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A phenetic approach to the genus *Limonium* (*Plumbaginaceae*) in Sicily

Abstract

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18 quantitative and 33 qualitative characters were investigated with statistical analyses on 43 accepted species of *Limonium* Mill. occurring in Sicily. Variance of each single quantitative character was tested. Furthermore an attempt of grouping the species through statistical methods was made.

Introduction

In Sicily and in the surrounding islands, including Malta, the genus *Limonium* is represented by 43 species; most of them are traditionally included in 5 or 6 clearly distinct groups (Brullo 1980, 1988; Brullo & Pavone 1981; Erben 1978, 1993; Greuter & al. 1989; Pignatti 1972, 1982) but, apart from some quite isolated ones, identification of each single taxon, as in the main part of the Mediterranean range, is hard owing to the lack of marked morphological characters.

In order to obtain a more objective taxonomic delimitation, a phenetic approach to the species occurring in Sicily and in the surrounding islands is presented here and compared with traditional treatments; in fact no previous studies were carried out using PCA or similar statistical analyses to assess the variability of this genus.

Materials and methods

All the characters were observed and measured in an exactly defined way on both dried and living material. As regards the material, the specimens kept in the herbaria of Palermo (PAL) and Catania (CAT), which contain the types of at least 2/3 of the Sicilian taxa, were analyzed. New exsiccata, prepared from plants collected in the *loci classici*, when possible, are housed in Palermo in the *Herbarium Mediterraneum* (PAL) and in München (herb. ERBEN).

The following 51 characters, chosen on the basis of their traditional importance as key characters or their inclusion in the relevant taxonomic descriptions, were observed and valued:

- 1) annual or perennial, 2) height*, 3) habit, 4) presence of sterile branches, 5) Stem

surface, 6) presence of wings, 7) stem colour when dry, 8) stem diameter (at the base)*, 9) basal internode length (measured on the first two internodes)*, 10) lower scale length (measured on the first two scales)*, 11) leaf presence, 12) leaf entire or lobed, 13) leaf shape, 14) leaf colour, 15) leaf habit, 16) leaf surface, 17) leaf surface hairy, 18) leaf margins (observed on dried material), 19) leaf tip shape, 20) leaf mucrone presence, 21) leaf length*, 22) leaf width*, 23) type of nerves, 24) number of nerves*, 25) panicle shape, 26) panicle width, 27) panicle branching, 28) spike length*, 29) number of spikelets per cm*, 30) number of flowers for spikelet*, 31) outer bract length*, 32) outer bract tip shape, 33) outer bract hyaline margin length, 34) outer bract mucrone presence, 35) middle bract length*, 36) middle bract tip shape, 37) middle bract hyaline margin length, 38) middle bract mucrone presence, 39) inner bract length*, 40) inner bract tip shape, 41) inner bract shape in section, 42) inner bract surface, 43) inner bract hyaline margin length, 44) inner bract mucrone presence, 45) inner bract mucrone length, 46) calix length*, 47) calix exceeding the inner bract*, 48) limb/tube, 49) tube hairy, 50) lobes shape, 51) corolla length (measured on fresh materials)*. Quantitative characters are marked with an asterisk. A set of variables, including binary, qualitative and quantitative measures, was collected on the basis of 31 observations. The arithmetic mean of each quantitative character was calculated. The similarity matrix was built up using binary, qualitative and quantitative standardized measures (Everitt 1980; Pankurst 1991).

All the statistical analyses were carried out on a personal computer using MINITAB version 13.3 (2000). The range of each continuous numerical character within the genus was tested with box-and-whiskers plots. A PCA (Principal Component Analysis) and a Cluster analysis were performed to group the species in OTUs (Operational Taxonomic Units). According to Rohl (1970), who considers them as complementary test of morphological affinity, the former estimates relationship among more distantly related taxa, the latter is useful for exploring the fine structure of relationship.

