P. Colombo, V. Spadaro & F. M. Raimondo

Morpho-anatomical analysis of *Viola tineorum* and *V. ucriana* (*Violaceae*) endemic to the mountains around Palermo (NW-Sicily)

Abstract

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Viola sect. *Melanium* is represented in Sicily by six species. Two of them, originally described as varieties of *Viola nebrodensis,* were later transferred at the species rank and named *V. tineorum* and *V. ucriana*. The study of micromorphological characteristics gives additional evidence agreeing with the independent specific status of *V. tineourum* and *V. ucriana*, that are confined to two very restricted areas south of Palermo: Rocca Busambra and Monte Pizzuta respectively.

Introduction

Taxa of the *Viola nebrodensis* group occur isolated on the calcareous mountains of the Madonie (Central N-Sicily), Rocca Busambra, and Monte Pizzuta (South of Palermo) (Pignatti 1982). Among these, *V. nebrodensis* C. Presl. since a long time had been considered as an independent species, including the Busambra and Pizzuta populations as varieties named *V. nebrodensis* var. *grandiflora* and *V. nebrodensis* var. *lutea*. This status was rather problematic from the taxonomical point of view. In fact, although morphologically mutual very close, and also with *V. nebrodensis* s. str., the Rocca Busambra and Monte Pizzuta populations have different anatomical characteristics, distribution ranges, and karyological numbers, consequently they have been treated at specific rank by Erben & Raimondo (1995). In this paper the results of a study on the leaf micromorphology and architecture, stem and petiole anatomy and the S.E.M. surface fine structure of leaves and seeds are presented in order to give additional data that agree with the specific rank of the taxa in question.

Materials and metods

Viola tineorum Erben & Raimondo is a dense caespitose hemicryptophyte, quite glabrous, with root slightly tickened, stems prostrate or ascending, leaves herbaceous, dark

green, glossy; blades, ovate obtuse or rounded; lower leaves, smaller and with shorter petioles than the upper ones.

Viola ucriana Erben & Raimondo is a dense caespitose hemicryptophyte hairy, with root slightly tickened, stems glabrous or scarcely hairy; prostrate or ascending; leaves herbaceous, green-greyish, blades rounded or broadly ovate, apex obtuse, basis emerginate or abruptly attenuate, midrib glabrous or hairy.

The study material was collected in the following localities:

Viola tineorum - Rocca Busambra, basis of the North facing slope, 900 m a.s.l.

Viola ucriana - Fratantoni slopes, near the Pizzuta top, 800 m a.s.l.

Soon after collection, fresh material, previously sectioned and coloured for the exact identification of tissues and their location, was fixed in F.A.A., then dehydrated and coloured with saphranine and light green, and finally, included in paraffine. The cross sections of 10-15 µm, obtained using a rotative microtome were mounted in the Canadian balsam. The number and size of epidermal cells, stomata, hairs, mean cross section of leaves, palisade and lacunose tissues and other structures on the leaf surfaces were measured on the epidermal replications. The xilematic pattern was studied on blades diaphanized according to Fuchs (1963), following the Hickey (1973) terminology. Concerning general terminology, Esaù (1965) is followed. The study material, pre-treated at the critical point was finally observed at the S.E.M.

Results

Viola tineorum – the leaves in the middle part of the stem have an average surface of 400 mm² and are amphystomatic with anisocytic stomata. From the morphological point of view, in the cross section, the leaf margin is linear, rounded (Fig. 1A-B); marginal cells are generally larger than epidermal on both adassial and abassial blades. The cross section of the leaf midrib is 355 µm (Fig. 1C), I order veins are generally 340 µm. 2-stratified palisade parenchyma (131 µm); the spongy parenchyma (123 µm), reveals many small intercellular spaces. Epidermis, including cuticle, is thicker than the lower one on the upper blade (Tab. 1).

The phytoderma (Vignal & Cherel 1983), examined in detail, shows several interesting epidermal details that, in the lack of marked morphological characteristics, are suitable for the taxonomic delimitation of *Viola tineorum* from *V. ucriana*. The most strongly differ-

Table 1. Leaf thickness (µm).

	Epidermis + upper cuticle	*Epidermis + lower cuticle*	*Whole leaf*	*Midrib*	*Upper palisade*	*Lacunose tissue*
V. tineorum	56	37	340	355	131	123
V. ucriana	55	35	284	352	125	136

entiated characteristic in both species is epidermis of medial and basal leaves. Here adassial cells are isodiametric; density is 173 per mm², the single cell being 116, 58, 56. Abassial cells are 261 per mm², 99, 51, 37. The most remarkable characteristic in the leaves of *V. tineorum* is represented by 5 large and holding out epidermal cells that are in linear succession on the upper surface along the midrib. Among these, the central cell is the largest (Fig. 1C). In the lower blade epidermal cells are more numerous, homogeneous in size, and larger than in the abassial. Epidermis is frequently mucilaginous (Metcalfe 1957) and single or groups calcium oxalate crystals are usually present in the mesophyll (Fig. 1D). In the lacunose tissue and near the vascular bundles, there are secretory channels, in some of which brown bodies of unknown nature are included (Skottsberg 1940). Mucilaginous cells are very frequent in the leaf surface (Fig. 1E). In diaphanized leaves (Fuchs 1963) these mucilages are similar to Hydropotes, that are epidermal multicellular structures active for water and mineral salt assumption (Metcalfe 1957; Watson & Dallwitz 1992). Hydropoten are evident in *Brasenia peltata* Pursh, *Cabomba acquatica* Aubl., *Nymhaea alba* L., *Nuphar luteua* Sibth. & Smith., *Ranunculus fluitans* Lam., *Caltha palustris* L., and, in many monocotiledons, and probably in a large number of plants (Riede 1920-1921). Observed at the S.E.M the upper blade appears consisting of isodiametric cells with slightly sinuose margins; cuticle is slightly sculptured except for some sporadic striatures located on the epidermal cells surrounding some stomata (Fig. 2A-B). Stomata, 48 per mm², on epidermis have a long front pore variable in diameter. The lower blade (Fig. 2C-D) consists of more numerous isodiametric cells with very sinuose margins and usually smaller than those in the upper blade. The stomatic patterns are of the same type (Fig. 3A-B), i.e. anisocytic, but more numerous (Tab. 2).

