

LIMONIUM CARVALHOI (PLUMBAGINACEAE), A NEW ENDEMIC SPECIES FROM THE BALEARIC ISLANDS*

by

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Resumen

ROSSELLÓ, J.A., L. SÁEZ & Á.C. CARVALHO (1998). *Limonium carvalhoi* (Plumbaginaceae), una especie nueva endémica de las Islas Baleares. *Anales Jard. Bot. Madrid* 56(1): 23-31 (en inglés).

Se describe *Limonium carvalhoi* Rosselló & L. Sáez de un marjal en una única localidad del sudoeste de Mallorca. Morfológicamente, la nueva especie es afín a otras tres también endémicas de las Baleares: *L. inexpectans*, *L. migjornense* y *L. magallufianum*. *Limonium carvalhoi* es apomíctica y presenta una combinación polen/estigma del tipo B/p. Los números cromosómicos encontrados van de $2n = 24$ a $2n = 26$. Sobre la base de la presencia de un cromosoma metacéntrico largo en todas las células examinadas, se sugiere que el complemento cromosómico original es $2n = 26$ y que los otros dos números se han derivado por aneuploidía descendente.

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

Abstract

ROSSELLÓ, J.A., L. SÁEZ & Á.C. CARVALHO (1998). *Limonium carvalhoi* (Plumbaginaceae), a new endemic species from the Balearic Islands. *Anales Jard. Bot. Madrid* 56(1): 23-31.

Limonium carvalhoi Rosselló & L. Sáez is described from a single salt marsh locality in southwest Majorca. Morphologically, *L. carvalhoi* is close to *L. inexpectans*, *L. migjornense* and *L. magallufianum*, all Balearic endemics. The new species has an apomictic breeding system and shows a single pollen/stigma combination (B/papillate type). Chromosome number ranges from $2n = 24$ to $2n = 26$. Based on the occurrence of a long metacentric chromosome in all cells examined, it is suggested that the original chromosome complement is $2n = 26$, the other chromosome numbers being derived by descending aneuploidy.

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

The great taxonomic diversification in the genus *Limonium* is readily noticeable in the flora of the Balearic Islands. If the comprehensive work of ERBEN (1993) is taken into account, only six out of forty-seven taxa occurring in the Balearic Islands, viz

L. echioides (L.) Miller, *L. virgatum* (Willd.) Fourr., *L. algarvense* Erben, *L. companyonis* (Gren. & Billot) Kuntze, *L. gibertii* (Sennen) Sennen and *L. girardianum* (Guss.) Fourr., are not endemic to the archipelago. The evolutionary basis of such diversity has not

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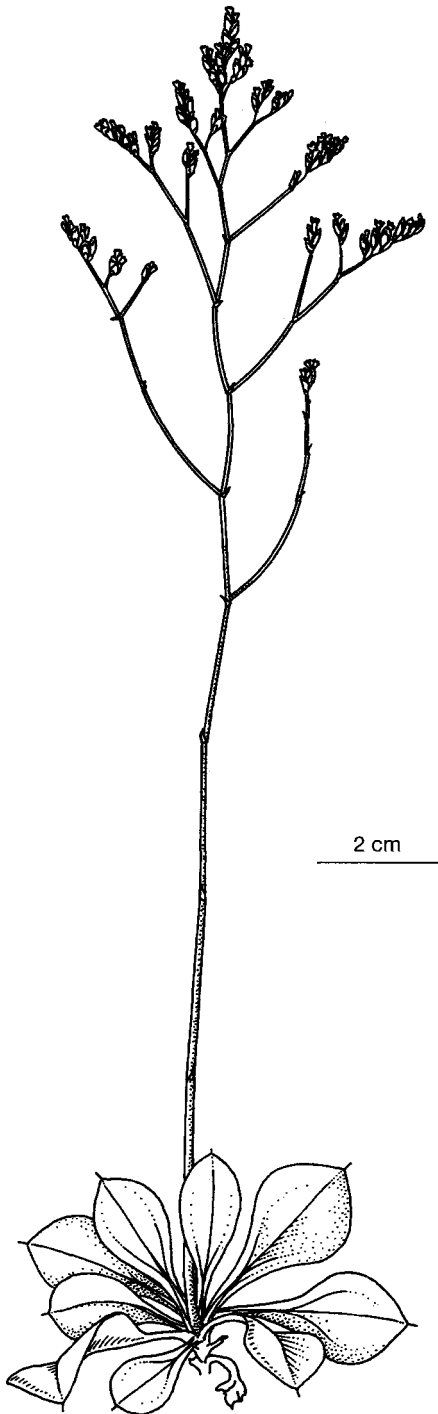