Well defined and isolated taxa with, at least, a distinctive character (*Limonium avei*, *L. ferulaceum* and *L. sinuatum*) were not included in the clustering techniques because being too distinct from the rest of the species and may have lead to a loss of detail in analyses.

Both a dichotomic and a multi-access key were set up using the DELTA program (DEscription Language for TAXonomy) which allowed for organizing and using the above amount of data in an easy and intuitive way.

Results

Box-and-whiskers plots allow a rough comparison of the range of each character in the 40 species.

The characters that show a wide range of variation should be considered the most suitable to discriminate between the taxa; outlying values, symbolized with an asterisk in the plots, could be used to identify single species. The lengths of the outer and of the inner bract show a wide range (Fig. 1) and should be used as discriminating between the taxa, also the length of the middle bract has a wide length of variability but unfortunately this character is inconstant inside the population, other characters such as the diameter of the stem measured at the base, the width and the length of the leaf and the spike length, do not

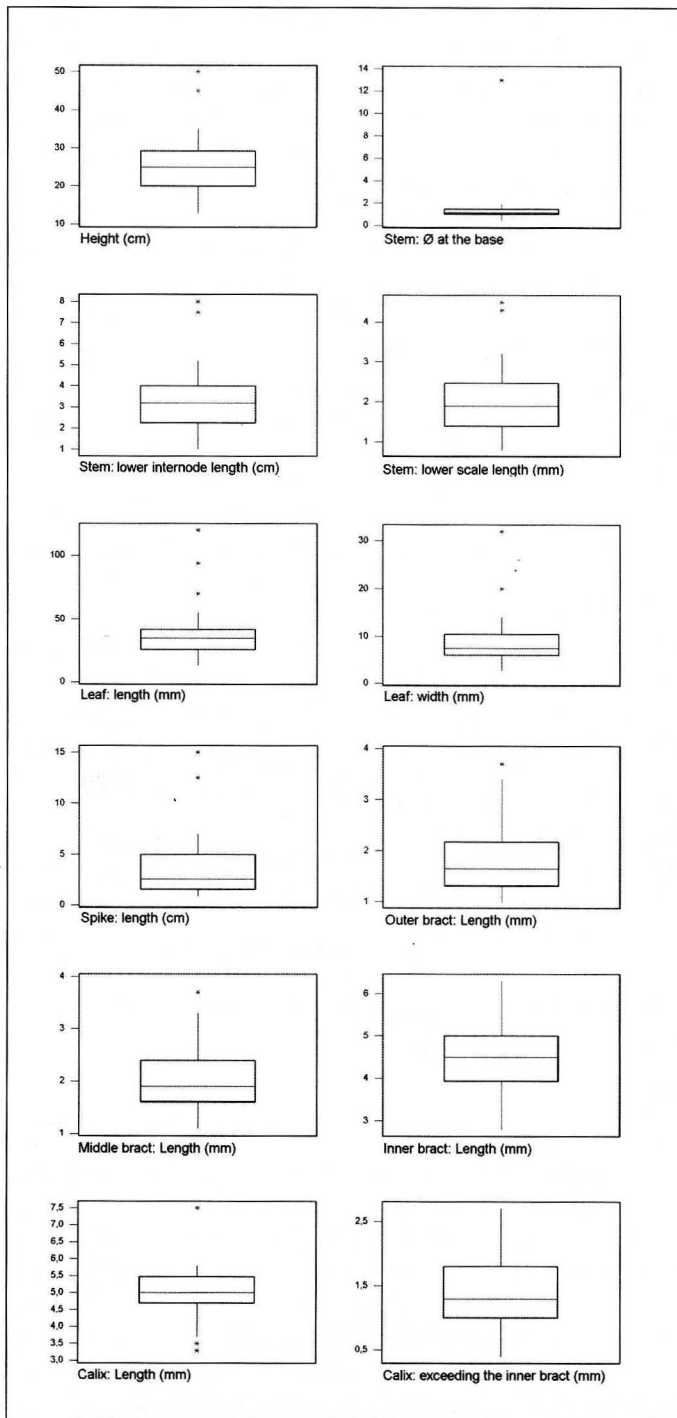


Fig. 1. Box-and-whiskers plots of continuous numerical characters.

