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Comparative anatomy and development in *Petagnaea, Lereschia,* and *Sanicula (Umbelliferae)*

Abstract

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A comparative anatomical study of leaves, stems and roots of three archaic umbel taxa found in S. Italy is presented: *Petagnaea gussonei,* representing a monospecific genus endemic to Sicily; *Sanicula europaea,* widespread in Europe; and *Lereschia thomasii,* again of a monospecific genus, endemic to Calabria. They have many traits in common, mostly reflecting their sciadophilous habit, but they also show significant structural differences that reflect upon their ecological versatility. Of the three, *Petagnaea* appears to be the least adaptable, which makes it particularly fragile and needful of protection.

Introduction

A comparative study of organ structure can provide valuable evidence in support of a systematic classification, by revealing phyletic regressions or progressions in structural organization. Thus, whereas gross morphology of the leaves may be useful for the delimitation of species and varieties, leaf anatomy may provide information on common descent and evolutionary pathways, and similarity of, e.g., the vascular architecture of leaves may be indicative of close relationship.

In the present study, we describe the comparative morphology, anatomy and development of young and mature leaves of three umbellifers: *Petagnaea gussonei* (Spreng.) Rauschert *(Saniculoideae, Lagoecieae), Sanicula europaea* L. *(Saniculoideae, Saniculeae)* and *Lereschia thomasii* (Ten.) Boiss. *(Apioideae, Ammieae);* the vascular skeleton and the spatial relationships of the various tissues of leaf, stem, and root will also be discussed. A better knowledge of their anatomy may help to understand their structural similarities and differences and evaluate their ability to adapt to the environment.

Petagnaea Caruel is a monospecific genus endemic to Sicily and is considered an archaic element (Brullo & al. 1976, Wolff 1913). It is a very isolated taxon, restricted to a

limited geographical area of the Nebrodi Mountains, showing similarity only with some species of *Sanicula* L.

Sanicula L., on the other hand, is represented in our study by its single widespread European species, which to the south reaches Sicily, usually occurring in beech and deciduous oak woods.

Lereschia Boiss., again monospecific, is endemic (probably palaeoendemic) to Calabria. It requires high humidity, a soil capable of retaining surface water, and partial shade (Codogno & al. 1984).

These three genera belong to the same biological type (scapose hemicryptophytes) and, in general, share a similar habitat. Ecologically they can be placed along a cline that runs from mesophilous woodland *(Sanicula)* through hygrophilous woodland *(Petagnaea)* to hygrophilous grassland *(Lereschia).*

Given the biological and systematic importance of the unique expressions of the genera *Petagnaea* and *Lereschia,* we undertook to investigate in depth some aspects of their morphological organization, comparing them with those of *Sanicula europaea,* a widespread species occurring within the ranges of both of the two narrow endemics, and thereby aiming to demonstrate their respective adaptability and to reveal further characteristics justifying their widely separate systematic position.

Materials and methods

Leaves, stems and roots were mostly gathered in the natural habitats of the plants (brooksides, springs and wet places in woods) during the vegetational optimum period, from five different individuals per species as follows: *Petagnaea gussonei* and *Sanicula europaea* from Tortorici (Nebrodi Mts, Sicily), at 400-800 m and 500-1500 m, respectively; *Lereschia thomasii* from Serra S. Bruno (Aspromonte, Calabria), at 800-1400 m. Vouchers were deposited at PAL.

Sections of fresh leaves and petioles, stems and roots were obtained with a hand microtome, and some were stained with IKI, fluoroglucine and HCl, or Sudan III (Johansen 1940).

Leaves were cleared by a modification of the method of Kurth (1978). Ten leaves at different stages of development were fixed in FAA (formalin : ethanol : acetic acid, 1 : 1 : 1), cleared of chlorophyll in 95 % ethyl alcohol, stained in 1 % safranin in 50 % ethanol, re-cleared in warm 2 % NaOH, rinsed, dehydrated, and mounted between glass slides, so as to preserve the veins in their natural position and arrangement.

Distances between the veins were measured from the centre of each of two parallel portions of adjacent veins, using an ocular micrometer under an Orthoplan (Leitz) microscope.

Stomata were studied on leaf replicas. The stomatal index was calculated as percent ratios of stomata number per total cell number (number of stomata plus epidermal cells). Descriptive statistics of means (with standard deviations) were made for all quantitative leaf characters.

The terminology follows Hickey (1973) and Kurth (1978).

Results

Table 1 displays the general and anatomical leaf features of *Petagnaea, Sanicula europaea,* and *Lereschia.*

Gross morphology

In *Petagnaea* the stem is brittle, with a strong odour of celery; the horizontal rhizome is white; the inflorescence is supported by two large bracts (40 × 40 mm). In *Sanicula europaea* the stem is firm, virtually scentless; the vertical rhizome is brown; the bracts are linear, up to 3 × 10 mm. *Lereschia* is similar to *Petagnaea* in general aspect; linear bracts are sometimes present.

