

LIMONIUM MARISOLII L. LLORENS (PLUMBAGINACEAE) REVISITED*

by

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Resumen

SÁEZ, L., Á.C. CARVALHO & J.A. ROSSELLÓ (1998). Reevaluación de *Limonium marisolii* L. Llorens (Plumbaginaceae). *Anales Jard. Bot. Madrid* 56(1): 33-41 (en inglés).

Se describe una nueva especie triploide y agamosperma, *L. leonardi-llorensii*, a partir de poblaciones costeras del suroeste de Mallorca. Desde el punto de vista morfológico, la nueva especie es afín a la también endémica *L. marisolii* L. Llorens, pero se distingue de ella por varios caracteres morfológicos (hojas sin papilas, cálices mayores, mayor número de flores por espiguilla), además de por el número de cromosomas ($2n = 26$, *L. leonardi-llorensii*; $2n = 27$, *L. marisolii*). Se discuten las relaciones entre ambas especies y se sugiere la posibilidad de que, a pesar del diferente número cromosómico, tengan una historia evolutiva común. En este sentido se considera la posibilidad de que *L. marisolii* se haya originado a partir de *L. leonardi-llorensii* por fisión cromosómica o hibridación.

Palabras clave: *Plumbaginaceae*, *Limonium*, taxonomía, endemismo, Islas Baleares.

Abstract

SÁEZ, L., Á.C. CARVALHO & J.A. ROSSELLÓ (1998). *Limonium marisolii* L. Llorens (Plumbaginaceae) revisited. *Anales Jard. Bot. Madrid* 56(1): 33-41.

A new triploid agamic species, *L. leonardi-llorensii*, is described from coastal populations of South-West Mallorca. The new species is closely related, on morphological grounds, to the endemic *L. marisolii* L. Llorens, from which it could be distinguished by several morphological characters (leaves without papillae, longer calyx, more flowers per spikelet) and by a different chromosome number ($2n = 26$, *L. leonardi-llorensii*; $2n = 27$, *L. marisolii*). The relationships between both species are discussed, and it is suggested that both taxa might share some common evolutionary history despite their divergent chromosome number. The origin of *L. marisolii* from *L. leonardi-llorensii* through chromosome fission or by a hybridization event is considered.

Key words: *Plumbaginaceae*, *Limonium*, taxonomy, endemism, Balearic Islands.

INTRODUCTION

The Balearic representatives of the genus *Limonium* have been the subject of a considerable amount of taxonomic work not

totally devoid of controversy (PIGNATTI, 1955, 1959, 1960, 1962, 1963, 1971; ERBEN, 1986, 1988, 1989, 1993; LLORENS, 1986a, 1986b; LLORENS & TÉBAR, 1988; ROSSELLÓ & *al.*, 1994; SÁEZ & ROSSELLÓ, 1996).

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Limonium marisolii L. Llorens is a triploid taxon endemic to the Balearic islands (LLORENS, 1986a; ERBEN, 1993), belonging in section *Limonium* subsect. *Dissitiflorae* Boiss. This species has a narrow discontinuous distribution along southern Mallorca, where it grows on the sandy slopes and on calcareous rock crevices near the seashore. The eastern populations (from which the plant was described) have more individuals than the western ones, which are very rare and contain scanty specimens as a consequence of habitat disturbances caused by touristic development. *Limonium marisolii* has been assigned to the *L. gibertii* complex (SÁEZ & ROSSELLÓ, 1996) on the basis of overall morphology, but this placement is currently tested by other cytological and molecular evidence (SÁEZ & *al.*, unpublished data). In the course of this revision, attention was paid to the morphological differences encountered between the western and eastern populations of *L. marisolii*, which were supported by different chromosome numbers and cuticular micromorphology. Evidence is presented here that supports taxonomic recognition for the western populations which are hereby described as *L. leonardi-llorensii*.

MATERIAL AND METHODS

Seeds, living plants and herbarium specimens were collected from all known populations, including the type locality of *L. marisolii*. Additional specimens were borrowed from the following herbaria: BCC, G, MA and MAF.