Fig. 1.—*Limonium carvalhoi*: habit.

been so far studied and the relationships between taxa are mainly based on macromorphological grounds and, to a lesser extent, on the ploidy level. Clear morphological discontinuities between populations, however, still remain the best way to build a practical classification on which further desirable biosystematic work should be carried out.

During field work devoted to improve the knowledge of *Limonium* from the brackish saltmarshes of Majorca, we found at Magalluf (southwest Majorca) a very small population of unknown identity. The Magalluf saltmarshes are one of the most striking places of the Balearic Islands, for *Limonium* flora not only for the species diversity but also for the percentage of the endemics occurring in them. Up to six Majorcan endemic species are recorded there (*L. validum* Erben, *L. camposanum* Erben, *L. ejulabilis* Rosselló & al., *L. magallufianum* L. Llorens, *L. inexpectans* L. Sáez & Rosselló, *L. boirae* L. Llorens) of which the latter four have not been found outside the Magalluf area. Indeed it was not surprising that the new population did not closely match any other species so far described. After a thorough study it became evident that the plants were related to, but in some floral features distinct from *L. inexpectans*, *L. magallufianum* and *L. migjornense*, three Majorcan endemic apomictic species of which the first two inhabit the same saltmarsh (SÁEZ & ROSSELLÓ, 1996). The combination of morphological and cytological features suggest that the plants belong to an undescribed species, which is here published as *L. carvalhoi*. The new species is dedicated to our late friend A. Carvalho for her outstanding effort and contribution to the knowledge of the Iberian and Balearic *Limonium* flora.

