

LIMONIUM VIGOI (PLUMBAGINACEAE), A NEW TETRAPLOID SPECIES FROM THE NORTHEAST OF THE IBERIAN PENINSULA*

by

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Resumen

SÁEZ, L., A. CURCÓ & J.A. ROSSELLÓ (1998). *Limonium vigo* (Plumbaginaceae), una nueva especie tetraploide del nordeste de la Península Ibérica. *Anales Jard. Bot. Madrid* 56(2): 269-278 (en inglés).

Se describe una nueva especie apomíctica (*Limonium vigo*) procedente del nordeste de la Península Ibérica (delta del Ebro). *Limonium vigo* presenta semejanzas morfológicas con *L. girardianum* (Guss.) Fourr. y *L. grosii* L. Llorens, pero se diferencia de éstos por presentar hojas retusas, las basales normalmente marchitas en la antesis, mucrón terminal poco desarrollado o ausente, indumento calicinal constituido por tricomas más largos y color de la corola más oscuro. Además, *L. girardianum* es triploide, mientras que *L. vigo* y *L. grosii* son tetraploides. *Limonium girardianum* y *L. grosii* presentan la misma combinación polínico-estigmática (A/cob), la cual difiere de la que posee *L. vigo* (B/papilosa). El material tipo de *Limonium girardianum* f. *retusum* Pignatti y *L. glaucophyllum* Pignatti, dos táxones descritos del delta del Ebro, resulta indistinguible de *L. girardianum* y presenta claras diferencias respecto a *L. vigo*.

Palabras clave: Plumbaginaceae, *Limonium*, taxonomía, táxones apomícticos, nordeste de la Península Ibérica.

Abstract

SÁEZ, L., A. CURCÓ & J.A. ROSSELLÓ (1998). *Limonium vigo* (Plumbaginaceae), a new tetraploid species from the Northeast of the Iberian Peninsula. *Anales Jard. Bot. Madrid* 56(2): 269-278.

A new tetraploid agamic species, *Limonium vigo*, is described from coastal populations of the Northeast of the Spain (Ebro delta). The new species is related, on morphological grounds, to *L. girardianum* (Guss.) Fourr. and *L. grosii* L. Llorens, from which it could be easily discriminated by its retuse leaves, the basal ones usually withered at anthesis, the very short (or even absent) leaf apiculum, the denser and longer (up to 0.7 mm) hairs of the calyx tube and the deeper colour of the corolla. In addition, *L. girardianum* is triploid, whereas *L. vigo* and *L. grosii* are tetraploid. *Limonium girardianum* and *L. grosii* show the same pollen/stigma combination (A/cob type) which differs from that exhibited by *L. vigo* (B/papillate type). The types of *Limonium girardianum* f. *retusum* Pignatti and *L. glaucophyllum* Pignatti, two taxa described from the Ebro delta, could not be distinguished from *L. girardianum*, but they clearly differed from *L. vigo*.

Key words: Plumbaginaceae, *Limonium*, taxonomy, apomictic taxa, Northeast of the Iberian Peninsula.

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INTRODUCTION

As a part of a biosystematic revision of the *Limonium girardianum* (Guss.) Fourr. complex some populations from the Ebro delta (NE Spain) were sampled. This place has rich and well preserved halophilous communities (DIJKEMA & *al.*, 1984) in which the genus *Limonium* is properly represented with 10 reported species (CURCO, 1992). Several populations related to *L. girardianum* but differing in some conspicuous foliar and floral characters were found. A detailed study revealed that plants from these places presented distinctive palynological and micromorphological features and a higher ploidy level than expected. All available data suggest that these populations represent a new species which is here proposed as *L. vigoi*.

MATERIAL AND METHODS

Seeds, living plants and herbarium specimens belonging to the *L. girardianum* complex were collected, with permission, from the Ebro delta. Additional specimens of *L. girardianum*, *L. grosii* and the types of *L. girardianum* f. *retusum* Pignatti and *L. glaucophyllum* Pignatti were studied at or borrowed from some herbaria (BC, BCC, BCF, G, MA, MAF, RO and VAB) and from the living *Limonium* collection of the authors held at the Valencia University greenhouses.

Morphology. Twenty-six vegetative and floral attributes were scored for *L. girardianum*, *L. grosii* and *L. vigoi* samples.

Breeding system. Flowers were removed from herbarium specimens and the stigma and pollen grains were stained according to the ALEXANDER (1980) technique.

