

Morpho-anatomy and histochemistry of European mistletoe (*Viscum album* L. subsp. *album*), a semi-parasite growing on *Malus domestica*

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Abstract

This paper provides detailed morpho-anatomical characteristics of the leaves, stems and berries of *Viscum album* L. subsp. *album* (Santalaceae) growing on the branches of *Malus domestica*. Additionally, microchemical analyses of all tissues and Energy Dispersive X-Ray Spectroscopy (EDS) analyses of the calcium oxalate crystals are provided for the first time. The plant grows as a semi-parasite on the branches of host trees and shrubs; it develops as dichasium with pseudo-dichotomous branching stems and well-developed green leaves with parallel veins; the dioecious inflorescences usually consist of three flowers, with female flowers generating white fleshy berries, in which a seed is embedded in the mucilaginous mesocarp, normally containing two embryos. The analyzed leaves were isobilateral, amphistomatic, and showed straight anticlinal epidermal cell walls, thick cuticles with epicuticular wax crystalloids, and paracytic stomata. The midrib is flat on both sides and has a single vascular bundle, whereas the petiole is concave-convex in shape and contains five bundles. The stems show a primary structure with a ring of nine vascular bundles enclosing the pith. Calcium oxalate druses and cubic and quadrangular prisms were observed in different parts of the plant. The microscopic features of the tissues and structures are illustrated with light and scanning electron micrographs.

Introduction

The genus *Viscum* (Santalaceae) comprises about 110–141 species^{1,2}. Nearly two-thirds of the species are found in Africa and Madagascar, and some in tropical Asia. A few species belonging to the *Viscum album* group have adapted to more temperate regions in Eurasia³, of which only two species, *Viscum cruciatum* ex Boiss. and *Viscum album* L., are found in Europe⁴. *Viscum* species, commonly called mistletoes, are shrubby semi-parasites growing on trees and shrubs. They embed into the host branches with a haustorium to nourish and obtain water⁵. These haustoria can extend over more than 5 cm within the host⁶. *Viscum album* grows on more than 450 different species of hosts through its three subspecies, *V. album* subsp. *album*, *V. album* subsp. *austriacum* (Wiesb.) Vollm., and *V. album* subsp. *abietis* (Wiesb.) Abrom., with distinct host preferences and, to some extent, specific geographic distribution⁷.

Viscum album subsp. *album* is commonly known as European mistletoe, gui, Mistel, vischio, or muérdago⁴. Its berries consist of a whitish exocarp, a thick mucilaginous mesocarp, a thin endocarp, and a seed that normally contains two embryos⁸. The dispersal of mistletoe seeds depends on birds, that feed from the berries during winter: mistle thrush (*Turdus viscivorus*) and waxwing (*Bombicilla garrulus*) gulp several fruits and after digestion excrete the seeds together with the exocarp and the sticky inner mesocarp; the blackcap (*Sylvia atricapilla*) digests only the exocarp and the outer mesocarp, after separating the seed and sticking it with the attached inner mesocarp on a thin branch. Separating seed and exocarp, the birds enable *Viscum album* embryos to germinate⁹.

In spring, the hypocotyl of the embryos elongates, turns to the bark of the host branch and flattens to a holdfast from which the primary haustorium grows with a tip meristem through the bark; when the meristem is embedded into the cambium of the host, it turns into an intercalary meristem, from which synchronously with the secondary thickness growth of the host's xylem the formation of the primary sinker starts. Originating from the primary haustorium, cortical strands grow longitudinally and circularly within the inner bark; whenever their meristems encounter the cambium, they give rise to the formation of secondary sinkers^{5,10}.

Viscum album is an important medicinal plant. Steiner and Wegman introduced its use in complementary oncological therapy in the early 1900s¹¹; since then, many reports have been published describing its medicinal properties^{12–13}. Besides carcinosis, mistletoe is used to treat spleen diseases, menstruation problems, infertility, cardiovascular diseases, ulcers and epilepsy^{13–15}. The plant is reported to contain several pharmacologically active compounds, such as amino acids, flavonoids, phenolic acids, polysaccharides, terpenoids, viscotoxins and mistletoe lectins^{16–21}.

As only limited information on the morpho-anatomy, micromorphology or histochemistry of *Viscum album* subsp. *album* growing on *Malus domestica* is available so far, the present study aimed to fill this gap. This study can also help in taxonomy, species identification, future comparisons with plants growing on other hosts, and quality control of the botanicals.

Materials And Methods

Plant materials

The use of plants in the present study complies with international institutional guidelines. *Viscum album* subsp. *album* was harvested from *Malus domestica* growing on a cultivated natural site in Switzerland (belonging to the Society for Cancer Research-VfK). Therefore, no permission for harvesting was needed. The plants were identified by Dr. Marcelo Guerra Santes (Universidade Estadual do Rio de Janeiro, Brazil) and a voucher specimen (C.H. Quaresma 18.332) was deposited at the Herbarium of the Faculdade de Formação de Professores, Universidade Estadual do Rio de Janeiro, Brazil. Fresh specimens of the leaves, stems, berries and seeds of *V. album* subsp. *album* were collected for the first investigations in July 2016 from plants growing on the branches of *Malus domestica* from the Basel area (Rüti, Himmelried) in Switzerland. To develop the present study, the leaves, stems, berries and seeds of *V. album* subsp. *album* were collected in April 2021 on the same location in Switzerland (Rüti, Himmelried).

Preparation of samples for light microscopy

Freshly collected stem, leaf, berry and seed samples were fixed in Formalin-Acetic Acid-Alcohol (FAA) for three days. The samples were then washed with water, transferred into 70% ethanol, and free-hand sections were prepared using razor blades. Selected sections were stained in toluidine blue²² and in a combination of Astra blue and basic fuchsin²³. Then the sections were mounted in a drop of glycerin (50%)²⁴ on glass slides, covered with a coverslip, and sealed with transparent nail polish. For the analysis of epidermal surfaces, small sections of the leaves were washed and then treated with sodium hypochlorite solution (50%) until translucent²⁵. The materials were then washed with distilled water and neutralized in an acetic acid solution (5%). The sections were rewashed with distilled water, stained in safranin²⁴ and mounted as described above.

Histochemical analyses

Free-hand cross-sections of fresh material were made and exposed to phloroglucinol/HCl to detect traces of lignin²⁶; Ellram, Wagner²⁷ and Dragendorff²⁸ reagents to evidence alkaloids; potassium dichromate (10%)²⁹ and ferric chloride (5%) for phenolic substances³⁰; hydrochloric vanillin solution (0,5%) to reveal condensed tannins³¹; Sudan III and Sudan black B were used to detect lipophilic compounds³²; Nile blue sulfate to expose neutral and/or acidic lipids³³; iodine solution (2%) to identify starch grains³⁰; ruthenium red solution (0,002%) to evidence pectins³⁰; PAS (periodic acid-Schiff reagent) test to expose polysaccharides³⁴; methylene blue solution (1%) to detect mucilage²⁶; Xylidine Ponceau³⁵ and Coomassie Brilliant blue³⁶ to evidence proteins. Appropriate controls were performed in parallel with the tests. Photomicrographs were captured using an Olympus CX 31 light microscope with the attached C-7070 control unit. The microscopic procedures were conducted in the Laboratory of Pharmacognosy at the State University of Ponta Grossa (UEPG, Brazil).

