Paolo Colombo & Salvatore Trapani

Morpho-anatomical observations on three *Limonium* species endemic to the Pelagic Islands.

Abstract

Colombo, P. & Trapani, S.: Morpho-anatomical observations on three *Limonium* species endemic to the Pelagic Islands. — Fl. Medit. 2: 77-90. 1992. — ISSN 1120-4052.

The results of morpho-anatomical studies on *Limonium albidum* (Guss.) Pignatti, *Limonium intermedium* (Guss.) Brullo and *Limonium lopadusanum* Brullo, all endemic to the Pelagic Islands, reveal xeromorphous characters in the root, flowering stem and leaf, indicating ecological adaptation to their respective habitat.

Introduction

The study of the morphological adaptations of plants to their environments confirms that anatomical structures evolve (Everi 1962; Fahn 1964, 1978) and change in plants that live in particular environmental conditions, such as an arid-windy climate (Stocker 1960).

During our research on endemic plants in Sicily and the surrounding islands, we have performed anatomical studies on the endemic species of the genus *Limonium* (Colombo & Trapani 1991). This paper describes our observations on *Limonium albidum* (Guss.) Pignatti, *Limonium intermedium* (Guss.) Brullo and *Limonium lopadusanum* Brullo, which are restricted to the Pelagic Islands.

Limonium albidum, endemic to Lampione, grows on rocks and cliffs; it is a chamaephyte 20-35 cm high, with non-pulvinate stems, and rugose-tuberculate scapes twinned, rarely only one. In each rosette oblanceolate-spatulate leaves, 28-50 x 5-12 mm, three-veined with a retuse apex and entire margin.; panicle branched in the upper part with straight, crowded fasciculate branches (2-6 per node). Spikes dense, 2-7 cm long with spikelets 6 to 8 flowered; inner bract 5 mm long; calyx 5.5 mm long.

Limonium intermedium, endemic to Lampedusa in brackish areas near the port, is a pulvinate chamaephyte, 20-30 cm high, with sturdy, single scapes. Leaves oblanceolate, 20-40 x 4-9 mm, three-veined, progressively narrowed at the petiole, more or less revolute at the edge with a mucronate apex and undulate margin. Panicle ramose with fasciculate branches. Spikes, 3-10 cm, with spikelets 3-to-4 flowered . Inner bract 4.5-5 mm long; calyx 5-5.5 mm long.

Limonium lopadusanum is found along the rocky coasts of Lampedusa and Linosa, in the areas nearest the sea. It is a pulvinate chamaephyte, 7-25 cm high, with single smooth or slightly rugose scapes. Leaves glaucous, obovate-spatulate, 5-20 x 4-10 mm, three-veined, with obtuse apex and entire margin.

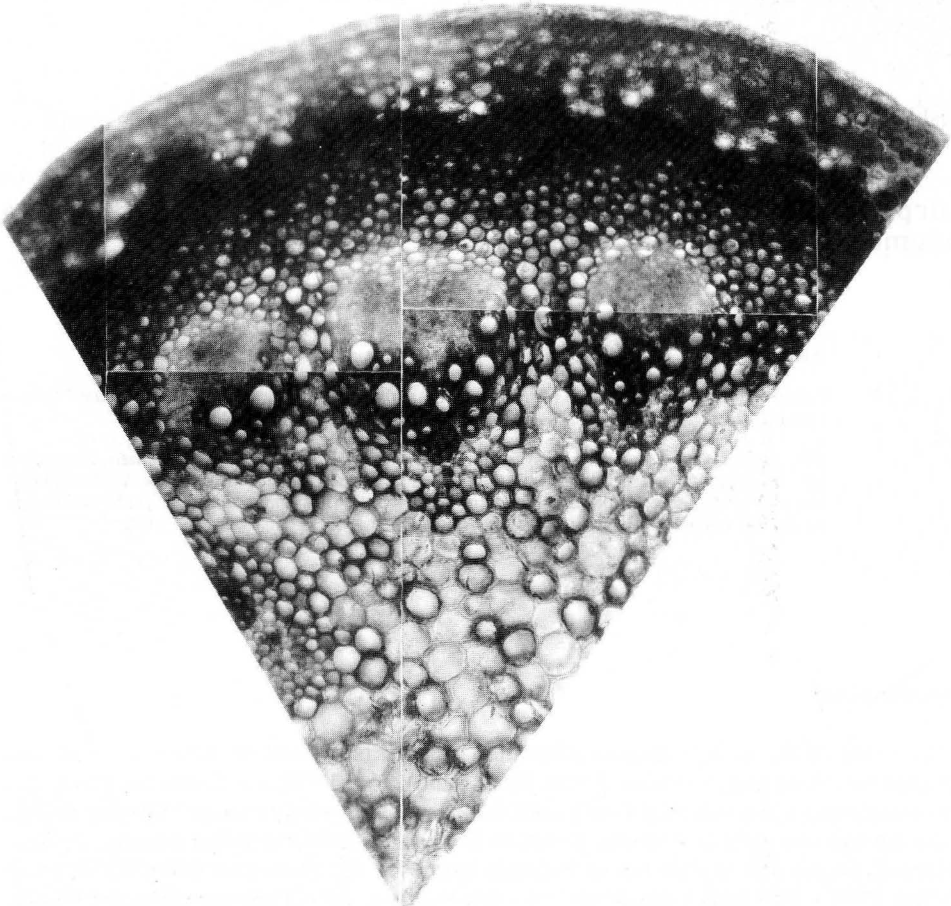

Fig. 1. Cross section (x 100) of the flowering stem of *L. intermedium*.