Viola ucriana – the leaves in the middle and basal parts of the stem are mostly larger, having 500 mm² surface; they are amphystomatic and have anisocytic stomata (Fig. 4A-B). Epidermal cells have marked sinuose margins, are more convex and bear a few cuticle

Table 2. Epidermal parameters.

	Upper blade		Lower blade	
	Viola ucriana	*V. tineorum*	*V. ucriana*	*V. tineorum*
N° epidermal cells × mm²	162	173	331	261
N° stomata × mm²	43	48	89	69
Epidermal cells length (μm)	138	116	112	99
Epidermal cells width (μm)	79	58	66	51
Epidermal cells thickness (μm)	55	56	35	37,0
Stomata length (μm)	42	45	38.5	39
Stomata width (μm)	27	33	28.5	31

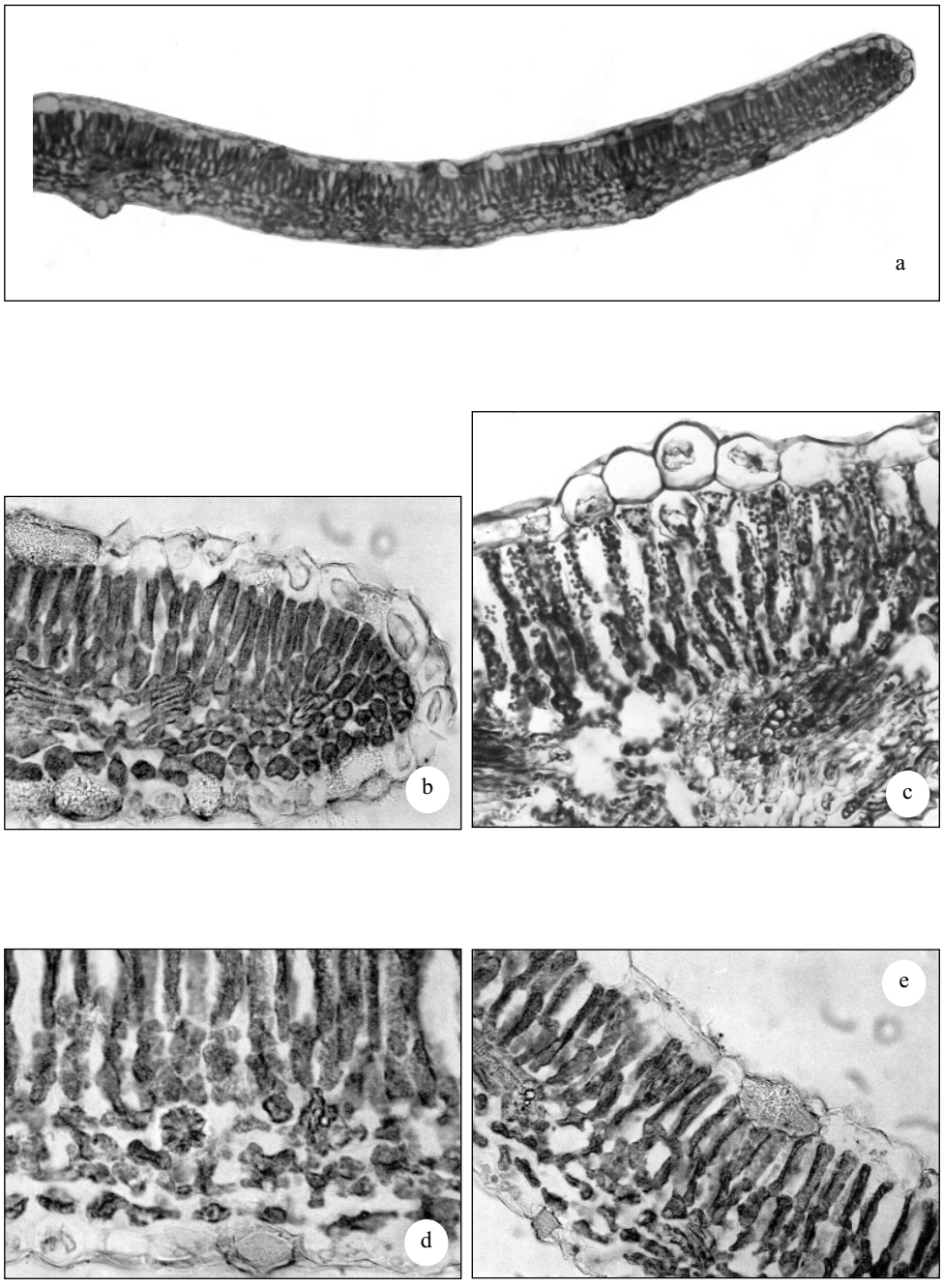


Fig. 1. *Viola tineorum*: a) cross section of the leaf (×100); b) detail of the cross section of the margin (×200); c) cross section near the midrib (×200); d) cross section of the leaf: detail of the palisade with mineral cristals; e) cross section of the leaf: detail of the palisade and of the muciparous glands.

ornamentations with the exception of the stomata in which dense cuticular strips perpendicular to the rime are found (Fig. 5A-B). Numeric data are included in Table 2.

In cross section the leaf margins are linear, rounded; cells are larger than those in both upper and lower blades (Fig. 6A). The leaves are on average 284 µm thick between the veinlets and reach 352 µm at the midrib level; the palisade parenchyma is two-layered in the area between veins; it is one-layered, 125 µm thick and provided with collecting cells above the midrib; the lacunose tissue is 136 µm, thickness of the adassial cuticle and epidermis include is about 55 µm, 35 µm the abassial (Tab. 1).

The peculiar structure of the leaves at the midrib level (Fig. 6B) represents a noteworthy difference from *V. tineorum.* Two superficial protrusions are visible on the foliar surfaces; in fact, 3 very large cells, among which the inner is smaller than the lateral ones, are found on the adassial surface. The protrusion on the abassial surface consists 6 or 7 large cells that are almost isodiametric, connected to one or two angular collenchyma centripetal layers.

