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## Variability in prickly sow-thistle (*Sonchus asper*) from western Mediterranean region

### Abstract

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We studied the morphological variability of *Sonchus asper* (L.) Hill in the western Mediterranean region by means of multivariate analysis. A two-step clustering method indicated that selection of a two-cluster model was optimal, with anther length and ligule length as the main characters contributing to cluster delimitation. These variables presented a general bimodal pattern of frequency distribution, denoting the presence of two floral morphotypes in the complex that, although partially sympatric, seem to show distinct geographical ranges and important differences in their reproductive systems. In addition, a preliminary karyological analysis yielded differences in chromosome morphology between the two morphs. Possible taxonomic implications of this variability are discussed. We propose the segregation of plants showing different flower morphotype as different taxa at specific level: large-flowered plants should be designated as *Sonchus nymanni* Tineo & Guss. in Guss., while small-flowered plants correspond to *Sonchus asper* (L.) Hill.

### Introduction

Prickly sow-thistle (*S. asper* (L.) Hill) is a common weed with a cosmopolitan distribution, although it appears to be introduced in the New World and some tropical areas (Reiche 1910: 39, Boulos 1973). At present, the variability of the species is commonly resolved by subdivision into two taxa: the typical subspecies and one more subspecies designated, depending on the authors, as *S. asper* subsp. *glaucescens* (Jord.) Ball (Boulos 1973; 1976) or *S. asper* subsp. *nymanni* (Tineo & Guss.) Hegi (Hegi 1928: 1110). However, delimitation of the two subspecies remains quite unclear at present. In the most recent worldwide revision of genus *Sonchus*, Boulos (1973) considered that subsp. *glaucescens* could be recognised by a biennial life cycle (annual in the typical subspecies), the presence of a basal rosette, leaf shape, flower heads arrangement and the margin of the fruits. Boulos also attempted to summarize the differences highlighted by V. Tineo and G. Gussone (in Gussone, 1844: 860), and C. T. A. Jordan (1847: 75-77) between typical *S. asper* and the taxa segregated by these authors, *S. nymanni* Tineo & Guss. and *S. glaucescens* Jord., but noted that distinguishing the subspecies was difficult and that hybridisation between them seems to be common, leading to the overlap of diagnostic characters. Strikingly, he failed to incorporate Jordan's indications of flower size and

flower head diameter during anthesis, features that have been suggested as clear diagnostic characters by recent authors (Matthews 1975; Zángheri 1976; Pottier-Alapetite 1981; Mejías 2002). At present, the general acceptance of the vague Boulos's criteria is responsible for imprecise and incongruous information in regional floras, which, in turn, may lead to the idea that there is no real need for taxonomic segregation.

In order to clarify and achieve some consistency for delimitation of taxa within the *Sonchus asper* complex, we have undertaken a general taxonomic revision of this species. Given its wide distribution, we selected the western Mediterranean area as a preliminary range of study. This choice is based on the variability of *S. asper* in the region, and the geographic origin of locality types of currently recognised subspecies: V. Tineo and G. Gussone described *Sonchus nymanni* from Sicilian plants, while C. T. A. Jordan proposed the name *Sonchus glaucescens* for plants collected near Toulon (southern France). Preliminary results for this revision are presented here.