RESULTS

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

Triploideum, agamospermum, simile speciebus Limonium migjornense, L. inexpectans

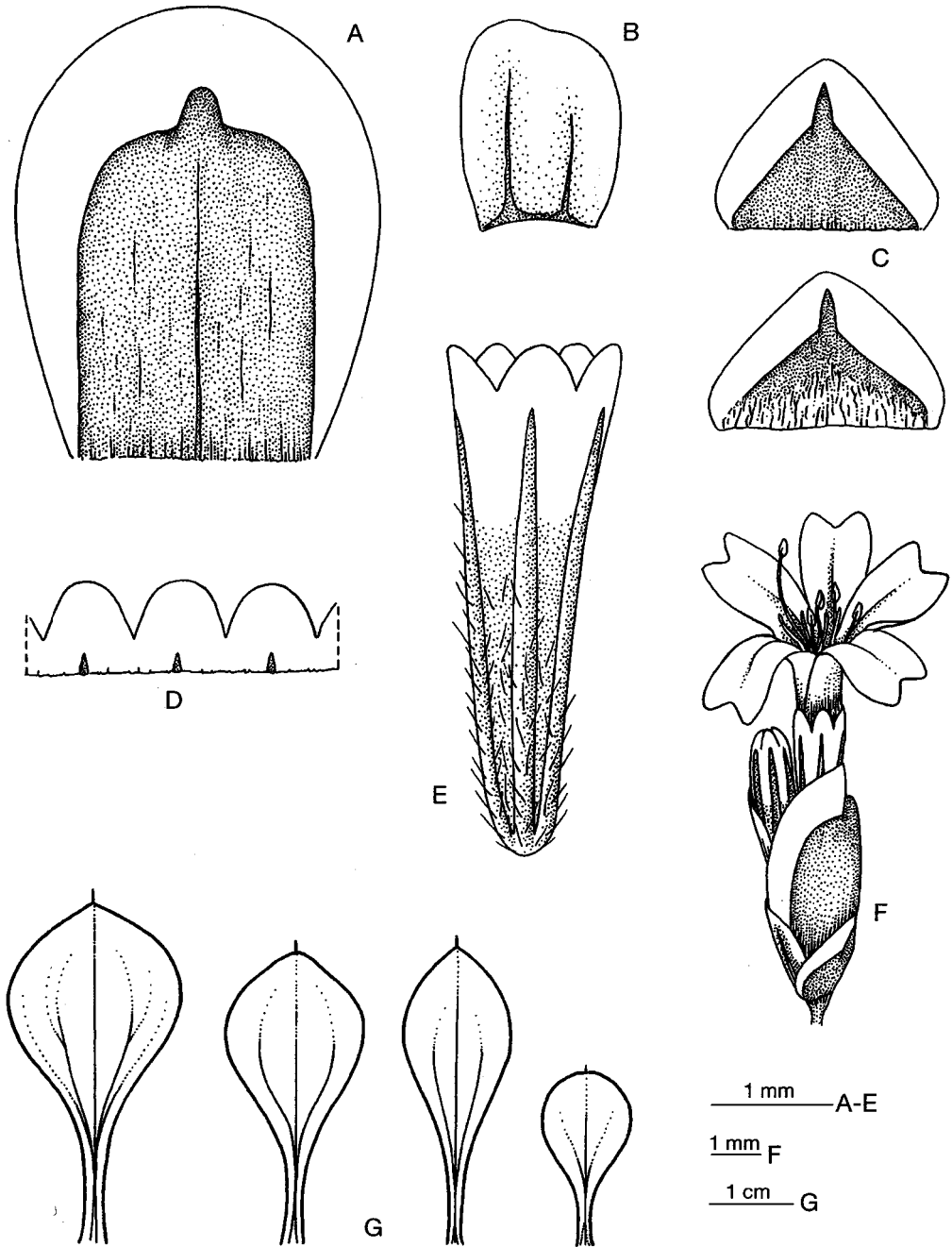


Fig. 2.—*Limonium carvalhoi*: A, inner bract; B, middle bract; C, outer bract; D, calyx teeth; E, calyx; F, spikelet; G, leaves.

pectans et *L. magallufianum*. *Folia spathulata vel subelliptica, mucronata, mucrone 1-2 mm longo, in petiolum attenuata laevem, 1/4-1/3(1/2) longitudinis laminae attingentem. Inflorescentia parce ramosa 6-30 × 4-16 cm, rami ut summum 12 cm longi, subdistichi atque subarcuati; spiculae 4,5-5,1 mm longae, 1-3 florum; bractea inferior 3,6-4,1 mm, obovata vel late obovata, obtusa, in parte centrali carnosula 2,7-1,9-2,3 mm, acuminata, acumine 0,3-0,5 mm longo marginem haud attingenti; calyx 3,6-4,3 mm longus, tubo sparse piloso, lobis c. 0,4-0,6 mm, semiellipticis.*

Holotype: Insulae Baleares, Majorica, loco dicto Sa Porrassa (31SDD57), in paludibus argillosis salsis, ubi M. Mus et J.A. Rosselló, 28-XII-1992, legerunt (BCC 5002).

Perennial with few stems, slender, glabrous. Caudicles 5-10 cm long, loosely branched, densely spirally leafy. Basal leaves patent, green at anthesis, 10-45 × 6-22 mm. Blade spathulate to slightly elliptical, tip obtuse to rounded with a short terminal apiculum 1-2 mm long; flattened at the margins, papillose on both sides, 3-(5) nerved. Petiole slightly canaliculate, 1/4 -1/3 (1/2) as long as the blade, 1-2 mm wide. Stem 15-42 cm long, erect; inflorescence paniculate, loosely branched in the half or upper third, 6-30 × 4-16 cm. Branches loosely distichous, up to 12 cm long, subcurved at the ends, obliquely inserted (45-70°), non flowering branches few or absent. Spikes 8-15 mm long, straight to subcurved, subpatent, with 5-7(8) spikelets per cm. Spikelets 4.5-5.1 mm long, 1-3 flowered. Outer bract 1.2-1.4 × 1.5-1.7 mm, triangular-ovate, apex acute to rounded, margin broadly membranous (0.3-0.5 mm); central part slightly subfleshy, shortly acuminate (the acumen not reaching the margin). Middle bract 1.4-1.7 × 1.2-1.4 mm, broadly elliptical to oblong-elliptical, blunt to subemarginate, membranous. Inner bract 3.6-4.1 × 2.8-3.1 mm, obovate to broadly obovate, blunt, with a broad membranous margin; central part subfleshy, 2.7-3 × 1.9-2.3 mm, oblong, acuminate, acumen 0.3-0.5 mm long, obtuse, not reaching the margin. Calyx 3.6-4.3 mm

