Resumen


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


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

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 × 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 *In* = 26, sed praesertim *foliis* non papillosis 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 x 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 × 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 × 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 subemarginate, 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.


**Material examined**


**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) Kunze, *L. minutum* (L.) Chaz and *L. gibertii* (Sennen) Sennen. Associated species were *Fagonia cretica* L., *Pistacia lentiscus* L. and *Sonchus tenerimus* L.
Fig. 2.—Limonium leonardi-llorensis, 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*

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

**PHYTODERMOLGY**

Both *L. leonardi-llorensii* and *L. marisolii* 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. marisolii*, 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. marisolii* (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 aneupomatic 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. marisolii* (B). (× 2500.)
both taxa. Chromosome size ranges from 2 (m) to 5 μm (sm) in L. marisolii, and from 1.2 (M) to 3.6 μm (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 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*

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

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