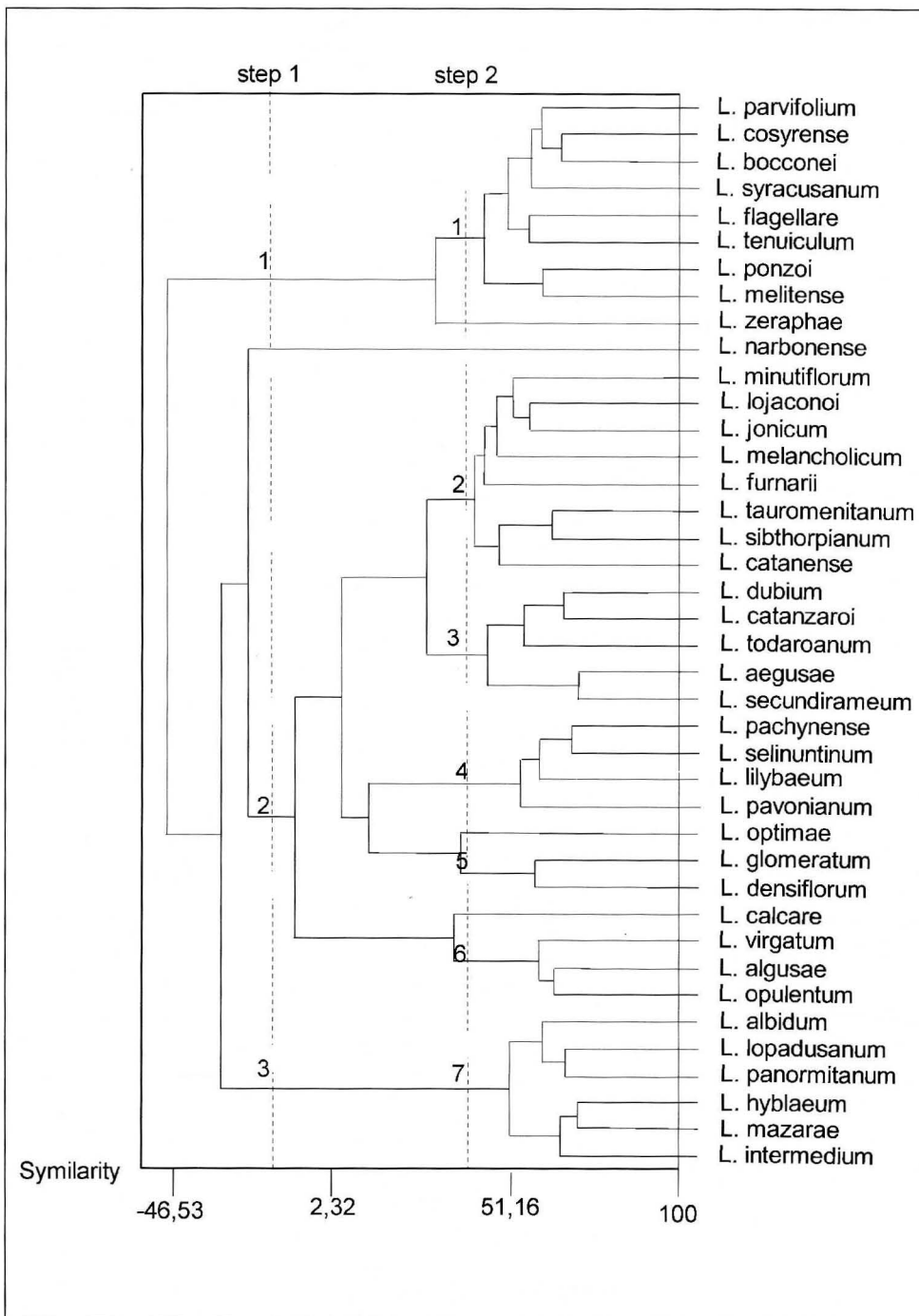


Fig. 3. Phenogram resulting from the cluster analysis; step 1 and 2 represent two different results owing to the similarity level selected.