Panicle ample with arcuate branches, simple, rarely fasciculate (2-3 per node). Spikes , 2-8 cm long with spikelets 2-to-3 flowered. Inner bract 4-4.5 mm long; calyx 4.5-5 mm, exserted 1 mm from the inner bract.

The average annual temperature is 19.5°C, with a maximum value of 26.1°C in August and a minimum of 13.4°C in January. The mean value of rainfall is 338 mm.

The distribution of rainfall is typically Mediterranean with a dry period of over 6 months; the bioclimate is of the thermo-Mediterranean semiarid kind (Rivas Martinez 1981).

From the systematic point of view these plants belong to the group of *Limonium albidum* (Pignatti 1982); phytosociologically *L. albidum* , *L. lopadusanum* fit in the *Crithmo-limonietalia*, *L. intermedium* in the *Sarcocornietalia-fruticosae*.

The aim of this research is to examine the anatomical characteristics of the root, of the flowering stem, of the leaf and of the xylematic architecture of the leaf of these species, which have adapted to particular environments.

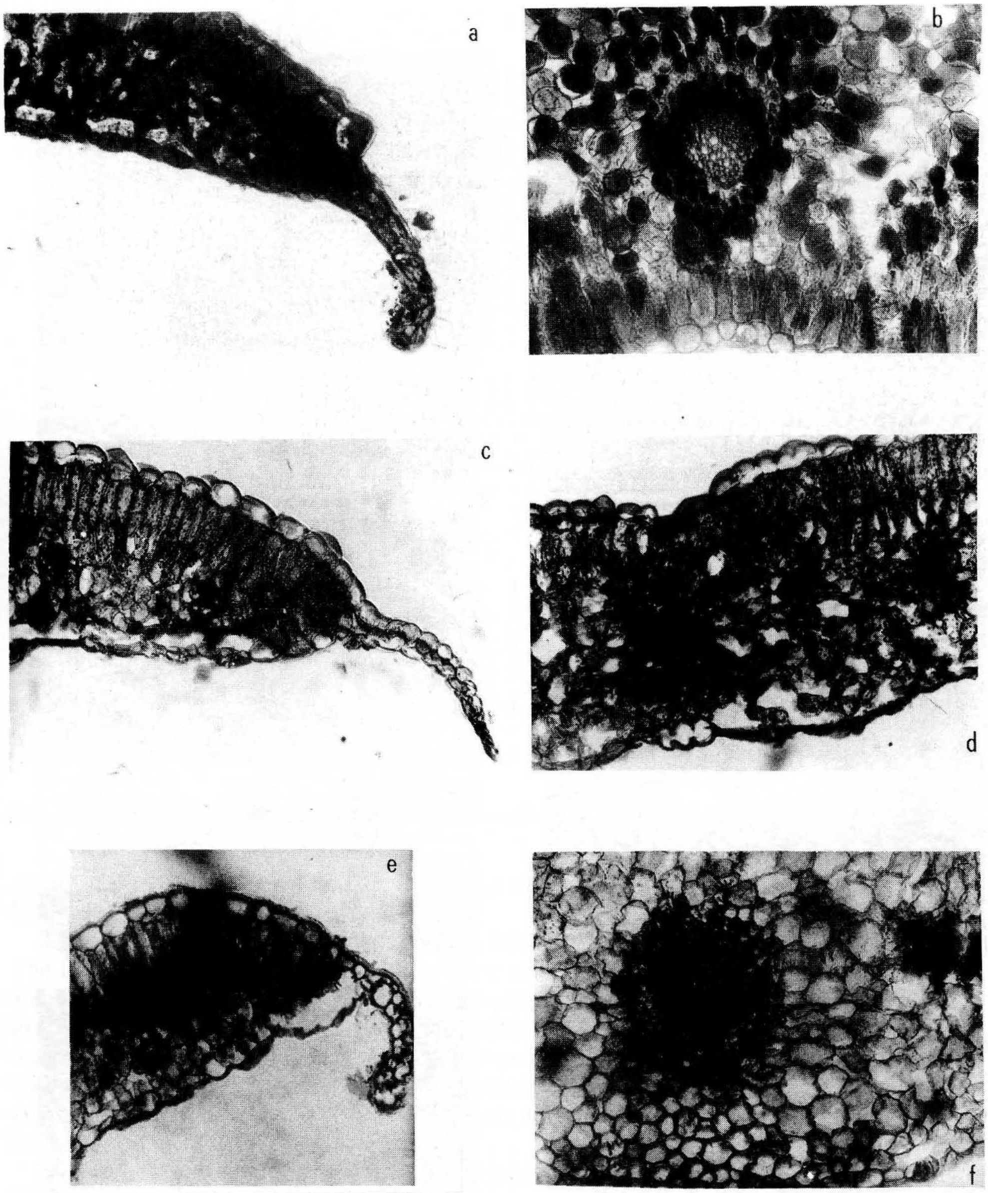

Fig. 2. Cross section (x 220): leaf of *L. lopadusanum* (**a**) and detail of the midvein (**b**); leaf of *L. intermedium* (**c**) and detail of the midvein (**d**); leaf of *L. albidum* (**e**) and detail of the midvein (**f**).