Crystals

Calcium oxalate crystals, isolated or less frequently grouped, occur between the palisade and the lacunose tissues (Melchior 1925; Solereder 1940) (Fig. 1D).

Secretory elements

Secretory elements, including brown mucilaginous bodies, have been observed in the mesophyll of some Hawaian species (Skottsberg 1940). Similar bodies variable in size have been found in the epidermal tissues (Fig. 1E, 6B) of both *Viola ucriana* and *V. tineorum*: secretory elements between epidermal cells on the adassial blade of *V. tineorum* are included between 85 and 115 µm, those on the abassial blade between 91 and 27 µm. In *V. ucriana* these secretory elements are smaller, ranging between 75 and 100 µm in the adassial bladed and between 63.5 and 38.5 µm in the abassial.

Architecture

Leaves, that are symmetric, obovate (Stearn 1966), rounded or slightly retuse, rounded at the basis, have been diaphanised according to Fuchs (1963). Terminology for the xylematic pattern follows Hickey (1979).

Viola tineorum midrib is 1.25-2 µm in diameter. It runs along the lamina up to the rounded apex (Fig. 7A); upper veins form an acute angle of divergence, while lower veinlets have less acute angle. Veinlets are generally thick, straight, ramified at some distance from the margin which bears 8-10 small incisions as many as spaced crenations. Parallel intermarginal veins at the leaf margin are also evident. They are resulting from the fusion and straightening of the lesser arched brachydodromous exmedian segments that appear independent venations. Irregular intercostal areas; III order veinlets reticulate and orthogonal form an angle toward the apex. Thin orthogonal IV e V order veinlets are partly distinguishable. At the margin of the leaf small free veinlets form terminal dichotomic ramifications (Fig. 7B). Areoles are well developed, large irregularly; quadrangular; branches are usually uniramified and bear evident terminal spiralate tracheids (Fig. 7C).

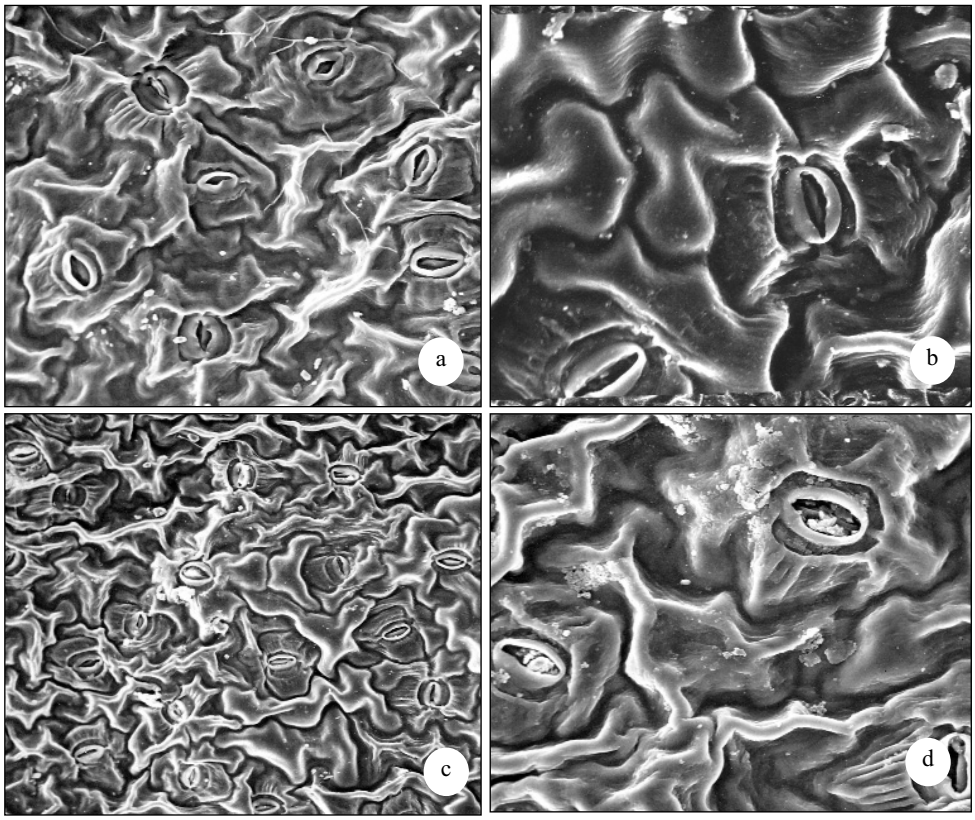


Fig. 2. *Viola tineorum*: a) adassial leaf surface at the S.E.M.; b) detail of the adassial surface at the S.E.M. with stomata; c) abassial leaf surface at the S.E.M.; d) detail at the S.E.M.of the abassial surface with stomata.

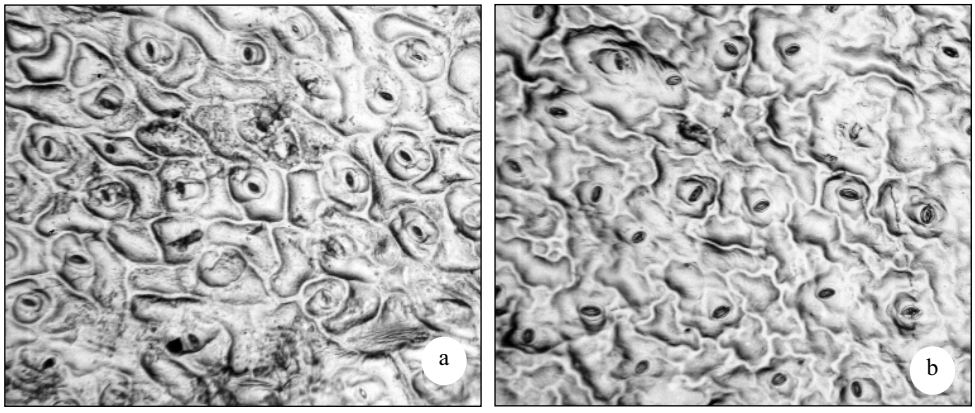


Fig. 3. *Viola tineorum*: a) epidermal replication of the leaf adassial surface (×200); b) epidermal replication of the leaf abassial surface (×200).

