Article

Morphometric characterization of *Eryngium viviparum* (Umbelliferae): description of a new subspecies from the Iberian Peninsula

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Abstract

Eryngium viviparum is an endemic plant of the Atlantic regions of Europe. Surveys carried out in recent years in the wetlands of northwest Spain have led to the identification of several previously undetected subpopulations of this species in inland areas with markedly Mediterranean bioclimatic characteristics that constitute the southern limit of this species. However, these populations overlap with the distribution of the Iberian endemic *E. galioides,* similar in size and morphology. We developed a biometric study on herbarium vouchers that has enabled us to identify a new subspecies, subspecies *bariegoi*, distributed in a limited geographical zone in Mediterranean areas of the northwest Iberian Peninsula.

Key words: taxonomy, aquatic plant, endemic plant, threatened plant, conservation, Spain

Introduction

Eryngium viviparum J.Gay (1848: 171) is a small plant growing in seasonally flooded sites (Cook 1983, 1990; Arts & Den Hartog 1990; Rodríguez-Oubiña *et al*. 1997). Since 1979 this species has been included in Annex I (strictly protected plant species) of the Berne Convention (1979), and is also listed as a priority species in Annex II of The Habitats Directive (1992). Today there is a single known subpopulation in France (in Morbihan, Brittany) (Annezo *et al*. 1995; Buord *et al*. 1999). The remaining subpopulations are all in northwest Spain, in the Region of Galicia, where it is considered threatened and has recently been rated as "*Endangered"* (Romero & Rubinos 2003; Romero *et al*. 2004).

Surveys carried out in recent years in the wetlands in the northwest Iberian Peninsula have led to the detection of several previously undetected subpopulations of this species in inland areas with markedly Mediterranean bioclimatic characteristics. These subpopulations, located in the Region of Castile-León, constitute the southern limit of this species' known range (see Fig. 1). Nevertheless, recent molecular data on this population has shown that the genetic distance between the Atlantic subpopulations (Galicia and Brittany) is lower than the distance between the Spanish subpopulations (Galicia and Castile-León) (Rodríguez-Gacio *et al*. 2009). Moreover, the monitoring of the northwest Iberian subpopulations has revealed morphological differences between them. In this paper we show that these differences are measurable. This information will be important for the preservation of the genetic and morphological variability of this rare species, which is associated to a very selective habitat (Menges 1986; Soltis & Gitzendanner 1998).

Material & methods

Morphometric data
This study was based on specimens from all three subpopulations deposited in the following herbaria: the Paris Herbarium (Herbier National de Paris-Muséum National d'Histoire Naturelle), the location of the original type

material, where we revised 16 vouchers with an average of 18 specimens per voucher; and the SANT Herbarium (University of Santiago de Compostela), where the Spanish subpopulation is very well represented, with 32 vouchers each with an average of six specimens.

Eryngium viviparum is a biennial plant that in its first year produces only a basal leaf rosette. In the second year, when the water disappears in early summer, the plant develops inflorescences with some basal leaves rosettes at the end of stoloniferous branches. Each individual can produce several inflorescences throughout its life and they form a series that can be followed from the initial inflorescence, identified here as R0, to the last inflorescence produced. The highest inflorescence order identified was R7.

From an initial inspection of the vouchers and our field experience, we determined the shapes of the leaves and bracteoles to be the morphological characters that showed the greatest differences between subpopulations. To quantify these differences, more than two thousand photographs of the specimens in the herbaria vouchers were taken with the aid of a digital camera connected to a stereomicroscope. Then, we measured the length and width of the leaves and the bracteoles with a specific software developed by one of the authors. The width measurements were taken at the widest part of the leaf or bracteole. With these data we also calculated length/width ratios for bracteoles and leaves.

FIGURE 1. Distribution of the population of *Eryngium viviparum*. Atlantic subpopulations: French (●), and Galician (■) subpopulations. Mediterranean subpopulation in the Region of Castile-León (▼).