Material and methods

Specimens of *Limonium albidum*, *Limonium intermedium* and *Limonium lopadusanum*, of uniform size, were collected from their natural habitat, during the period of their maximum vegetative development.

The observations were made on ten plants, taking comparable segments of the roots and of the flowering stems, at the same level, and five leaves of different age from the

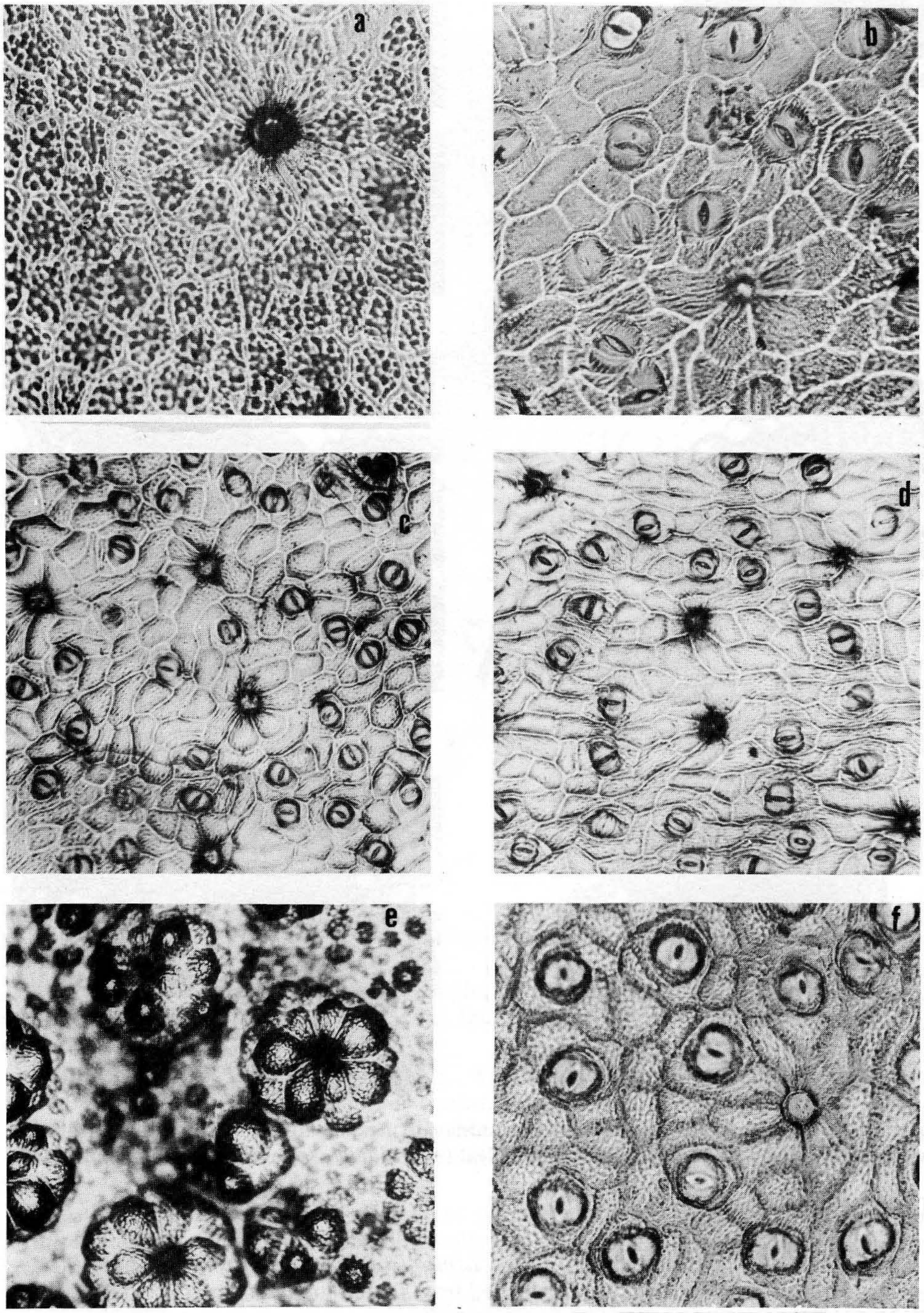

Fig. 3. Leaf prints (x 100): the upper epidermis of *L. albidum* (**a**), *L. intermedium* (**c**) and *L. lopadusanum* (**e**); lower epidermis of *L. albidum* (**b**), *L. intermedium* (**d**) and *L. lopadosanum* (**f**).

