

## **IS *LIMONIUM CAVANILLESII* ERBEN (PLUMBAGINACEAE) REALLY AN EXTANT SPECIES?\***

by

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### **Resumen**

SÁEZ, L. & J.A. ROSSELLÓ (1999). ¿Ha sido realmente relocalizado *Limonium cavanillesii* (Plumbaginaceae)? *Anales Jard. Bot. Madrid* 57(1): 47-55 (en inglés).

Se describe una nueva especie apomíctica triploide, *Limonium perplexum*, de una localidad del litoral de la provincia de Castellón, España. La nueva especie había sido confundida hasta el momento con *L. cavanillesii* Erben, de la que se conocen únicamente escasos ejemplares de herbario. La morfología de *L. perplexum*, el número cromosómico y la combinación polínico-estigmática permiten relacionarla con el complejo de *L. duriusculum*. Sobre la base del estudio morfológico se establece la hipótesis de que *L. cavanillesii* Erben podría haber sido originado por una hibridación entre *L. perplexum* y *L. dufourii*.

Palabras clave: *Plumbaginaceae*, *Limonium*, táxones apomícticos, endemismo, flora mediterránea.

### **Abstract**

SÁEZ, L. & J.A. ROSSELLÓ (1999). Is *Limonium cavanillesii* Erben (Plumbaginaceae) really an extant species? *Anales Jard. Bot. Madrid* 57(1): 47-55.

A new triploid apomictic species (*Limonium perplexum*) is described from a single coastal locality of E Spain. The new species has been previously confused with *L. cavanillesii* Erben, which is only known from few herbarium specimens and it is believed to be extinct. Overall morphology, chromosome number, and the pollen/stigma combination suggest that *L. perplexum* belongs to the *L. duriusculum* complex. Based on morphological grounds it is suggested that *L. cavanillesii* Erben could have been originated through a crossing between *L. perplexum* and *L. dufourii*.

Key words: *Plumbaginaceae*, *Limonium*, apomictic taxa, endemism, Mediterranean Flora.

### **INTRODUCTION**

The *Limonium duriusculum* complex is a group of triploid and tetraploid agamic taxa restricted to coastal and inland places in the Western Mediterranean basin (E Spain, SE France, Sardinia, Balearic islands; PIGNATTI,

1976; GREUTER & al., 1989; ERBEN, 1978, 1993). The taxonomic treatment of the complex is mainly based on the work of ERBEN (1978, 1980, 1981, 1989, 1991), who recognized up to 9 species, most of which show a very narrow distribution. Members of this complex can be easily adscribed to the

\* Part seven of the series "A taxonomic and biosystematic revision of the genus *Limonium* (Plumbaginaceae)". For part six see *Anales Jard. Bot. Madrid* 56(2): 269-278 (1998).

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group on the basis of some reproductive features like the few flowered spikelets, and the appearance of the calyx after anthesis.

One of the narrow endemics of the *L. duriusculum* complex is *L. cavanillesii* Erben. This taxon was first described (SENEN, 1913) as *Statice duriuscula* var. *valentina* Sennen & Pau from plants collected at Benicarló (E Iberian Peninsula) few years before. Later on, the taxon was described as *L. cavanillesii* (ERBEN, 1980) on the basis of the same material collected by Sennen. *L. cavanillesii* has not been found again in the type locality (ARAN, 1996), but some authors (AGUILERA & al., 1984; SAMO LUMBRERAS, 1994) claimed that plants coming from a coastal locality at Sierra de Hirta (not far away from Benicarló) should be adscribed to *L. cavanillesii*. This finding prompted the study of these plants for conservation purposes. It was shown that the population of Sierra de Hirta was monomorphic regarding RAPD markers (PALACIOS & GONZÁLEZ CANDELAS, 1997), but by means of AFLP markers several intrapopulational genotypes could be detected (PALACIOS, 1997). Micropropagation of plants from this population was successfully achieved using inflorescence stem pieces as initial explants (AMO-MARCO & IBÁÑEZ, 1998).

When plants from Sierra de Hirta were compared to the type material of *L. cavanillesii* several noteworthy differences were found. The study revealed that the population from Sierra de Hirta belongs to an undescribed taxa of the *L. duriusculum* complex, which is here described as a new species.