Statistical analysis

The data were analysed as an ANOVA with two crossed factors: subpopulations (three levels: 1) France, 2) Galicia, and 3) Castile-León); and inflorescence order (eight levels from R0 to R7, although not all were included in the analysis; see below).

The design, however, was not balanced since low-order inflorescences were less abundant due to the desiccation and death of the oldest parts of the plant, and because many plants did not produce inflorescences of a very high order. Table 1 shows the number of samples (*n*) in each group. Owing to the considerable differences in the quantity of samples among the groups, we did not include groups with a small number of samples in the statistical analysis, i. e. R6 and R7 (bracteoles) and R0, R6 and R7 (leaf). However, Fig. 2 includes all the data.
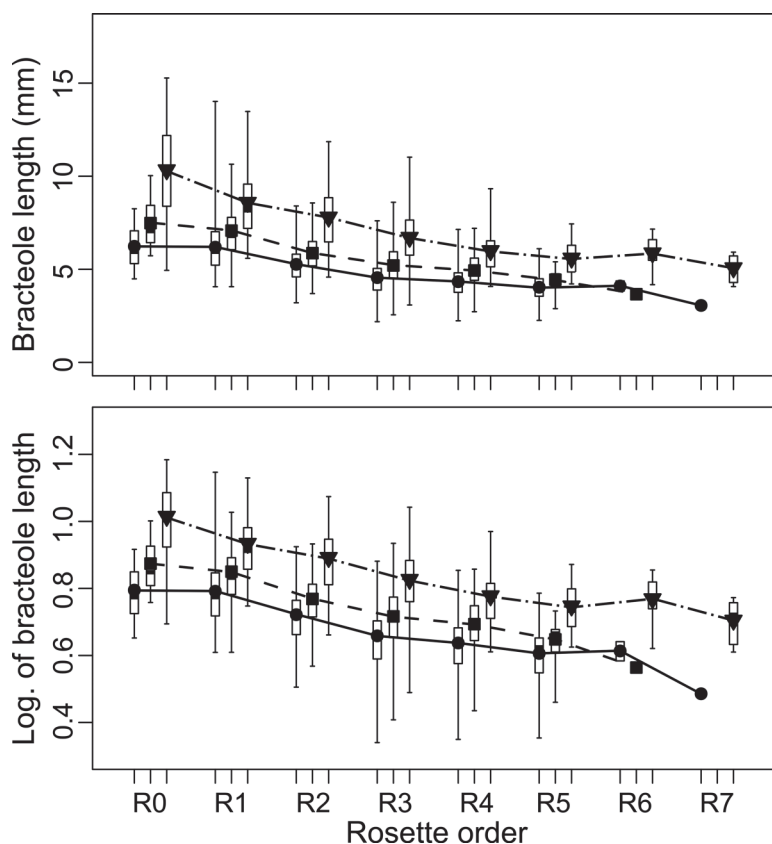

FIGURE 2. Means and distributions of the bracteole length data. The box-plots summarize the distribution of the data grouped by subpopulation and rosette order. The overlayed symbols represent the means for those groups (France: solid line and dots, Galicia: dashed line and squares, Castile-León: dash-dot line and triangles). The upper graph shows the original data and the lower graph the data after logarithmic transformation. The distributions of the transformed data showed improvements in symmetry and homoscedasticity. The parallel, descendent, non-overlapping mean lines for the three populations indicate the existence of significant effects due to both factors (subpopulation and rosette order) and the lack of significant interaction between them.

As a first step in the analysis, we carried out normality and homoscedasticity tests in order to determine whether the data met the conditions needed for a formal ANOVA test. To do this we applied the Shapiro Wilks test of normality and the Fligner-Killeen test of homoscedasticity to the groups with sufficient data. This is one of the most efficient tests in the presence of non-normal data distributions (Conover *et al*. 1981). As will be explained in the results section, we applied a logarithmic transformation to improve both the normality and homoscedasticity of the data.