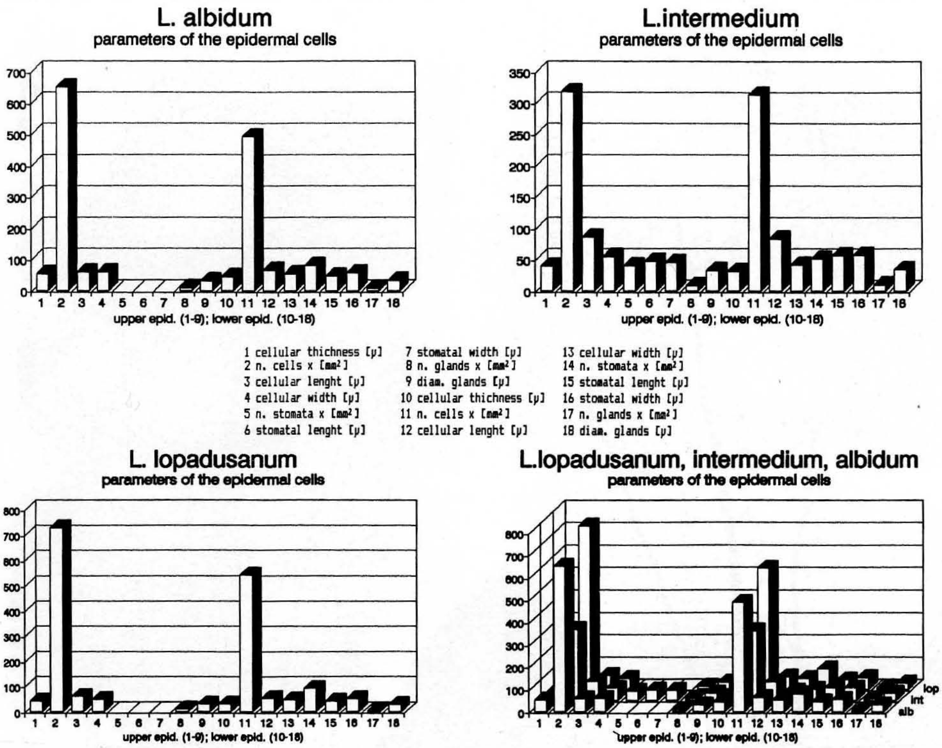

Fig. 4. Parameters of the epidermal cells of *L. albidum*, *L. intermedium* and *L. lopadusanum*.

medium part of the rosette. The flowering stem was sectioned below the flowering branches.

The material used was observed both as fresh sections and with paraffin preparations. The fresh sections were stained with specific stains (safranin, zinc chloro-iodide, Lugol's solution, sudan III, ferric chloride); the ones prepared with the paraffin method were fixed in FAA, dehydrated and stained with alcoholic safranin. The observations with the SEM were made after pretreatment at the critical point. A group of leaves was fixed, cleared with 6% NaOH and stained with a 1% alcoholic solution of safranin to study the xylem architecture. Some samples were covered with nail-polish to make prints that would show the morphological characters.

Measurements were taken with a micrometric ocular inserted on a Reichert Jung Microstar microscope. The anatomical terms are used according to Esau (1965), while the leaf vascular terminology reported is that of Hickey (1973).

Results

Root. — In the specimens examined the root is formed by a multilayered periderm with thick-walled cells containing tannins. The cortex is composed of parenchymatous cells with thin walls, which in *L. lopadusanum* penetrate irregularly into the lower continuous sclerenchymatous layer, which has remarkably thickened cells and evident porocanals.
In the other two species, instead, the cortex is wider and among the parenchyma cells there are groups of sclerenchymatous cells, of varying consistency and dimensions.

