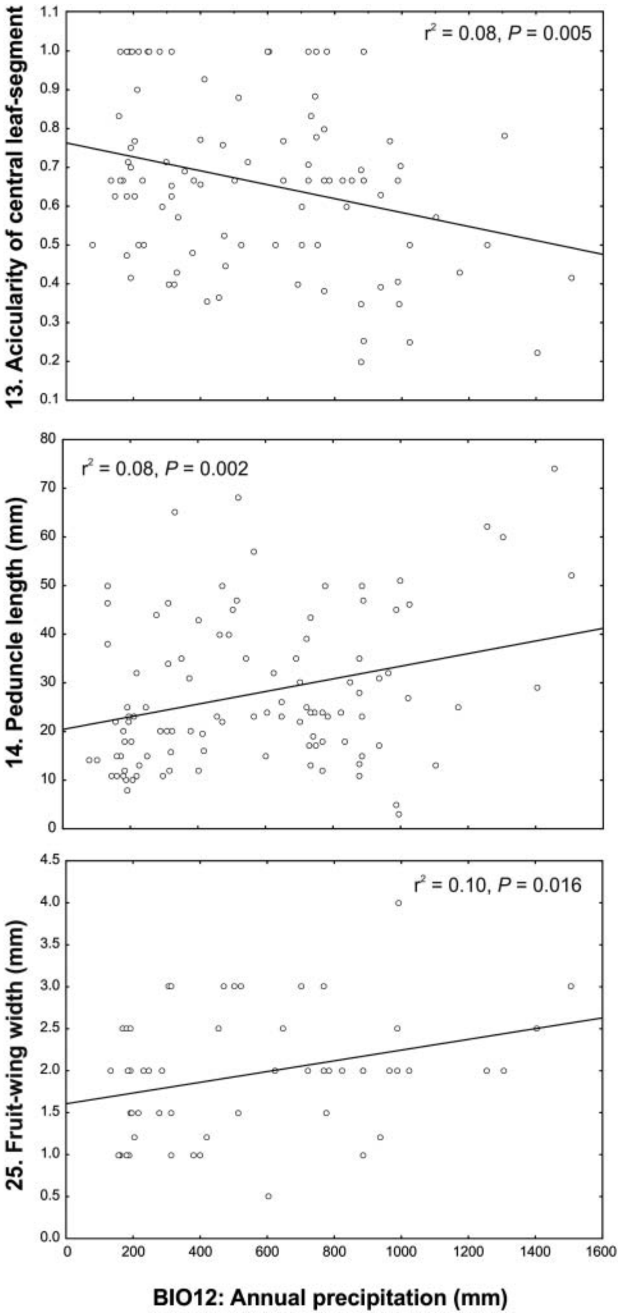

Fig. 6. Relationships between annual precipitation and morphometric variables (peduncle length, leaf acicularity, fruit-wing width) for specimens of *Mulinum spinosum s.l.*, with linear regression fits and associated r^2 and P values.

identification keys (Constance, 1988; Martínez, 2003) have not worked satisfactorily. Moreover, cpDNA phylogenies published to date (Nicolas & Plunkett, 2012) show that these species form part of a larger polytomy together with other *Mulinum* species. This problem subsists in phylogenies based on more rapidly evolving cpDNA markers and/or the nuclear ribosomal DNA ITS region (Fernández et al., submitted). However, the univariate and multivariate morphometric analyses presented here show that *M. leptacanthum* differs morphologically from the rest in its shorter inflorescences and its smaller leaves. In addition, the bioclimatic analyses indicate that *M. leptacanthum* is found in regions with lower temperatures and higher precipitations, and although partly sympatric with the areas of *M. spinosum* and *M. echinus*, it has a more limited distribution generally restricted to high altitudes of the northern Patagonian Andes, in Chile and Argentina. Together with morphological and genetic traits, climatic differences have recently been considered important characters in relation to species delimitations (Raxworthy et al., 2007; Rissler & Apodaca, 2007). Climatic factors are essential for the distribution of plants, and since even closely related species can have their own unique ecological preferences (Grossenbacher et al., 2014), climatic characteristics can be useful to discriminate between taxonomically difficult or cryptic taxa (e.g., Nicola et al., 2014; Piedra-Malagón et al., 2016; Poudel, Möller, Gao, Ahrends, & Baral, 2012). Therefore, based on the morphological distinctiveness and restricted bioclimatic preferences of *M. leptacanthum*, it is considered a distinct species and is treated as such in the recent revision of *Mulinum* (Fernández et al., 2017) taking into account the morphological and climatic differences presented here.