To show the differences among subpopulations in the variables analysed we employed the means of ten individuals instead of the original data themselves, thereby reducing the variability of the data and making the differences between the subpopulations clearer. To do this we resampled the data with replacement to form groups of ten randomly selected individuals, and calculated their means. We repeated this procedure 10,000 times (Jackson and Somers 1989). The resampling was done within each subpopulation independently (i.e. 30,000 means were calculated). Finally, we applied kernel smoothing in its uni- and bivariate variants to estimate the density distribution functions of the means (Wand and Jones 1995).

All the calculations and graphs were done with the R language (R Development Core Team 2011), and Package KernSmooth (Wand 2011) used for kernel smoothing procedures.

Results

We found that a logarithmic transformation of the data greatly improved their homoscedasticity. This can be seen in Fig. 2, which shows the original and transformed data for bracteole length. It also produced a general improvement in the normality of the data. However, a small number of groups with normal distributions prior to the transformation become not normally distributed afterwards. It should be noted, however, that we set a probability limit of $p<0.05$, which made our tests conservative. A lower limit –say 0.01– would have identified fewer groups as non-normal. Despite this, we decided to apply the transformation to all the data, since the sensitivity of the ANOVA to heteroscedasticity is greater than its sensitivity to departures from normality. Fig. 2 shows the original data for bracteole length and the same data after transformation and clearly demonstrates the advantages of the transformation, particularly with regard to the stabilisation of the variances. The Mediterranean samples had greater variances than the Atlantic samples prior to the transformation.

The results of the ANOVAs are presented in Appendix I. Most of them detected significant effects ($p<0.001$) for both factors but none detected interaction effects. The cases for which a factor effect was found less significant ($0.050 > p > 0.001$) were the subpopulation factor for leaf width and the rosette factor for leaf length/leaf width.

Although the results of the ANOVA showed differences between subpopulations, the dispersion of the individual measurements was large, thus producing a substantial overlap among the data distributions. This makes it quite difficult to assign an individual to any of the three subpopulations based on a single measurement. Fortunately, this problem can be solved by using the mean of several measurements as the basis for comparison. We used the resampling procedure described in the methods section to construct the distribution of the means of groups of ten measurements. We also tested this method using the means of smaller groups, but we found that the resolution power was not sufficient to achieve a clear separation between subpopulations.

We found that the most resolutive variables were those related to bracteole shape. Fig. 3 shows a contour graph representing the bivariate density function estimated with kernel smoothing for the variables bracteole length and bracteole width (means of ten measurements). It shows that the Mediterranean subpopulation can be clearly differentiated from the two Atlantic subpopulations, although these two subpopulations – while different – show a large degree of overlap which makes them more difficult to differentiate.
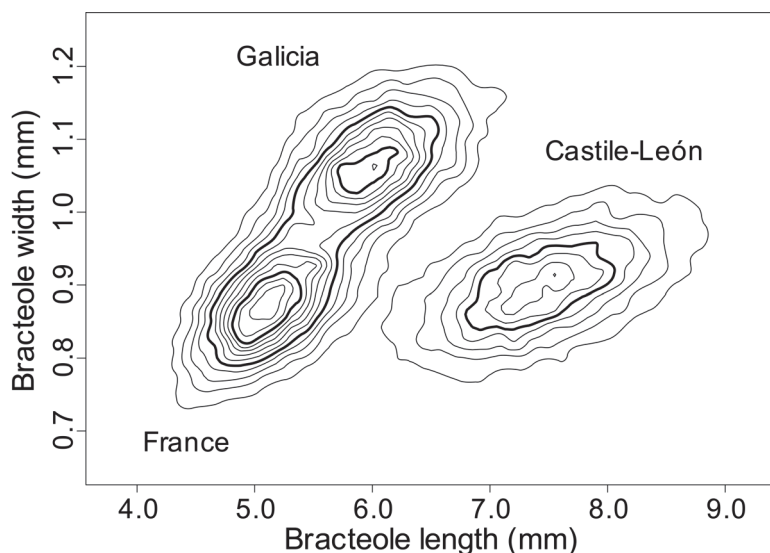

FIGURE 3. Bivariate probability density plot for the variables bracteole length and bracteole width. The isolines are 0.2 probability units apart, and those with a thicker line are the 1.0 and 2.0 levels. Note that although the three modes are clearly distinguishable, there is a large overlap between the Galician and French subpopulations.

