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Notes on the taxonomic and ecological significance of bark structure in the genus *Virgilia* (Fabaceae, Podalyrieae)

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ABSTRACT

A comparative study of bark anatomy of all three taxa of the genus *Virgilia* has revealed interesting differences relating to the taxonomic relationships of the species and subspecies and also to the marked ecological differences (as reflected in the bark anatomy) between *Virgilia oroboides* subsp. *oroboides* (exposed to high fire frequencies in the western parts of the Cape Province of South Africa, from Cape Town to Swellendam) and *V. oroboides* subsp. *ferruginea* and *Virgilia divaricata* (growing in areas of relatively low fire frequency along the southern Cape coast from George to Port Elizabeth). The conspicuous difference in the appearance of the bark (thick and corky in *V. oroboides* subsp. *oroboides*; thin and smooth in *V. oroboides* subsp. *ferruginea* and *V. divaricata*) results from a distinct difference in the sclerification of periderm cells. Detailed descriptions of the bark anatomy of all three taxa are presented, showing a pattern of variation that supports the delimitation of species and subspecies. The discovery of crystals in the trichome cells of *V. oroboides* subsp. *ferruginea* appears to be a first record of the presence of crystals inside trichomes in the Fabaceae and is a useful diagnostic character for the subspecies. A remarkable feature of the bark of *Virgilia* is the common occurrence of acicular crystals arranged in radial (sheath-like) aggregates.

1. Introduction

Virgilia Poir. is a small genus of trees endemic to the Cape coastal region of South Africa. The species have become popular garden trees and are of considerable ecological interest as forest margin pioneers, closely associated with afromontane forest. Three taxa have been distinguished in a revision of the genus by Van Wyk (1986), namely *Virgilia oroboides* (P.J. Bergius) T.M. Salter subsp. *oroboides* (Cape Peninsula to Swellendam), *V. oroboides* subsp. *ferruginea* B.-E.van Wyk (restricted to the George district in the southern Cape) and *Virgilia divaricata* Adamson (George to Grahamstown). *Virgilia oroboides* subsp. *ferruginea* was considered to be intermediate between the two species of *Virgilia* because it shares large bracts and densely hairy leaves with *V. oroboides* subsp. *oroboides* but dark pink flowers and smooth bark with *V. divaricata*. A graphical summary of the most conspicuous differential characters between the three taxa was provided by Greinwald et al. (1989), who concluded that the distribution of alkaloids supported the latest circumscription of taxa.

Based on genetic distance data, Van der Bank et al. (1996a,b) suggested that the isolated populations of *V. oroboides* subsp. *oroboides* in the western part of the distribution range is consistent with the fragmentation and isolation of afromontane forest during the last glacial period (80,000 to 30,000 years ago). The resultant forest patches were probably subjected to higher fire frequencies as a result of the fire-prone fynbos that surrounded them. This may have led to the development of thick corky bark, which is a diagnostic character for *V. oroboides* subsp. *oroboides*.

The aim of this study was to examine the bark anatomy of *Virgilia* in order to gain insights into the underlying anatomical basis for the conspicuous morphological differences in stem and bark characters. Only a few bark characters had previously been studied (Metcalfe and Chalk, 1950) and the data reported are inconsistent with our results.

2. Materials and methods

The origin of the material used is listed in Table 1. For each sample, three stages in the bark development were used: (1) from branch tips without a visible periderm layer; (2) from lower parts of the stem where periderm was starting to form; (3) from thicker stems with mature bark that had more or less thick periderm. These were fixed in FAA (Johansen, 1940). Transverse, radial, and tangential sections of bark were made with a freezing microtome (Ernst Leitz GMBH, Wetzlar, Germany) and then stained with a 1:1 alcian blue/safranin mixture (Jansen et al., 2004). Macerations of material using Jeffrey's solution were also prepared for mature bark. Measurements were made with an Olympus Analysis Imaging Solutions (OASIS) programme version 5.1. For photographs we used Olympus ColorView Soft Imaging System and a Jeol JSM 5600 Scanning Electron Microscope (SEM).

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Table 1
Bark characters of *Virgilia* species and subspecies (voucher specimens: KK = K. Kotina, AO = A. Oskolski, BEVW = B.-E. van Wyk).

Characters and character states *character states of potential taxonomic value; **character states apparently correlated with the diameter of the sample studied	*V. divaricata*		*V. oroboides* subsp. *oroboides*		*V. oroboides* subsp. *ferruginea*
	KK02-10 (JRAU)	AO16-09 (LE)	KK01-10 (JRAU)	BEVW5722 (JRAU)	KK44-11 (JRAU)
Diameter of sample (mm)	28	43	15	55	23
**Thickness of the bark (mm)	1.7	2.2	1.3	4.0	1.6
Length of sieve tubes (mean/min–max, μm)	341 ± 11 237–479	265 ± 10 174–339	322 ± 16 129–431	323 ± 11 233–522	284 ± 9 202–367
*Diameter of sieve tubes (μm)	28 ± 1 21–32	23 ± 1 20–29	19 ± 1 12–28	21 ± 1 15–25	25 ± 1 20–30
Length of phloem fibres (μm)	1272 ± 49 800–1944	873 ± 48 509–1557	1068 ± 46 620–1560	950 ± 38 611–1340	1119 ± 50 594–1653
*Width of multiseriate rays in phloem (mean, min–max, μm)	44 ± 2 25–73	41 ± 2 24–68	49 ± 2 34–70	49 ± 2 35–68	49 ± 2 31–75
**Height of multiseriate rays in phloem (min–max, μm)	295 ± 20 147–585	288 ± 19 124–482	452 ± 48 195–774	294 ± 16 177–477	441 ± 21 203–670
*Height of uniseriate rays in phloem (min–max, μm)	147 ± 8 84–266	150 ± 10 79–291	173 ± 14 66–363	173 ± 11 77–351	193 ± 15 103–299
*Mean size of uniseriate ray cells	46	34	61	51	35
**Number of multiseriate rays per mm in phloem (mean, min–max, μm)	3.0	3.8	3.0	4.2	2.0
Number of uniseriate rays per mm in phloem (mean)	1.9	2.4	1.3	1.9	2.5
**Total number of rays per mm in phloem (mean)	4.9	6.2	4.5	4.3	6.1