## Material and Methods

The present morphological variability revision is based on 357 exsiccata from the following herbaria: BC, BCN, BM, C, COI, FCO, FI, G, GDAC, JACA, L, LY, and SEV. Among them, 107 herbarium sheets including complete individuals were used for a statistical analysis. Eighteen quantitative characters (Table 1) were recorded and measured using a COMECTA digital caliper for each specimen. For ligules, flower tubes and anthers characters, as well as fruit length (FLO, FWO, FTO, FAO, FAI and FRL; see Table 1) the mean of three measurements per plant was used; remaining values were obtained as a single measurement. Two-step cluster analysis was performed based on Schwarz Bayesian Information Criterion (BIC) since this method allows the automatic determination of the optimal number of clusters (Pérez López 2005; Garson 2010). To avoid high-level redundancy in the data sets, two characters: ligule width in outer flowers (FWO), and anther length in outer flowers (FAO) were removed from the statistical analysis because of the high correlation coefficients ( $R \geq 0.80$ ,  $P < 0.05$ ) obtained with the following variables: ligule length in outer flowers (FLO), tube length in outer flowers (FTO), and anther length in inner flowers (FAI). All variables were typified in order to facilitate normality and homoscedasticity. Statistical calculations and analysis were carried out using the SPSS package version 17.0. Remaining 250 herbarium specimens were measured for the following characters: ligule length and ligule width in outer flowers (FLO, FWO), tube length in outer flowers (FTO), anther length in outer flower (FAO), and anther length in inner flower (FAI) to elucidate the geographical pattern of floral morphotypes in the area studied.

Mitotic chromosome studies were performed with root tips of plants obtained from seeds collected in the field. The root tips were treated with 0.002 M 8-hydroxyquinoline for about 3.5 h at room temperature (Tjio & Levan 1959), fixed in Farmer's fluid (Löve & Löve 1975) and stained with alcoholic-hydrochloric carmine solution. Four to six mitotic plates from at least three individuals per population were used for setting up karyotype idiograms. Measurements and ratios recommended by Teppner & Wetsching (1980) were used to characterize the idiograms. The terminology of Levan & al. (1964) was used for describing chromosome morphology. Karyotype asymmetry was estimated according to Stebbins (1971) and Romero (1986).

Table 1. Characters used in the cluster analysis of *Sonchus asper* (L.) Hill, and their acronyms.

Character	Acronym
Number of stems	STN
Stem width (mm)	STW
Basal leaf length (mm)	LBL
Biggest cauline leaf length (mm)	LLB
Biggest cauline leaf width (mm)	LWB
Length of a mid stem leaf (mm)	LLM
Width of a mid stem leaf (mm)	LWM
Highest leaf division depth (mm)	LDD
Biggest tooth in leaf margins (mm)	DTL
Number of synflorescences per plant	FCN
Number of flower heads per synflorescence	FHN
Ligule length in outer flowers of the flower head (mm)	FLO
Ligule width in outer flowers of the flower head (mm)	FWO
Tube length in outer flowers of the flower head (mm)	FTO
Anther length in outer flowers of the flower head (mm)	FAO
Anther length in inner flowers of the flower head (mm)	FAI
Fruit length (mm)	FRL
Number of ribs in each fruit side	FRN

## Results

### *Morphological variability*

A two-step clustering method indicated that a two-cluster model was optimal for the grouping of our dataset, since it gave the lowest value for the BIC coefficient (BIC = 1972.877, range = 1972.877-4340.94) and showed largest “ratio of distance measure” among centroids (1.894, range = 1.008-1.894). Variable importance plots (Fig. 1) indicated that anther length in inner flowers (FAI) and ligule length in outer flowers (FLO) were the main characters contributing to cluster delimitation. Values for these variables were  $1.79 \pm 0.63$  mm,  $4.72 \pm 0.92$  mm for cluster 1 and  $3.70 \pm 0.55$  mm,  $9.78 \pm 1.88$  mm for cluster 2, respectively. Both characters showed a general bimodal pattern of frequency distribution (e.g., see Fig. 2 for anther length of inner flowers), indicating they could be used as predictors for discriminant functions. Similar bimodal patterns were also shown by anther length in outer flowers (FAO) and ligule width in outer flowers (FWO), as could be expected from the high correlation found between FAO and FAI ( $R = 0.874$ ,  $P < 0.001$ ), and FWO and FLO ( $R = 0.816$ ,  $P < 0.001$ ). Depth of leaf divisions was also found to be meaningful in cluster delimitation, mean values being  $3.23 \pm 2.45$  mm and  $9.12 \pm 5.54$  mm for cluster 1 and cluster 2, respectively. Figure 3 shows that the long anther morph is virtually absent in the Iberian Peninsula and large areas of France, but it is quite common in southeast France. In the Italian Peninsula, Sicily and North Africa both morphs seem to be present.