Phytodermology. Dried leaves were rehydrated, decolored and stained with Bismarck brown using standard techniques. At least twenty five stomatal guard cells from both leaf surfaces were measured for each accession.

Karyology. Seeds were germinated in Petri dishes on moistened filter paper and root tips were pretreated for about 4 h 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 tip squashes were made in 45 % acetic acid.

RESULTS

***Limonium vigoi* L. Sáez, Curcó & Rosselló, sp. nov.** (figs. 1, 2)

Planta tetraploidea, agamosperma, Limonio girardiano et Limonio grosii similis; foliis autem magis papillois, apice obtusis vel emarginatis, nonnumquam mucronatis—mucrone at plurimum 0,2 mm longo—, sub anthesi emarcidibus; apice partis internae bractearum longo (0,6-1 mm); calycibus dense indutis, costis quidem usque ad apicem pilosis, pilis 0,5-0,7 mm longis; floribus intense violaceis; denique, sed praecipue, combinatione pollen/stigma "B/papillate".

Holotypus. TARRAGONA: Delta de l'Ebre, platja dels Eucalyptus, circa lacunam dictam la Tancada, 31TCF1102, 2 m s.m., in arenosis maritimis, ubi die 30-V-1997, M. Mayol & L. Sáez legerunt, MA 611684 (isotypi adsunt in BC, BCC atque in herb. L. Sáez).

Derivatio nominis. *Species magistro et amicis J. Vigo ex animo dicata.*

Perennial with many stems. Caudices 1-5 cm, loosely branched, spirally leafy in the upper part. Basal leaves usually withered at anthesis, 1.9-7.1 × 0.8-1.9 cm. Blade spatulate to subelliptical, papillose, tip obtuse to subemarginate, with a short, 0-0.2 mm apiculum; 1-3(5) nerved. Petiole slightly canalicate, 1/3-1/2 as long as the blade, 1-2.2 mm wide. Stem 17-70 cm long, erect, robust, specially papillose in the lower third. Inflorescence paniculate, branched in the upper half or third, 9.5-30 × 4-23 cm. Branches loosely distichous, up to 17 cm long, erect to erect-patent, obliquely inserted;