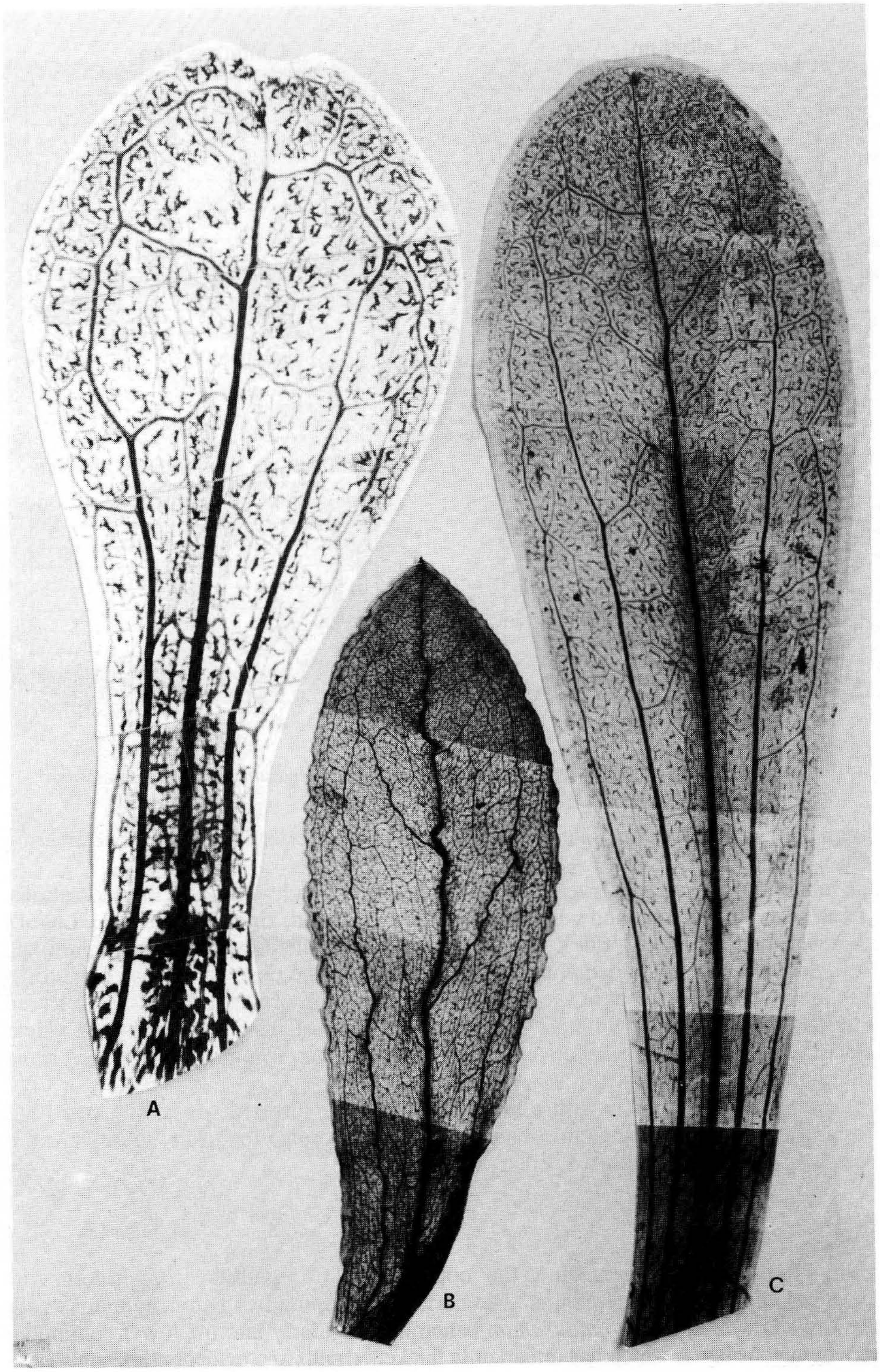

Fig. 5. Leaf architecture of *L. lopadusanum* (x 32) (**A**), of *L. intermedium* (x 23) (**B**) and of *L. albidum* (x 20) (**C**).

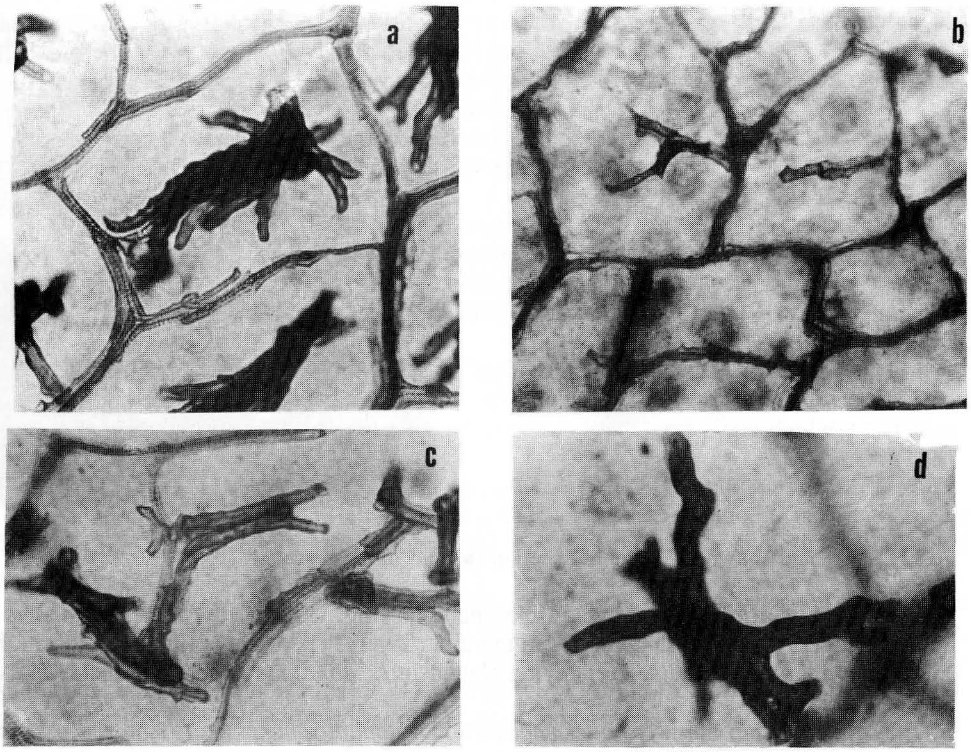

Fig. 6. Areoles with sclereidal idioblasts in *L. lopadusanum* (x 175) (**a**) and in *L. albidum* (x 200) (**c**); without idioblasts in *L. intermedium* (**b**); detail of the idioblasts in *L. lopadusanum* (**d**).