Breeding system and pollen fertility. 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, decolorized and stained with Bismarck Brown, using standard techniques. Thirty stomatal guard cells from both leaf surfaces were measured for each accession.

Karyology. Seeds were germinated in Petri dishes on moistened filter paper. 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 tip squashes were made in 45% acetic acid. Photographs of metaphase plates were taken at a final magnification of $\times 2500$. In order to make the idiograms comparable, the length of the short and long arms of the chromosomes in each taxon was expressed in relative values (chromosome set = 100 %). The description of the chromosome sets follow the nomenclature of LEVAN & *al.* (1964).

RESULTS

***Limonium leonardi-llorensii* L. Sáez, Carvalho & Rosselló, sp. nov.** (figs. 1, 2)

Species nova Limonio marisolii L. Llorens affinis, a qua vero differt calyce longiore (4-4,6 mm), spiculis 2-5-floris, numero chromosomatico $2n = 26$, sed praesertim foliis non papillois et stomatibus 33-47 μm longis.

Derivatio nominis: Named after L. Llorens, who was the first to find a population of the new species and included it under *L. marisolii*.

Perennial with many stems, glabrous. Caudices 5-30 cm, loosely branched, spirally leafy in the upper part. Basal leaves green at anthesis, 3.3-9 \times 1.2-2.5 mm. Blade spatulate to elliptical, tip obtuse to rounded, with a short, 0.1-0.2 (0.3) mm apiculum; smooth on both faces, 3-5 nerved. Petiole slightly canaliculate, 1/3-1/2 as long as the blade, 2-4 mm wide. Stem 20-120 cm long, erect. Inflorescence paniculate, branched in the upper half or third, 11-60 \times 9-42 cm. Branches loosely distichous, up to 40 cm long, erect to erect-patent, obliquely inserted; non flowering branches few or absent. Spikes 10-25 mm long, with 5-8 spikelets per cm. Spikelets 4.8-5.6 mm long, 2-5 flowered. Outer bract 1.5-2 \times 1.8-2 mm, triangular-

ovate, acute to obtuse; margin broadly membranous, central part subfleshy, long acuminate, the acumen nearly reaching the margin. Middle bract 1.7-2.3 × 1.3-1.7 mm, oblong-elliptic, blunt to submarginate, membranous. Inner bract 3.9-4.2 × 2.8-3.4 mm, obovate to elliptical, obtuse to rounded, with a broad membranous margin; central part subfleshy, 2.6-3.2 × 1.7-2.2 mm, oblong, triangular acuminate, the acumen

(0.5)0.6-0.8(0.9) mm, not reaching the margin. Calyx 4-4.6 mm, tube with long eglandular hairs; teeth ca. 0.4-0.7 × 0.6-0.8 mm, semielliptic; midrib not reaching the calyx lobes. Corolla funnel-shaped. Petals 7.1-7.7 × 1.9-2.4 mm, cuneate, violet. Pollen-stigma combination: A/cob.

Holotypus: *Insulae Baleares, Majorica, ubi loco dicto Punta Negra, Calvià, 31S DD6175, in saxosis maritimis, die 18-VIII-1995 L. Sáez legit, BCC-5015 (isotypi: herb. L. Sáez).*

Material examined

L. leonardi-llorensii: Punta Negra, Calvià, 31S DD6175, 7-I-1995, J.M. González, J.A. Rosselló & L. Sáez, BCC, herb. Sáez; ibidem, 22-IV-1995, J.A. Rosselló & L. Sáez, BCC, herb. Sáez; ibidem, 18-VI-1995, L. Sáez, BCC, herb. Sáez. Cala Major, Palma de Mallorca, 31SDD6678, 7-I-1995, J.M. González, J.A. Rosselló & L. Sáez, BCC, herb. Sáez; ibidem, 24-VIII-1995, L. Sáez, BCC, herb. Sáez; ibidem, 25-X-1996, J.A. Rosselló & L. Sáez, BCC, herb. Sáez.