Preparation of samples for scanning electron microscopy (SEM)

The FAA-fixed samples were dehydrated by passing through increasing concentrations of ethanol in water (70%, 80%, 90%, and 100%). The samples were then dried in a Balzers CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein) supplied with liquid CO₂ and then coated with gold using a Quorum (model SC7620) sputter coater. Photomicrographs were recorded using a Mira 3 field-emission scanning electron microscope (Tescan, Brno-Kohoutovice, Czech Republic).

Energy Dispersive X-Ray Spectroscopy (EDS)

During the SEM procedure, EDS was performed to obtain the chemical composition spectra of the crystals. This analysis was made for the crystals as well as the cells devoid of crystals as a control, using an EDS detector on the same variable pressure

microscope at 15 kV. SEM and EDS analyses were performed at the Multiuser Laboratory Complex (C-Labmu) of the State University of Ponta Grossa (UEPG, Brazil).

Results And Discussion

Morphology

Viscum album subsp. *album* is a semiparasite growing on the branches of deciduous trees such as *Malus domestica* (Suckow) Borkh. (Fig. 1a). It has several articulated, glabrous and green stems (Fig. 1b, 1c). The leaves are leathery, opposite, simple, oblong, with an obtuse apex, attenuated base, entire margins, with 3–5 parallel veins, green to yellowish-green and measuring 2–6 cm long and 0.3–2 cm wide (Fig. 1b, 1c). The fruit is a pseudo berry, globular, whitish, translucent, sessile, 0.8–1 cm in diameter (Fig. 1d, 1e, 1f), crowned by the remains of the dried stigma and remains of the perigone leaves (Fig. 1e); they contain a seed coated by a skinny endocarp and embedded in a very viscous translucent mesocarp. The berries ripen during autumn before winter solstice, with the exocarp changing from green to white or yellowish-white when ripe. Berry color supports the differentiation of *V. album* (European mistletoe) from the closely related *Viscum coloratum* (Kom.) Nakai (Korean mistletoe) of Eastern Asia³⁷.

Leaf anatomy

The surface view of the clarified leaf evidences straight and thin anticlinal cell walls on both adaxial and abaxial epidermises (Fig. 2a,d), covered by a thick cuticle (Fig. 2e). Paracytic stomata (Fig. 2a,d) are located at the same level as the adjacent epidermal cells, and are found on both sides of the lamina (Fig. 2a,b,c,d,f). Epicuticular wax crystalloids were observed on both epidermises (Fig. 2f).

Anatomical features such as epidermis with straight anticlinal cell walls, amphistomatic leaves, the presence of paracytic stomata, epicuticular wax crystalloids and thick cuticle, are commonly observed in *Viscum* species^{38–44}. However, anomocytic stomata were evidenced in *V. album* subsp. *austriacum*. This subspecies also evidenced glandular trichomes on the adaxial side of the leaves⁴⁴; this feature was not observed in the present study. The type of stomata and trichomes are might be used as anatomical markers of *V. album* subsp. *austriacum*.

In cross-section, the leaf epidermis is unilayered with strongly cutinized external cell walls on both sides (Fig. 2e,i). Although the leaves are photosynthetic, the mesophyll is undifferentiated and comprises polygonal cells (Fig. 2g,h). Minor collateral vascular bundles are distributed in the mesophyll (Fig. 2h). A dorsiventral mesophyll was found in *V. album* subsp. *album* collected from a host plant *Tilia cordata* Mill.⁴¹ and *V. album* subsp. *austriacum* growing on *Pinus* sp.⁴⁴.

Metcalf and Chalk³⁸ stated that the mesophyll of the biennial leaves of *V. album* is formed by isodiametric cells during the first year, yet a single layer of palisade parenchyma develops towards both sides in the second year. From May until August the shoots of *V. album* have both types of leaves: in the first and in the second year of growth, as can be seen in Fig. 1c.

The mesophyll cells have several starch grains (Fig. 2i,k). They are small, rounded or elongated, and found solitarily or in groups. The presence of starch grains has been reported in various species and subspecies of *Viscum*⁴⁴. Also, different morphotypes of crystals are distributed in the mesophyll (Fig. 2i) and in the vascular bundle (Fig. 2j). They are rectangular prisms, druses (Fig. 2l), platy aggregations of cluster crystals (Fig. 2m), and cubic-shaped crystals (Fig. 2n). Under light microscopy, the druses have a central thick and black region surrounded by many polygonal small crystals (Fig. 2i,j). While cubical, prismatic and druse crystals were observed in the mesophyll of *V. album* subsp. *album* in the present study, only druses were reported in *V. album* subsp. *golestanicum*⁴³. The presence of crystals is commonly reported in various species and subspecies of *Viscum*^{41,42,45}. However, no crystals were reported in a previous study of *V. album* subsp. *album* by Khan *et al.*⁴². Also, the presence of platy aggregation cluster crystals in *V. album* subsp. *album* is reported here for the first time. The

grouping of more than one crystal morphotype can be present as the feature of the subspecies, species, section, subgenus or genus, giving support to the taxonomy⁴⁶.

The midrib is flat on both sides (Fig. 2g). The vascular system is represented by a central collateral vascular bundle (Fig. 2g). Fibers are abutting the xylem and phloem (Fig. 2g,j). Bicolateral vascular bundles were found in *V. album* and in *V. cruciatum* Sieber ex Boiss. In a study by Khan *et al.*⁴².

The strongly shortened and weakly differentiated petiole of *V. album* subsp. *album*, sectioned transversely at the medial portion, had a concave-convex shape with two wings on adaxial side (Fig. 3a). The epidermal cells are covered by a thick cuticle (Fig. 3b). The ground tissue is undifferentiated as in the lamina. The vascular system is collateral and was represented by five bundles organized in an open arc (Fig. 3a). A cap of perivascular fiber adjoins the xylem and phloem (Fig. 3c). The same crystal morphotypes previously described for lamina were observed in the ground parenchyma (Fig. 3b). However, Khan *et al.*⁴² have not reported any crystal in the petiole of *V. album*. Although the outline of the petiole is a general diagnostic feature for higher plants⁴⁷, there are only few studies involving the anatomy of the petiole of *Viscum* species. The same features were found in *V. album* subsp. *album* and *V. album* subsp. *golestanicum*⁴³. However, 6–7 vascular bundles were observed in *V. album* and *V. cruciatum* in the study by Khan *et al.*⁴².

Stem anatomy

The stem is circular in cross-section (Fig. 3d). The epidermis is unilayered with tangentially elongated cells (Fig. 3g). The external wall is particularly thick and fully cutinized (Fig. 3g). Over each external wall, in the middle of the cell, a projecting monticule or papilla, half-moon or elliptical shaped, could be observed (Fig. 3e,g). The cortex is discreetly collenchymatous and formed by several layers of parenchyma cells that increase in size towards the vascular system (Fig. 3e). The vascular system is represented by nine vascular bundles forming a ring and delimiting the pith (Fig. 3d). The row and number of cells in the xylem are higher than in the phloem (Fig. 3e). One cap of perivascular fibers is attached to the xylem and another to the phloem (Fig. 3d,e,f,i), the latter being more developed. The pith is at the center of the stem and is composed of parenchyma cells (Fig. 3d). Idioblasts containing brownish substances corresponding to phenolic compounds are present in the phloem (Fig. 3h). Cortical, vascular and medullary parenchyma are filled with starch grains (Fig. 3g,h,i,j,k) with the same features previously described for leaves; however, these are not present in the first layers of the cortex.