#### MATERIAL AND METHODS

**Morphology.** Twenty-six vegetative and floral attributes were scored on the type material of *L. cavanillesii* (MA, holotype; BC, isotype), and on plants from Sierra de Hirta. Herbarium specimens and living material from some related taxa of the *L. duriusculum* complex (*L. duriusculum* (Girard.) Fourr., *L. companyonis* (Gren. &

Billot) Kuntze, *L. thiniense* Erben, *L. rigualii* M.B. Crespo & Erben, *L. geronense* Erben, *L. validum* Erben, *L. bianorii* Erben, *L. orellii* Erben, and *L. connivens* Erben) were examined for comparative purposes.

**Breeding system.** Flowers were removed from herbarium specimens and pollen grains were stained following the ALEXANDER (1980) technique.

**Karyology:** Seeds were germinated in Petri dishes on moistened filter paper and root tips were pretreated for about 4h with 0.2 % colchicine, fixed in ethanol: glacial acetic acid (3:1) at 4 °C for 24h, hydrolysed in HCl 1N for 3 minutes at 60 °C, and stained with acetic orcein overnight. Root tips squashes were made in 45 % acetic acid.

#### RESULTS

#### *Limonium perplexum* L. Sáez & Rosselló, sp. nov. (figs. 1, 2a)

*Triploideum, agamospermum, simile speciebus Limonio duriusculo et Limonio cavanillesii. Planta annualis, ± glabra. Folia basalia florendi tempore persistentia, 10-100 × 3-20 mm, obtusa, mucronata, in petiolum 1/3-1/2 longitudinis laminae attingentem, 1-2 mm latum attenuata. Caules usque 50 cm longi, erecti, ramosi. Inflorescentia paniculata, 10-30 × 8-24 cm. Rami omnes fertiles, usque ad 17 cm longi, sub angulis 50-90° abeentes, longiores dense ramosi. Spicae 2-14 cm longe, rectae. Spiculae 5-6 mm longae, 2-3 floriae, ad 1-3(4) in 1 cm dispositae. Bractea inferiora 1,3-1,6 × 1,5-1,6 mm, triangulari-ovata, acuta vel obtusa, margine late membranaceo, parte centrali subcarnosula, longe acuminata, acumine marginem non attingente. Bractea media 1,6-1,8 × 1,1-1,3 mm, oblongo-elliptica, rotunda ad subemarginata, membranacea. Bractea superiora 4,3-5 × 2,9-3,1 mm, obovata ad elliptica, rotunda ad obtusa, margine late membranaceo; parte centrali carnosula 3,1-4,1 × 1,8-2 mm, oblonga, triangularis acuminata, acumine 0,7-1 mm longo, marginem*