L. marisolii: In saxosis maritimis l. d. Punta dels Esparrals (Punta Llobera, Marina de Llucmajor), 15-VII-1971, A. & L. Llorens, MA 327928, *isotypus*. Pas de Sa Senyora, región de la Punta Llobera, rochers maritimes, 10-VI-1985, A. Charpin, AC 19476 & al., G 287741. Pas de Sa Senyora, Llucmajor, 31SDD8059, 7-I-1995, J.M. González, J.A. Rosselló & L. Sáez, BCC, herb. Sáez; ibidem, 9-VIII-1996, J.A. Rosselló & L. Sáez, BCC, herb. Sáez. Es Cap Blanc, Llucmajor, 31SDD8257, M. Mayol, J.A. Rosselló & L. Sáez, BCC, herb. Sáez; ibidem, 23-X-1996, J.A. Rosselló & L. Sáez, BCC, herb. Sáez. A prop de Cala Beltràn, Llucmajor, 31SDD8456, 23-XII-1995, M. Mayol, J.A. Rosselló & L. Sáez, BCC, herb. Sáez.

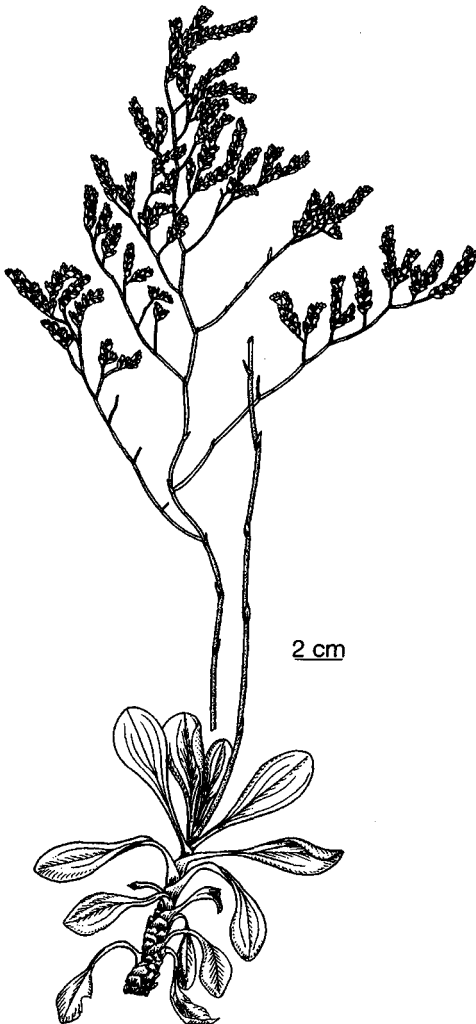


Fig. 1.—*Limonium leonardi-llorensii*, Punta Negra (Mallorca) (holotype, BCC): habit.

ECOLOGY AND DISTRIBUTION

Limonium leonardi-llorensii grows on maritime slopes on calcarenite rocks of two South-West Majorcan localities, whereas *L. marisolii* is restricted to several coastal places located on the southeastern part of the Palma bay (fig. 6). Few other *Limonium* species have been noted growing with *L. leonardi-llorensii* viz, *L. companyonis* (Gren. & Billot) Kuntze, *L. minutum* (L.) Chaz and *L. gibertii* (Sennen) Sennen. Associated species were *Fagonia cretica* L., *Pistacia lentiscus* L. and *Sonchus tenerrimus* L.