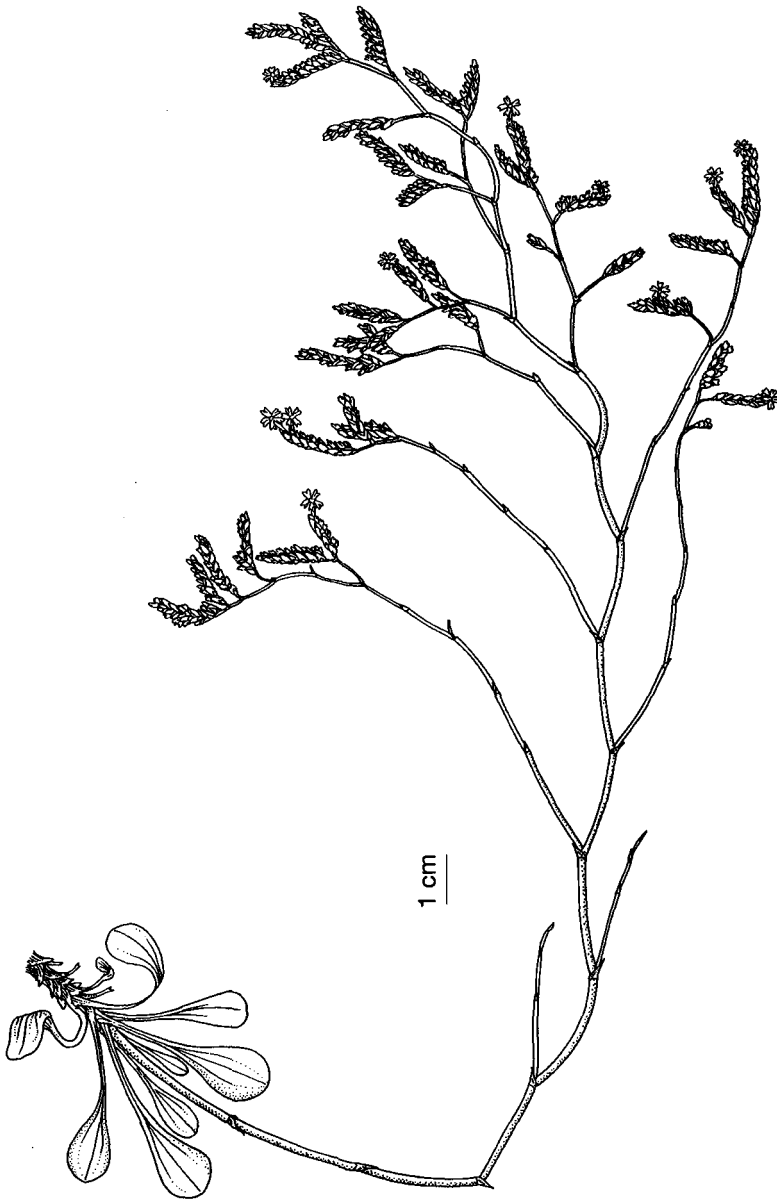


Fig. 1.—*Limonium vigoi* (holotype, MA): habit.

non flowering branches few or absent. Spikes 9-25 mm long, with 5-9 spikelets per cm. Spikelets 5.5-6.2 mm long, 2-7 flowered. Outer bract 1.9-2.5 × 2-2.8 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 1.8-2.1 × 1.4-1.6 mm, oblong-elliptic, blunt to submarginate, membranous. Inner bract 4.2-4.9 × 3.4-4.3 mm, obovate to elliptical, obtuse to