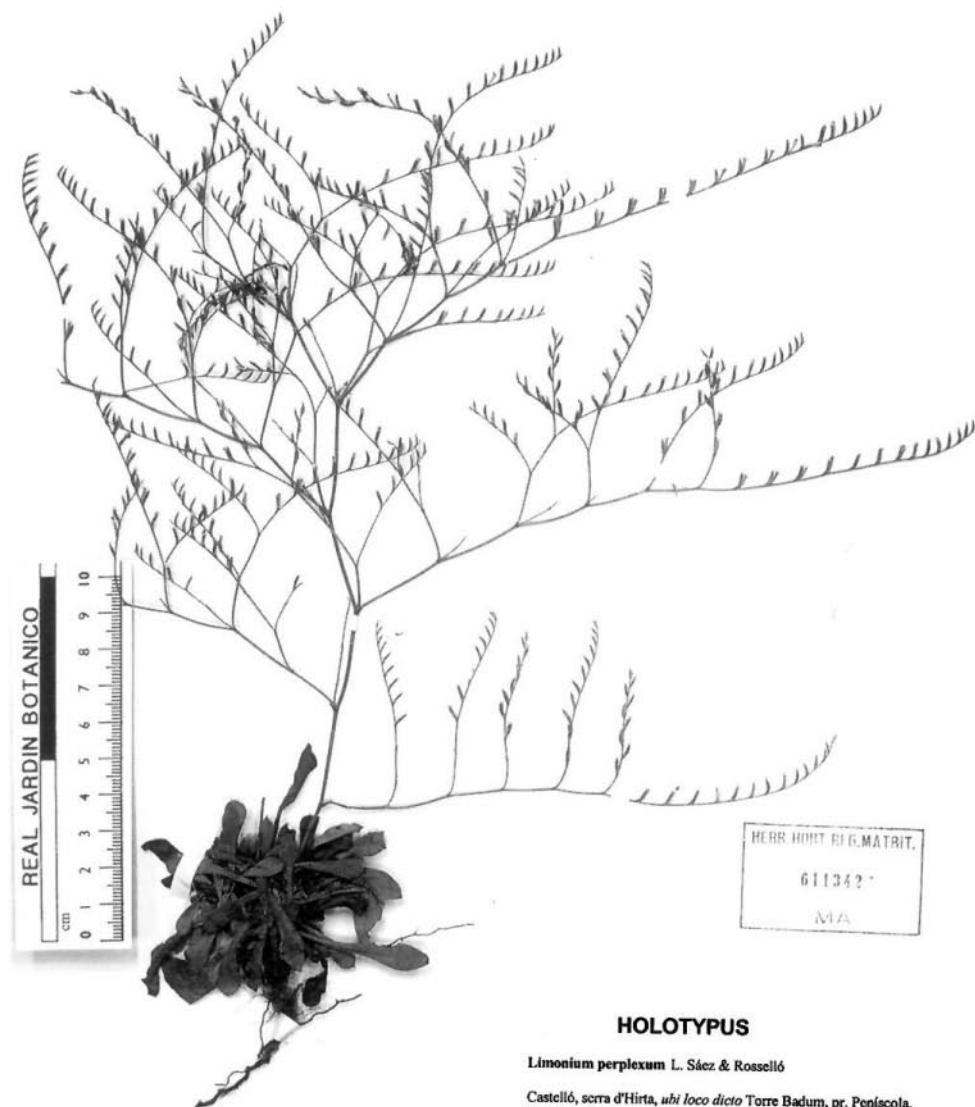


Fig. 1.—*Limonium perplexum* (holotype, MA).

*non contingente. Calyx 4,5-4,8 mm longus, tubo plerumque dense longae piloso; dentes calycis 0,4-0,6 × 0,5-0,6 mm, triangulares ad triangulari-elliptici; costae calycis supra partem basalem dentium desinentes. Corolla infundibuliformis. Petala 7,3-7,8 × 2,3-2,7 mm, cuneata, pallide violacea. Combinatione pollen/stigma "B/cob".*

*Holotypus:* Castelló, serra d'Hirta, ubi loco dicto Torre Badum, pr. Peníscola, 31T BE7667, 10 m in arenosis maritimis, ubi die 30-V-1997, M. Mayol & L. Sáez legerunt, MA 611342 (isotypi adsunt in W, BCC atque in herb. L. Sáez).

Perennial with many stems. Caudices 1-5 cm, loosely branched, spirally leafy in the upper part. Basal leaves usually green at anthesis, 1-10 × 0.3-2 cm. Blade spatulate to subelliptical, smooth, tip obtuse, with a short, 0.2-0.3 mm apiculum; 1-3 nerved. Petiole slightly canaliculate, 1/3-1/2 as long as the blade, 1-2 mm wide. Stem 15-40 cm long, erect, smooth. Inflorescence paniculate, branched near the base, 10-30 × 8-24 cm. Branches loosely distichous, up to 17 cm long, erect-patent to spreading; non flowering branches absent. Spikes 2-14 cm long, with 1-3(4) spikelets per cm. Spikelets 5-6 mm long, 2-3 flowered. Outer bract 1.3-1.6 × 1.5-1.6 mm, triangular-ovate, acute to obtuse, occasionally with a few short eglandular hairs; margin broadly membranous, central part subfleshy, long acuminate, the acumen nearly reaching the margin. Middle bract 1.6-1.8 × 1.1-1.3 mm, oblong-elliptic, blunt to subemarginate, membranous. Inner bract 4.3-5 × 2.9-3.1 mm, obovate to elliptical, obtuse to rounded, with a broad membranous margin; central part subfleshy, 3.1-4.1 × 1.8-2 mm, oblong, triangular-acuminate, the acumen 0.7-1 mm, not reaching the margin. Calyx 4.4-4.8 mm, tube densely hairy, with long eglandular hairs; teeth ca. 0.4-0.6 × 0.5-0.6 mm, triangular-ovate to semi-elliptic; midrib exceeding the calyx lobes. Corolla funnel-shaped. Petals 7.3-7.8 × 2.3-2.7 mm, cuneate, emarginate, deep violet. Pollen-stigma combination: B/cob.