show a wide variation, but are suitable to identify single taxa with outlying values (e.g. in *L. optima*e for the measures of the leaf and for the diameter of the stem, in *L. syracusanum* and *L. furnari* for the length of the spike).

In the plot of the first two components of the PCA, performed on a correlation matrix, the traditional groups fall in distinct regions of the Cartesian plan (Fig. 2). These groups are not clearly separated in the plot where only a little part of the total variation (0,263) is represented (Table 1).

Table 1. Eigen-analysis of the correlation matrix.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	6,7695	6,1281	4,74	3,8896	3,5697	2,6447
Proportion	0,138	0,125	0,097	0,079	0,073	0,054
Cumulative	0,138	0,263	0,36	0,439	0,512	0,566

The set of data was further analyzed with the cluster analysis using the Ward's linkage method and the Euclidean distance measure. The phenogram obtained from the entire coded data matrix is shown in Figure 3.

As a first step it is possible to distinguish 3 principal branches (Fig. 3 step 1): a first one that includes the species that in traditional treatments belong to the *L. cosyrense* group plus *L. parvifolium* and *L. zeraphae*, a second one with the species that in traditional treatments belong to the *L. albidum* group and a third one with all the remaining Sicilian species. Finally *L. narbonense* results isolated.

In a further detail (Fig. 3 step 2) it is possible to distinguish 7 OTUs:

The OTU n. 1 includes the species of the *L. cosyrense* group plus *L. parviflorum* that is usually considered isolated. The OTU n. 2 comprises the species traditionally included in the *L. minutiflorum* group and, in addition, *L. catanense* and *L. sibthorpiatum*, two species traditionally considered isolated. The OTU n. 3 comprises *L. dubium* and *L. catanzaroi* included by Pignatti (1982) and Greuter & al. (1989) in the *L. virgatum* group but considered as isolated species by Brullo (1980, 1981), *L. aegusae* and *L. secundirameum* usually included in the *L. ramosissimum* group and the traditionally isolated *L. todaroanum*; this OTU is made up of taxa that have little discriminating characters in common. The OTU n. 4 includes taxa traditionally belonging to the *L. ramosissimum* group. The OTU n. 5 contains only *L. glomeratum* and *L. densiflorum* that are here distinctly separated from *L. aegusae* and *L. secundirameum*; this is in accordance with Greuter & al. (1989) and Pignatti (1982), both splitting the *L. densiflorum* group (Brullo 1981) in two. The OTU n. 6 comprises species traditionally belonging to the *L. virgatum* group. The OTU n. 7, already emphasized at a lower level of similarity (step 1), is made up of the species traditionally belonging to the *L. albidum* group.

L. zeraphae, *L. narbonense*, *L. optima*e and *L. calcare* resulted to be quite isolated species morphologically easily distinguishable.

Conclusions

This contribution represents an operative step for the study of the morphological variability of the genus founded on numerical basis.

The branching of the phenogram (Fig. 3) shows how it is possible to group these taxa in different way owing to the similarity level selected (e.g. step 1 and 2). This phenetic treatment, in comparison with the traditional ones, has been used to produce the following identification key.