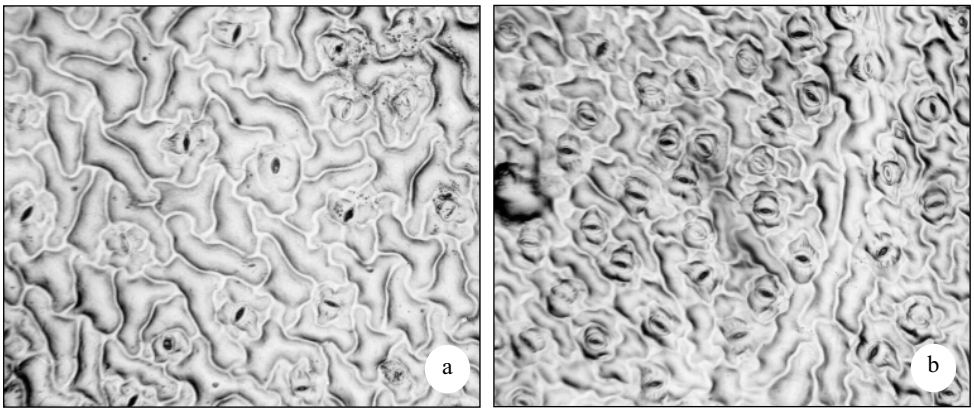


Fig. 4. *Viola ucriana*: a-b epidermal replication with anisocytic stomata (×200).

Viola ucriana - all leaves are provided with camptodrome or cladodrome veins; more or less large midrib runs straigt between the petiole and the rounded an sligthly retuse apex (Fig. 8A). The angle of divergence between the veins and the midrib is acute. II order veins are generally slight, straight and ramified near the margin crenations. Intermarginal veins provided with terminal branches running into crenations are also evident. Among these, the most developed is connected to the next crenation. Intercostal are quite regular. III order veins originate from the lower side of veins. The angle of divergence is acute, ramified reticle is irregular. Thin and almost undistinguishable IV and V order veinlets are also present. Short terminal veinlets are also ramified at the leaf margin. Areoles are well developed, irregular, polygonal, small.

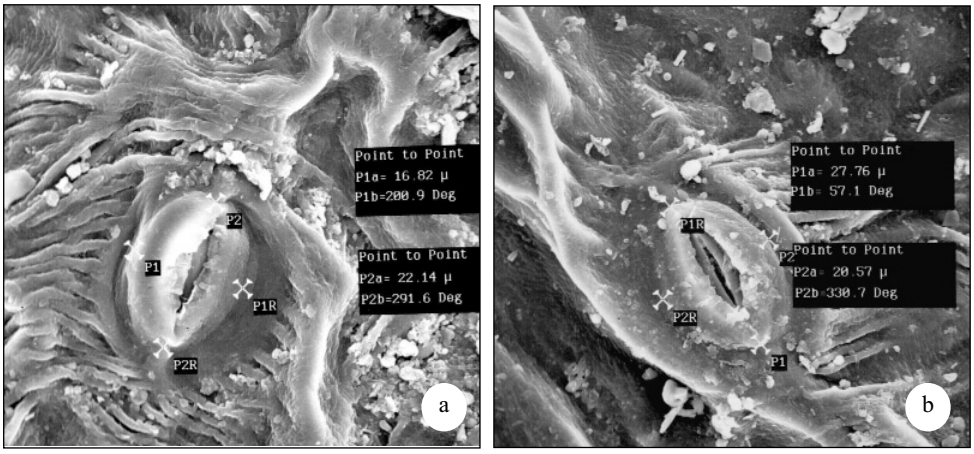


Fig. 5. *Viola ucriana*: a-b cuticular stripes at the S.E.M.

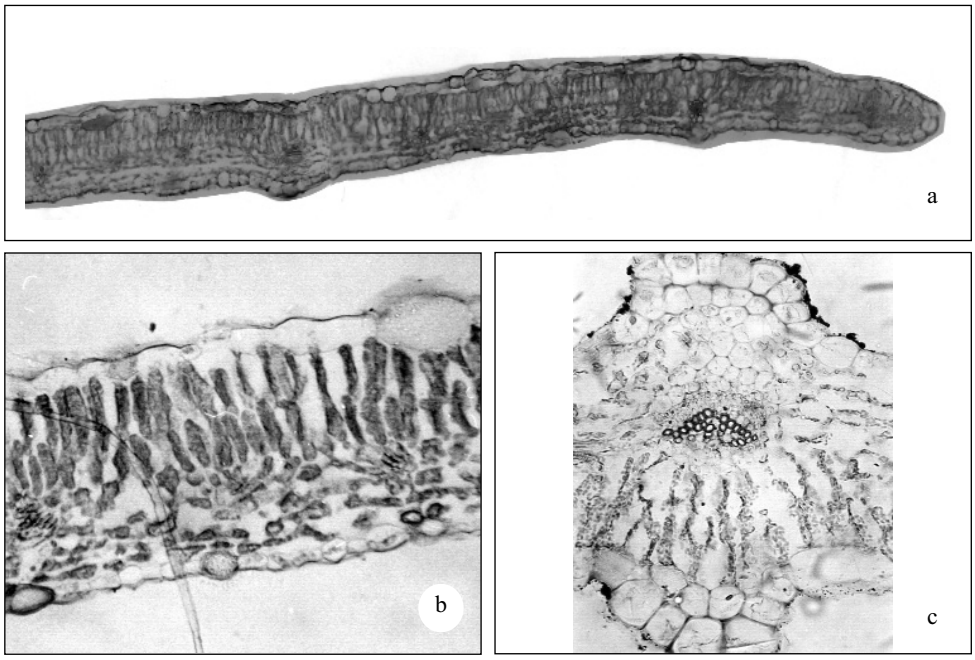


Fig. 6. *Viola ucriana*:a-b) cross section of the leaf and detail of the mesophyllous (×200); c) detail of the midrib in cross section (×200).