## KARYOLOGY

Plants from Sierra de Hirta showed a chromosome number of  $2n = 27$ , which suggests a triploid level for *L. perplexum*. This result agrees with the cytological knowledge of the *L. duriusculum* complex. Nearly all studied taxa are triploid with  $2n = 26$  (*L. thiniense*; ERBEN, 1981) or  $2n = 27$  (*L. validum*, *L. bianorii*, *L. connivens*, *L. companyonis*, *L. duriusculum*; ERBEN, 1978, 1981, 1989, 1991), whereas a tetraploid count ( $2n = 35$ ) has been reported for *L. geronense* (ERBEN, 1978).

## BREEDING SYSTEM

*Limonium perplexum* presents a single pollen/stigma combination (B/cob) in all studied plants. Fifteen individuals were scored for pollen stainability. Many of the pollen grains show shape irregularities and the mean of pollen stainability is 4.3 % (sample size: 393 pollen grains). Both data strongly suggest that *L. perplexum* is a predominant or exclusive apomict, as the related members of the *L. duriusculum* complex (Sáez & Rosselló, unpublished data). Few flowers from the type of *L. cavanillesii* were available for study due to the scarcity of the material and the poor conservation of some structures. The pollen has a small reticulum (B type), but it has not been possible to observe the stigma morphology. Therefore, the pollen/stigma combination of *L. cavanillesii* could be B/papillate or B/cob.

## ECOLOGY AND DISTRIBUTION

The new species is known from a single population of ca. 200 individuals on the litoral belt (10 msm) of the the sierra de Hirta (GÓMEZ & al., 1999). The species grows on Jurassic (Malm) sandstones (pH: 8.15) on East exposed slopes. The only associated *Limonium* species is *L. girardianum* (Guss.) Fourr., which grows in the vicinity.