Druses (Fig. 3i,j,l), cubic and quadrangular prismatic (Fig. 3m) crystals are also spread in the stem tissues. Abundant crystals are present in the cortex and in the phloem of *V. cruciatum*, yet are absent in *V. album* in a study of Khan *et al.*⁴². Mehrvarz *et al.*⁴³ have reported that the distribution and morphotype of the calcium oxalate crystals could provide valuable support in delimitation of subspecific taxa in *V. album*. The use of calcium oxalate crystals in solving taxonomic problems has been suggested in previous studies of other plant groups such as *Baccharis*^{46,48}, *Eucalyptus*⁴⁹ and *Piper*⁵⁰.

Most characteristics observed in the present study have been described for *Viscum* species^{41–44}. The vascular bundles showed poorly developed phloem but well-developed xylem. It means that they can resourcefully take up water and prepared inorganic and organic nutrients from the host plant⁵¹.

Berry anatomy

The berry, in frontal view, presents an epidermis with straight anticlinal cell walls. In cross-section, the exocarp is formed by an unilayered epidermis covered by a smooth cuticle and 3–4 layers of angular collenchyma (Fig. 4a,b). In the medial region, small collateral vascular bundles are present (Fig. 4a). The mesocarp, which surrounds the seed, contains an outer fleshy layer and an inner sticky viscin tissue (Fig. 4a,c). The fleshy layer is not sticky.

The viscin tissue is composed of strongly vacuolated globular cells (Fig. 4c,d,e) and highly viscous, sticky and extensible, long cells with spirally thickened walls located towards the endocarp (Fig. 4f). These cells contain druses and rectangular prism crystals, and a few starch grains. The endocarp is thin and encloses the seed. Grazi & Urech⁵² reported that there is no

information of extensible filaments between the seeds and the outer mesocarp in *V. album* subsp. *abietis* and *V. album* subsp. *austriacum* growing on conifers.

Seed anatomy

The seed is green and does not have a seed coat. Heide-Jørgensen⁵³ stated that in *Viscum* genus no seed coat is formed since the integuments are lacking. In cross-section, the seed has an unilayered epidermis covered by a cuticle (Fig. 4h). The epidermal cells have several starch grains (Fig. 4h). The endosperm cells are large, parenchymatous and contain chlorophyll and large amounts of starch grains (Fig. 4g,h,i,j). The seed usually has two embryos, each with two cotyledons and a hypocotyl (Fig. 4i,j,k,l). The number of embryos per berry varies in *Viscum album*. Commonly, *V. album* subsp. *album* has two embryos, sometimes only one embryo, and rarely three or even four embryos, while the percentage of monoembryonal berries is higher in subsp. *abietis* and *V. album* subsp. *austriacum*^{8,40}.

Elemental chemical composition of crystals

The chemical composition of the crystals occurring in plants can be identified using EDS^{54,55}. In the present study, four morphotypes of calcium oxalate crystals were found in various tissues of *V. album* subsp. *album* (Figs. 2i,j,l,m,n, 3b,i,j,l,m). The EDS spectra of druses (Fig. 5a) exhibited prominent peaks for calcium (Ca), carbon (C), and oxygen (O). However, in addition, other elements such as magnesium (Mg), phosphorous (P), and potassium (K) were also found in minor concentrations in the cubic (Fig. 5c) and rectangular prisms (Fig. 5b), and platy aggregation crystals (Fig. 5d). The most common minerals formed by plants are calcium oxalate, calcium carbonate and silica⁵⁶. This is the first study of the elemental chemical composition of crystals of *Viscum* species using EDS.

Histochemical tests

Microchemical analyses using specific reagents and stains under light microscopy are helpful for the characterization of chemical compounds present in plant tissues. They can also be used to detect, within specific cells and tissues, the distribution and accumulation of the compounds or groups of secondary metabolites, such as lipophilic material, protein, mucilage, lignified elements, and phenolic compounds^{26–36}. In the present study, reagents such as Dragendorff, Ellman, Wagner, Sudan III, Sudan black, Nile blue sulfate, potassium dichromate, ferric chloride, hydrochloric vanillin, phloroglucinol/HCl, iodine solution, ruthenium red, PAS, methylene blue, Xylidine Ponceau and Coomassie Brilliant were used to evidence specific chemical compounds and groups of secondary metabolites and the results are summarized in Table 1. No histochemical tests were previously reported for *V. album* subsp. *album*.

Sudan III stains the lipids red or red-orange while Sudan black gives a dark blue or dark color in the microchemical test³². *V. album* subsp. *album* reacts positively with Sudan III and Sudan black, showing lipophilic compounds in cuticles of the leaves (lamina (Fig. 6a) and petiole (Fig. 6b)), stems (Fig. 6c), berries (Fig. 6d) and seeds (Fig. 6e). Oil bodies also react with this reagent⁴⁶ and are found in the epidermis and mesophyll (Fig. 6a) of the lamina, in the epidermis (Fig. 6b) and ground parenchyma (Fig. 6b) of the petiole, and in the exocarp cells of berries (Fig. 6d). Oil bodies (Fig. 6f) stain with Nile blue as well, indicating that the bodies contain neutral fats. Nile blue is a basic dye in the oxazine group that stains neutral fats and fatty acids in red and blue, respectively⁵⁷.

Phenolic compounds can be evidenced using different reagents: ferric chloride solution through iron precipitation produces dark brown or black coloration; potassium dichromate solution produces a brown or reddish-brown color; and vanillin-hydrochloric acid gives rise to a bright red vanillin-tannin condensate⁵⁸. *V. album* subsp. *album* reacts positively with potassium dichromate (Fig. 6g,h) and ferric chloride (Fig. 6i,j) solutions, and the cells containing phenolic compounds were found in the vascular bundles of leaves and stems. However, no condensed tannins were found.

Lignified elements can be detected using phloroglucinol/HCl, which stains lignin in the cell walls pink to red. Lignin is a compound present in several or all the secondary wall layers that contribute to the lignification process resulting in the

modification of cell wall properties⁴⁹. In the present study, lignified elements were evidenced in fibers and vessel elements in the leaves and stems (Fig. 6k,l).

Plant polysaccharides are macromolecules comprised of several identical or different monosaccharides with α or β -glycosidic bonds. In microchemical tests, Schiff reagent is frequently used to distinguish certain mucins and other carbohydrates in a staining sequence called the PAS (periodic acid-Schiff) test. Polysaccharides that comprehend a pair of adjacent hydroxyl groups can be oxidized to aldehydes by periodic acid. The aldehydes react with colorless Schiff reagent and the positive tissue sites become magenta. Neutral polysaccharides are spread in the cell walls in the epidermis, ground parenchyma in the midrib (Fig. 6m), and phloem and pith in the stem (Fig. 6n), exocarp and mesocarp of berry and all the cell walls in the seed, especially near the embryo.