The phloem is made up of a thin continuous layer with few proto- and metaphloem elements externally, and of many secondary vascular elements, with parenchyma cells and few fibres internally. Next, there is the cambium layer, not very visible, and the secondary xylem, which in *L. lopadusanum* is made up of large vessels, surrounded by fibres that increase progressively towards the centre, with a consequent reduction in the number and size of the vessels; the pith is lignified and parenchyma and pith rays are absent. The secondary xylem in *L. albidum* and *L. intermedium* has small vessels arranged in cords, alternated with rows of multiseriated sclerenchyma fibres. The pith present many elements containing tannins and a central islet of cells with strongly lignified walls.

Flowering stem. — The flowering stem of all three species consists of a single- layer epidermis, with a thick rugose cuticle which penetrates between the cell walls, small stomata and narrow and elongate salt glands. Beneath the epidermis, there is the chlorenchyma, made up of layers of roundish cells with few intercellular spaces.

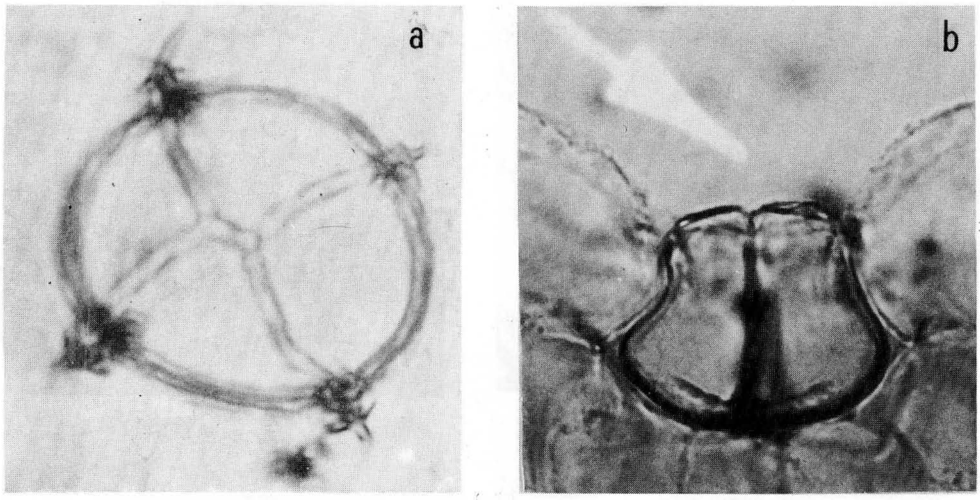

Fig. 7. Salt gland shown in tangential section (**a**) and in cross section (**b**).

Below this is a thick multilayeredsclerenchyma ring, with narrow lumen elements in the outer layers and wide lumen ones in the inner layers. There are cortical bundles in the chlorenchyma outside the sclerenchyma sheath in *L. albidum*, close to or inside it in *L. intermedium*.

The sclerenchyma ring in *L. intermedium* and *L. lopadusanum* occurs sinously between the bundles, forming cords of wide lumen cells with poorly lignified walls. The vascular system consists of a circle of closed collateral bundles, surrounded by a parenchymatous sheath, rich in tannins. In *L. intermedium* and *L. lopadusanum* the bundles are numerous, of different sizes, with xylem elements arranged in a V- shaped, the proto elements toward the centre, the meta ones along the sides of the V-shaped; the phloem elements are between the sides of the V-shaped.

In *L. albidum* the phloem elements are arranged in a ring with a slightly sinusoid pattern and the poorly defined xylem elements are separated by very thin 1 to 2-seried pith rays. In the three plants the pith consists of large parenchyma cells, among which there are some rich in tannin, others lignified (Fig. 1).

Leaf anatomy. — The leaf, with a dorsiventral blade, has a single layer epidermis, formed by a layer of polygonal, almost isodiametric cells; the upper epidermis has a very convex surface, covered by a cuticle with a very irregular and wrinkled profile in *L. albidum* and *L. lopadusanum* (Fig. 3 a, e), a slightly convex or flat surface with light cuticle ornamentations in *L. intermedium* (Fig. 3 c). In the lower epidermis of *L. albidum* and *L. lopadusanum* (Fig. 3b, f) the cuticle ornamentations are not very pronounced, while in that of *L. intermedium* (Fig. 3 d) the cells are flat and poorly cutinized. The stomata, situated among the epidermal cells, are of the anisocytic kind, and are present on the lower epidermis in *L. albidum* and *L. lopadusanum*, and on both surfaces in *L. intermedium*. They are more numerous in *L. lopadusanum* and larger in *L. intermedium*.

The presence of the glands of Mettenius is a characteristic of the epidermis of *Limonium*; the glands in these three species are distributed on the whole surface of the leaf blade (Fig. 7 a, b). In *L. lopadusanum* the glands are enclosed in particular formations of cells arranged in a rosette, which protrude quite evidently from the upper epidermis, while they are scarcely evident in the lower epidermis; they are irregularly distributed, numerous and large in the upper part of the blade, small and fewer in the lower part.