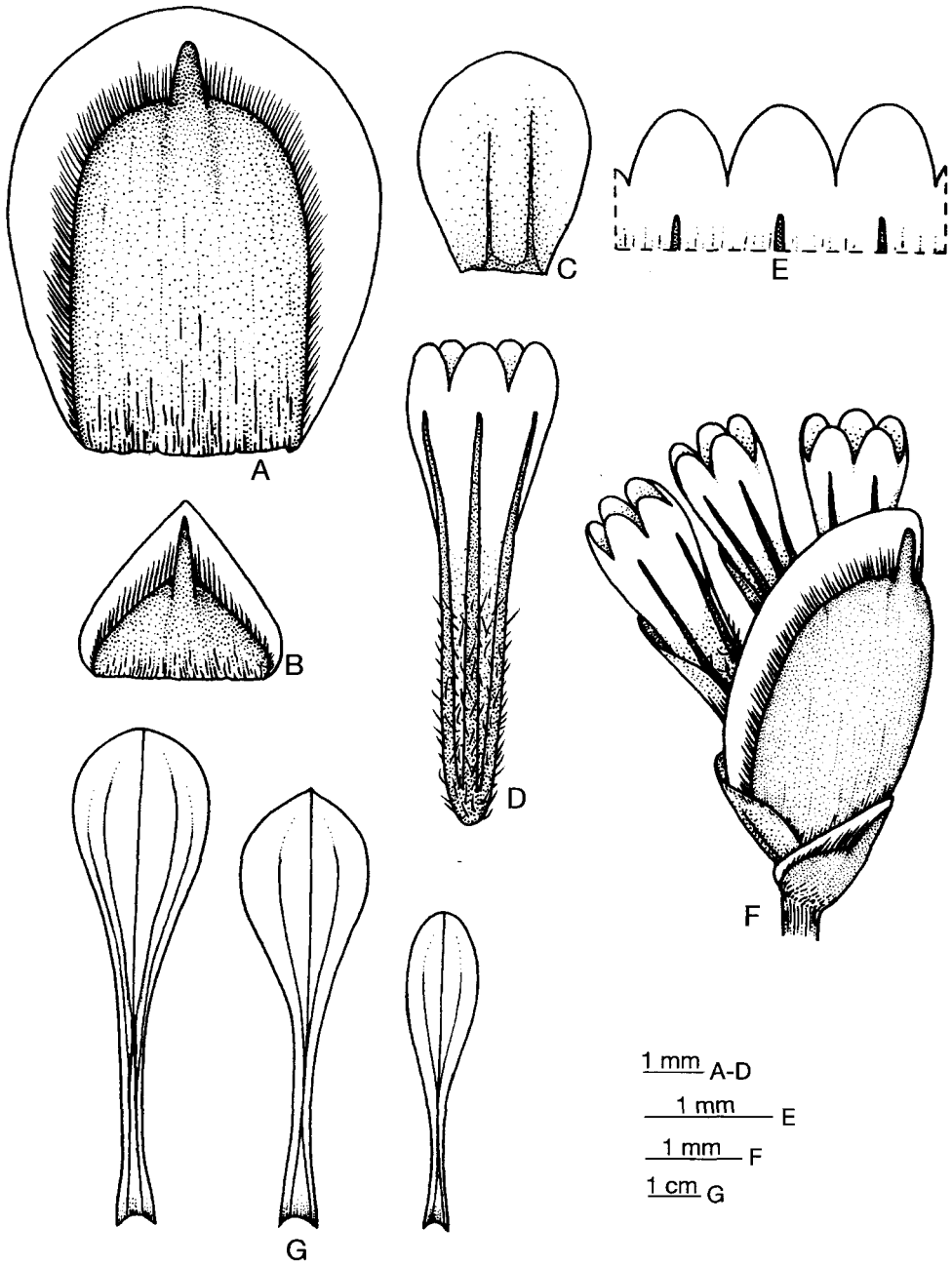


Fig. 2.—*Limonium leonardi-llorensii*, Punta Negra (Mallorca) (holotype, BCC): A, inner bract; B, outer bract; C, middle bract; D, calyx; E, calyx teeth; F, spikelet; G, leaves.

TABLE 1

KARYOLOGICAL FEATURES OF *LIMONIUM LEONARDI-LLORENSII* AND *L. MARISOLII*

	Locality	Chromosome number	Marker chromosome	Sample size: individuals (cells)
<i>Limonium leonardi-llorensii</i>	Punta Negra Cala Major	2n = 26	1	11(33)
		2n = 26	1	7(39)
		2n = 27	1	1(1)
<i>L. marisolia</i>	Cap Blanc Pas Senyora	2n = 27	0	5(8)
		2n = 27	0	6(16)
		2n = 26	0	1(1)

PHYTODERMOLOGY

Both *L. leonardi-llorensii* and *L. marisolia* show subpolygonal epidermal cells in the leaves. Salt glands are uniformly scattered in the adaxial and abaxial surfaces. Anticlinal walls [(5)6-8(9) μm wide] present pits of irregular width. In *L. marisolia*, papillae are irregularly scattered along the periclinal walls of both leaf surfaces, whereas they are absent in *L. leonardi-llorensii*. Both species have amphistomatic leaves, with anisocytic (WILKINSON, 1979) stomata regularly distributed along the leaf blade (fig. 5). The length of stomata guard cells is significantly longer in *L. leonardi-llorensii* than in *L. marisolia* (table 2).