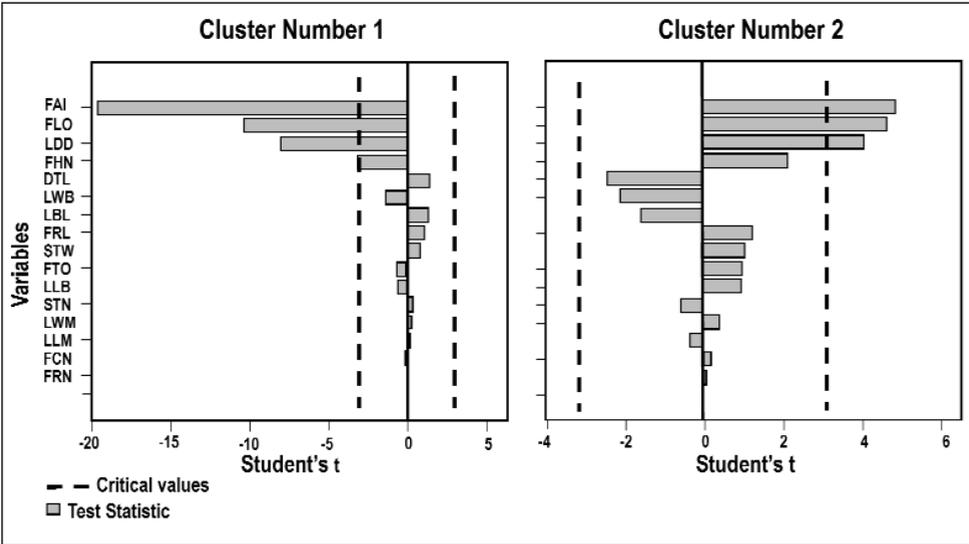


Fig. 1. Importance of the variables registered for two-step clustering in *Sonchus asper* (L.) Hill. For variable acronyms, see Table 1.

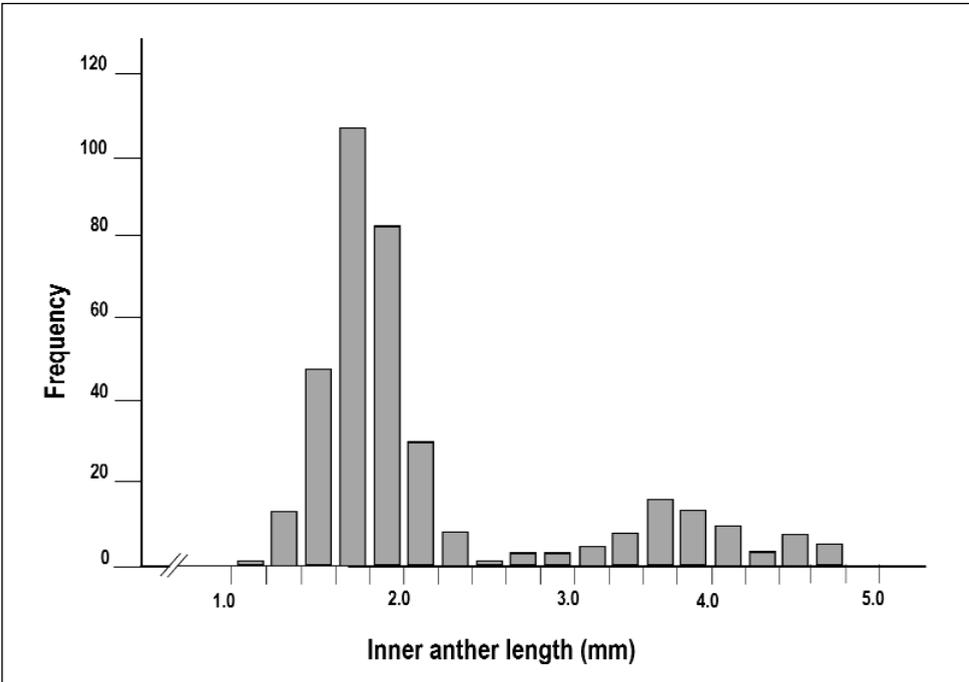


Fig. 2. Frequency distribution of anther length in inner florets of the flower heads in *Sonchus asper* (L.) Hill (N=357).