Regarding the identities and differences between *M. spinosum* and *M. echinus*, it is important to note that the concept of *M. echinus sensu* Constance (1988) was based on a specimen collected by Haenke in Chile (HAL 26839) which had been erroneously labelled as isotype of *M. echinus* by Mathias and Constance in 1969–1970 (explained in Fernández et al., 2017). This specimen corresponds to the generally smaller, glaucous plants with smaller leaves that traditionally have been named *M. echinus* (Constance, 1988; Martínez, 2003), and this is the concept that we have followed here in order to evaluate if this entity deserves treatment at species level. The statistical results showed that *M. echinus* and *M. spinosum* presented significant differences in the means of most morphometric variables, however all of them showed considerable overlap, reflecting the difficulty to separate the entities in practice. Moreover, the morphological multivariate analyses showed superposition of the taxa and low morphological distances between them. Even though *M. echinus* has traditionally been diagnosed as being smaller plants with smaller leaves, the morphometric overlap is also evident in diagnostic dilemmas of keys to identify these species (Constance, 1988; Martínez, 2003). Other characters used in these keys such as number of flowers in umbels did not show differences between these species in our analyses. Plant size and foliage colour were not measured in this work because of the difficulties of obtaining this information from herbarium specimens, but we have observed in the field that these characteristics vary within these species, as well. Additional evidence of the two being the same entity is that 40% of the specimens originally considered as *M. echinus* were classified *a posteriori* by DA as *M. spinosum*, which is considerably higher than the 15% threshold often used in taxonomic studies (e.g., Lehnebach, 2011; Lopez Laphitz et al., 2015). Besides, the climatic PCA presented here shows an overlap in climatic preferences between both entities; both are sympatric and found in a wide array of semiarid climates in the Andes of central Chile and bordering areas of Argentina, and in Patagonia. Therefore, the extended and continuous morphological variation of *M. spinosum* and *M. echinus*, together with the amplitude and high overlap in their climatic niches and geographic distributions, supports them as part of the same taxonomic entity. The same is true for the varieties of *M. spinosum*, as shown by the position of the type specimens in the PCA analyses. Thus, the generally smaller plants once recognized as *M. echinus* have already been treated within the variation of *M. spinosum* in the recent taxonomic treatment of *Mulinum* (Fernández et al., 2017) where the name *M. echinus* has been included as a heterotypic synonym of *M. spinosum* for the first time. Similarly, the varieties of *M. spinosum* have also been included as synonyms, except for two based on qualitative characters associated with geographic distributions (Fernández et al., 2017). To avoid ambiguities for the purpose of this study, we refer to the specimens of *M. spinosum* plus *M. echinus* as *M. spinosum s.l.*

The statistical analyses of this work also provided diagnostic characters to differentiate *M. leptacanthum* from *M. spinosum s.l.* Leaf size and inflorescence length were shown to be the most important characters to separate these species, but peduncle length was the only one that did not present superposition and therefore is very important for taxon identification. Consequently *M. leptacanthum* can be identified because it presents shorter umbels and generally smaller and flatter leaves than *M. spinosum s.l.* (Fernández et al., 2017). Although not considered in these morphometric analyses, another morphological character that separates both species is plant height, as *M. leptacanthum* forms flat and compact cushions less than 12 cm high, whereas *M. spinosum s.l.* forms lax, hemispheric cushions more than 20 cm high. Moreover, *M. leptacanthum* is found exclusively in high altitudes with a colder and wetter climate (i.e., with more snow accumulation) than *M. spinosum s.l.*

Morphometric and environmental variation within the widespread *Mulinum spinosum*