KARYOLOGY

Metaphase plates from individuals belonging to eastern populations consistently yield a chromosome count of $2n = 27$ (fig. 3a), with a chromosome formula of $4M + 15m + 8sm$. One aneusomatic cell with an aneuploid karyotype ($2n = 26$) was observed in one individual (#4) from one of the two examined accessions. In contrast, the two western populations had a $2n = 26$ complement (fig. 3b), with a chromosome formula of $7M + 10m + 8sm + 1st$. Also, one cell with a deviating chromosome number ($2n = 27$) was encountered in a $2n = 26$ individual (#5). These chromosome numbers are in accordance with a polyploid (triploid) level for

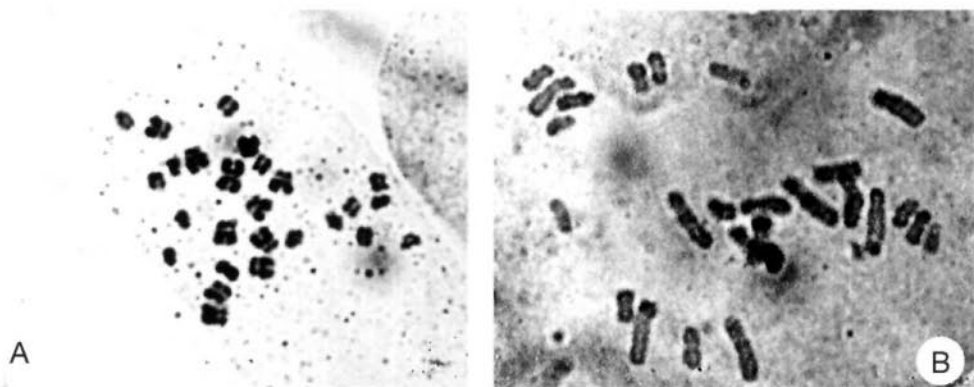
Fig. 3.—Metaphasic plates of *Limonium leonardi-llorensii* (A) and *L. marisolia* (B). ($\times 2500$.)

TABLE 2

PHYTODERMOLOGICAL FEATURES OF *LIMONIUM LEONARDI-LLORENSII* AND *L. MARISOLII*

	Locality	Papillae	Stomate guard cells length (Mean \pm SD)		Sample size (individuals, cells)
			Adaxial	Abaxial	
<i>Limonium leonardi-llorensii</i>	Punta Negra	–	40.01 \pm 1.78	41.13 \pm 1.79	(3.84)
	Cala Major	–	38.53 \pm 2.08	39.60 \pm 2.69	(7.195)
<i>L. marisolii</i>	Cap Blanc	+	31.64 \pm 1.86	33.70 \pm 2.20	(6.49)
	Pas Senyora	+	32.51 \pm 1.92	33.54 \pm 3.83	(7.198)

both taxa. Chromosome size ranges from 2 (m) to 5 μ m (sm) in *L. marisolii*, and from 1.2 (M) to 3.6 μ m (M) in *L. leonardi-llorensii*. The complement of the latter has a long metacentric chromosome, which is assumed to be the marker chromosome characteristic of the $x = 8$ genomes (ERBEN, 1979); this chromosome is absent in *L. marisolii* (figs. 3, 4). Idiograms of both taxa show nine pairs of chromosomes and eight (*L. leonardi-llorensii*) or nine (*L. marisolii*) unpaired ones. The paired chromosomes are similar in both species, whereas the unpaired ones show a striking difference in both karyotypes.