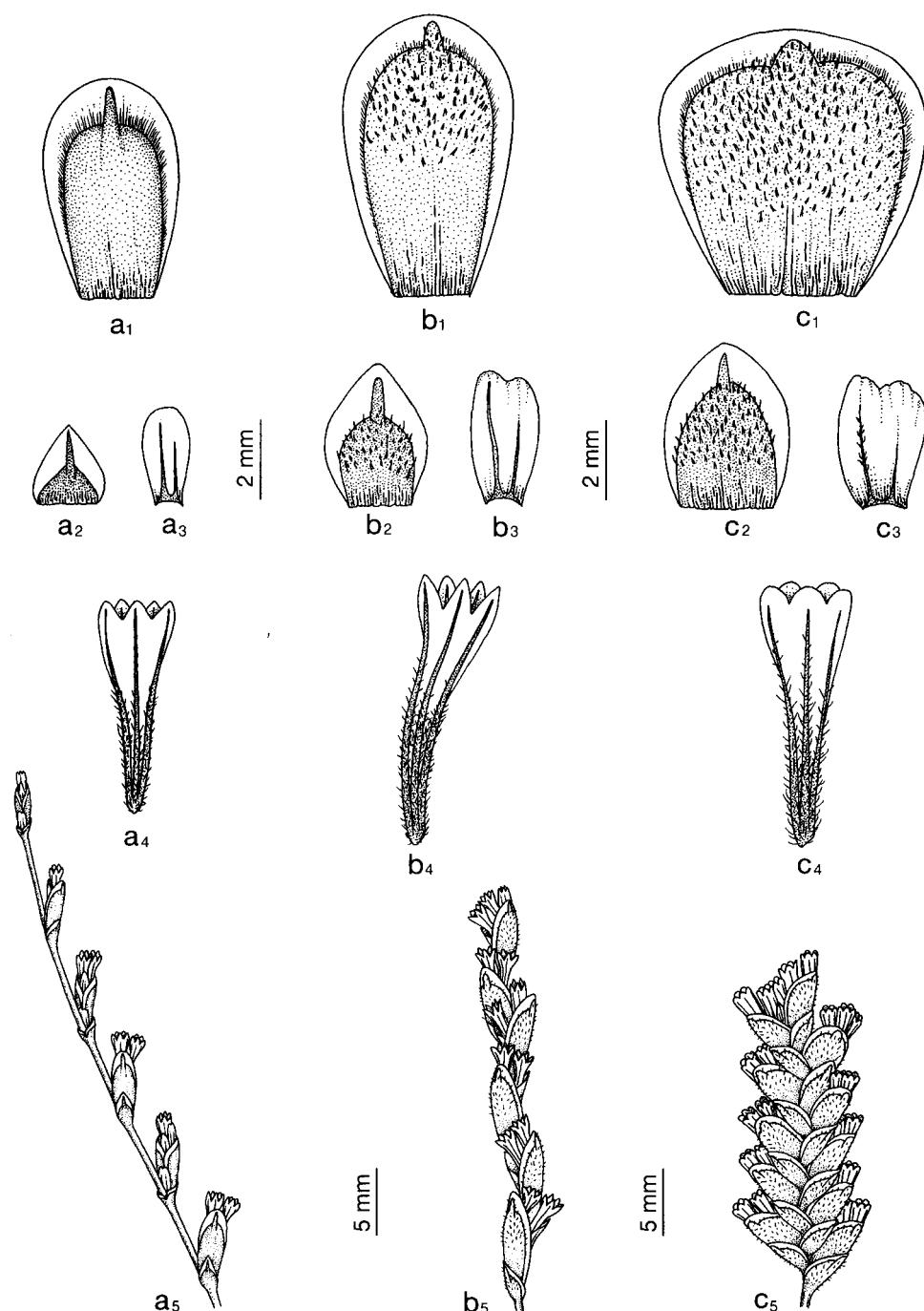


Fig. 2.-a, *Limonium perplexum*; b, *L. cavanillesii*; c, *L. dufourii*; 1, inner bract; 2, outer bract; 3, middle bract; 4, calyx; 5, spike.

## DISCUSSION

*Taxonomic relationships of L. perplexum.* Overall morphology and the chromosome number suggest that *L. perplexum* should be included within the *L. duriusculum* complex. Additional evidence is given by the pollen/stigma combination (B/cob), which is present in all members of the *L. duriusculum* complex but has been rarely recorded in *Limonium* taxa belonging to section *Limonium* (ERBEN, 1979). It is thought (ERBEN 1978, 1980, 1981, 1989, 1991), that *L. duriusculum* may be differentiated from the other members of the complex [*L. bianorii*, *L. companyonis*, *L. connivens*, *L. geronense*, *L. orellii*, *L. rigualii*, *L. thiniense*, *L. validum*] by its annual life cycle, all other related taxa being perennial. However, field observations have shown (Sáez & Rosselló, unpublished data) that some taxa considered to be perennial (*L. thiniense*, *L. vallidum*, and most Balearic plants identified as *L. companyonis*) have in fact an annual or biennial cycle. Plants of *L. perplexum* did not survive after fructification in the field and in the greenhouse, but it should not be excluded that some individuals may exceptionally survive the first flowering period and last several flowering seasons. *Limonium cavanillesii* has been reported to be perennial (ERBEN, 1980, 1993; AGUILERA & al., 1994), but the study of the available material of this taxon is not conclusive. We think that probably *L. cavanillesii* is annual or shortly perennial, but, clearly, it is not a suffruticose camephyte as has been erroneously reported (SAMO LUMBRERAS, 1994). *Limonium perplexum* shows several common features with either *L. duriusculum* and *L. thiniense*, but it may be easily distinguished from them by several diagnostic reproductive characters (Table 1). The base of the scape of *L. duriusculum* is papillate (ERBEN, 1978), but it is usually glabrous in all remaining members of the complex, including *L. perplexum*. Nevertheless, some individuals of this taxon may show scattered papillae in the lowest part of the scape. By contrast, the type material of *L. cavanillesii* shows scapes with many