Key to species

1. Annual (in the Sicilian climate), inner bract tuberculate *L. avei* Brullo & Erben
- Perennial, inner bract smooth..... **2**
2. Outer bract surrounded by numerous aristate bracts, leaves absent
..... *L. ferulaceum* (L.) Chaz.
- Few not aristate bracts, leaves present **3**
3. Leaves lobed, stem winged *L. sinuatum* (L.) Mill.
- Leaves entire, stem not winged **4**
4. Sterile branches abundant **5**
- Sterile branches few or absent **19**
5. Inner bract with a conspicuous mucrone **6**
- Inner bract with a short mucrone **12**
6. Inner bract 4,6-5,5 mm long, calix 5,5-6,5 mm long **7**
- Inner bract 3,0-4,6 mm long, calix 3,5-5 mm long **8**
7. Leaves obovate-spathulate, 30-50 mm long, 9-25 mm wide, 5-6 spikelets per cm
..... *L. ponzoii* (Fiori & Bég.) Brullo
- Leaves oblanceolate, 10-30 mm long, 3-7 mm wide, 3-4 spikelets per cm
..... *L. melitense* Brullo
8. 2-4 spikelets per cm **9**
- 4-7 spikelets per cm **10**
9. Inner bract dorsally curved, keeled in section, calix 4-5 mm long
..... *L. syracusanum* Brullo
- Inner bract straight, circular in section, calix 3,5-3,8 mm long
..... *L. tenuiculum* (Tineo) Pignatti
10. Stems thin (\varnothing at the base 0,4-0,8 mm), calix 3,5-4 mm long exceeding the inner bract
1-1,5 mm *L. cosyrense* (Guss.) O. Kuntze
- Stems stronger (\varnothing at the base 1,1-2,1 mm), calix 4-5 mm long exceeding the inner bract
1,5-2 mm **11**
11. Leaves smooth, spatulate, the wider 3 nerved *L. flagellare* (Lojac.) Brullo
- Leaves rough or rugose, linear-spathulate, uninerved *L. bocconei* (Lojac.) Litard.
12. Panicle elliptic, leaves revolute, spikes 0,5-2 cm long
..... *L. parvifolium* (Tineo) Pignatti
- Panicle angular-ovate, leaves flat, spikes more than 2 cm long **13**
13. Leaves smooth not mucronated **14**
- Leaves rough or rugose lightly mucronated **15**

14. Panicle very much branched, inner bract 3,2-4,0 mm long, calix 5,4-5,7 mm long
 *L. calcarae* (Tod.) Pignatti
 - Panicle not very much branched, inner bract 4,5-5,0 mm long, calix 4,7-5,2 mm long
 *L. zeraphae* Brullo
15. Bracts with wide hyaline margin (> 1 mm) *L. catanzaroi* Brullo
 - Bracts with thin hyaline margin (< 1 mm) **16**
16. Leaves oblanceolate-spathulate, spikelets with 1-2 flowers
 *L. dubium* (Andrz. ex Guss.) Litard.
 - Leaves linear spatulate, spikelets with 2 or more flowers **17**
17. Outer bract 2,5-2,8 mm long, 2-3 spikelets per cm, stem dark when dry
 *L. albusae* (Brullo) W. Greuter
 - Outer bract 1-2,5 mm long, 3-6 spikelets per cm, stem pale when dry **18**
18. Panicle very much branched, spikelets with 2-5 flowers *L. virgatum* (Willd.) Fourr.
 - Panicle not very much branched, spikelets with 5-11 flowers
 *L. opulentum* (Lojac.) Brullo
19. Leaves pinnately nerved, stems strong (\emptyset at the base 2-25 mm) *L. narbonense* Mill.
 - Leaves parallel-nerved or uninerved, stems thinner (\emptyset at the base 2 mm max.) **20**
20. Leaves obovate-spathulate, wide an half than long **21**
 - Leaves narrow, wide 1/3 or less than long **29**
21. Inner bract 5,9-6,2 mm long, outer bract 2,7-3,1 mm long
 *L. secundirameum* (Lojac.) Brullo
 - Inner bract max 5,5 mm long, outer bract max 2,4 mm long **22**
22. Calix 7-8 mm long *L. todaroanum* Raimondo & Pignatti
 - Calix shorter up 6 mm long **23**
23. Leaves green, stems strong (\emptyset at the base 1,6-2,1mm), calix 3,5-4 mm long.....
 *L. catanense* (Tineo ex Lojac.) Brullo
 - Leaves glaucous or glaucescent, stems thinner (\emptyset at the base 1,6 mm max.), calix 4,5-
 6 mm long **24**
24. Leaves flat **25**
 - Leaves revolute **26**
25. Leaves erect, erect-spreading, stems \emptyset at the base 0,7-1,5 mm *L. hyblaeum* Brullo
 - Leaves spreading, spreading-reflexed stems thinner (\emptyset at the base 0,4-0,7 mm)
 *L. mazarae* Pignatti
26. Spikelets with 6-10 flowers *L. albidum* (Guss.) Pignatti
 - Spikelets with 1-4 flowers **27**
27. Inner bract 3-3,5 mm long *L. panormitanum* (Tod.) Pignatti
 - Inner bract 4-5 mm long **28**
28. Panicle angular-ovate, stems smooth, dark when dry *L. lopadusanum* Brullo
 - Panicle umbel shaped, stems rugose, pale when dry *L. intermedium* (Guss.) Brullo
29. Inner bract 2,6-2,9 mm long, leaves glaucous *L. sibthorpiatum* (Guss.) O. Kuntze
 - Inner bract > 3,5 mm long, leaves green or glaucescent **30**
30. Outer bract 2-3,6 mm long **31**
 - Outer bract until 2 mm long **37**
31. Calix 4-5 mm long **32**
 - Calix 5-6 mm long **34**