Viola ucriana - all leaves are provided with camptodrome or cladodrome veins; more or less large midrib runs straigt between the petiole and the rounded an sligthly retuse apex (Fig. 8A). The angle of divergence between the veins and the midrib is acute. II order veins are generally slight, straight and ramified near the margin crenations. Intermarginal veins provided with terminal branches running into crenations are also evident. Among these, the most developed is connected to the next crenation. Intercostal are quite regular. III order veins originate from the lower side of veins. The angle of divergence is acute, ramified reticle is irregular. Thin and almost undistinguishable IV and V order veinlets are also present. Short terminal veinlets are also ramified at the leaf margin. Areoles are well developed, irregular, polygonal, small.

Petiole

In *Viola tineorum* the petiole cross section shows that it is provided with a main central semicircular bundle and smaller lateral ones. The vascular cylinder is flottered and concave on the adassial side; on the convexe abassial side and in the pith there are many secretory channels. Chlorophyllous parenchima, 2-3 stratified, is covered with a single epidermal strate. Large oxalate crystals are formed between the pith and chlorophyllous parenchima. In *Viola ucriana* the petiole cross section shows a large central bundle in which V-shaped xylem is open to words the adassial surface. Phloem is in front to the abassial surface. Here,

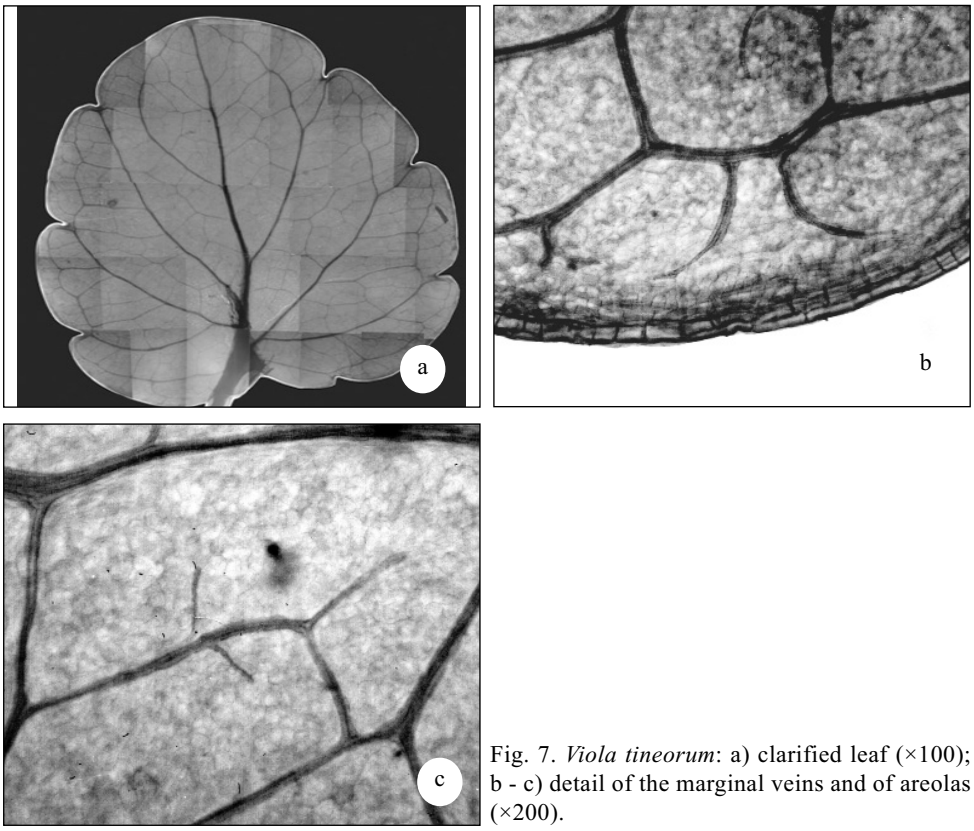


Fig. 7. *Viola tineorum*: a) clarified leaf (×100); b - c) detail of the marginal veins and of areolas (×200).

few cribrosous elements are found togheter with many secretory channels in contact with the endodermal, petiole therefore has three nodes (Metcalfe 1979). Epidermis is mucilaginous and chlorophyllous parenchyma is less developed. In a section out next to the lamina, petiola is quite different, revealing an proximately pentogonous shape epidermal layer with a dense trichome covering 2 or 3 lamellar collenchyima layers thickening around bundles where flexible collenchyma keels (Erben & Raimondo 1995). Two more marked parenchyma prominences are by the sides of the adassial surface. In the middle of petiole are 5 bundles, of wich the main, I order, is central, surrounded by the II and III order ones that are displayed in a semicircle. Petiole is multilacunose nodule since from it stipular venations originate. Each bundle is collateral with 9 secretory channels near the phloem: among these, 5 are surrounding the main bundle, 3 and 1 are displayed next to the III and II order bundles, respectively. Two collenchima sheets include both large and small bundles like a sandwich. Other secretory structure and sporadic, small oxalate crystals are found sparse in the pith.

Stem

In both species prostate and ascending stems are found in the same individual.