long, overtopping 0.5-1.1 mm the inner bract, tube irregularly hairy, with eglandular hairs 0.3-0.4 mm long; tooth c. 0.4-0.6 mm, semielliptic; midrib not reaching the calyxlobes base. Petals 6.5-7.2 × 1.5-1.8 mm, cuneate, pale violet.

Material examined: Ibidem, 4-VIII-1995, leg. L. Sáez (BCC).

Breeding system

A single pollen-stigma combination (B/papillate type) has been found in all known individuals of *L. carvalhoi*. Most pollen grains did not stain using ALEXANDER'S (1980) method and showed both shape irregularities and a variable number of colpi. These facts strongly suggest that *L. carvalhoi* is an apomictic taxon.

Cytology

The chromosome numbers $2n = 24$, $2n = 25$ and $2n = 26$ have been found in plants of *L. carvalhoi* (table 1), which is in accordance with a polyploid (triploid) level. The

TABLE 1
CHROMOSOME NUMBER VARIATION FOUND
IN *LIMONIUM CARVALHOI*

Individual	Chromosome number	Cells
1	$2n = 25$	4
2	$2n = 24$	5
	$2n = 25$	1
	$2n = 26$	1
3	$2n = 24$	2
	$2n = 25$	1
4	$2n = 25$	2
5	$2n = 25$	3
	$2n = c. 25/26$	1
6	$2n = 25$	3
7	$2n = 25$	1
8	$2n = 24$	1
	$2n = c. 24/25$	1
	$2n = 25$	9
9	$2n = 25$	2

chromosome number $2n = 25$ is present in all plants examined and is, by far, the most common number counted (70.3 %). In contrast, three out of nine individuals had $2n = 24$ (21.6% of cells) and in only one plant the $2n = 26$ cytotype (fig. 3) was found, with a frequency of 2.7%. The chromosome complement could not be exactly determined in two cells due to chromosome overlapping. Chromosome size ranges from 1.5 to 5 μm . Two idiograms were elaborated from the $2n = 25$ cytotype (fig. 4). A long metacentric chromosome, present in all cells analyzed, is assumed to be the chromosome marker characteristic of the $x = 8$ genomes (ERBEN, 1979).

Habitat

The two populations so far known of *L. carvalhoi* grow on the remains of the original Magalluf saltmarsh. One of them, with fewer individuals, is situated on the ecotone of the saltmarsh and a limestone shrubland, under a *Pinus halepensis* canopy. Several *Limonium* species were noted in the vicinities of *L. carvalhoi*: *L. magallufianum*, *L. validum*, *L. boirae* and *L. virgatum*. Associated species were *Inula crithmoides* L., *Juncus maritimus* Lam., *Arthrocnemum glaucum* Ung.-Sternb., *Atriplex prostrata* DC. and *Pistacia lentiscus* L. One of the populations has over 200 adult individuals whereas the other is restricted to a small disturbed area not exceeding 25 square meters and currently contains five plants. According to the UICN (1994) recommendations *L. carvalhoi* should be included in the CR (critical) category.