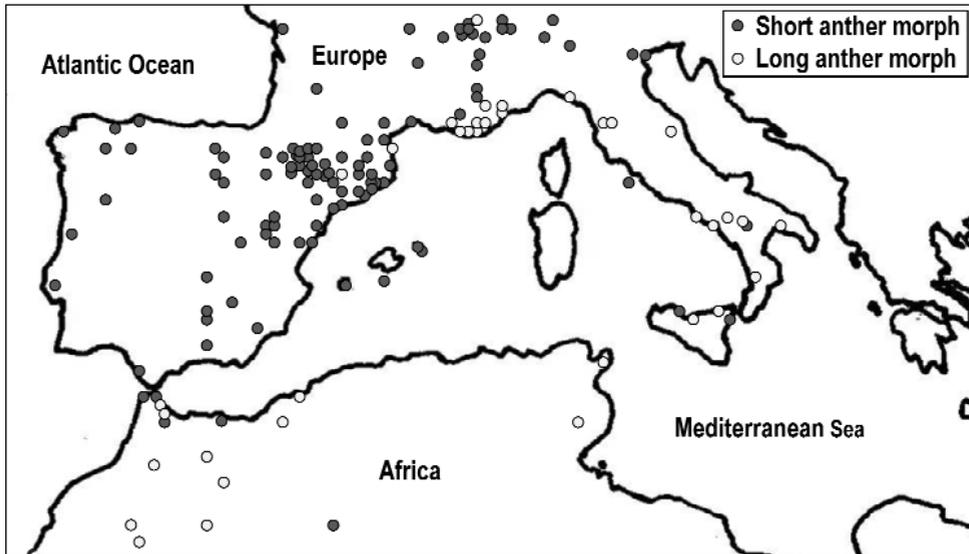


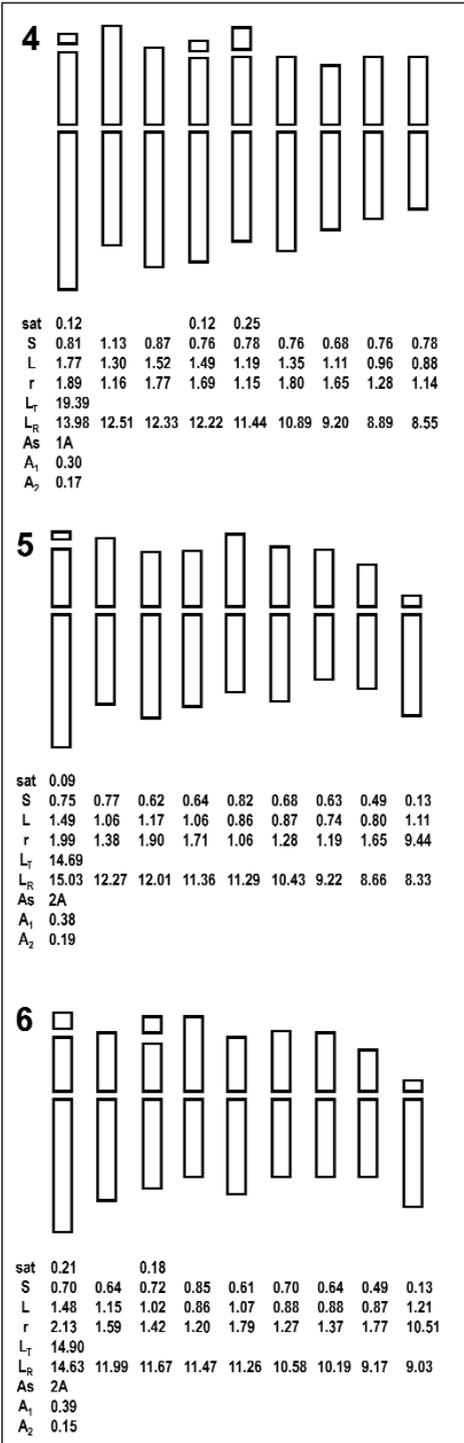
Fig. 3. Geographical pattern of distribution of anther length morphs in *Sonchus asper* (L.) Hill from Western Mediterranean area.