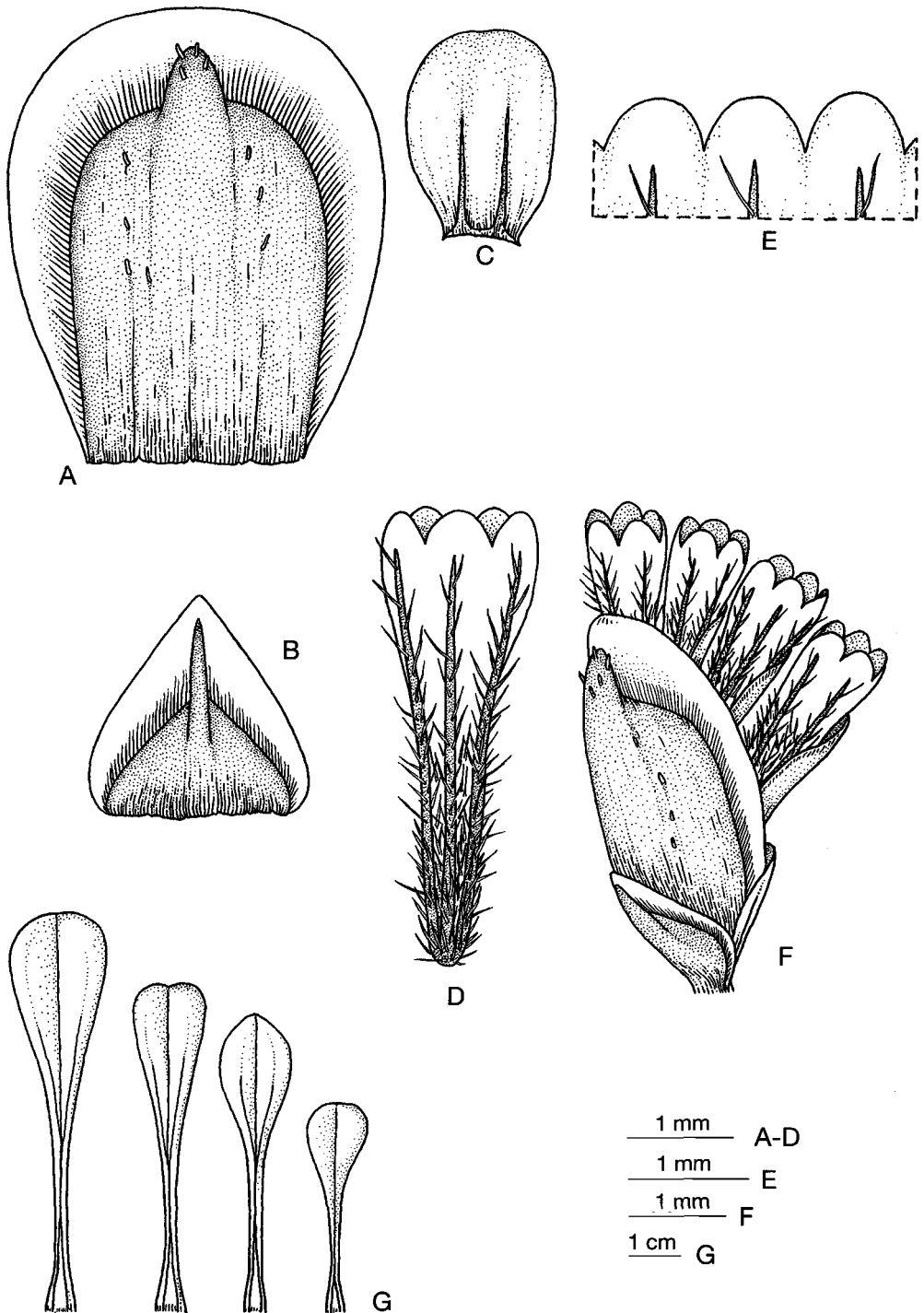


Fig. 2.—*Limonium vigoi*: A, inner bract; B, outer bract; C, middle bract; D, calyx; E, calyx teeth; F, spikelet; G, leaves.

rounded, with a broad membranous margin; central part subfleshy, 2.7-3.5 × 2.5-3 mm, oblong, triangular acuminate, the acumen (0.6)0.7-0.8(1) mm, usually with a few short eglandular hairs, not reaching the margin. Calyx 4.7-5.2 mm, tube densely hairy, with long eglandular hairs which can attain the midribs tops; teeth ca. 0.5-0.8 × 0.7-1 mm, semi-elliptic; midrib not reaching the calyx lobes. Corolla funnel-shaped. Petals 7.0-8.1 × 2.2-2.6 mm, cuneate, emarginate, deep violet. Pollen-stigma combination: B/papillate.

Material examined

ESP, TARRAGONA: Platja de l'Alfacada, *Zygophyllo-Limonietum*, CF10, 14-VI-1989, A. Curcó, Herb. L. Sáez; ibidem, 30-VI-1989, A. Curcó, Herb. L. Sáez. Entre els Eucaliptus i l'Alvet, CF10, 28-VII-1989, A. Curcó, Herb. L. Sáez. Delta de l'Ebre, platja dels Eucaliptus, c. La Tancada, 31SCF1102, 2 m, 18-VI-1996, A. Curcó, M. Mayol & L. Sáez, Herb. L. Sáez; ibidem, 30-V-1997, L. Sáez, Herb. L. Sáez; ibidem, 30-V-1997, M. Mayol & L. Sáez, Herb. L. Sáez. Torre de Sant Jordi, CF1731, 6-VI-1998, L. Sáez, Herb. L. Sáez.

KARYOLOGY

In the nine individuals sampled of *Limonium vigo* we have found the same chromosome number $2n = 36$ (fig. 3) Cytological counting in *L. vigo* is somewhat difficult since many chromosomes of the complement are small and tend to clump together. Therefore, chromosomal counts other than $2n = 36$, like $2n = 34$ and $2n = 35$, could be overlooked. Our experience with

Mediterranean *Limonium* suggests that most triploid and tetraploid taxa may display infraspecific (and intraindividual) aneuploidy. In any case, our results unequivocally show that *L. vigo* is a tetraploid. A single metacentric chromosome, the longest of the complement, could be the same that ERBEN (1979) considers a marker of the $x = 8$ genomes. However, it should be stressed that it has a lower size when compared to the marker chromosomes of the other diploid and polyploid taxa having one or more $x = 8$ genomes. According to Erben's hypothesis a large metacentric marker chromosome would not be expected in a $2n = 36$ tetraploid species.

PHYTODERMOLOGY

Limonium girardianum, *L. grosii* and *L. vigo* have leaves with anisocytic stomata, subpolygonal cells and conspicuous salt glands regularly distributed on both surfaces. These results agree with previous phytodermological data reported on the genus (WILKINSON, 1979). The mean length of the stomatal guard cells of the triploid taxon (*L. girardianum*) is significantly lower (table 2) than that in tetraploids (*L. grosii* and *L. vigo*). All samples of *L. vigo* have epidermal cells with elongate (2-3 times longer than wider) cylindrical striate papilla (a single papillae per cell) up to 115 μm long. On the contrary, the epidermal cells

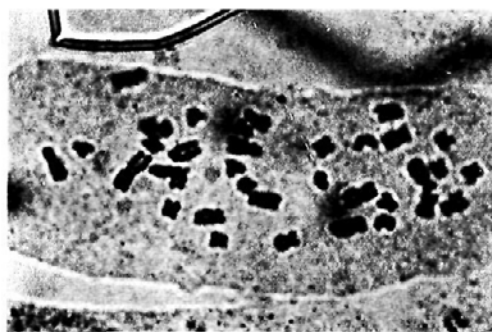


Fig. 3.—Metaphasic plates of *Limonium vigo*, $2n = 36$.