The leaves of *Petagnaea, Sanicula europaea,* and *Lereschia* all have a palmately divided lamina. In *Petagnaea* the lamina is divided for ½ to ⅘ into 3-5 segments; in the basal leaves of *Sanicula* it is divided for 9/10; and in *Lereschia,* it is 3-foliolate.

In *Petagnaea* the leaf segments have 1-2 coarse teeth and 3-5 smaller ones in their distal half; the central segment has 4-6 teeth per side. *Sanicula europaea* is similar, but the central segment has 10-20 teeth per side. In *Lereschia* the leaf segments are irregularly serrate.

Vascular architecture of the leaf

The three taxa studied, while fundamentally different in some respects, have many features in common: loose architecture, irregular with typical xylematic "diverticula" and bundle sheaths; dorsiventrality of the blades, and a very loose spongy mesophyll.

The xylematic "diverticula", or anastomosed xylematic arches, form minor (secondary) connections between the main veins at the junction between leaf septa (segments), which are organized differently in each species. The main vascular skeleton of the leaf is seen to be more or less uniform in the three taxa, and it is stable, that is to say it is not modified by the environment. There is a linear and thin median vein in every structural component (segment or leaflet) of the palmately divided laminae, as well as 4-5 pairs of thin secondary, lateral veins, and tertiary veins both oblique and transversal; intersecondary veins can also be seen.

The leaves are actinoid, and 4-5 secondary veins diverge from either side of the median vein at an acute angle. However, the angles of divergence of the lateral veins vary, as do the connections and the minor diverticula between the segments of the laminae and the thickness and lignification of all veins, which are more pronounced in *Sanicula europaea* and *Lereschia.* The acuminate serrations or teeth of the serrate-concave leaf margins are of different depth, and are differently vascularized and organized, in the three taxa.

The tertiary veins are clearly recognizable, but the higher-order, minor veins form a network that is not consistent, nor is it easily observable. The marginal veins of the first order, fine and linear, are almost continuous. The smallest subdivisions of the laminae, delimited by minor veins, are the large-sized areoles, which are almost imperfect, that is to say, their form varies in the three species, and they usually contain only a few loose straight or curved branches. The internerval distances are more than 0.5 mm, and the den-

Table 1. Leaf features of *Petagnaea gussonei, Lereschia thomasii,* and *Sanicula europaea.*

	Petagnaea	*Lereschia*	*Sanicula*
Leaf			
division	palmate	palmate	palmate
margin	serrate-concave	serrate-concave	serrate-concave
teeth spacing	irregular	irregular	irregular
apex	acute	acute	acute
thickness (µm)	246	124	229
length (cm)	11	4.0	4.1
width (cm)	11	4.8	3.3
length/width ratio	1	0.8	1.2
Upper leaf epidermis			
thickness (µm)	22.0 ± 3.8	29.6 ± 3.2	32.2 ± 4.2
cell number (mm^{-2})	430 ± 30	1154 ± 59	634 ± 34
cell length (µm)	79.4 ± 10.5	51.5 ± 6.9	63.7 ± 15.8
cell width (µm)	37.5 ± 8.5	34.9 ± 3.1	54.4 ± 10.2
stomata number (mm^{-2})	14 ± 16	18 ± 8	2 ± 7
stomata length (µm)	40.9 ± 42.6	28.4 ± 3.1	6.1 ± 12.1
stomata width (µm)	28.5 ± 29.4	21.0 ± 1.3	5.5 ± 10.9
glands	absent	absent	absent
Lower leaf epidermis			
thickness (µm)	14.5 ± 1.7	20.3 ± 1.4	21.8 ± 1.7
cell number (mm^{-2})	1562 ± 115	1534 ± 110	1010 ± 72
cell length (µm)	59.0 ± 8.1	38.8 ± 6.4	46.6 ± 10.0
cell width (µm)	32.6 ± 5.8	23.9 ± 5.1	46.8 ± 11.2
stomata number (mm^{-2})	161 ± 28	333 ± 32	175 ± 25
stomata length (µm)	36.9 ± 20.9	22.6 ± 3.2	29.8 ± 1.7
stomata width (µm)	35.3 ± 6.9	18	15
stomatal index (see text)	9	18	15
glands	absent	absent	absent
Mesophyll			
thickness (µm)	188.0	75.0	124.0
palisade layers (no.)	2	1	2
palisade cells, lenght (µm)	32.0	27.5	32.1
palisade cells, widht (µm)	13.5	12.5	15.1
palisade cells, l-w ratio	2.4	2.2	2.1
palisade layers, thickness (µm)	65.3	28.0	53.0
spongy mesophyll, thickn. (µm)	123.0	55.5	122.0
palisade-mesophyll ratio	0.5	0.5	0.4
Bundle sheath	evident	evident	evident
Leaf venation	reticulate	reticulate	reticulate
1st order veins, diam. (µm)	90.0	150.0	134.0
1st order veins, course	straight	straight	straight
2nd order veins, angle	acute	acute	acute
2nd order veins, angle variation	random	different	different
2nd order veins, diam. (µm)	71.0	100.0	75.0
2nd order veins, course	not regularly branched	not regularly branched	not regularly branched
intersecondary veins	present	present	present

Table 1 (continued).