Results of the PCA analyses corroborate that *M. spinosum s.l.* is a morphologically very variable species that presents highest variations in leaf size, leaf acicularity, inflorescence length and fruit size. The species has an extended distribution in the southern Andes and Patagonia, being generally found in arid and semiarid areas of the Patagonian steppe, southern Monte desert, mountains of central Chile, and neighbouring areas of Argentina. Although it is generally found in semiarid temperate sites (average annual mean temperature and annual precipitation of all specimens studied *c.* 7.6 °C and 565 mm), it is distributed through a great diversity of climates with wide ranges of temperature and precipitation (−3–16 °C; 79–1505 mm), extending to high latitudes (28–52 °S). Throughout its distribution, annual mean temperature results correlated to other important bioclimatic variables such as maximum and minimum temperatures of the warmest and coldest months, or mean temperatures of the wettest period, all of them with high contributions to PC1. In addition, annual precipitation is correlated to precipitation of the wettest and driest months, and of the warmest and coldest periods, with high contributions to PC2. These seasonal bioclimatic variables can be extreme or limiting for plant growth and/or survival in temperate semiarid regions, and therefore could have exerted selective pressure in the evolution of morphological traits of *M. spinosum s.l.*, resulting in ecotypic differentiation as has been suggested for other species in this area (e.g., Quiroga, Premoli, & Ezcurra, 2002). But the wide temperature and precipitation ranges in the high latitudinal distribution of *M. spinosum s.l.* could also have selected a high phenotypic plasticity in this species, as has been described for other taxa that attain southern high latitudes (Molina-Montenegro & Naya, 2012). Plasticity can act as a quick buffer under climatic changes (Valladares et al., 2014), and the apparent persistence of *M. spinosum s.l.* in its present range during the last glacial maximum (Sede et al., 2012) is certainly suggestive of plastic responses to climatic variation.

Results of the regressions between the two most informative bioclimatic variables (annual mean temperature and annual precipitation) and the most variable morphometric traits of *M. spinosum s.l.*, point to significant associations between morphology and climate in this species. These type of associations have also been found for other southern South American ecologically diverse and geographically extended species (e.g., *Cerastium*, Quiroga et al., 2002; *Calceolaria*, Mascó, Noy-Meir, & Sérsic, 2004; *Embothrium*, Chalcoff et al., 2008; *Anarthrophyllum*, Paiaro et al., 2012; *Embothrium*, Souto & Smouse, 2013; *Calceolaria*, Cosacov, Coccucci & Sersic, 2014; *Monttea*, Baranzelli, Johnson, Cosacov, & Sérsic, 2014; *Quinchamalium*, Lopez Laphitz et al., 2015). For *M. spinosum s.l.*, in general, leaf size increased with temperature, inflorescence size increased with precipitation, leaf acicularity increased with temperature and lower precipitations, and fruit-wing increased with precipitation and lower temperatures. These types of associations suggest the influence of climate on morphological variation. For example, in temperate latitudes, an increase of leaf size with higher temperatures (i.e., at generally lower altitudes) can be attributed to a more extended growing period, with less duration of snow cover or freezing temperatures that arrest plant growth (e.g., Givnish, 1979; Royer, Wilf, Janesko, Kowalski, & Dilcher, 2005 and references therein). An increase in inflorescence size with precipitation could be attributed to increased growth in rainier sites, as rainfall is an important determinant of shoot extension in *M. spinosum* (Damascos, Barthélémy, Ezcurra, Martínez, & Brion, 2008). Leaves that are more acicular in warmer and drier places (i.e., more arid sites) are optimal to reduce transpiration and leaf heating (Ezcurra, Ruggiero, & Crisci, 1997, and references therein). Wider fruit-wings in wetter, cooler sites could improve floating capabilities of the fruits of *M. spinosum s.l.* favouring dispersion by water, a dispersal mechanism that could be important in wet high-altitude environments during thaw periods. A lignified endocarp, a plesiomorphic character within Azorelloideae (Calviño, Martínez, & Downie, 2008a) may prevent seed putrefaction in water. Moreover, water dispersal has repeatedly been estimated as an important mechanism for colonization of new territories within Apiaceae (Calviño, Martínez, & Downie, 2008b; Calviño, Teruel, & Downie, 2016). All these associations found between climate and morphology in *M. spinosum s.l.* could both result from evolutionary adaptations to climatic differences, or from phenotypic plasticity. Although to distinguish between these two possibilities common-garden experiments should be performed (Franks, Weber, & Aitken, 2014), the significant associations between climate and some vegetative and reproductive traits certainly suggest the influence of the environment on the morphology of *M. spinosum s.l.*