The graph also suggests that a more synthetic method of differentiating the individuals belonging to the Mediterranean subpopulation would be the ratio between bracteole length/bracteole width (B_l/B_w). We calculated this quotient for the resampled data (means of ten measurements as before), and Fig. 4 shows the density function for these data. The value $B_l/B_w = 7$ can be considered the limit between the values obtained for the Atlantic subpopulations (which are lower than this value) and the Mediterranean subpopulation. It should be noted that all

these calculations were done with untransformed data. An important point to note is that the resampling procedure did not distinguish between rosette orders. As a consequence, any group of ten measurements could serve to identify the subpopulation of origin without any need to take into account the order of the rosettes from which the data were collected.
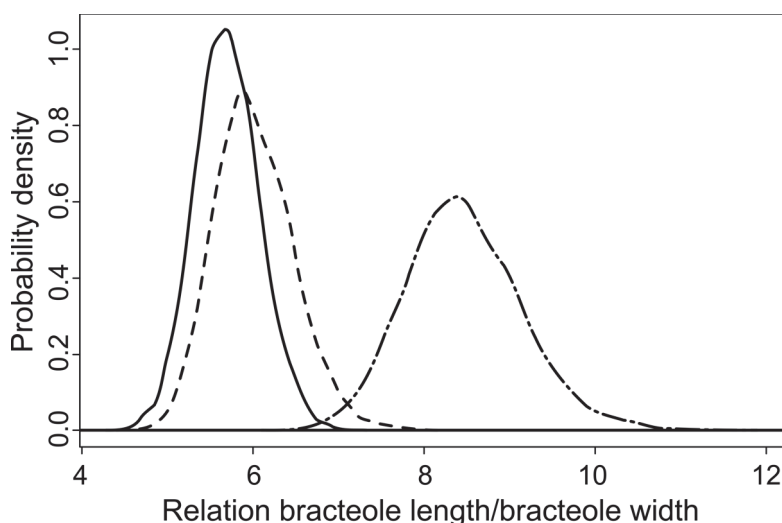

FIGURE 4. Probability density functions of the means ($n = 10$) of the ratio between bracteole length and width for the three subpopulations. Solid line: Brittany, dashed line: Galicia, dash-dot line: Castile-León. Each distribution was estimated by resampling the data for the corresponding subpopulation.

Discussion

The results showed that there were significant differences between subpopulations and between inflorescence orders, and also that there were no significant interactions between factors. The interpretation of these results can clearly be observed in Fig. 2. The mean bracteole length values in the Mediterranean subpopulation were consistently higher than those in the other two subpopulations for all rosette orders. Likewise, a reduction in the mean size was observed with the inflorescence order, and this occurred simultaneously and in parallel in the three subpopulations, which justifies the lack of interaction between the two factors. The patterns shown in Fig. 2 are shared by the other variables, and we thus omitted these graphs. Although all the ANOVAs showed that there were significant differences between subpopulations, these were harder to appreciate in the case of the leaf variables, due to their greater variability. This fact justified the use of bracteole characteristics to differentiate the subpopulations. Moreover, leaves tend to disappear throughout the growing season, whereas the bracteoles persist.

The significance of the inflorescence order factor for all the variables is the result of a decrease in both the width and length of the leaves and bracteoles as the inflorescence order increases. The length/width ratio also diminishes with order. These relationships are consistently higher for the Mediterranean subpopulation, which have narrower leaves and bracteoles compared to the other two subpopulations. In addition to these features, the appearance of the Mediterranean plants was spiny and coriaceous, similar to a small thistle, while the plants from the Atlantic subpopulations had a soft and fleshy appearance.