DISCUSSION

The chromosome number of L. carvalhoi

According to ERBEN (1978, 1979) the triploid taxa in *Limonium* arise through the various combinations of reduced and unreduced gametes from individuals with $n = 8$ and $n = 9$. Erben stated that the basic number of $x = 8$ has one characteristic long



Fig. 3.—Metaphasic plate of *Limonium carvalhoi*, $2n = 26$ ($\times 1850$).

metacentric chromosome, absent in $x = 9$ taxa, derived by centric fusion of two telocentric chromosomes of the latter. According to Erben's statement it could be possible to determine the chromosome complements involved in a triploid through the number of these long metacentric marker chromosomes: triploids $2n = 24$ should have three, $2n = 25$ two, $2n = 26$ one, while those $2n = 27$ should have none. Interestingly, *L. carvalhoi* has one long metacentric chromosome in all 37 cells examined, despite variation in chromosome number; so, according to Erben's hypothesis, it should originally belong to the triploid $2n = 26$ cytotype and not to the $2n = 25$ one, where two such chromosomes should be found. Somehow contrasting evidence is derived from the analysis of one class of $2n = 25$ idiograms (fig. 4) which showed nine pairs of chromosomes and seven unpaired ones. This is in accordance with a supposed origin through a $n = 18$ and a $n = 8$ gametes. Three different chromosome numbers, with divergent frequencies, have been found in the 37 cells analyzed. It could be envisaged that the most common number, i.e. 25, should be the typical of the taxon, the others being derived by aneuploidy.

However, the question to be addressed is to what extent there is a typical chromosome number in polyploid *Limonium*. CHOUDHURI (1942) noted that, in interspecific *Limonium* hybrids, structural chromosome rearrangements could be found, and eventually they could give rise to new

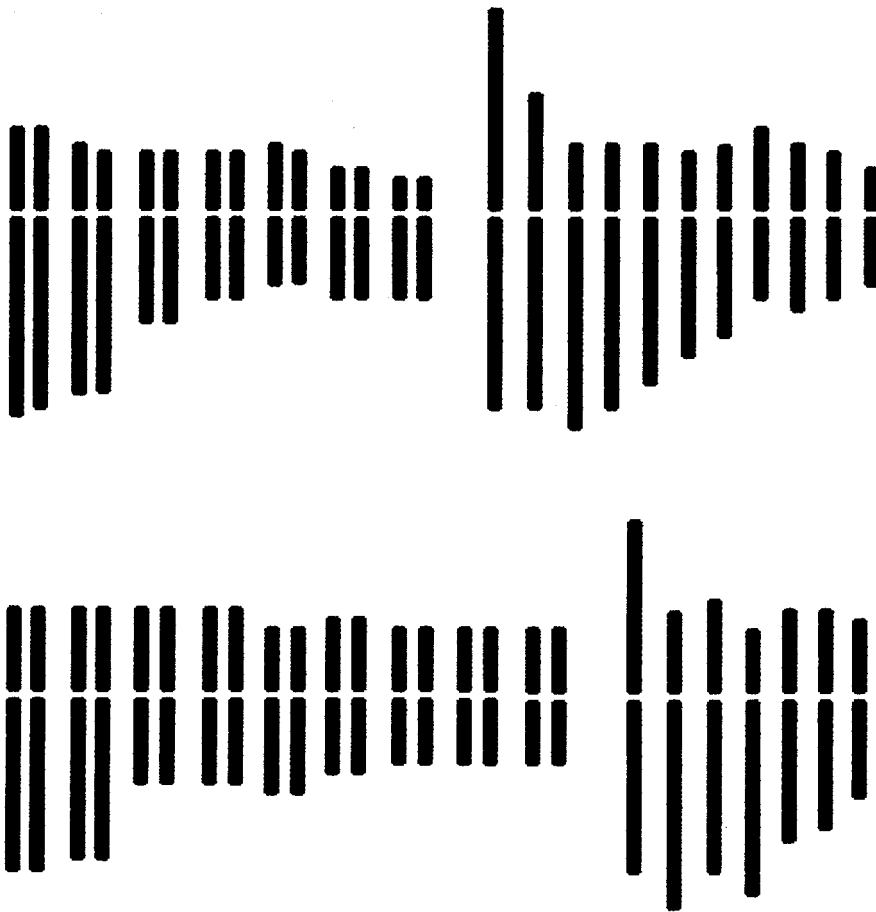


Fig. 4.—Idiograms of the $2n = 25$ cytotype of *Limonium carvalhoi*.