Iodine solution is used to stain starch in dark blue to black. Almost all other structures stain yellow, but this color has no specific meaning. Starch is one of the main ergastic substances of the protoplast and contains a long chain of polysaccharides grouped around a hilum and forming characteristic granules. Starch grains are found in epidermis and mesophyll cells (Fig. 7a) of the lamina and ground parenchyma of the midrib. They are widespread in the cortex, medullary rays, and pith of the stem (Fig. 7b), in some mesocarp cells in the berry (Fig. 7c), and in the endosperm of the seed (Fig. 7d). They are small, rounded and found in groups.

Xylidine Ponceau and Coomassie Brilliant Blue react positively with *V. album* subsp. *album*. The presence of protein globular corpuscles is observed occasionally in the leaves (Fig. 7e), stems (Fig. 7f) and berries (Fig. 7g,i). However, they are commonly found in seeds, especially in the endosperm (Fig. 7h,j). The protein globular corpuscles are vacuolated structures that accumulate reserve protein in the seeds and are called protein bodies or aleurone grains.

Ruthenium red is a polycationic stain that reacts with pectic substances, mucilage, and gums⁵⁹. These substances are detected in almost all cells in the leaves, stems, berries (Fig. 7k) and seeds, yet not in fibers and xylem elements. Azuma *et al.*⁶⁰ analyzed the cellulose system of the viscous fibrous cellulosic polysaccharide (viscan) in the viscin tissue of *V. album* and reported that it is formed by cellulose and hemicellulose together with a minor amount of pectic substance. The viscin tissue assists in attaching the mistletoe berries to the host branches.

Dragendorff, Ellram and Wagner are reagents that stain alkaloids from orange to reddish-brown^{27,28}. These secondary metabolites were not detected in any of the organs of the *V. album* subsp. *album*. Methylene blue was used in order to reveal mucilaginous content in the cells, which swell and exhibit a sharper blue color when positive²⁶. In the present study, no mucilaginous contents were detected in *V. album* subsp. *album*.

Table 1
Results of microchemical tests with *V. album* subsp. *album*. ND: not detected.

Microchemical reagents	Reaction	Occurrence in plant organs					
		Leaf			Stem	Berry	Seed
		Lamina	Midrib	Petiole			
Sudan III	Staining lipids red or red-orange	Cuticle and oil bodies (epidermis and mesophyll)	Cuticle and oil bodies (epidermis and ground parenchyma)	Cuticle	Cuticle and oil bodies in the exocarp cells	Cuticle	
Sudan black	Staining lipids black						
Nile blue	Stains neutral fats in red and fatty acids in blue	Oil bodies	Oil bodies	ND	Oil bodies	ND	
Potassium dichromate	Gives phenolics a brown or reddish-brown color	Minor vascular bundles	Vascular bundles	Cortex and vascular bundles	ND		
Ferric chloride	Turns phenolics to dark brown						
Vanillin/HCl	Gives rise to a bright red vanillin-tannin condensate	ND					
Phloroglucinol/HCl	Reveals lignified elements in pink to red color	Fibers and vessel elements		Fibers and vessel elements	Elongate cells	Endocarp cells	
PAS (periodic acid-Schiff)	Neutral polysaccharides become magenta	Epidermis and mesophyll cells	Epidermis, ground parenchyma and phloem		Phloem and pith	Exocarp and mesocarp	All the cell walls, especially near the embryo
Iodine solution	Stain starch in dark blue to black	Epidermis and mesophyll cells	Ground parenchyma	ND	Epidermis, cortex and pith	Mesocarp (globular cells region)	Endosperm
Xylidine Ponceau	Reveals protein bodies in red color	Protein globular corpuscles					Endosperm, hypocotyl and embryo
Coomassie Brilliant Blue	Turns protein bodies blue	Protein globular corpuscles					
Ruthenium red	Reacts with pectins, mucilages and gums turning them pink to red	Epidermis, phloem and mesophyll cells	Epidermis, ground parenchyma and phloem			Epidermis and exocarp cells	
Methylene blue	Stain mucilage in blue	ND					
Dragendorff, Ellram and Wagner	Gives alkaloids an orange to reddish-brown color	ND					

Conclusion

This study provides valuable anatomical, micromorphological and microchemical information about *Viscum album* subsp. *album*. The anatomy features of the leaf, stem, berry and seed are significant, and this study reveals several attributes of potential taxonomic importance at the genus and species levels. The present study will also provide a basis for future studies involving other taxa in the family for a better understanding of the morpho-anatomy, interaction with host plants, and phylogeny of this interesting group of semi-parasitic plants.

Declarations

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Author contributions

V.P.A. and I.T.M. performed the experiments. J.V.C.B. and M.G. collected and prepared the plant material conservation. Conceptualization and supervision were done by J.M. and C.H. The manuscript was written by V.P.A., J.V.C.B. and J.M. V.R., H.R., C.H. and S.B. revised and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