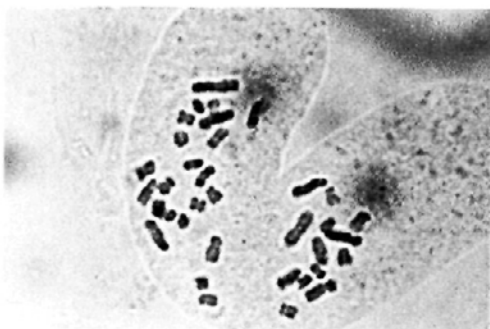


TABLE 1

MAIN MORPHOLOGICAL DISCRIMINANT FEATURES BETWEEN *LIMONIUM GIRARDIANUM*, *L. GROSII* AND *L. VIGOI*

[Measures are given in mm. Values between brackets and noted with an asterisk are taken from ERBEN (1993)]

	<i>Limonium girardianum</i>	<i>Limonium grosii</i>	<i>Limonium vigoii</i>
Leaves: acumen	0.2-0.9	0-0.6	0-0.2
Outer bract length	1.8-2.8	2-2.4[2.4-2.9]*	1.9-2.5
Inner bract length	3.8-4.6	4-5	4.2-4.9
Inner bract width	3.6-4.9	3.4-4.1[3.9-5.1]*	3.5-4.2
Inner bract acumen	0.4-0.7	0.6-0.8	0.5-1
Calyx length	4.1-4.8	4.5-5.5(5.7)	4.7-5.2
Calyx tube	scarcely hairy	scarcely hairy	densely hairy
Petals	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

of *L. girardianum* and *L. grosii* show the periclinal walls with obtuse dome-like protuberances which are covered by a reticulate (exceptionally striate) sculpture (fig. 4).

or exclusive apomict, as the related *L. girardianum* and *L. grosii* (SÁEZ & al., unpublished data).

ECOLOGY AND DISTRIBUTION

BREEDING SYSTEM

Limonium vigoii presents a single pollen/stigma combination (B/papillate) in all studied plants. Many of the pollen grains show shape irregularities and the percentage of pollen fertility is lower than 1 % as inferred from the staining procedure used. Both data strongly suggest that *L. vigoii* is a predominant

Limonium vigoii grows on psammophilous salt-marshes belonging to the Mediterranean alliance *Limonium galloprovincialis* Br.-Bl. 1931. It could be a characteristic species of the *Zygophyllo-Limonietum* Br.-Bl. 1935 association, a plant community limited to the Ebro delta and rich in xero-halophilous elements, specially *Limonium* species. In topographical terms, the community is

TABLE 2

PHYTODERMOLOGICAL FEATURES OF *LIMONIUM GIRARDIANUM*, *L. GROSII* AND *L. VIGOI*

	Stomatal guard cells length (Mean ± SD)	Papillae	Papillae length	Sample size (individuals, cells)
<i>Limonium girardianum</i>	35.18 ± 2.0	Usually absent	20-35	(34, 1020)
<i>L. grosii</i>	43.96 ± 2.45	Absent or present	25-80	(16, 480)
<i>L. vigoii</i>	42.08 ± 1.88	Present	70-115	(8, 240)