short papillae or papillate hairs (20-80 mm) throughout their length. Within the complex, the new species has the smallest outer bract (1.3-1.6 × 1.5-1.6 mm). This feature allows *L. perplexum* to be distinguished not only from *L. duriusculum* (1.7-2.4 × 1.5-2.3 mm) but from *L. cavanillesii* (2.6-3.6 × 2.2-2.8 mm). Also, the length of the inner bract of *L. perplexum* differs from *L. thiniense* (4-4.4 mm) and from nearly all other taxa of the complex (*L. duriusculum* excepted; cf. Table 1). In addition, calyx length, calyx teeth and petal dimensions are useful features to discriminate the new species from the related *L. duriusculum*. The number and nature of the differential characters between *L. perplexum* and related taxa, support the microspecific distinction of the population of sierra de Hirta within the *L. duriusculum* complex.

*The status of L. cavanillesii.* Taking into account the clear cut morphological differentiation between *L. perplexum* and *L. cavanillesii* (Table 1), it is difficult to envisage why both entities have been confused in the past. Admittedly, the recognition of *L. perplexum* and the other taxa of the *L. duriusculum* complex at the specific level may be questioned if a broader specific concept is used for the agamic complexes within *Limonium*. Nevertheless, no matter what taxonomic criterion is followed in the *L. duriusculum* complex, the plants recently found at the litoral belt of sierra de Hirta should not be identified with *L. cavanillesii* (Table 1, fig. 3). The presence of non flowering branches, the length of spikelets, the length and width of the outer and inner bracts, the length of the inner bract acumen, the calyx length and the dimensions of the calyx teeth and petals, and the presence of papillae and hairs in the scapes and the surface of the inner bracts are enough discriminant features to reject the inclusion of the sierra de Hirta population under *L. cavanillesii*. The type of this taxon could be easily distinguished not only from *L. perplexum*, but from all recognized taxa of the *L. duriusculum* complex. However, some of the morphological features present in