In *L. intermedium* the glands are surrounded by epidermical cells neatly arranged on the upper surface, irregularly distributed on the lower one.

In *L. albidum*, the glands are found randomly among the epidermic cells. Each gland is made up of a cutinized capsule with the convex part facing the mesophyll and the upper surface flat or slightly concave.

As a whole the structure of each gland resembles a cup with a circular rim; from this rim 4 small columns originate; these columns join together in the basal part, which resembles an anchor. Inside there are 12 cells grouped in the following way: 4 secreting cells, each with a characteristic pore, 4 internal calyx-like cells, and 4 external collector cells.

The mesophyll consists of two palisade layers and a spongy mesophyll with rounded cells separated by intercellular spaces. The closed collateral conducting bundles, present in the central part of the leaf, are numerous, arranged in linear series and the larger ones are surrounded by a sclerenchymatous sheath.

The smaller bundles are protected by sclerenchyma cells in the phloem zone (Fig. 2).

A peculiar characteristic, in almost all *Limonium*, is the presence of sclereids in the form of idioblasts, located in the areoles near the free branches or sometimes connected with them.

In *L. lopadusanum* (Fig. 6 a, d) the sclereids are linear and grouped at the end of the free branches in the middle and apical part of the leaf; in the basal part they are thick and short, closer together and more numerous. In *L. albidum* (Fig. 6 c) they are linear, not very numerous, isolated or in groups of 2-3, connected with the free branches in the central-apical part; isolated and with shorter elements in the basal part. They are totally absent in *L. intermedium* (Fig. 6 b).

Leaf xylem architecture. — The leaf blade in the three individuals (Fig. 5) is three-veined with a large midvein and two commissural veins running parallel to the midvein and diverging towards the margins so as to originate the thinner secondary veins.

These secondary veins, imperfectly basal acrodromous, form acute angles that enlarge from the base to the apex and they branch towards the margins, delimiting non homogeneous intercostal areas; both the primary and the secondary veins run straight in the three *Limonium* species.

The tertiary veins are thinner than the secondary ones and they anastomose with these or with other tertiary veins with a transverse branched pattern. Fourth and fifth order veins, are randomly oriented and easily visible.

The submarginal veins are incomplet; the free branches are 1- or 2- branched and only slightly lignified. The sclereid idioblasts are close to the free branches, which have short xylem elements, and are present in *L. albidum* and *L. lopadusanum*; the differentiation of these idioblasts precedes that of the free branches.

The areoles, irregular in form and of medium size, have an incomplete development with a random distribution.

The architecture of the three plants develops gradually,the midvein differentiating first, then the lateral veins, the secondary and the marginal ones, and successively the intersecondary ones, those of higher order and the free branches.

Table 1. - Ecological data and foliar organization of *L. lopadusanum*, *L. intermedium* and *L. albidum*.

	L. lopadusanum	*L. intermedium*	*L. albidum*
Locality	Lampedusa	Lampedusa	Lampione
Metres a.s.l.	0-50	0-50	0-50
Average rainfall (mm) (years 1965-1985)	338	338	338
Average temperature (°C) (years 1965-1985)	19.5	19.5	19.5
Climatic zone	thermo-medit. semiarid	thermo-medit. semiarid	thermo-medit. semiarid
Substrate	Limestone and dolomite rock	Limestone and dolomite rock	Limestone and dolomite rock
Biological form	CH-PULV	CH-PULV	CH
Chromosome number (2*n*)	18	32	18
Leaf			
Shape	obovate-spatulate	oblanceolate	oblanceolate-spatulate
Margin	entire	undulate	entire
Apex	retuse	mucronate	obtuse
Thickness (without midvein) (μ)	409	238	341
Length (cm)	1.61	3. 15	3.36
Width (cm)	0.72	0.60	1,00
Length/width ratio	2.24	5.25	3.36
Upper leaf epidermis			
Thickness (μ)	41.9	39.3	53.1
Nr. of cells x (mm^2)	728	318	650
Length (μ)	60.1	85.3	58.1
Width (μ)	47.4	54.3	59.3
Nr. of stomata x (mm^2)	absent	40	absent
Stomatal index		11.2	
Stomata length (μ)		47.0	
Stomata width (μ)		45.2	
Nr. of glands x (mm^2)	11	8	8
Diameter of glands (μ)	28.7	32.1	30.1
Lower leaf epidermis			
Thickness (μ)	30.1	31.0	43.3
Nr. of cells x (mm^2)	541	313	491
Length (μ)	53.6	82.6	64.2
Width (μ)	46.5	41.3	52.7
Nr. of stomata x (mm^2)	91	51	79
Stomatal index	14.4	14.0	13.9
Stomata length (μ)	41.1	56.3	44.9
Stomata width (μ)	51.3	56.7	56.3
Nr. of glands x (mm^2)	4	9	6
Diameter of glands (μ)	28.3	33.5	31.8