chromosomes. Since then, other workers have found different chromosome numbers not only in the same taxon, but in the same population and, even, in the same individual, rendering virtually impossible a precise count (DOLCHER & PIGNATTI, 1967, 1971; INGRUILLE & STACE, 1985; DAWSON, 1990; ARRIGONI & DIANA, 1993). In contrast, no such variation has been reported in many Mediterranean triploid and tetraploid taxa (ERBEN, 1978, 1979, 1988; BRULLO & PAVONE, 1981; ARTELARI, 1989a, b, c, 1992) and each species has been characterized by a unique chromosome number. DAWSON (1990) suggested technical reasons to explain the eight cytotypes found in populations of *L. humile* from Great Britain

($2n = 36, 38, 48, 49, 50, 51, 52, 54$). Excessive pressure during squashing, mixing of the contents of two adjacent cells and small size of the smaller chromosomes were regarded as plausible reasons to explain this chromosomal variability. The quality of our $2n = 26$ and $2n = 24$ plates, although enough for accurate chromosome counting, did not allow a detailed study of all the triploid complement, and conservation priorities prevented the study of more individuals. Therefore, it was not possible to assess the chromosomal basis of the aneuploid cytotypes. Chromosomal rearrangements were evident in some cells (fig. 4), those having only seven pairs of chromosomes.

These results and those obtained from other Balearic and Spanish *Limonium* (Carvalho & Rosselló, unpublished data) are in agreement with the earlier published view that in polyploid taxa cytological instability (both numerical and structural) is the rule rather than the exception. At the western mediterranean basin many *Limonium* taxa grow together in single localities as is the case of the Magalluf area. If gene flow is not restricted among apomictic taxa, as it is suspected, the use of seeds for caryological investigations could be problematic since they may be originated by a hybridisation event. However, and as far as overall morphology is a good predictor, we have not noted extant hybridisations among the *Limonium* flora present at Magalluf. In the absence of further data careful observations of many cells should be performed before the cytological variation of apomictic taxa can be fully appraised.

In the case of *L. carvalhoi*, the reason why there is a predominance of the $2n = 25$ cells instead of the original $2n = 26$ complement remains unknown. The technical reasons, presented by DAWSON (1990), seem too unrealistic to explain the overall cytological variation so far found in apomictic taxa. Chromosomal unbalanced gametes, mitotic irregularities, cytomixis and gene flow between different cytotypes are larger avenues to explore the problem.

Taxonomic relationships

On morphological grounds, *L. carvalhoi* belongs to sectio *Limonium* subsect. *Dis-sitiflorae*. The most closely related taxa are the balearic *L. migjornense*, *L. magallufianum* and *L. inexpectans* from which the new species, however, can be easily discriminated (table 2). *Limonium migjornense* has oblanceolate leaves with longer petioles, a higher number of flowers per spike, bigger outer bracts and wider inner bracts. *L. magallufianum* differs from it by having a more robust habit, a more branched inflorescence, leaves with shorter mucro, shorter inner bracts with a conspicuous longer

mucro on the subfleshy central part, and shorter calyx. *Limonium inexpectans* shares the overall inflorescence shape with *L. carvalhoi* but differs from it in its bigger habit, leaves with shorter mucro, longer spikes and narrower inner bracts with a longer mucro on the subfleshy central part. *Limonium ejulabilis* Rosselló & al., which also grows at Magalluf differs in many morphological features and by no means is it related to *L. carvalhoi* (table 2).

The chromosome number $2n = 26$ has been reported for *L. magallufianum* (ERBEN, 1988) and *L. inexpectans* (Carvalho & al., unpublished data); *L. migjornense* has $2n = 25$ (ERBEN, 1988). If the hybrid origin of *L. carvalhoi* through a diploid ($2n = 18$) and a reduced gamete ($n = 8$) is taken into account, three hypothesis could be drawn.