This species is morphologically very similar to *E. galioides* Lam. (1798:757), an aquatic plant endemic to the western Iberian Peninsula and with which it has been confused (Nieto Feliner 2003). Both species seem to be close relatives (Chater 1968; Nieto Feliner *op cit*.), but unfortunately, there are not data to support a discussion of their taxonomic relationship. We have included *E. galiodes* in the key at the end of the paper in order to facilitate its differentiation from the subspecies of *E. viviparum*.

The morphological data presented here, in conjunction with the aforementioned genetic data (Rodríguez-Gacio *et al*. 2009), lead us to conclude that the Mediterranean subpopulation can be considered as a distinct subspecies from the Atlantic ones (see the keys of Chater (1968) and Nieto Feliner (2003)).

Taxonomy treatment

Eryngium viviparum J. Gay subsp. *bariegoi* M. I. Romero Buján & C. Real *subsp. nov.* (Fig. 5)

Eryngium viviparum J.Gay subsp. *bariegoi* is similar to *E. viviparum* subsp. *viviparum,* from which it is easily distinguished by the bracteoles of the flowers which are very narrow and long, presenting appear nearly awned. As a result the new subspecies is a spiny plant, like a tiny thistle.

Type:—SPAIN. Zamora: Otero de Bodas 29TQG3347, 828 m, 1 January 2003, *MI. Romero, P. Ramil & P. Bariego 2424* (holotype SANT! [SANT 50009 specimen on the top left], isotypes: remaining specimens).

Herbaceous spiny plant with procumbent stems 1(−2) mm; basal leaves linear-lanceolate, dentate, with lamina decurrent on petiole and present at the time of flowering. Inflorescence cylindrical, capitula with similar bracts and bracteoles, rigid and lanceolate-acuminate with 1 spine. Bracteoles > 7 times longer than wide.

Distribution and habitat:—Endemic subspecies from the Mediterranean areas of the Northwest of the Iberian Peninsula. The plant grows in very shallow temporary ponds which exist only in winter. The habitat is indicated as a priority habitat type (3170 * Mediterranean temporary ponds) in the Annex I of The Habitats Directive (1992) into the freshwater habitats, standing water.

Etymology:—In recognition of his dedication and contribution to the study of the local flora, the subspecies is dedicated to Patricio Bariego, Technical Staff of Natural Parks Service of Castile-León Community.

Additional specimens examined:—FRANCE (vouchers from the Paris Hebarium [ID N° of voucher]). Morbihan. Carnac; August 1939, *P. Jovet* [P00271714]; Between Ploemel and Erdeven, 09 September 1966, *H. Bouby* [P00271715]; Carnac, August 1849, *J. Lloyd* [P00272716]; Auray, 17 September 1847, *Maire* [P00272717]; Ploëmel, 29 August 1895, *R. Ménager* [P00271718]; Erdeven, 17 September 1848, *Grenier* [P00271719]; Ploëmel, 17 August 1928, *J. Charrier* [P00271720]; Ploemel, 15 August 1883, *E. Préaubert & Ch. Bouvet* [P00271721]; Carnac, September 1869, *Toussaint* [P00271722]; Vannes, August 1849, *Lloyd?* [P00271723]; Carnac, 12-13 July 1928, - [P00271724]; Glaucharnel, 16 August 1907, *G. Hibon* [P00271725]; Carnac, Konaz, 27 August 1873, *Bureau* [P00271726]; Carnac, 12 October 1885, *D. Luizet* [P00271727]; Between Ploemel and Erdeven, 01 September 1866, *G. Gallée* [P00271728]; Erdeven, *Toussaint* [P00271729];