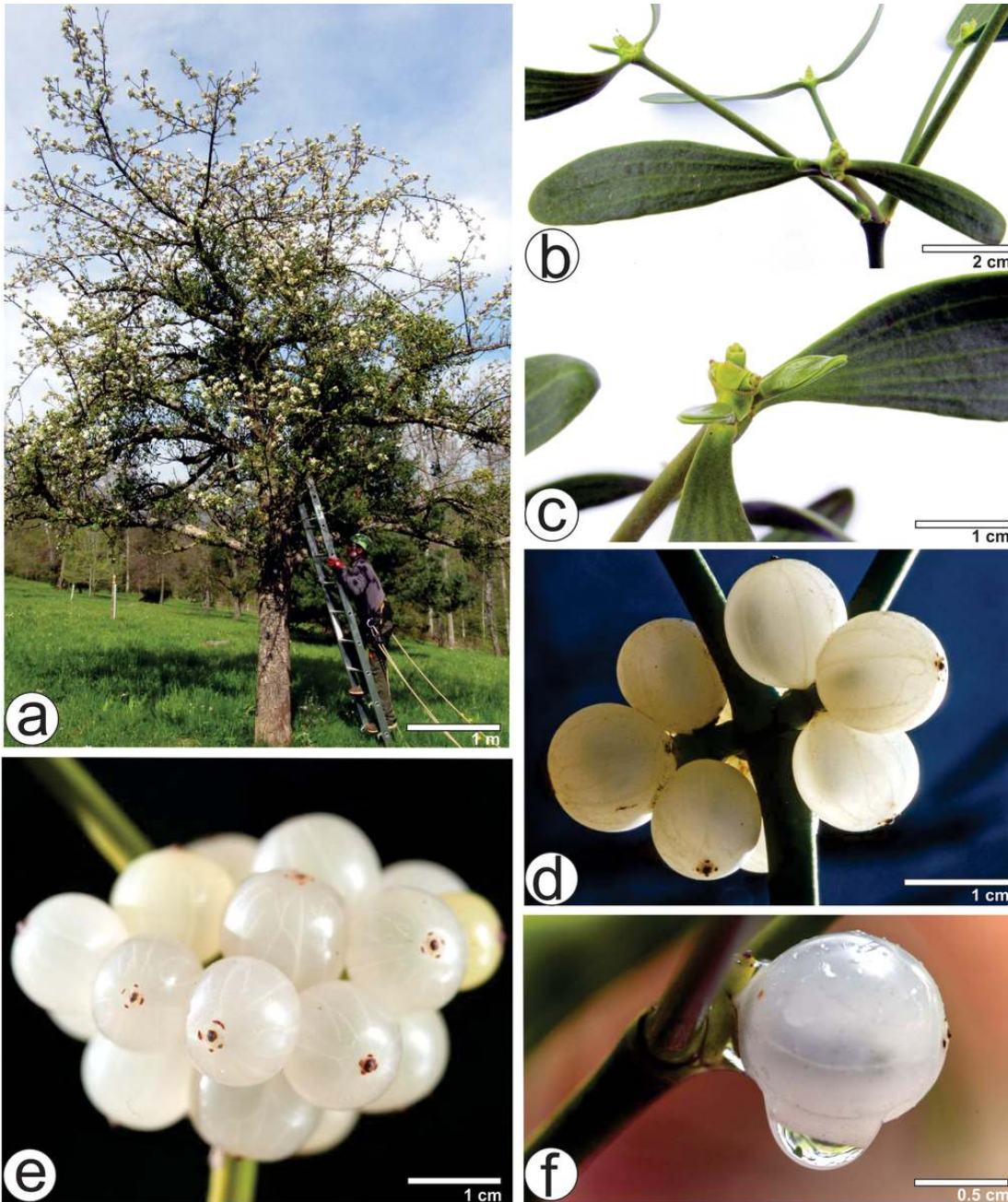


Figure 1

Morphology of *Viscum album* subsp. *album*. **a** – Plants growing on host branches of *Malus domestica*. **b**. Several shoots originate from the same node. **c** – Stem with two opposite leaves. **d** – Berries. **e** – Infructescence with berries showing terminal ring of four lateral (tepalar) and one central (stigmatal) scars. **f**. Berry with a raindrop.

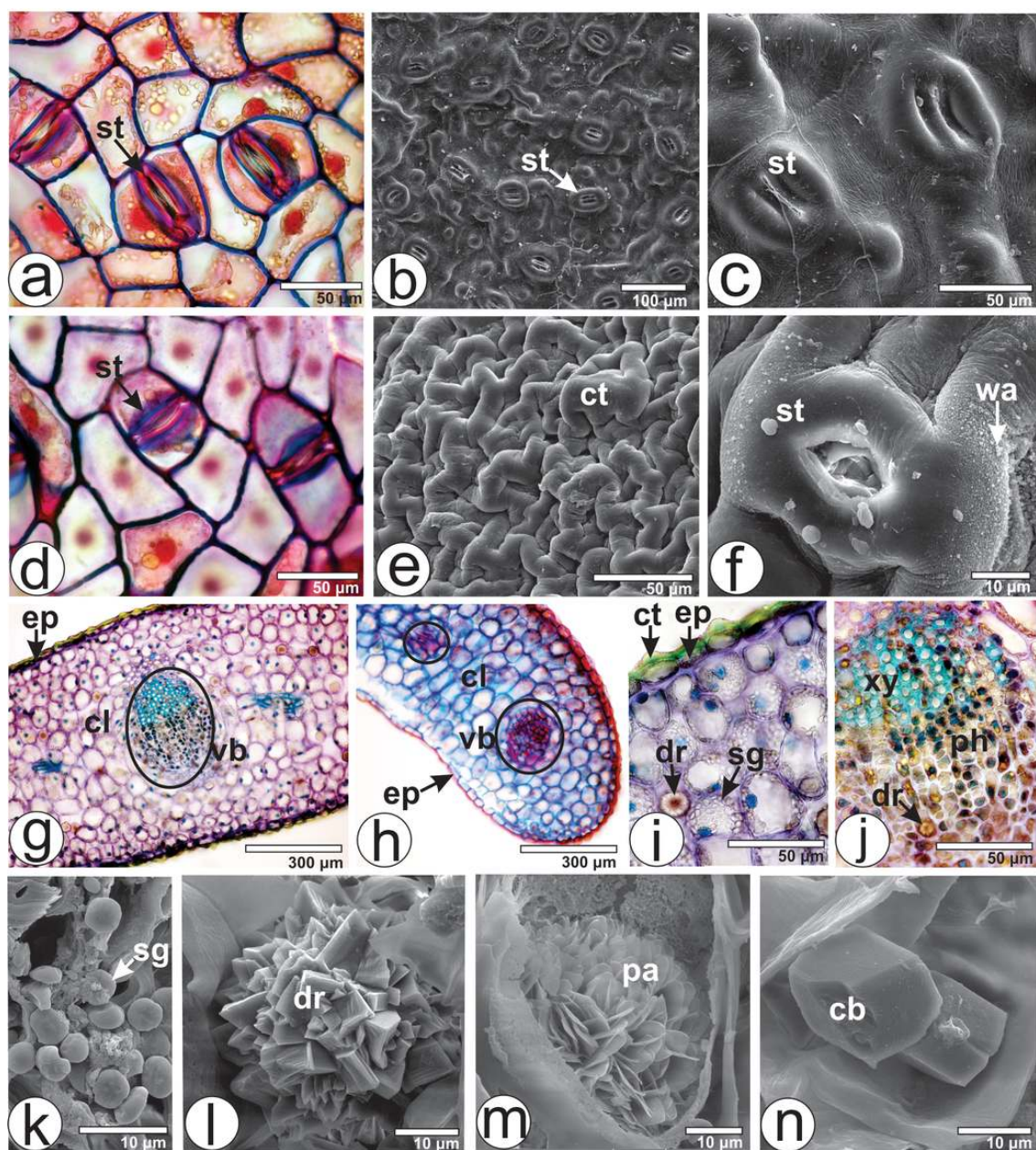


Figure 2

Anatomy of the leaves of *Viscum album* subsp. *album*. (a,d,g-j) light microscopy and (b,c,e,f,k-n) SEM. (a-c) Adaxial and (d-f) abaxial sides on the leaf. (a-f) Leaf in surface view. (g-n) Transverse sections. [cb, cubic-shaped crystal; cl, chlorenchyma; ct, cuticle; dr, druse; ep, epidermis; ph, phloem; pa, platy aggregation cluster crystal; sg, starch grain; st, stomata; vb, vascular bundle; wa, epicuticular wax; xy, xylem].