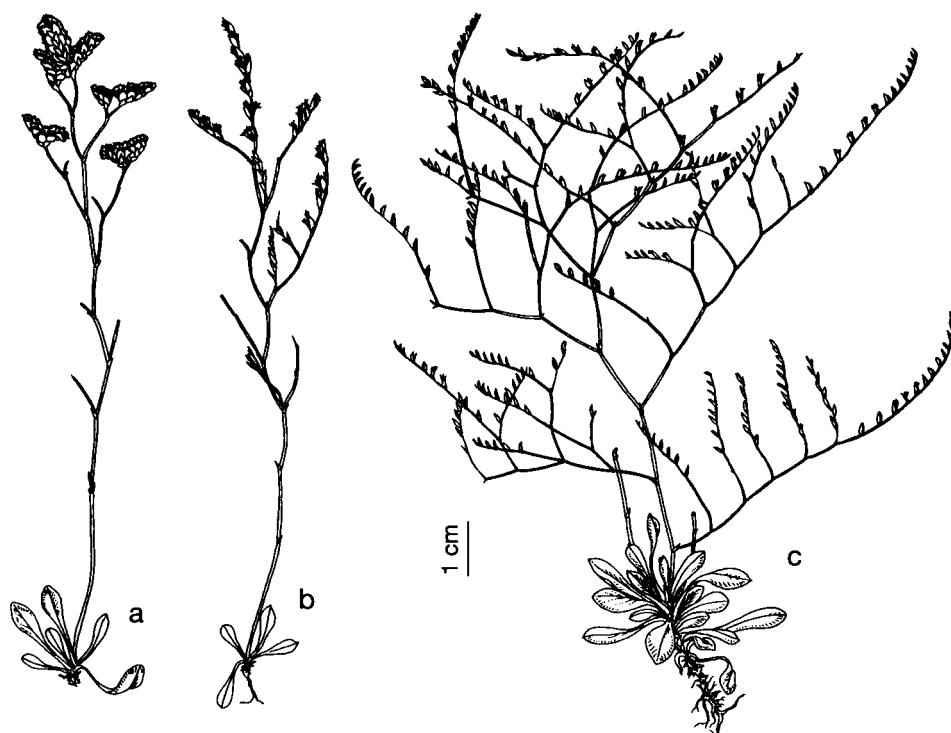


Fig. 3.—a, *Limonium dufourii* (from El Saler, Valence; L. Sáez herb. pers.); b, *L. cavanillesii* (Holotype, MA 91959); c, *L. perplexum* (Holotype).

*L. cavanillesii* apparently relate it to the *L. duriusculum* complex, like the (probably) annual habit, the few-flowered spikes, and the lacerate calyx at the fruiting stage. In addition, the presence of non flowering branches, the greater length of the spikelets and several reproductive features, and the presence of papillae and hairs at the scape and the inner bract, resembles the *L. dufourii* morphology. *Limonium dufourii* is an agamic triploid taxon endemic to E Spain whose nearest populations to the localities where *L. cavanillesii* was recorded are less than 50 km apart. The available evidence suggests an hybrid origin for *L. cavanillesii*. It may have been originated through a cross between *L. dufourii* and a member of the *L. duriusculum* complex, probably *L. perplexum* or a taxon closely related to it. The intervention of *L. dufourii* in the genesis of some coastal taxa from E Spain, like *L. castellonense* Erben

(an extinct plant which is believed to have originated from a cross between *L. dufourii* and *L. angustibracteatum* Erben; ERBEN, 1993) and the tetraploid *L. vigoi* L. Sáez, Curcó & Rosselló (SÁEZ & al., 1998), has been suggested on morphological grounds. This hypothesis implies that the breeding system of the putative parental triploid taxa is not exclusively apomictic, and that some gene exchange is possible between sympatric populations. The hypothesis that *L. cavanillesii* is of hybrid origin was anticipated by SENNEN (1913), and ERBEN (1980: 560) pointed out some common features shared by this taxon and *L. dufourii*. In fact, an isotype specimen of *L. cavanillesii* (MA 92305) which was examined for the *Flora iberica* project was annotated by M. Erben as *L. dufourii* × *L. girardianum* (8-X-1993). Unfortunately, plants resembling *L. cavanillesii* have not been found again at the type

TABLE 1  
MAIN MORPHOLOGICAL DISCRIMINANT FEATURES BETWEEN *LIMONIUM DURIUSCULUM*,  
*L. PERPLEXUM*, *L. CAVANILLESII* AND *L. DUFOURI*  
[Measures are given in mm (except for papillae)]

	<i>L. duriusculum</i>	<i>L. perplexum</i>	<i>L. cavanillesii</i>	<i>L. dufouri</i>
Habit	anual	anual	perennial?	perennial
non flowering branches	absents	absents	presents	presents
Spikelet length	5.5-7	5-6	7.5-8	7-8
Spikelets/cm	3-4	1-3	2-4	6-8
Outer bract length	1.7-2.4	1.3-1.6	2.6-3.6	2.8-3.9
Outer bract width	1.5-2.3	1.5-1.6	2.2-2.8	2.8-3.5
Inner bract length	4.3-5.5 glabrous	4.3-5 glabrous	6.5-7.5 hairy	5.2-6.2 densely hairy
Inner bract width	2.7-3.5	2.6-3.1	3.4-4	4.7-5.8
Inner bract acumen	0.7-1.1	0.7-1	0.6-0.8	0.5-1
Calyx length	4.7-5.5	4.4-4.8	6-6.8	5.4-6
Calyx teeth	0.5-0.7 × 0.7-1	0.4-0.6 × 0.5-0.6	0.8 × 0.7	0.5 × 1.3
Papillae/hairs length (μm)	presents up to 20	absents or scarcely papillous up to 20	presents 25-80	densely hairy 70-115
Petals	pale violet 6.5-6.8 × 1.4-1.6	pale violet 7.3-7.8 × 2.3-2.7	pale violet 7.8-8.4 × 2.5-2.7	deep violet 7.0-8.1 × 2.2-2.6

locality and elsewhere to corroborate this hybridization scenario. Recent explorations to the locality made by several teams of local botanists failed to find the plant (ARAN, 1996). We have noted dramatic changes in the littoral ecosystems from where *L. cavanillesii* was described, and it is likely that this species is extinct.