Mesophyll			
Thickness (μ)	337.0	167.0	245.0
Nr. palisade layers (upper surface)	2	2	2
Length palisade cells (upper surface) (μ)	84.5	56.5	56.0
Width palisade cells (upper surface) (μ)	22.5	20.0	19.5
Palisade cells (length/width upper surface)	3.8	2.8	2.9
Thickness palisade (upper surface) (μ)	116.0	83.0	109.0
Thickness palisade (lower surface) (μ)	absent	absent	absent
Thickness spongy mesophyll (μ)	221.0	84.0	136.0
Thickness palisade / Thickness spongy mesophyll	0.5	1.0	0.8
Bundle sheath	present	present	present
Leaf venation kind	three-veined	three-veined	three-veined
I order venation			
a) thickness (μ)	242.0	116.0	268.0
b) course	straight	straight	straight
II order venation	imperfect-basal-acrodomous	imperfect-basal-acrodomous	imperfect-basal-acrodomous
a) divergence angle	acute	acute	acute
b) variations of the divergence angle	almost uniform	almost uniform	almost uniform
c) thickness (μ)	46.0	20.0	46.5
d) course	straight	straight	straight
e) intersecondary	present	present	present
III order veins pattern	transverse-branched	transverse-branched	transverse-branched
Resolution of the higher order veins	distinguished	distinguished	distinguished
Orientation of the higher order veins (IV-V)	random	random	random
Last marginal veins	incomplete	incomplete	incomplete
Free branches	1-2 ramif. with idioblasts	1 ramif. seldom 2 ramif.	1-2 ramif. with idioblasts
Areolation			
a) number of free branches	1-2	1-2	1-2
b) development	incomplete	incomplete	incomplete
c) course	random	random	random
d) shape	irregular	irregular	polygonal-irregular
e) size	medium	medium	medium
Anastomoses	absent	absent	absent

Discussion

The anatomical studies carried out on the three species of *Limonium* have shown the relationship existing between the structural characters of the plants and the environmental conditions in which they live. The root in the three cases presents sclerenchymatous cortical bundles, secondary xylem with diffused porosity in the pith, with large vessels in *L. lopadusanum*. The pith is lignified in the three *Limonium*, so the structures adapts well to the respective habitats. The size of the vessels and the narrow phloem zone are related to the kind of conduction present in these plants. The predominance of the fibres in the xylem and the presence of sclereids in the cortex give the organ great tensile strength and incompressibility (De Fraine 1916).

The flowering stem is characterized by the remarkable presence of sclerenchyma elements protecting the central cylinder, where the vascular part is made up of by conduction elements with large tracheids for the massive conduction of water.

The microscope study of the histological components of the leaf (Table 1, Fig. 4) shows that the epidermis, with many cells, present cuticular ornamentations; the thickness of the upper epidermis varies in the three species from 39 to 53 µm, that of the lower epidermis from 30 to 43 µm and their sum varies from 70 to 96 µm. The upper/lower epidermis ratio values are between 1.23 and 1.29. The thickness of the upper and lower epidermis varies from 17.5 % to 29.8 % of that of the lamina. The number of epidermal cells per mm^2 is higher in the upper epidermis then in the lower one in *L. albidum* and *L. lopadusanum*, while in *L. intemedium* the cells are larger in size, fewer , and their number is almost the same in both surfaces. The salt glands, present in the upper and lower epidermis of the three species are more numerous on the upper surface of *L. lopadusanum* and on the lower surface of *L. intermedium*.

The stomata, present in the lower epidermis of *L. albidum* and *L. lopadusanum*, and in upper and lower epidermis of *L. intermedium*, are more numerous in *L. lopadusanum*. Their frequency and their larger dimensions reflect the adaptation of rosette plants to arid environments (Stober 1917), and the stomatal index confirm that on the lower surface of species of the same genus this value is almost uniform, as referred by Rowson (1964). The mesophyll thickness in *L. albidum* and *L. intermedium* is 72 % of that of the lamina, while in *L. lopadusanum* it is the 82 %.

The palisade tissue consist of two layers and its thickness is 35 % of that of the mesophyll in *L. lopadusanum*, 44 % in *L. albidum* and 49 % in *L. intermedium*. The length/width ratio of the cells of the palisade is rather high and this is characteristic of plants exposed to strong insolation. The primary and secondary veins, protected by sclerenchymatous sheaths, run straight; the thickness of the midvein in *L. intermedium* is 38 % of that of the lamina zone in which it runs, in *L. lopadusanum* it is 40 % and in *L. albidum* 44 %.

The free branches have short xylem elements and many sclereidal idioblasts in *L. albidum* and *L. lopadusanum*. There are many tannin-rich cells in the subepidermal region and around the midvein.

The histological components of the leaf seem to be the most satisfactory parameters for the study of the relations between specific xeromorphous structures of plants and their habitat, although the anatomy of the root and flowering stem could give additional information, possibly, in some cases, significantly.

These species of *Limonium* in the same section, grow in similar habitats and form a group of xeromorphic plants adapted to the microclimates of their respective environments.

Acknowledgments

Financial support by Ministero della Università e della Ricerca Scientifica e Tecnologica (60%) is gratefully acknowleged.

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Addresses of the authors:
Prof. P. Colombo & Prof. S. Trapani, Dipartimento di Scienze Botaniche, Università di Palermo, Via Archirafi 38, I - 90123 Palermo, Italy.