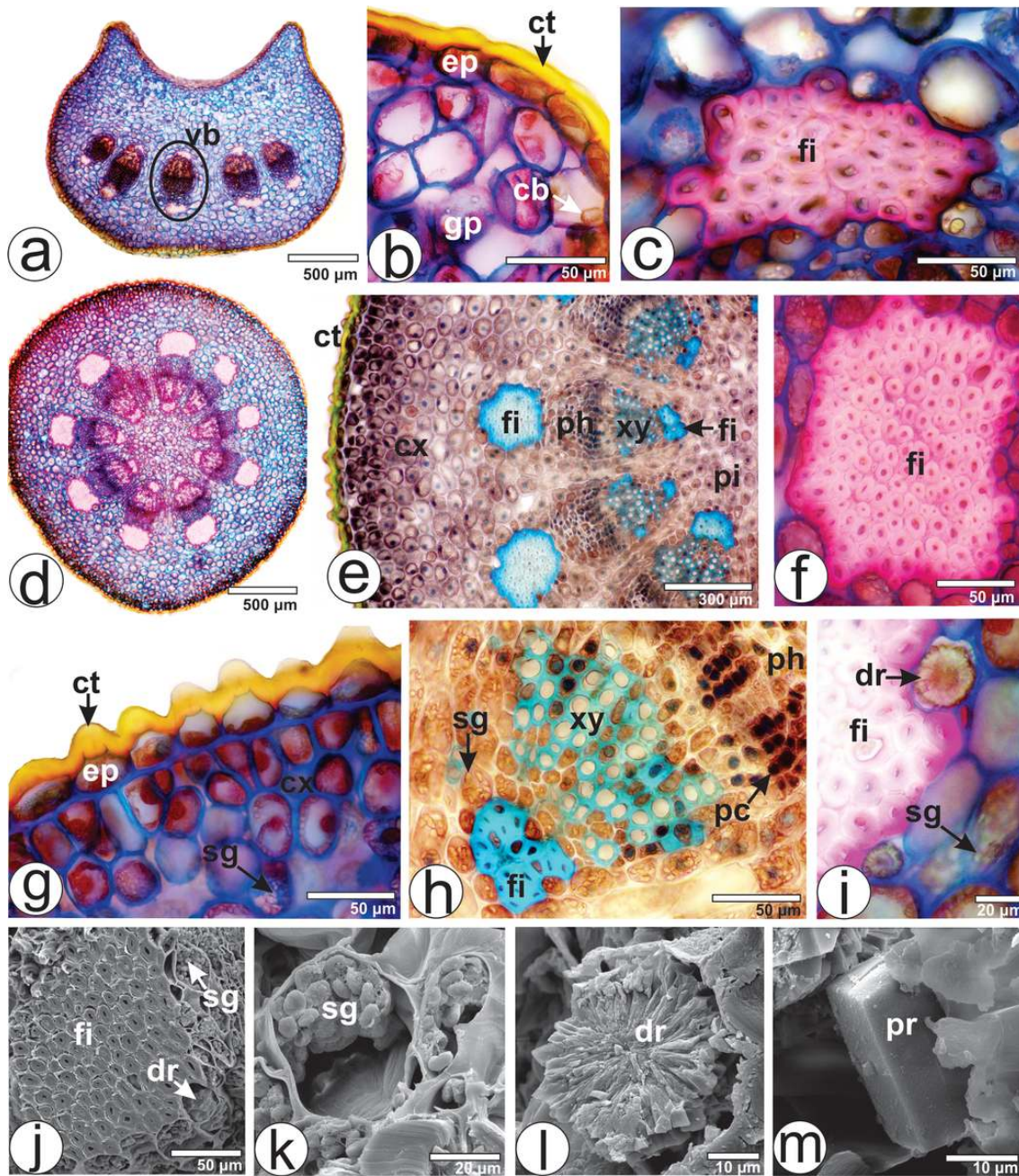


Figure 3

Leaf (petiole) and stem anatomy of *Viscum album* subsp. *album* in cross-section: (a-c) Petiole. (d-m) Stem. (a-i) light microscopy and (j-m) SEM. [cb, cubic-shaped crystal; ct, cuticle; cx, cortex; dr, druse; ep, epidermis; fi, fiber; gp, ground parenchyma; pc, phenolic compounds; ph, phloem; pi, pith; pr, quadrangular prismatic crystal; sg, starch grain; vb, vascular bundle; xy, xylem].

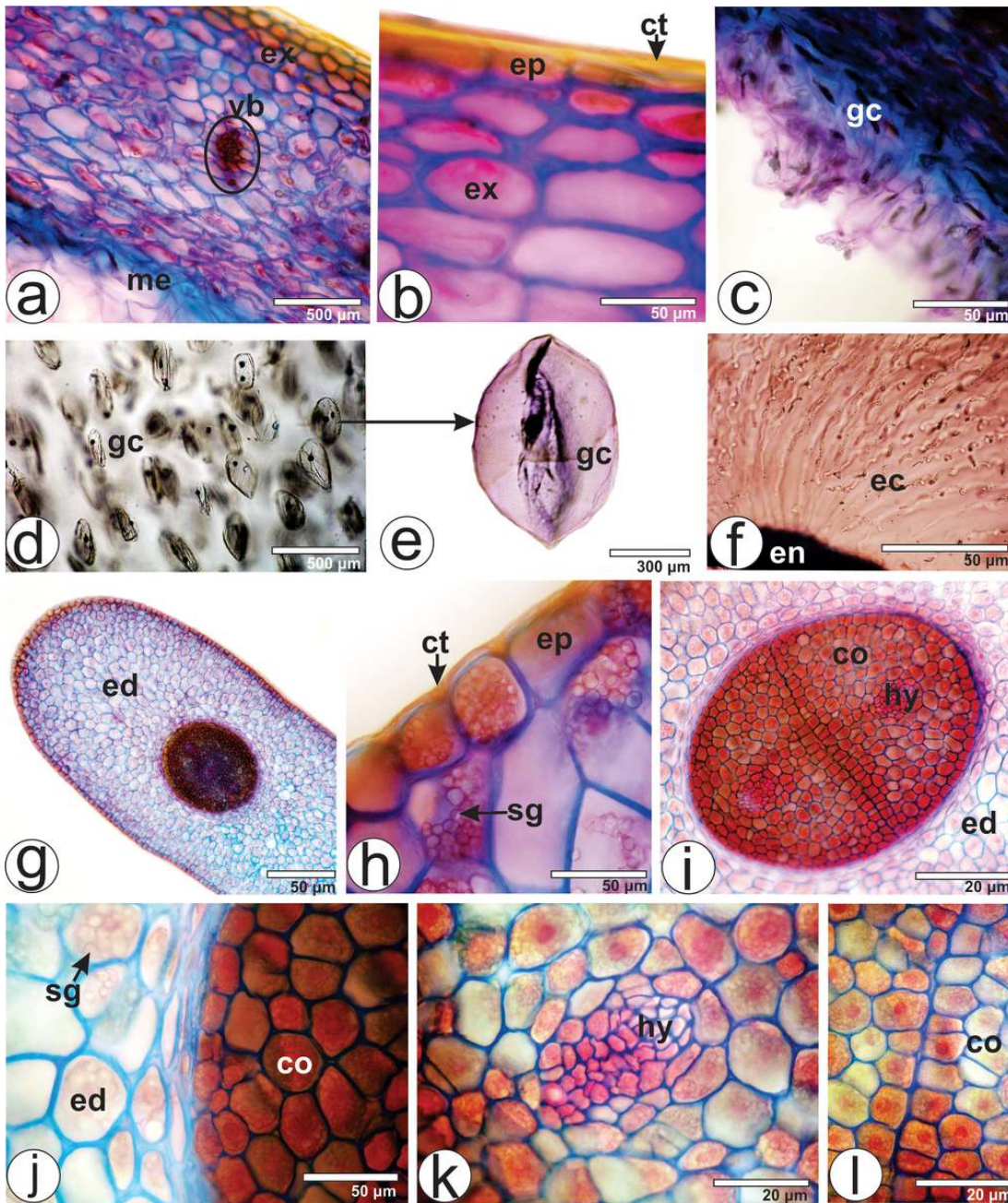


Figure 4

Berry and seed anatomy of *Viscum album* subsp. *album* in cross-section. (a-f) Berry. (g-l) Seed. [ct, cuticle; co, cotyledon; ec, elongated cells with spiral thickening; ep, epidermis; ed, endosperm; en, endocarp; ex, exocarp; gc, globular cells; hy, hypocotyl; me, mesocarp; sg, starch grain; vb, vascular bundle].