32. Spikelets with 4-5 flowers, outer bract 3-3,6 mm long *L. pavonianum* Brullo
 - Spikelets with 2-3 flowers, outer bract 2-2,7 mm long 33
33. Panicle wide, calix 4,8-5 mm long, middle bract 2,5-3 mm long *L. lilybaeum* Brullo
 - Panicle narrow, calix 4-4,5 mm long, middle bract 1,7-2,1 mm long
 *L. pachynense* Brullo
34. Leaves green, rough or rugose, linear-spathulate 35
 - Leaves glaucescent, smooth, oblanceolate-spathulate 36
35. Leaves 3-5 nerved, panicle not very much branched, outer bract 2,5-3 mm long.....
 *L. ionicum* Brullo
 - Leaves uninerved, panicle very much branched, outer bract 2-2,2 mm long
 *L. melancholicum* Brullo, Marcenò & Romano
36. Sterile branches absent, spikes with 10-12 spikelets per cm
 *L. densiflorum* (Guss.) O. Kuntze
 - Sterile branches present, spikes with 6-9 spikelets per cm
 *L. glomeratum* (Tausch) Erben
37. Spikelets with 4-6 flowers *L. selinuntinum* Brullo
 - Spikelets with 1-2 flowers 38
38. Leaves glaucescent 39
 - Leaves green 41
39. Leaves 3-5 nerved, stem smooth, calix with limb < tube *L. optima* Raimondo
 - Leaves uninerved, stem rough, calix with limb > tube 40
40. Leaves flat, panicle very much branched, spikes with 4-7 spikelets per cm
 *L. furnarii* Brullo
 - Leaves revolute, panicle not very much branched, spikes with 8-10 spikelets per cm
 *L. minutiflorum* (Guss.) O. Kuntze
41. Stems rugose, calix 5-5,5 mm long
 *L. aegusae* Brullo
 - Stems smooth, calix 4-5 mm long 42
42. Leaves linear-spathulate with acute tip, calix with limb = tube
 *L. tauromenitanum* Brullo
 - Leaves oblanceolate-spathulate with rounded tip, calix with limb < tube.....
 *L. lojaconoi* Brullo.

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