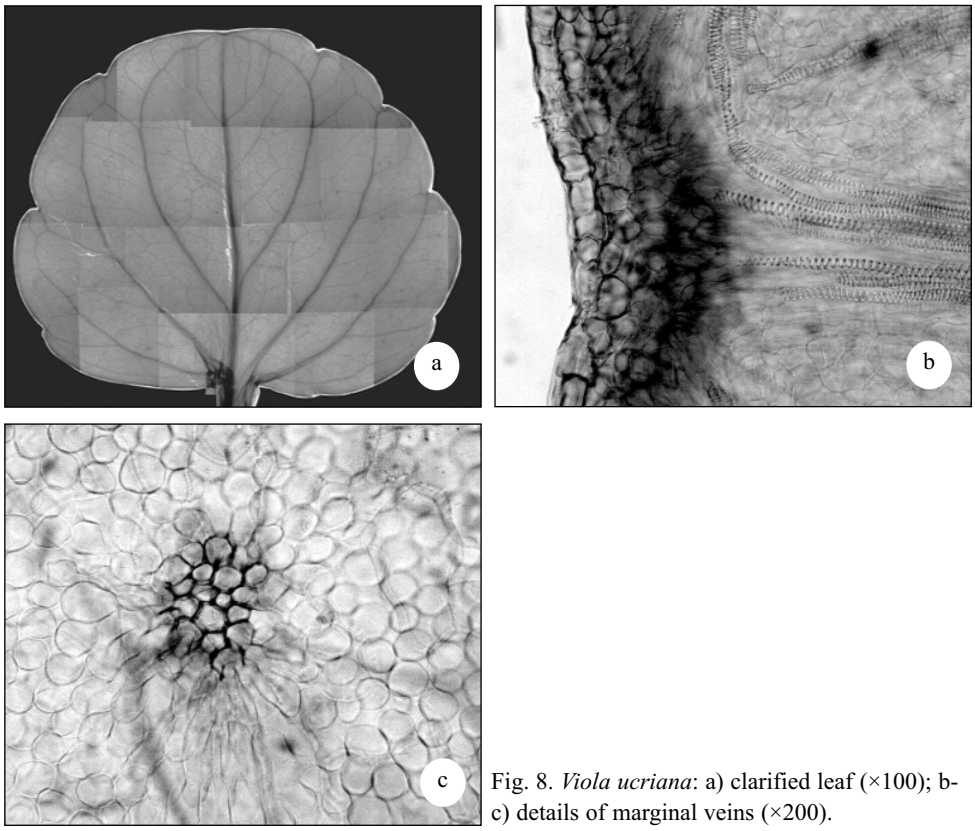


Fig. 8. *Viola ucriana*: a) clarified leaf (×100); b-c) details of marginal veins (×200).

In the apical cross section of stem, epidermis is layered with cell walls more tickened outside. Beside collenchyma on the epidermal side is located especially near the veins and cuttings (Fig. 12A). In the parenchymatous bark frequent large oxalate crystals are included. Secretory channels are found next to endodermis or are bordering on phloem. Pericycle is parenchymatous. Each open collateral bundle is distinct, separated by a parenchymatous band in which thin xylem segments reveal some starting cambium activity. Vessel are simple or sometimes provided with scalar plates. Pith, usually allow, includes abundant isolated or grouped crystals. In prostrate stems, epidermis is more thickened and rather suberized. Cortex is always parenchymatous; but cells gradually decrease while crystals are increasing in size.

Endoderma is rich in starch with storage function. Phloem forms a continuous ring rich in cells including brown bodies. Xylem also forms a continuous ring.

Vessels more or less small in size (30-50 μm), are displayed in rays separated by uniseriate rays. Sclerenchymatous fibers are septate and strongly lignified. Pith cells are roundish, smaller and with thinner wall than the cortex ones. Numerous large crystals are included in the pith. In *Viola ucriana* (Fig. 12B) outer parts of the stem rare earlier suberified: cortex is thinner, parenchymatous, and cells are larger and more thin-

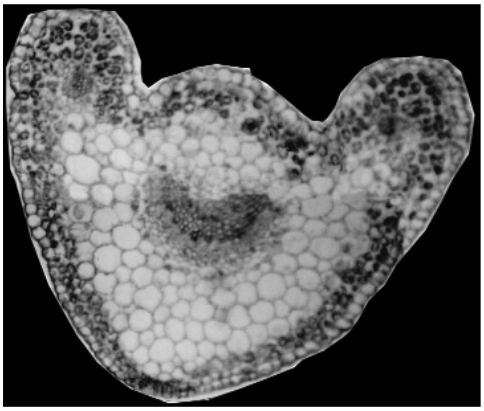


Fig. 9. *Viola tineorum*: cross section of the petiole (×100).

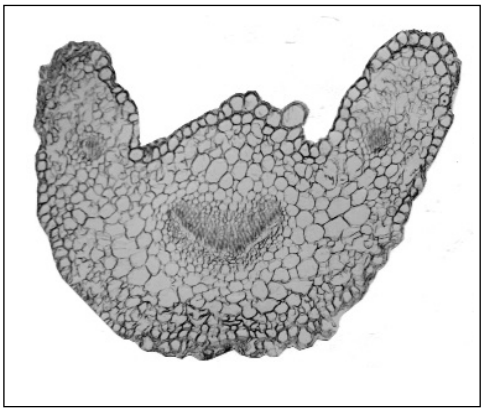
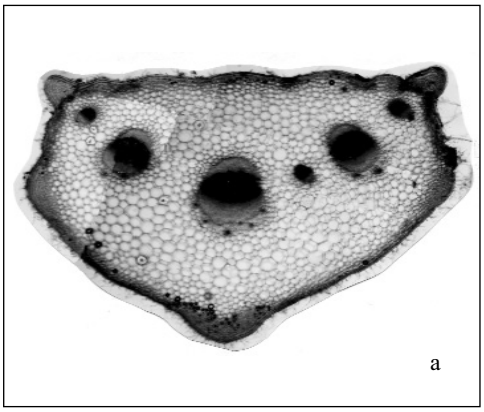
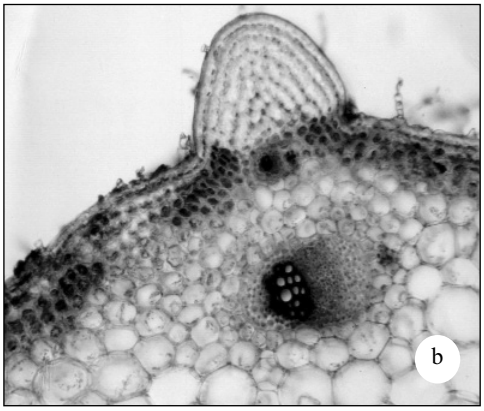
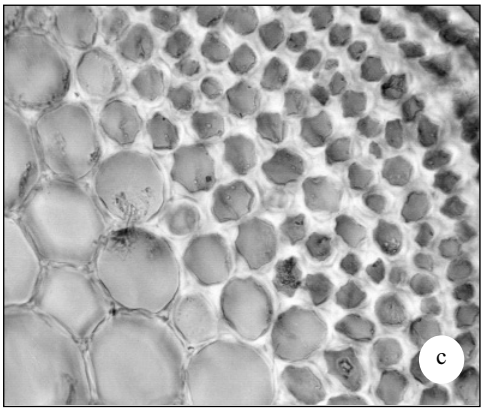


Fig. 10. *Viola ucriana*: cross section of the petiole (×100).