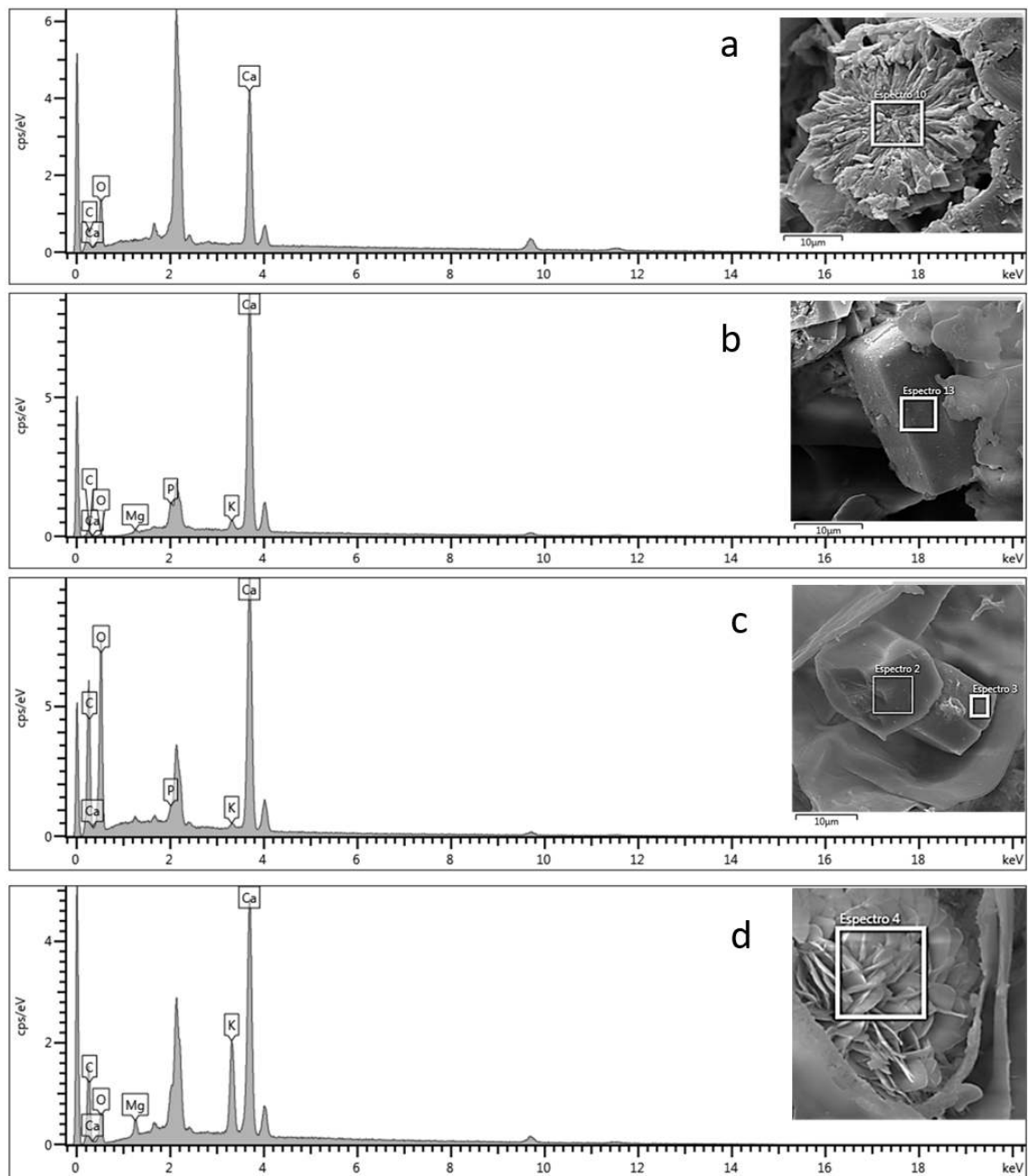


Figure 5

SEM image and EDS spectra of the druse (a), rectangular prism (b), cubic prism (c) and platy aggregated (d) crystals from *Viscum album* subsp. *album*. The prominent unlabeled peak at 0 keV is the noise peak, and the peak near 2.1 keV is for gold (Au) used for sputter-coating the samples for SEM analysis.