SPAIN (vouchers from the SANT Hebarium [ID N° of voucher]). A Coruña: Melide, 27TNH8350, 449 m elev., 26 October 2000, *MI. Romero, M. Rubinos & P. Ramil 2102* [SANT 46310]; León: Chozas de Arriba, 30TTN7711, 855 m elev., 21 September 2001, *J. Amigo 50101*[SANT 45316]; Villadangos del Páramo, 30TTN7011, 21 September 2001, *J. Amigo 50102* [SANT 45312]; Lugo: Cospeito, 29TPH1888, 440 m elev., 10 October 1997, *J. Amigo, P. Ramil, M. Rodríguez & J. Izco 39497* [SANT 38490]; Rabade, 1 August 1951, *A. Penas* [SANT 06228]; Begonte, 09 August 1951, *A. Penas* [SANT 05909]; Begonte, 24 July 1951, *A. Penas & F. Bellot* [SANT 05361]; Begonte, 29TPH0481, 395 m elev., 17 June 1999, *MI. Romero, Rodríguez Oubiña, P. Ramil & M. Rubinos 2033* [SANT 41397]; Cospeito, 29TPH1685, 395 m elev., 31 July 1987, *F.J. Silva-Pando & G.B.G. 11161* [SANT 17595]; Cospeito, 29TPH1882, 390 m elev., 17 June 1999, *MI Romero, Rodríguez Oubiña, P Ramil & M. Rubinos 2032* [SANT 41391]; Rábade, 01 July 1951, *A. Penas* [SANT 06228]; Ourense: Porqueira, 29TNG9655, 615 m elev., 25 October 2001, *I. Pulgar* [SANT 45414]; Rairíz de Veiga, 29TNG5956, 617 m elev., 09 October 2001, *MI. Romero, I. Pulgar & J. Izco 2122* [SANT 46390]; Rairiz de Veiga, 29TNG5759, 620 m elev., 13 September 2001, *I. Pulgar* [SANT 45319]; Sandiás, 29TPG0663, 619 m elev., 25 October 2001, *I. Pulgar* [SANT 45413]; Sarreaus, 29TPG1065, 618 m elev., 13 September 2008, *I. Pulgar* [SANT 59746]; Sarreaus, 29TPG1065, 619 m elev., 14 October 2001, *I. Pulgar* [SANT 45969]; Vilar de Santos, 29TNG9658, 29 September 1995, *I. Pulgar* [SANT 32524]; Villar de Santos, 1 August 1987, *E. Rico* [SANT 34962]; Xinzo de Limia, 29TNG9967, 640 m elev., 27 October 2001, *I. Pulgar* [SANT 45415]; Xinzo de Limia, 29TPG1358, 640 m elev., 27 October 2001, *I. Pulgar* [SANT 45416]; Junquera de Ambía, 29TPG0468, 630 m elev., 25 October 2001, *I. Pulgar* [SANT 45417]; Zamora: Ayoó de Vidriales, 29TQG3668, 911 m elev., 01 November 2003, *MI. Romero, P. Ramil & P. Bariego 2423* [SANT 50008]; Ayoó de Vidriales, 29TQG3769, 905 m elev., 29 September 2003, *P. Bariego 2835* [SANT 60809]; Cubo de Benavente, 29TQG3668, 895 m elev., 29 September 2003**,** *P. Bariego 2834* [SANT 60810]; Ferreruela, 29TQG4730, 785 m elev., 21 June 2003, *P. Bariego 2482* [SANT 60814]; Fonfría, 29TQG4209, 820 m elev., 06 July 2003**,** *P. Bariego 2481* [SANT 60807]; Melgar de Tera, 29TQG4547, 810 m elev., 22 May 2004, *P. Bariego 2571* [SANT 60812]; Melgar de Tera, 29TQG4447, 810 m elev., 09 August 2003, *P.*

Bariego 2480 [SANT 60815]; Melgar de Tera, 29TQG4347, 820 m elev., 09 August 2003, *P. Bariego 2479* [SANT 60813]; Otero de Bodas, 29TQG3347, 828 m elev., 01 november 2003, *MI. Romero, P. Ramil & P. Bariego 2102* [SANT 50009]; Otero de Bodas 29TQG3347, 810 m elev., 29 September 2003, *P. Bariego2836* [SANT 60808]; Peque, 29TQG2756, 820 m elev., 03 July 2004, *P. Bariego 2542* [SANT 60811]; Vigo de Sanabria, 29TPG9066, 900 m elev., 24 July 2002, *MI.Romero & P. Ramil 2335* [SANT 47268].