DISCUSSION

Limonium marisolii and the new proposed species, *L. leonardi-llorensii*, share a similar

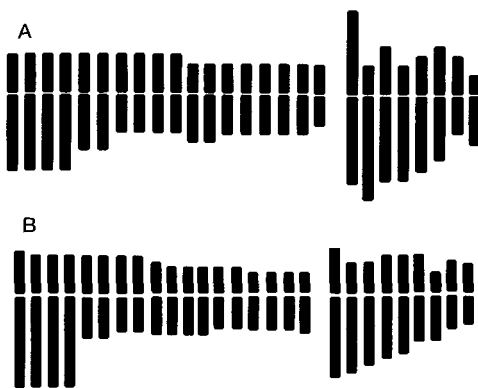


Fig. 4.—Idiograms of *Limonium leonardi-llorensii* (A) and *L. marisolii* (B).

overall morphology, which has favoured the confusion of both taxa under a single entity. However, conspicuous differences in morphology (table 3), cuticular ornamentation pattern, stomatal guard cells length and chromosome number support the view that the western and eastern allopatric populations of *L. marisolii* deserve taxonomic recognition at the specific level. The divergent chromosome number and the presence, in one taxon, of a long metacentric marker chromosome, strongly support the idea that both taxa are not so closely related as their macromorphology could suggest. In fact, according to ERBEN's (1978, 1979) hypothesis about the origin of polyploid taxa in the genus, *L. marisolii* and *L. leonardi-llorensii* should not share a common evolutionary pathway. If Erben's hypothesis is correct, then *L. leonardi-llorensii*, or its ancestor, originated through a cross between a reduced gamete of a diploid taxa of basic chromosome number $x = 8$ with an unreduced gamete of a species having $2n = 18$ chromosomes (thus with basic basic number $x = 9$). According to the same hypothesis, *L. marisolii* should have arisen in a similar way, by a combination of gametes of plants of different ploidy level belonging solely to taxa with $x = 9$. Erben's explanation of the formation of the polyploid agamic taxa assumes that the long metacentric marker chromosomes are homologous and, therefore, their presence in the triploid and tetraploid taxa comes from diploid ancestors belonging to $x = 8$ chromosome lineages. However, no

TABLE 3

MORPHOLOGICAL DISCRIMINANT FEATURES OF *LIMONIUM LEONARDI-LLORENSII* AND *L. MARISOLII*

	<i>Limonium leonardi-llorensii</i>	<i>Limonium marisoliai</i>
Leaves: acumen (mm)	0.1-0.2(0.3)	0.2-0.4
Outer bract (mm)	1.5-2 × 1.8-2	1.4-1.9 × 1.5-1.9
Inner bract: length × width (mm) acumen length (mm)	3.9-4.2 × 2.8-3.4 (0.5)0.6-0.8(0.9)	3.9-4.4 × 2.9-3.1 (0.7)0.8-0.9(1)
Spikes length (mm)	10-25	8-16
Flower per spikelet	2-5	1-3
Calyx: length (mm) teeth length × width (mm)	4-4.6 0.4-0.7 × 0.6-0.8	3.8-4.4 0.3-0.6 × 0.6-0.9
Calyx midrib	Not reaching teeth basis	Reaching or depassing teeth basis