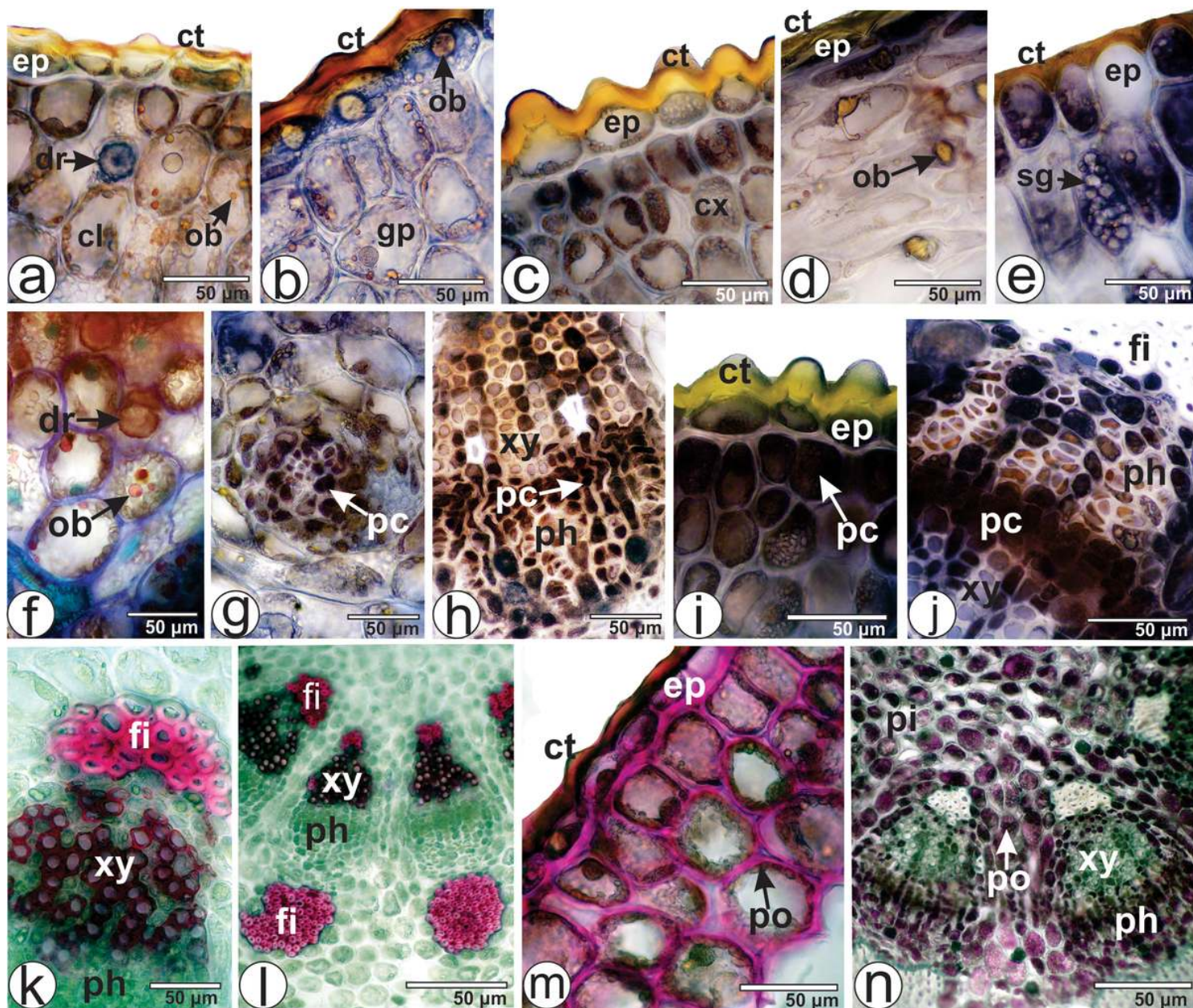


Figure 6

Microchemical tests of *Viscum album* subsp. *album* in cross-section. (a,b,g,h,k,m) Leaf [(a,g) Lamina, (f,m,k) Midrib, (b,h) Petiole]. (c,i,j,l,n) Stem. (d) Berry. (e) Seed. Reagents: (a-e) Sudan III. (f) Nile blue. (g,h) Potassium dichromate. (i,j) Ferric chloride. (k,l) Phloroglucinol/HCl. (m,n) PAS (periodic acid-Schiff reagent). [cl, chlorenchyma; ct, cuticle; cx, cortex; dr, druses; ep, epidermis; fi, fibers; gp, ground parenchyma; hy, hypocotyl; ob, oil bodies; pc, phenolic compounds; ph, phloem; po, neutral polysaccharides; sg, starch grain; xy, xylem]

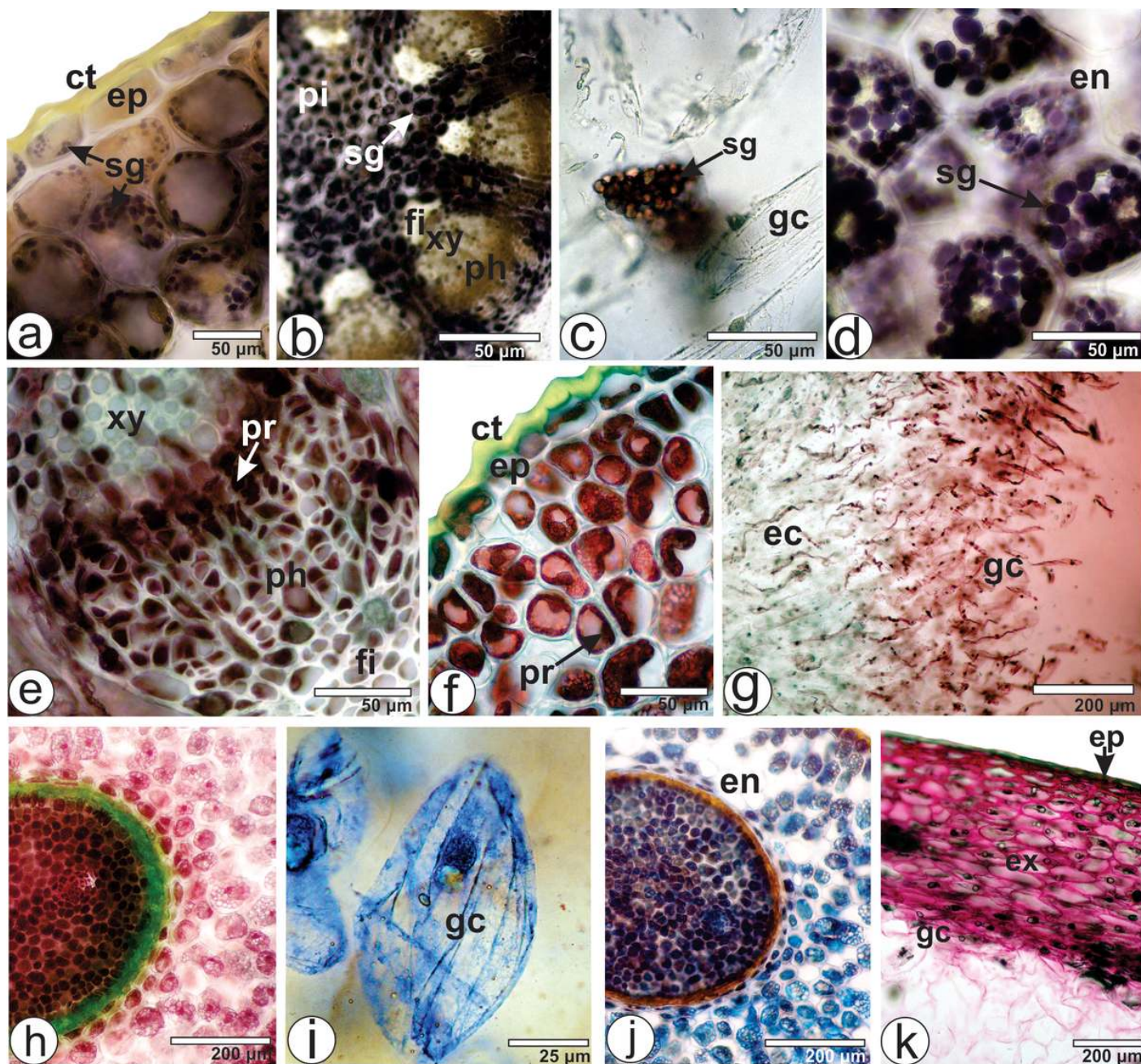


Figure 7

Microchemical tests of *Viscum album* subsp. *album* (Cross-sections of (a) Lamina, (e) Midrib, (b,f) Stem, (c,g,i) Berry, and (d,j,k) Seed. Reagents: (a-d) Iodine solution. (e-h) Xylidine Ponceau. (i,j) Coomassie Brilliant blue. (k) Ruthenium red. [ct, cuticle; ec, palisade-like elongated cells with spirally thickened walls; en, endosperm; ep, epidermis; ex, exocarp; fi, fiber; gc, globular cells; ph, phloem; pi, pith; pr, protein; sg, starch grain; xy, xylem].