Despite the significant linear associations observed between morphology and climate, the great dispersion of the morphological data when regressed on the bioclimatic variables (reflected in the low r^2) shows that the variance explained by these models is relatively low. A possible source of dispersion could be inaccuracy in the Worldclim data due to methodological problems in extrapolations on dissected terrain (Bedia, Herrera, & Gutiérrez 2013). Another source could be the effect of local variation in environmental characteristics that cannot be measured in herbarium material, such as edaphic factors, site conditions determined by slope aspect, or local differences in periodic fires history, and/or herbivory pressure. In plants of *M. spinosum s.l.* from specific skeletal soil types such as rock outcrops, a smaller leaf size associated to higher acicularity and smaller plant-form has often been observed (authors' pers. obs.). Also, differences in slope aspect determine differences in insolation and windiness that produce microclimatic dissimilarities that can affect plants, as has been found in mountains and rock outcrops of the region (e.g., Ferreyra et al., 1998; Speziale & Ezcurra, 2015). In addition, high herbivory pressure can produce morphological changes in leaf and other plant traits (Brown, Lawton, & Grubb, 1991), and in southern South America large mammals such as native guanacos (*Lama guanicoe*) or introduced domestic cattle and European hare can act synergistically with abiotic factors such as fire on plant species (Raffaele, Veblen, Blackhall, & Tercero-Bucardo, 2011). Therefore, local and regional environmental factors could be acting in a complex fashion to produce the great dispersion of the morphological traits in relation to the environment, detected in this work.

Even so, the partial incongruence between morphological and environmental variables as reflected in the low r^2 of the regressions could also be due to other mechanisms, such as mutations, genetic drift, or restrictions of gene flow (e.g., Grant, 1991). Indeed, according to phylogeographic studies in *M. spinosum*, the northern and southern Patagonian populations differentiated *in situ* by isolation (with limited gene flow) for long periods of time due to historical barriers such as the Deseado or Chubut basins (Sede et al., 2012). In our work, the morphological comparisons of specimens of *M. spinosum s.l.* of northern vs. southern Patagonia resulted in differences in inflorescence characteristics, decoupled of any vegetative trait. This apparent correlation between reproductive morphology and genetic structure suggests the selection in isolation of morphotypes, probably mediated by different pollinator assemblages. However, the northern versus southern populations also showed differences in annual precipitation, a climatic variable also associated with inflorescence size in this species. Therefore, the differences in reproductive traits between northern and southern populations could also be simply attributed to present or historical climatic effects.

Conclusions

The results of our morphological and climatic studies within the species-group of *M. spinosum*, *M. echinus*, and *M. leptacanthum* support that the entity traditionally considered *M. echinus* is part of the large morphological and climatic variation of *M. spinosum*, so its treatment as a distinct taxon is not justified at any rank. On the other hand, the morphological distinctiveness and restricted bioclimatic preferences of *M. leptacanthum*, support its treatment as a distinct species.

The great morphological variation in vegetative and reproductive characteristics of the widespread *M. spinosum* is likely related to regional climatic gradients that have probably been important in the evolution of these species plasticity, diversification and differentiation in arid and semiarid habitats of southern South America. But local environmental characteristics and evolutionary mechanisms such as mutations, genetic drift, or restrictions of gene flow may have also influenced this variation.

Our work confirms the taxonomic value and possible evolutionary implications of using analyses of environmental data together with morphometrics in species delimitation, especially for sympatric species-groups with low levels of phylogenetic resolution.

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Supplemental data

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