### Cytotaxonomy

Using cluster analysis results and taxonomic literature indications, three populations were selected for karyological observations: a) Jbel Tazzeke (Morocco, RNG SL Jury 11810), which was composed of large flower heads plants with subrosulate habit; b) El Burgo - Álora (Spain, SEV 126466), that included small flower heads plants showing the main characteristic reported by Boulos (1973) for *S. asper* subsp. *glaucescens* (Jord.) Ball; i.e., subrosulate habit, umbel-like synflorescences and narrow-wing fruits; and c) Murcia, Calasparra (Spain, SEV 126488) comprising typical *Sonchus asper* (L.) Hill subsp. *asper* individuals, with leaves all along the stem and small flower heads. The three populations showed  $2n = 18$  as a somatic number as is the rule for the species (Mejías & Andrés 2004). Karyotypes were quite similar in chromosome morphology and chromosome size (Figs. 4-6), but two main differences should be highlighted. First, the number of satellited pairs ranged from one to three; and second, a pair of small telocentric chromosomes was detected in the populations from El Burgo - Álora (Fig. 5) and Calasparra (Fig. 6), which was absent in the population from Jbel Tazzeke (Fig. 4). This feature determined that Stebbins's type of symmetry and Romero's intrachromosomal asymmetry index were 1A and  $A_1 = 0.30$ , respectively, in this last population; while a 2A symmetry type and  $A_1 = 0.40, 0.41$  were deduced from the El Burgo - Álora and Calasparra populations.

### Discussion

The analysis of morphological variability in *Sonchus asper* complex shows the existence of two major groups of plants mainly characterised by flower size. Geographical seg-



Figs 4-6. Haploid idiograms in *Sonchus asper* (L.) Hill. 4. Morocco: Jbel Tazzeka (RNG, SL Jury 11810). 5. Spain: El Burgo - Álora (SEV 126466). 6. Spain: Calasparra (SEV 126488). sat: satellite length ( $\mu\text{m}$ ); S: short arm length ( $\mu\text{m}$ ) L: long arm length ( $\mu\text{m}$ ); r: L/S; L<sub>T</sub>: total length of the haploid karyotype ( $\mu\text{m}$ ); L<sub>R</sub>: relative length of each chromosome (%); As: Stebbins's asymmetry index; A<sub>1</sub> and A<sub>2</sub>: Romero's asymmetry indexes).

regation of the two morphotypes (Fig. 3) reinforces the importance of clustering analysis and the interest of taxonomic segregation. It has commonly been considered that *S. asper* shows inconspicuous flower heads with high incidence of selfing (Mejías 1992). However, our results indicate the existence of another group of plants within the complex, bearing attractive flower heads that show higher incidence of outcrossing (unpublished data, José A. Mejías). For example, in plants from Jbel Tazzeqa, the diameter of fully expanded flower heads during anthesis ranged from 23.1 to 41.5 mm ( $34,1 \pm 5,5$ ), while values varied between 18.0 and 20.2 mm ( $18,6 \pm 1,2$ ) for plants from Calasparra (Mejías *l.c.*). It should be noted that anther and outer ligule length seem to be the most discriminative characters for the groups since they clearly show bimodal frequency distribution patterns. Leaf division depth has also been considered for cluster delimitation, but the high standard deviation and a non-bimodal frequency distribution pattern indicate it is of low interest as discriminant function.