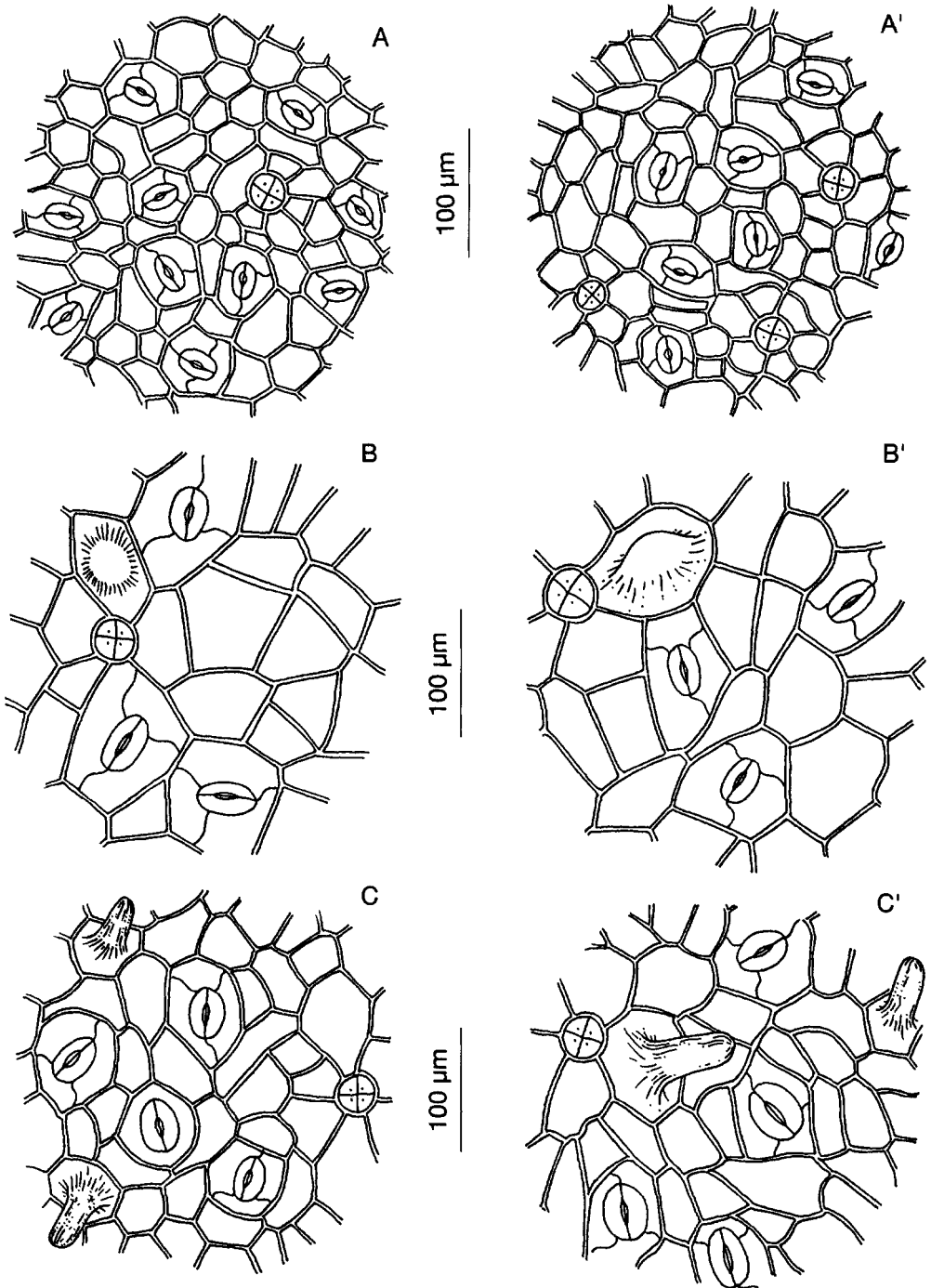


Fig. 4.—Cell pattern of adaxial (left) and abaxial (right) leaf epidermis: A, *Limonium girardianum*; B, *L. grosii*; C, *L. vigoii*.

limited by the psammophilous rushes of the *Schoeno-Plantaginion* Br.-Bl. 1931 alliance and by hygrophilous salt-marshes of *Arthrocnemion fruticosi* Br.-Bl. 1931 alliance. Associated plants growing with *Limonium vigoii* are *Zygophyllum album* L., *Helichrysum stoechas* (L.) Moench, *Limonium girardianum* and *L. bellidifolium* (Gouan) Dumort. Usually, *Limonium vigoii* can be found in small sandy hillocks not subjected to an eolian motion, which have enough elevation to be flooded during sea storms. The new species is restricted to several coastal sites along the littoral belt of the southern hemidelta, from Buda island to the La Banya Spit, forming scattered populations in several places. Also, *L. vigoii* forms small populations in the rocky-cliff shore near the Sant Jordi d'Alfama castle (north of Ametlla de Mar village). There, it grows in the *Crithmo-Limonion* community, with other species such as *Limonium gibertii* (Sennen) Sennen *L. virgatum* (Willd.) Fourr. and *Crithmum maritimum* L.

DISCUSSION

Overall morphology suggests the inclusion of *Limonium vigoii*, and the related *L. girardianum* and *L. grosii*, within Sect. *Limonium* subsect. *Densiflorae* Boiss., which is probably a polyphyletic assemblage not worthy of taxonomic recognition. *Limonium vigoii* can be easily distinguished from the aforesaid species by its retuse leaves, the basal ones usually withered at anthesis, the very short (or even absent) leaf apiculum, the denser and longer (up to 0.7 mm) indument of the calyx tube and the deeper colour of the corolla (table 1). On the other hand, *L. girardianum* is a triploid with $2n = 26$ (ERBEN, 1978) whereas *L. grosii* (ERBEN, 1988) and *L. vigoii* are tetraploid. In addition, *Limonium girardianum* and *L. grosii* share, through their whole area, the same pollen/stigma combination (A/cob type) which differs from that exhibited by *L. vigoii* (B/papillate type). All the available evidence strongly support the view that *L. vigoii* is

distinct from the related *L. girardianum* and *L. vigoii* and should be recognized at the specific level.