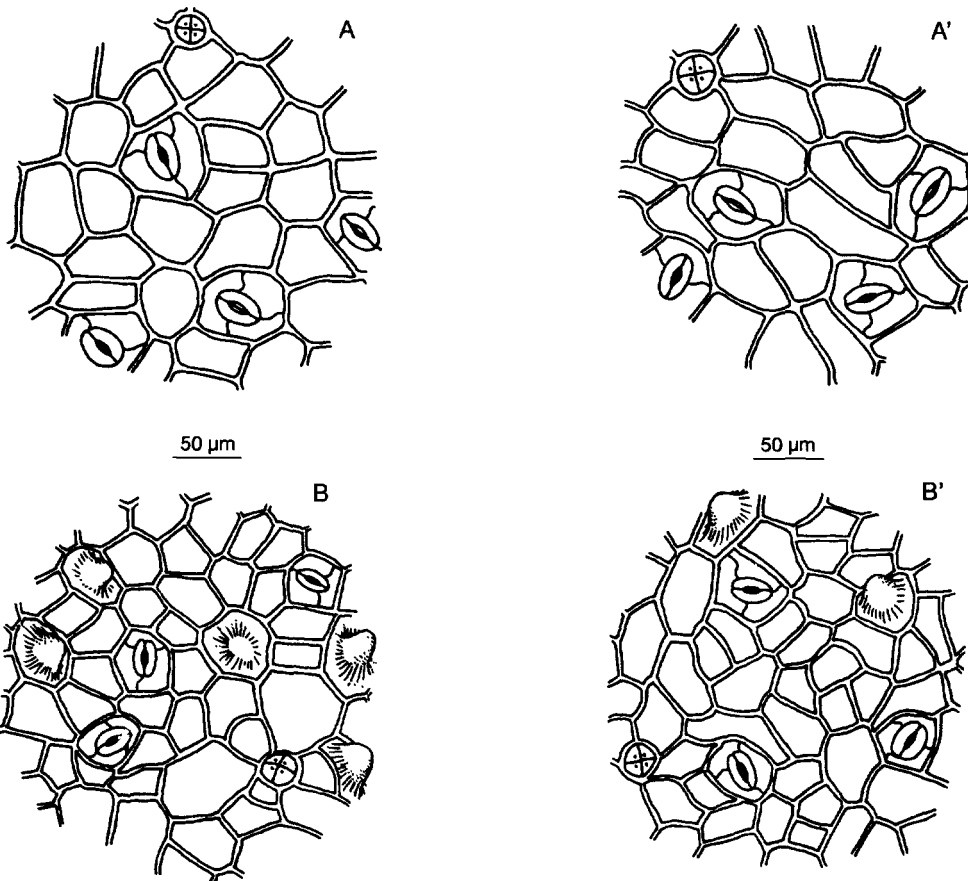


Fig. 5.—Cell pattern of leaf epidermis. *Limonium leonardi-llorensii* (A, adaxial; A', abaxial); *L. marisoliai* (B, adaxial; B', abaxial).



Fig. 6.—Distribution of *Limonium leonardi-llorensii* (▼) and *L. marisolii* (●).

conclusive evidence other than cytological observation of chromosome morphology is available, and some authors have proposed alternative hypotheses for the origin of the agamic taxa in other groups on the basis of somatic mutations and chromosomal rearrangements (INGROUILLE, 1984; INGROUILLE & STACE, 1985). Patterns of aneuploid speciation have never been advocated in *Limonium*, but on a theoretical basis this possibility should not be ruled out. By a centromeric fission of the long metacentric chromosome present in the $2n = 26$ taxa, new cytotypes with $2n = 27$ could arise. If chromosomal rearrangements also did occur, then the similarity between both karyotypes would not be as close as expected. If *L. marisolii* arose from *L. leonardi-llorensii* through this chromosomal event then the genetic similarity between both taxa should be high. Interestingly, *L. leonardi-llorensii* and *L. marisolii* share the same pollen-stigma combination (A/cob) and identical alleles at twelve out of thirteen isozymic loci so far analyzed, including a rare putative FDH gene duplication, which is absent in all other taxa of the *L. gibertii* complex (Carvalho, unpublished data). This high allelic similarity would not be present in two taxa of such presumed different evolutionary pathways, as inferred from ERBEN's (1978) data, at least if one considers the moderate level of isozyme differentiation

found within and between the taxa of the *L. gibertii* complex (Carvalho, unpublished data). On the other hand, RFLPs of non-coding chloroplast DNA (trnC-trnD) show that *L. leonardi-llorensii* and *L. marisolii* have different haplotypes, a noteworthy feature if both taxa have an ancestor-descendent relationship. These conflicting molecular data could be reconciled assuming an hybrid origin of *L. marisolii* through a cross between *L. leonardi-llorensii* and another still unidentified taxa, which should be the ovule donor (chloroplast DNA transmission is assumed to be maternal in *Limonium*; HARRIS & INGRAM, 1991). Karyological rearrangements and chromosomal losses in unstabilized zygotes could account for the low chromosome number found in *L. marisolii*. Genomic in situ hybridization (GISH) is currently underway, to further explore both compelling hypotheses about the genomic relationships and evolution between *L. leonardi-llorensii* and *L. marisolii*.