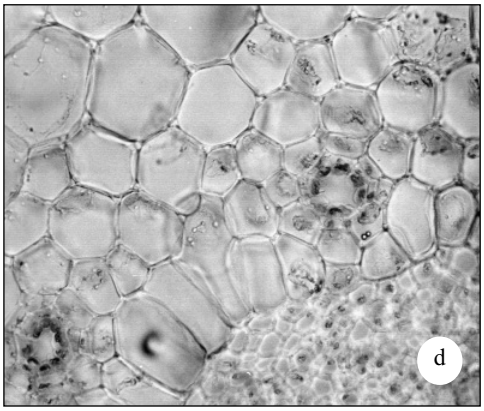


Fig. 11. *Viola ucriana*: a) cross section of the petiole near the blade (×100); b-c) details of collenchyma (×100, ×200); d) secretory structures in the petiole (×200).

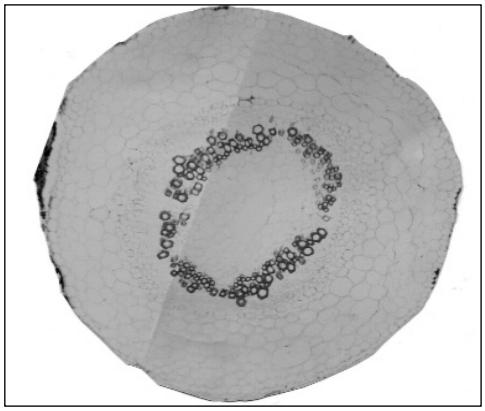


Fig. 12. *Viola ucriana*: stem cross section (×100).

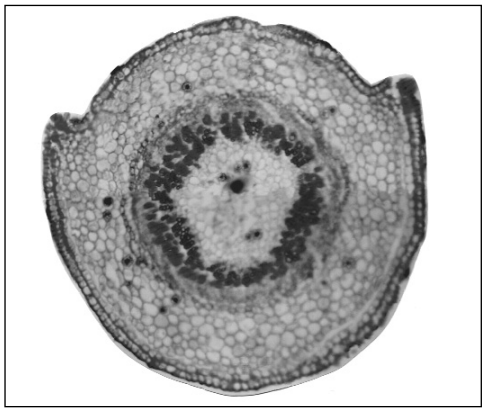


Fig. 13. *Viola ucriana*: stem cross section (×100).

walled than *V. tineorum*. Besides, oxalate crystals are rare or absent. Very small secretory channels are contiguous with phloem. Periclycle is parenchymatous. Vessels bear simple piths, small protoxylem and more or less large meta-xylem elements, markedly lignified. Parenchima is paratracheal. Thin medullary rays are present. Pith becomes empty in the inner part.

Root

Viola tineorum and *V. ucriana* are very similar as far as roots are concerned. Indeed both species grow on calcareous substrates, and roots are deeply wedged in the rock. Owing to this condition and a protective thickened periderm wich appears rather worm-out in the suberified parts is formed. Cortex is more thickened and more abundant siliceous druses

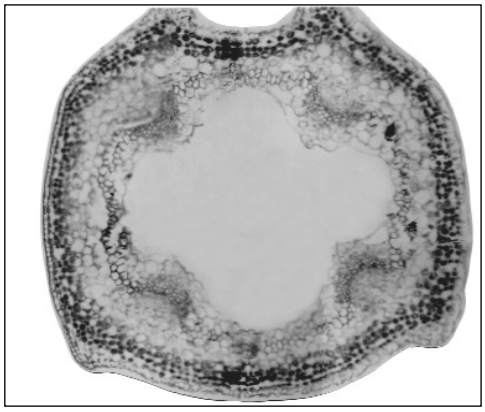


Fig. 14. *Viola tineorum*: cross section of the petiole (×100).

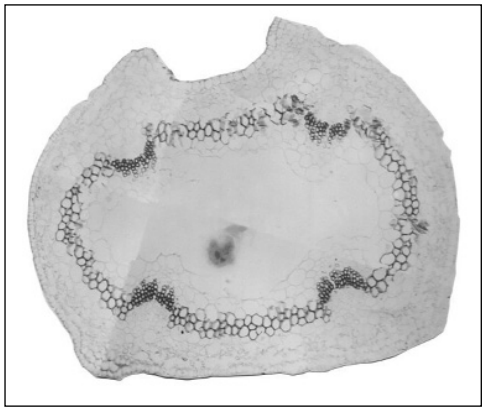


Fig. 15. *Viola ucriana*: cross section of the petiole (×100).

and minute crystals, in comparison with *V. ucriana*. The vascular cylinder is dense with larger and more lignified tracheae. The pith is lacking.

Viola ucriana: moderately thickened and divided in the upper part, the root periderm is suberified, displayed in several thin layers rather inconsistent. Cortex partly consists of a storage parenchyma and partly of other parenchymatous cells provided with large intercellular spaces. Structure is secondary, with a solid stele consisting of exarch protoxylem surrounded by a very dense xylem provided with very large vessels that are 25-30 μm, mixed with lignified fibers. Traces of numerous lateral roots starting from the central cylinder are also evident.

Flower stalk

Viola tineorum pedicels are very light. The cross section cut at the medium height appears somewhat quadrangular bearing two collenchima ribs on the abassial surface and two more prominent ones on the adassial surface (Fig. 14). Epidermis, 1-layered, is composed of small, thin-walled cells. Sub-epidermal gelatinous tissue is surrounding the 3-4 layered chlorophyllous parenchyma which is formed by small roundish shaped cells; secretory channels are found in the inner cortex around the central cylinder. The cylinder, sinusoid outlined, consists of 4 large vascular bundles separated by pith rays that include a cambium layer forming a sketch of secondary structure. This structure is incomplete since the stalk degenerates soon after anthesis; small minor bundles are found among the pith rays. Pith is large and empty.