Plants resembling *L. girardianum* but having retuse leaves were earlier reported from the Ebro delta (PIGNATTI, 1953). This author described his *L. girardianum* f. *retusum* from plants collected in a single locality from the north of the delta. We have studied the type specimen held at RO (La Cava, Foci d'Ebro, 25-VIII-1952, leg. S. Pignatti) and although the leaves are slightly divergent (fig. 5) all other features are diagnostic of *L. girardianum* (indument of calyx tube, length of the apical tip of leaves, lack of papillae in the leaves, etc.). The mean length of the stomatal guard cells is $36.61 \mu\text{m} \pm 1.41$ which falls near the mean of *L. girardianum* and support a triploid level for the plant. In addition, the pollen-stigma combination of the type specimen is A/cob, like that in *L. girardianum*. Therefore *L. girardianum* f. *retusum* should be viewed as a local variant of *L. girardianum* and should not be ascribed to *L. vigoii*.

Another taxon described from this area is *Limonium glaucophyllum* (PIGNATTI, 1953). It was suggested that its origin is a cross between *L. densissimum* (Pignatti) Pignatti and *L. girardianum* (ERBEN, 1993). To examine the possibility that the plants we are hereby describing are referable to *L. glaucophyllum*, we have studied the type material (RO) of the latter. The pollen-stigma combination of the type specimen is A/cob, like that exhibited by *L. girardianum*. The mean length of the stomatal guard cells is $35.17 \mu\text{m} \pm 1.41$, suggesting that the plant was a triploid; the epidermis lacks papillae. Overall morphology of *L. glaucophyllum* is indistinguishable from *L. girardianum*. This agrees with PIGNATTI (1953) who reported that the features in flowers and inflorescences match those of *L. girardianum*. In fact the type material of *L. glaucophyllum* can be distinguished from *L. vigoii* by the longer apiculum at leaf apex (0.3-0.8 mm), shorter (c. 10 mm) and denser spikes, shorter inner bracts (3.9-4.2 mm), and the shorter acumen (0.4-0.6 mm) of the subfleshy central part