In the first scenario, *L. carvalhoi* arose in situ through the hybridization of two diploid unrelated taxa of different basic chromosome number. But, if this was correct, then it should be pointed out that, at present, in the Balearics no species with $2n = 16$ chromosomes are known. On the other hand, only the *L. minutum* complex is available as the donor of the $2n = 18$ gamete. This is in contrast with the several triploid taxa ($2n = 24, 25, 26$) endemic to the Balearic islands which should have the same postulated origin through the ERBEN (1978) hypothesis. An unlikely selective extinction of several diploid taxa from the archipelago would be required to fit the current triploid diversity into Erben's hypothesis.

In the second scenario, it could be conceived that *L. carvalhoi* arose through somatic mutations from triploid apomictic ancestors. This idea has been discussed by INGROUILLE (1984) and INGROUILLE & STACE (1985) to explain the origin of the British agamospecies of the *L. binervosum* aggregate. At the moment, no closely related taxa to *L. carvalhoi* from which it could be derived by minor morphological changes through this process can be found. Too many conspicuous morphological discontinuities exist between *L. carvalhoi* and its presumably related species *L. migjornense*, *L. magallu-*

TABLE 2

MORPHOLOGICAL FEATURES OF *LIMONIUM CARVALHOI* COMPARED TO *L. MAGALLUFIANUM*,
L. MIGJORNENSE, *L. INEXPECTANS* AND *L. EJULABILIS*

	<i>L. magallufianum</i>	<i>L. migjornense</i>	<i>L. carvalhoi</i>	<i>L. inexpectans</i>	<i>L. ejulabilis</i>
Leaves shape	obovate-spatulate to spatulate	oblanceolate	spatulate to slightly elliptical	spatulate to elliptical	elliptical to ovate-elliptical
petiole / blade mucro length (mm)	1/2-3/4 1	1/2-3/4 0.6-1.5	1/4-1/3(1/2) 1-2	(1/3)1/2 0.2	(1/2)3/4-1 3
Scape	branched at 1/4	branched at 1/3	branched at 1/2-2/3	branched at 1/3	branched at 1/4-1/3
Spike length (mm) density (spike/cm)	10-30 4-7	5-15 6-9	8-15 5-7(8)	15-25 4-6	7-20 6-7
Spikelets number of flowers	2-4	2-5	1-3	1-3(4)	2-4
Outer bract (mm)	1.3-1.8 × 1.4-1.8	1.3-2 × 1.6-2	1.2-1.4 × 1.5-1.7	1.1-1.5 × 1.3-1.6	1.2-1.5 × 1.1 × 1.5
Inner bract shape	elliptical to obovate-elliptical	broadly obovate	obovate to broadly obovate	broadly elliptical to obovate	obovate to elliptical
dimensions (mm) tip (mm)	3-3.5 × 2.1-2.6 0.8-1	3.7-4.1 × 2.8-3.8 0.3-0.7	3.6-4.1 × 2.8-3.1 0.3-0.5	3.5-4 × 2.4-2.8 0.4-0.7	2.8-3.1 × 2.3-2.7 0.8-1
Calyx tube length (mm) pubescence	3.2-3.7 densely hairy in half part	3.7-4.1 densely hairy in half part	3.6-4.3 densely hairy in half part	3.8-4.4 sparsely hairy	2.8-3.2 densely hairy in half part
Chromosome number	2n = 26	2n = 25	2n = 26	2n = 26	2n = 25-26

fianum and *L. inexpectans* to support a common origin of the four taxa through non sexual mutations.

Lastly, the fact that in the Magalluf area many endemic *Limonium* taxa grow which are restricted to this place, could be partially explained by hybridization between some apomictic taxa. Putative hybrids between triploid or between diploid and triploid taxa have been detected at the Balearic islands (ERBEN, 1993; Carvalho, Rosselló & Sáez, unpublished data). Karyological analysis of such hybrids is now in progress, although preliminary data show that their chromosome number is not the sum of the parents complements. Instead, lower chromosomal values are attained. If *L. carvalhoi* has arisen through this process, no clear candidates as parents can be firmly suggested on morphological grounds. However, similar

inflorescence architecture in *L. carvalhoi* and *L. inexpectans* could be something more than a point of convergence.

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