The taxonomic evaluation and delimitation of putative endangered and threatened species should be clarified prior to the execution of recovery strategies. Unfortunately, this is not always the case. Recently, it has been shown (BAYER & LÓPEZ GONZÁLEZ, 1991) that a species that has received legal protection by Spanish laws does not deserve taxonomic recognition. As has been aptly stated (HAIG, 1998), these cases can free resources that could otherwise be spent on more critical needs. *Limonium cavanillesii* could have been another case-study in which a taxonomic mistake resulted in an unjustified waste of resources. Fortunately, our study has only

revealed a *systematic* mistake having no further implications for the conservation management of the plant. This could have been detected earlier provided that a proper taxonomic knowledge of local endangered *Limonium* be prioritized.

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## MATERIAL EXAMINED

### *Limonium cavanillesii*

CASTELLÓN: Benicarló ["Valence"], terrains maritimes, 1-VII-1909, *Sennen* 747, MA 91959, 92305. Peñíscola, terrains maritimes, 24-VI-1909, *Sennen* 746, MA 91962.

### *Limonium duriusculum*

BOUCHES-DU-RHÔNE: Fos-sur-Mer, bords de l'étang de Lavalduc, le long de la voie ferrée, VIII-1891, *Roseron*, MA 91957. Sigean, Gebiet der Saline Grimaud bis zum Canal du Grand salin, 22-VII-1960, s.r., MA 581493. Sète, Hérault, Francia, 16-IX-1974, *Erben*, MA 310881.

### *Limonium dufourii*

CASTELLÓN: Torreblanca, pr. Clot. De Tomás, 1 m, 31TBE6553, 4-VIII-1996, C. *Fabregat* & S. *López*, VAB 96/3232. VALENCIA: In pasquis marítimis Dehesa de la Albufera, 9-V-1897, 1-VII-1898, *Pau*, MA 91899. Valencia, VII-1898, *Pau*, MA 91900. Dehesa Albufera, 25-VII-1909, *Moroder*, MA 458698. Castellón de la Plana, VII-1915, *Gil*, MA 91903; ibidem, VI-1921, *Gil*, MA 91901. La Albufera, VIII-1945, *Borja*, MA 169003. Dehesa de la albufera, saladas, VI-1976, *G. Mateo*, MA 382533.

### *Limonium rigulai*

ALICANTE: Dénia, Les Rotes, 31SBD5201, 0,5 m, 17-VII-1992, A. *Barber*, VAB 92/3495. Xàbia, la Barraca, 31SCD59, 1 m, 2-VIII-1993, J.X. *Soler*, VAB 95/5504. Dénia, les Rotes, 31SBD50, 5 m, 5-VIII-1993, J.X. *Soler*, VAB 95/0691.

### *Limonium thiniense*

ALBACETE: Entrada al pueblo de Pétrola, 30SXH2598, 22-VII-1976, S. *Cirujano* & S. *Castroviejo* 58, MA 458775. Corral Rubio, laguna Hoya Rasa, 30S XH3695, 23-VI-1995, S. *Cirujano* & al., MA 593320. Pétrola, laguna de Pétrola, 30SXJ2500, 18-IX-1996, S. *Cirujano* & al., MA 593319. Los Llanos 30SWJ9113, 18-IX-1996, S. *Cirujano* & al., MA 593321. Higueruela, laguna de Salobrejo, 30SWJ0817, 24-IX-1996, S. *Cirujano* & al., MA 593318. ALICANTE: Los Balsares del Altet (El Altet), Salicornietalia, 8-V-1956, A. *Rigual*, MA 374679. ALMERÍA: Mojácar, pr. locum dictum Lomos cantal, 30S XG0308, ad 5 m, secus viam, *Fernández Casas* 1086 & *García Guardia*, 16-IV-1976, MA 394531.