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REFERENCES

- ALEXANDER, M. P. (1980). A versatile stain for pollen, fungi, yeast and bacteria. *Stain Tech.* 55: 13-18.
- ERBEN, M. (1978). Die Gattung *Limonium* im Südwestmediterranean raum. *Mitt. Bot. Staatssamml. München* 14: 361-631.
- ERBEN, M. (1979). Karyotype differentiation and its consequences in Mediterranean *Limonium*. *Webbia* 34: 409-417.
- ERBEN, M. (1986). Bermerkungen zur Taxonomie der Gattung *Limonium*. IV. *Mitt. Bot. Staatssamml. München* 27: 381-406.
- ERBEN, M. (1988). Bermerkungen zur Taxonomie der Gattung *Limonium*. IV. *Mitt. Bot. Staatssamml. München* 27: 381-406.
- ERBEN, M. (1989). Bermerkungen zur Taxonomie der Gattung *Limonium*. V. *Mitt. Bot. Staatssamml. München* 28: 313-417.

- ERBEN, M. (1993). *Limonium*. In: S. Castroviejo & al. (eds.), *Flora iberica* 3: 2-143. Real Jardín Botánico (CSIC). Madrid.
- HARRIS, S.A. & R. INGRAM (1991). Chloroplast DNA and biosystematics: the effects of intraspecific diversity and plastid transmission. *Taxon* 40: 393-412.
- INGROUILLE, M.J. (1984). A taxometric analysis of *Limonium* (Plumbaginaceae) in Western Europe. *Pl. Syst. Evol.* 147: 103-118.
- INGROUILLE, M.J. & C.A. STACE (1985). Pattern of variation of agamosperous *Limonium* (Plumbaginaceae) in the British Isles. *Nord. J. Bot.* 5: 113-125.
- LEVAN, A., K. FREDGA & A.A. SANDBERG (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
- LLORENS, L. (1986a). Revisión sistemático-taximétrica del género *Limonium* Miller en la isla de Mallorca (I). *Lazaroa* 8: 11-68.
- LLORENS, L. (1986b). El género *Limonium* Miller en la isla de Formentera (Balears). *Lazaroa* 8: 69-83.
- LLORENS, L. & J. TÉBAR (1988). *Limonium escarrei* y *Limonium boirae* Llorens & Tébar, dos nuevas especies de la isla de Mallorca (Balears). *Anales Jard. Bot. Madrid* 45: 173-180.
- PIGNATTI, S. (1955). I *Limonium* della flora balearica. *Arch. Bot. (Forlì)* 31: 66-100.
- PIGNATTI, S. (1959). Studi sui *Limonium*, IV. Sulle sottospecie di *Limonium virgatum* ed in particolare sulla "*Stalice dictyoclada*" di Boissier. *Nuovo Giorn. Bot. Ital.* 66: 560-563.
- PIGNATTI, S. (1960). I *Limonium* della flora Balearica. (Supplemento). *Arch. Bot. (Forlì)* 36: 206-210.
- PIGNATTI, S. (1962). Studi sui *Limonium*, V. Note sulla sistematica delle specie iberiche di *Limonium*. *Collect. Bot. (Barcelona)* 6: 293-330.
- PIGNATTI, S. (1963). Über die Beziehungen zwischen italienischen und iberischen Arten der Gattung "*Limonium*" (Plumbaginaceae). *Webbia* 18: 73-93.
- PIGNATTI, S. (1971). Studi sui *Limonium*. VIII. *Bot. J. Linn. Soc.* 64: 361-370.
- ROSSELLÓ, J.A., M. MUS & J.X. SOLER (1994). *Limonium ejulabilis*, a new endangered endemic species from Majorca (Balearic Islands, Spain). *Anales Jard. Bot. Madrid* 51: 199-204.
- SÁEZ, L. & J.A. ROSSELLÓ (1996). *Limonium inexpectans* (Plumbaginaceae), a new apomictic species from Mallorca (Balearic Islands). *Anales Jard. Bot. Madrid* 54: 285-289.
- WILKINSON, H. (1979). The plant surface (mainly leaf). In: C.R. Metcalfe & L. Chalk (eds.), *Anatomy of dicotyledons*, 2.^a ed., vol. I. Clarendon Press. Oxford.

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