Present findings contrast with Boulos's description (Boulos 1973, 1976) of morphological variability within *S. asper*. Currently, common acceptance of Boulos's approach has determined that arrangement of leaves and inflorescences are the major characters involved in subspecies diagnosis. Strikingly, however, variability in flower size within the *Sonchus asper* complex had long been revealed as a potential diagnostic character. Tineo and Gussone (in Gussone 1844: 860) indicated that flower heads in *S. nymanni* [*Sonchus nymanni* Tineo & Guss. in Guss., Fl. Sic. Syn. 2: 860 (1844)] doubled in size to that of *S. asper*. Jordan (1847: 75) proposed the name *S. glaucescens* [*Sonchus glaucescens* Jord., Observ. Pl. Nouv. 5: 75 (1847)] for plants in southern France bearing large flowers, and he also indicated that diameters of flower heads <<... pendant l'anthèse égalent environ 4 ou 5 cent. [are about 4 or 5 cm during anthesis] ...>>, and pointed out that *Sonchus asper* flowers are <<... d'un jaune fort pale et de moitié plus petites [quite pale yellow and half the size] ...>>. According to Jordan (1847: 77), *S. nymanni* and *S. glaucescens* differ in the number of fruit ribs and habit, with the former showing five ribs instead of three (as is the rule in the complex) and perennial roots. During the twentieth century, some authors also recognised the taxonomic interest of flower size and flower head diameter and reported distinct taxa at specific (Halácsky 1902: 206, Rouy & Foucaud 1905: 202-203, Coste, 1903: 436) or subspecific (Hegi 1928: 1110, Matthews 1975: 691, Zángheri 1976: 797, Pottier-Alapetite 1981: 1118, Mejías 2002: 675) levels, but other proposed a taxonomic segregation based on different criteria (Coutinho 1913: 673, Pignatti 1982: 263).

Although a more extensive prospection is necessary, cytotaxonomy appears to reinforce the convenience of taxonomic segregation within the current *Sonchus asper* complex. The karyotype of plants from Jbel Tazzeqa (Morocco) lacks a telocentric chromosome pair that is present in the other two populations from the Iberian Peninsula and seems to be common in the group (Mejías & Andrés 2004). This reflects differences in the organization of chromosomes that are probably responsible for possible interfertility barriers among populations (Grant, 1966; Chandler & al. 1986; Levin 2002: 72-74). Interestingly, the first population consists of large-flower plants, while the other two represent the small-flower morphotype. Other differences based on the number of satellites or small variations in chromosome asymmetry are not necessarily indicators of speciation processes. Contrary to the general indication of annual or biennial cycles in *S. asper*, the Moroccan population studied shows long life cycle (plants were cultivated in the greenhouse for five years) as

did several others from the area. This and some other characters (e.g., presence of more than three ribs in the fruits) fit the description of *Sonchus nymanni* Tineo & Guss. However, taxonomic value of characters proposed to separate *S. nymanii* and *S. glaucescens*, as well as the differentiation of other large-flower taxa within the complex described in the literature [i.e., *S. graecus* Reut. ex Weiss in Verh. Zool.-Bot. Ges. Wien 19: 45 (1869), *S. giganteus* Rouy, Fl. Fr. 9: 203 (1905) and *S. kralikii* Rouy, Fl. Fr. 9: 203 (1905)] must be evaluated carefully. A karyological and DNA phylogenetic analysis within the group would be of interest in order to elucidate if all representatives constitute a single lineage or several ones, which can be of taxonomic relevance. A similar study should be desirable within the small-flower group since a remarkable variability in habit and vegetative characters is also present within.

In our view, the present results clearly indicate that the *Sonchus asper* complex comprises at least two taxa in the western Mediterranean, which is reflected in the existence of two floral morphs. Both morphs are also present in the eastern Mediterranean, where probably large-flower morphotype is most common (Halácsy 1902; Matthews 1975, Mejías personal observations). We propose the separation of two taxa at species level: *Sonchus asper* (L.) Hill for small-flower plants, and *Sonchus nymanni* Tineo & Guss. for large-flower individuals. Anyway, a parallel integrative study dealing with karyology, phylogeny, biological and morphological variability within the complex now in progress will hopefully more clearly elucidate the number of taxa involved and their taxonomic range.

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