Comparison with the type subspecies:—The new subspecies can be differentiated from *E. viviparum* subsp. *viviparum* by the bracteoles of the flowers (Fig. 5), which are narrower and longer than in the subspecies *viviparum*. This can be done by measuring the length and width of ten bracteoles and then calculating the quotient of these variables. Samples with a mean value greater than seven belong to *E. viviparum* subsp. *bariegoi*. When making the selection of bracteoles the rosette order is not relevant, and data from different individuals can be pooled if needed.
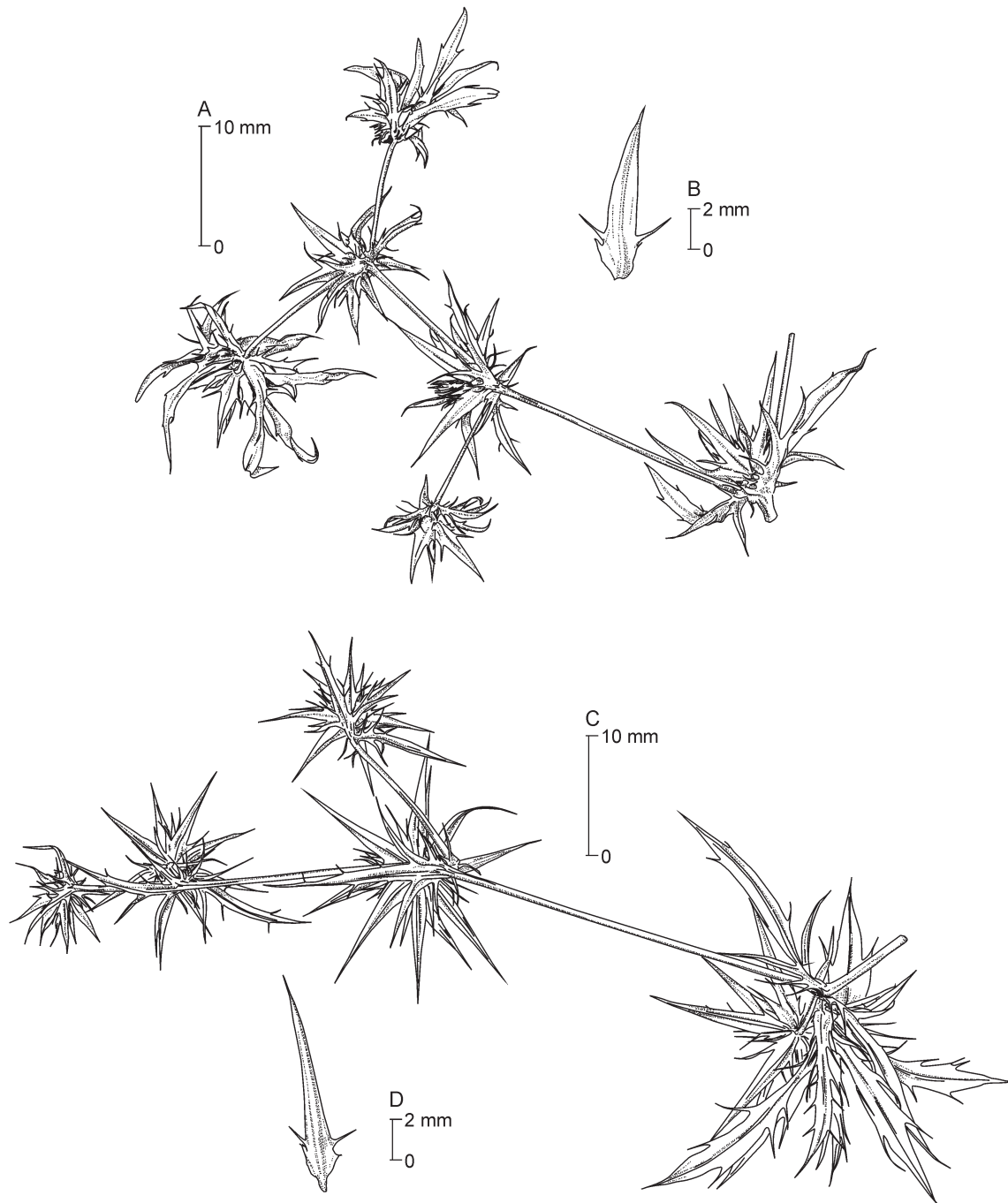