	Petagnaea	*Lereschia*	*Sanicula*
3rd order veins, pattern	random, reticulate	random, reticulate	random, reticulate
higher order veins, resolution	distinguished	more distinguished	distinguished
higher order veins, orientation	transverse-oblique	transverse-oblique	transverse-oblique
last marginal veins	straight	straight	straight
vein endings	simple	simple	simple
Areolation	imperfect	relatively well developed	imperfect
number of vein endings	very few	few	very few
arrangement	random	random	random
shape	polygonal-irregular	polygonal-irregular	polygonal-irregular
size	large	large	large
Anastomoses	with diverticula	with diverticula	with diverticula

sity of the minor and major veins (total length per surface unit) is about 23.5 cm^{-1} and 3 cm^{-1}, respectively. The network of minor veins has a direct connection not only with the smaller tertiary and quaternary veins, but also with the secondary ones.

Petagnaea has a loose leaf architecture, irregular, slightly marked, and with characteristic diverticula. *Sanicula europaea* and *Lereschia,* on the other hand, have more clearly marked and more regular veins. In *Lereschia* in particular, the vascular skeleton is more articulated, with more completely delineated marginal veins, larger connections between the veins, and a clear, polygonal intercostal delimitation.

Characters of the stomata and mesophyll as well as the presence of parenchymatous bundle sheaths were also taken into consideration (see Table 1). The tabulated data relating to the presence of xylematic diverticula have been evaluated from the photographic evidence. The diverticula and bundle sheaths, together with the thickness of the veins, their primitive skeleton, and the presence of marginal serrations, are the most saliently diagnostic leaf structural aspects.

Anatomy of the petioles, stems, and roots

The petioles, stems, and roots of *Petagnaea,* in transect, show a regular, simple pattern, complicated only by the occasional presence of canals close to the vascular bundles, e.g. in the petioles. In the rhizome there are up to 8 bundles, and supporting tissue is absent. In the petiole there are 3 bundles. The root is tetrarchic and regular, the endodermis barely evident.

In the rhizome of *Sanicula europaea* the bundles are more numerous than in *Petagnaea,* and many canals are seen, running along the bundles, in the petioles and the collenchymatous tissue. The root shows secondary elements, and a characteristic endodermis made up of irregular cells of different shape.

The rhizome and petiole in *Lereschia* are hollow, there are many bundles with a protective covering and sclerenchyma in the rhizomes. The root is not hollow. It seems to be triarchic, with secondary elements, an endodermis of monomorphic cells, and characteristic air-filled spaces in the 3 organs.

Conclusions

The three taxa examined show a tendency towards sciaphilous habit: thin, glabrous, relatively large leaf blades with scarce supporting tissue not forming hypodermic layers. From an ecological point of view the architectural characteristics of the leaves illustrate their adaptation to a high level of humidity. In terms of phylogeny, the leaves show primitive, unspecialized vascular features, whose development and spatial relationship with the mesophyll we have closely followed.

The study of leaf architecture has revealed that each of *Petagnaea, Sanicula europaea,* and *Lereschia* has a distinct leaf morphotype showing different anatomical patterns, but that they all have in common a marked sciaphilous tendency that is evident in the dorsiventral leaves with very loose lacunal tissue, reduced thickness, and limited supporting tissue. Undoubtedly *Lereschia* has the largest amount of supporting tissue; it has a strongly marked and articulated vascular leaf skeleton, a characteristic that is correlated with good environmental adaptability.

Leaf venation pattern is difficult to measure or describe. The observed differences between the three taxa concern the thickness and lignification of the veins and their connections and diverticula. The veins are relatively fine in all three taxa and delimit large areoles, more or less sharply defined and forming a pattern that we consider as primitive.

There is no supporting tissue in the rhizomes of *Petagnaea,* and there are but few vascular bundles; the latter are more numerous in *Sanicula europaea* and *Lereschia.*

In conclusion, the structural characteristics of *Sanicula europaea* and *Lereschia* reflect a reasonably good environmental adaptability and a relatively great structural flexibility, expressed, for example, in a well articulated and fairly dense vascular architecture of the leaves. *Petagnaea* on the other hand, due to the extremely simple and primitive nature of its anatomy, appears to be unable of adapting to other than its present environment. Therefore its natural habitat in the Calagna stream area (Tortorici), while not under immediate threat, should nevertheless be carefully protected (Pignatti 1982).

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