In *Viola ucriana* stalk is quite similar to *V. tineorum* (Fig. 15), but it is more flattened; pith rays are broader and curved. Finally it is more markedly lignified.

Seeds

Ovule is bi-tegmented, crassi-nucellate in both species. Testa in the seed consists of a dense reticle with sclerotic cells and stomata near the calaza. In *V. ucriana* tegmen observed at the S.E.M. looks like a drop (Fig. 16A), almost smooth on the surface convex. Highly magnificated (660 ×) its structure is still smooth but some sculptures polyhedric delimiting numerous areoles 40 μm long (Fig. 16 B) are evident.

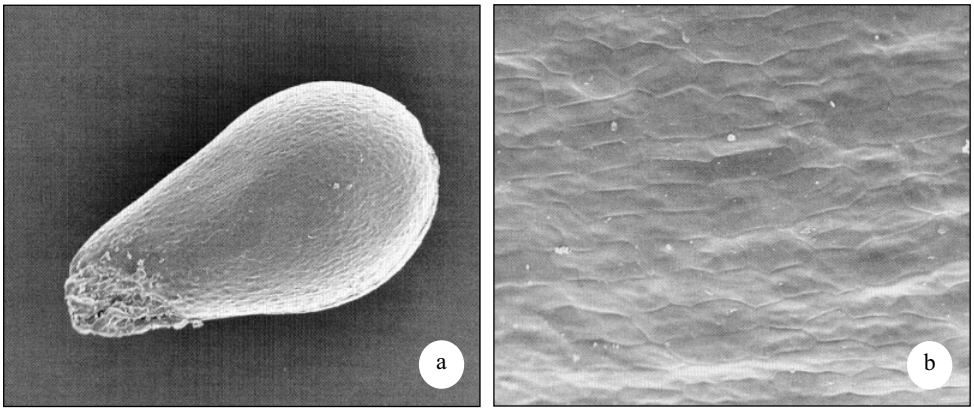


Fig. 16. *Viola ucriana:* a) a seed at the S.E.M.; b) tegmen detail.

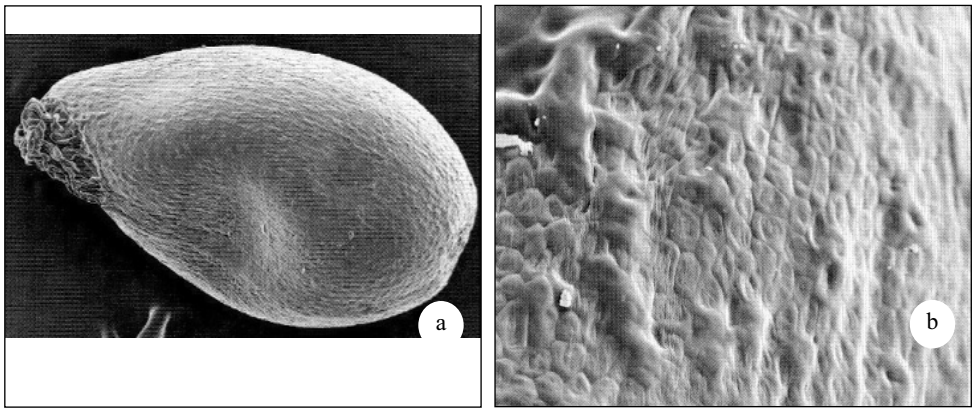


Fig. 17. *Viola tineorum*: a) seed at the S.E.M.; b) tegmen detail.

Viola tineorum seeds are somewhat larger, ovate, convex, with the eso-tegmen almost smooth (Fig. 17A); at high magnification (660 ×) numerous small (20 μm) slightly centrally depressed polyhedric sculptures are evident (Fig. 17B). On the abassial side a light concavity is evident (Fig. 17C).

Discussion and conclusion

Anatomic comparison points out several structural characteristics suitable for significantly distinguishing *Viola tineorum* from *V. ucriana*. In particular, significant differences concern the leaf architecture, the dermatologic pattern of both the adassial and abassial sides near the midrib. Differences are also evident in the leaf epidermal parameters, in the venation arrangement and the relevant angle of divergence, in the size and shape of the areoles and free ramifications; in the fine leaf epidermal structure at the S.E.M., especially of stomata; in the anatomy of petiole; in the composition and size of secretory elements in the lignification degree and diameter of vessels in both stems and roots. Further remarks concern environmental adaptation of both studied species. Edaphic and environmental conditions are rigorous, especially referring to altitude, that in the study case is above 1000 m and the winter temperature which frequently reaches 0 °C. Another factor is represented by the violent north wind especially when it is associated with low temperature. Both species under study are herbaceous, therefore they are sensitive to the above mentioned factors. Their adaptations typically concern the slopy habitats: minimal habitus, short stem internodes, deepened into the calcareous substrate roots, reduced foliar surface on long, flexible petioles, epidermis provided with sub-epidermal mucilage or rich in muciparous cells and channels active in the direct water assumption in winter or when supplies are scarce (Francini Corti 1967).

In *Viola tineorum* which occurs between 1300-1400 m a.s.l., owing to the high altitude rigours, all venations of the leaves are considerably lignified; the xylematic pattern is scarcely reticulated since leaves can absorb H_2O through the mucilage and the number of

mucous cells.

Viola ucriana, which occurs at lower altitudes, smaller leaves that are rather independent, from rigours, show xylematic pattern less lignified, uniramified terminal, thinner than in *V. tineorum* tracheids; a higher number of cells and muciparous channels per mm², that are larger on average. Mesophilous adaptations can be observed in the leaves of both species.

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Addresses of the authors:
Paolo Colombo, Vivienne Spadaro & Francesco M. Raimondo, Dipartimento di Scienze botaniche dell'Università, via Archirafi 28, 90123 Palermo, Italy. – E-mail: pcolombo@unipa.it.