FIGURE 5. A) *Eryngium viviparum* subsp. *viviparum*. A, B drawn from voucher P00271721 of Paris Herbarium [A, branches with basal leaves and inflorescences (capitula); B. bracteole detail]. *Eryngium viviparum* subsp. *bariegoi*. C, D drawn from SANT Herbarium, SANT 60809 [C, branches and D, bbracteole]. Drawing by Luis Gómez-Orellana.

Conservation status:—According to IUCN (2012) the species can be assigned as endangered (EN, criterion and subcriteria B2ac), due to its highly restricted area of occupancy (less than 500 km²) and to the severe fragmentation of its population, which is linked to a very selective habitat "seasonal pools or temporary Mediterranean pools".These habitats are affected by different types of disturbances: changes in land use (drainage, water pollution, etc.), or human or animal pressure, which result in habitat loss. In addition, the number of mature individuals is subject to extreme annual fluctuations because summer heat waves do not allow the development of fruits. As a consequence, climatic change is also a potential risk for this species.

Key for the european aquatic taxa of the genus *Eryngium*
1.   Width of the stems 1(−2) mm, basal leaves green when the plant is in flower: Soft in texture, biennial plant 2
-    Width of the stems (2−) 3−4 mm, dry basal leaves when in flower, spiny annual plant .... *E. galioides*
2.   Bracteoles ≤ 7 times longer than wide, finely denticulate or with spinescent teeth .... *E. viviparum* subsp. *viviparum*
−   Bracteoles > 7 times longer than wide, spiny plant .... *E. viviparum* subsp. *bariegoi*

Acknowledgements

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Appendix I. ANOVA tables.

Table 1: Logarithm of bracteole length.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	6.269	3.134	39.1876	<0.001
Inflorescence order	5	5.129	1.026	12.8239	<0,001
Interaction	10	0.090	0.009	1.128	0.337
Residuals	1235	9.878	0.008		

Table 2: Logarithm of bracteole width.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	1.569	0.784	90.907	<0.001
Inflorescence order	5	1.045	0.209	24.225	<0.001
Interaction	10	0.102	0.010	1.187	0.295
Residuals	1235	10.654	0.009		

Table 3: Logarithm of the bracteole length/ bracteole width ratio.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	7.784	3.892	441.718	<0.001
Inflorescence order	5	1.662	0.333	37.733	<0.001
Interaction	10	0.121	0.012	1.378	0.185
Residuals	1235	10.882	0.009		

Table 4: Logarithm of leaf length.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	1.969	0.984	90.292	<0.001
Inflorescence order	4	1.654	0.414	37.933	<0.001
Interaction	8	0.110	0.014	1.263	0.262
Residuals	346	3.772	0.011		

Table 5: Logarithm of leaf width.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	0.123	0.062	4.434	0.013
Inflorescence order	4	0.950	0.238	17.123	<0.001
Interaction	8	0.116	0.015	1.046	0.401
Residuals	346	4.800	0.014		

Table 6: Logarithm of the leaf length/leaf width ratio.

	D. f.	Sum sq.	Mean sq.	F value	Pr (>F)
Subpopulation	2	1,459	0.730	70,072	<0.001
Inflorescence order	4	0.110	0.028	2.645	0.034
Interaction	8	0.064	0.008	0.767	0.632
Residuals	346	3.603	0.010		