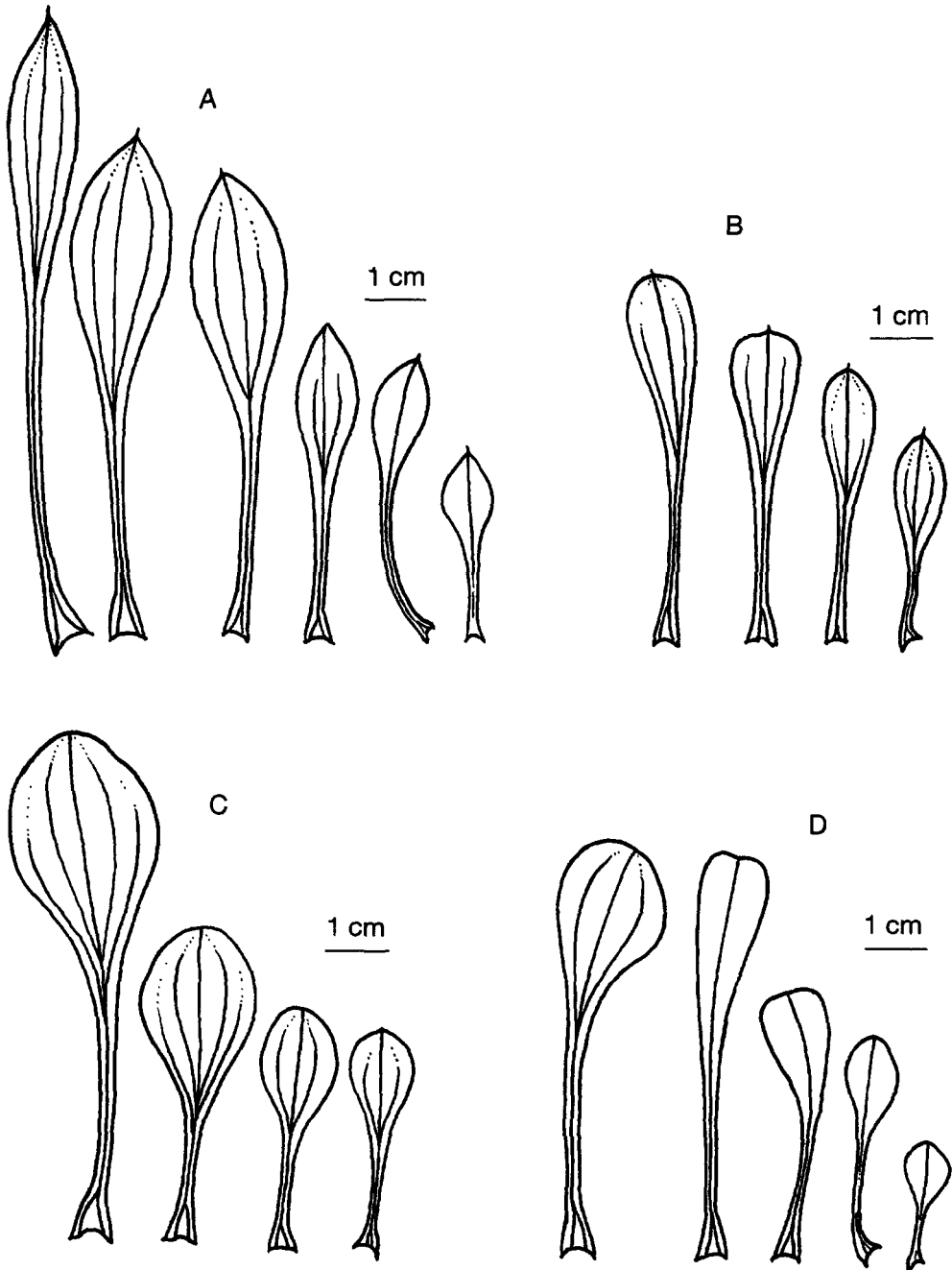


Fig. 5.—Representative leaves of *Limonium girardianum* (A), *L. girardianum* f. *retusum* (B), *L. grosii* (C) and *L. vigoii* (D).

of the bract. Taking all the evidence into account, we conclude that: i) *L. vigoii* differs from the type of *L. glaucophyllum* in

morphological, phytodermological and reproductive features, and ii) we have not been able to trace any single feature which

could distinguish the *L. glaucophyllum* type from *L. girardianum*. We do not find supporting evidence for the hypothesis that *L. glaucophyllum* is a hybrid between *L. densissimum* and *L. girardianum* and, instead, we are confident that it should be considered a mere synonym of the latter.

Several morphological features of *L. vigoi*, notably the presence of scattered long papillae on bracts and leaves, the whiteness of leaves at anthesis and the shape of leaves relates it to *L. dufourii* (Girard) Kuntze, a triploid species endemic to the Castellón and Valencia provinces whose northern populations are not far from the Ebro delta. However, *L. dufourii* is a densely hairy plant, with non flowering sterile branches, bigger outer (2.8-3.9 × 2.8-3.5 mm) and inner bracts (5.2-6.2 × 4.7-5.8 mm) and longer calyx (5.4-6 mm) than *L. vigoi*.

It is tempting to suggest that *L. dufourii* and another species also belonging in subsect. *Densiflorae* Boiss. could be the ancestors of *L. vigoi*. However, no further evidence on the origin of the tetraploid *L. vigoi* is available and all attempts to put it on a hybridization scenario solely on morphological grounds should be viewed as rather speculative. ERBEN (1978, 1979) postulated that tetraploid taxa in *Limonium* should have been originated through interspecific hybridizations between triploid (with $2n = 25$, $2n = 26$ or $2n = 27$) and diploid taxa ($2n = 16$ or $2n = 18$) but other evolutionary scenarios have been also proposed (DOLCHER & PIGNATTI, 1971; INGROUILLE, 1984, INGROUILLE & STACE, 1985). On the basis of several pieces of cytological and morphological evidence we favour the working hypothesis that some triploid and tetraploid species could arise through hybridization between triploid taxa through fusion of unbalanced gametes (reduced or not). Clearly, all these hypotheses should be tested with more powerful methods than conventional karyology, such as molecular ones. However, preliminary work using isozymes, RFLP of cp DNA and ITS sequences of ribosomal DNA on diploid and polyploid Iberian and Balearic *Limonium* species (ROSSELLÓ et al., unpublished data) is

not conclusive about the origins of polyploidy in the genus.

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