The descriptive terminology follows Trockenbrodt (1990) and Junikka (1994).

3. Results

The surface of young stems is smooth, green and covered by a prominent indumentum (Fig. 1C). The epidermis is formed by a single layer of isodiametric to round or radially-flattened, thin-walled cells, with simple uniseriate trichomes of two or three short basal cells and an elongated terminal cell. Crystals occur in the terminal cells of the trichomes of *V. oroboides* subsp. *ferruginea* (Fig. 1D).

The cortex is composed of five to 10 layers of isodiametric to radially-flattened, thin-walled parenchyma cells, 8–50 μm in tangential diameter (Fig. 1E), containing chloroplasts, brown contents or acicular crystals in sheaf-like aggregates.

Dilatation of the cortical tissue is effected mostly by tangential stretching of cells and also by anticlinal division, thus forming strands of two to four (up to nine in *V. divaricata* [AO16-09]) cells. No sclerification of cortical cells was observed. The number of cells containing acicular crystals in sheaf-like aggregates (Fig. 1J, K) or brown contents increases with age.

Primary phloem fibres are arranged in an almost continuous ring, two to six cells wide (Fig. 1E), that is broken only by medullary rays. Cells of the medullary rays become sclerified with age and form a ring of mechanical elements.

The surface of mature bark is deeply furrowed in *V. oroboides* subsp. *oroboides* (Fig. 1B), or more or less smooth with small furrows in *V. divaricata* and *V. oroboides* subsp. *ferruginea* (Fig. 1A).

First-formed periderm is initiated subepidermally or deeper in the cortex and persists for several years in all studied species. The phellem is composed of 10 to 15 layers of radially-flattened cells with slightly thickened walls. The phelloderm comprises one to three (up to five in *V. oroboides* subsp. *oroboides*) layers of radially-flattened, thin-walled cells. Acicular crystals in sheaf-like aggregates occur in the phelloderm cells in all samples examined and, in *V. oroboides* subsp. *ferruginea* [KK44-11], also in the phellem cells. Subsequent periderms are initiated in the outer region of the cortical parenchyma as scales. In *V. divaricata* sclerified phellem cells are arranged in uni- or biseriate tangential rows of 10 to 80 (occasionally more than 100) cells and in *V. oroboides* subsp. *oroboides* rare sclerified phellem cells into uniseriate rows only up to six cells. No samples of mature bark with sclerified phellem were available for *V. oroboides* subsp. *ferruginea*. Sclerified phelloderm cells occur in mature stems of *V. divaricata* [AO16-09]. Acicular crystals in sheaf-like aggregates were found in the phelloderm cells of all studied species.

The rhytidome is scaly. In *V. divaricata* and *V. oroboides* subsp. *ferruginea*, the rhytidome consists of one or two rows of fugacious narrow scales formed by one to eight rows of markedly compressed cells (Fig. 1G). Subsequent periderms together with the dilated tissue rapidly and regularly flake off due to the formation of strands of sclerified cells (Fig. 1G, H). In contrast, the rhytidome scales in *V. oroboides* subsp. *oroboides* persist for a long time. These scales are eight to 25 cells wide, arranged in four to eight rows (Fig. 1I) and their cells are not compressed. Phelloderm cells and parenchyma cells adjacent to the periderms contain chloroplasts.

The secondary phloem is composed of tangential zones comprising sieve elements and companion cells which alternate with axial parenchyma cells and are permeated by a network of phloem rays (Fig. 1F) Sieve tube members are 12–30 μm (up to 32 in *V. divaricata* [KK02-10]) wide; their mean length varies between 265 and 341 μm. Sieve plates are simple. The mean length of secondary phloem fibres varies from 873 to 1272 μm.

Axial parenchyma cells are fusiform, septate and occur in strands of mostly two to six cells. Secondary phloem rays are uniseriate to three-seriate. Uniseriate rays are composed mostly of upright cells while multiseriate rays have procumbent and square cells, as well as upright cells forming one to five marginal rows.

The transition from non-collapsed to collapsed secondary phloem is gradual. In collapsed secondary phloem, sieve elements and companion cells are obliterated. Axial parenchyma cells occur as thin-walled, chambered crystalliferous cells or strands of sclerified cells. Dilatation of secondary phloem is mostly radial. Dilated rays are extensively enlarged, mostly by tangential expansion and also by anticlinal divisions of ray cells resulting in rays of up to 10 cells wide (Fig. 1F, I). Ray cells are sometimes sclerified, and often contain acicular crystals in sheaf-like aggregates. A summary of the salient anatomical characters of the three taxa studied is presented in Table 1.

4. Discussion

Although the two species and two subspecies of *Virgilia* are rather uniform in their bark structure, there are some notable differences. The presence of crystals in the trichome cells of *V. oroboides* subsp. *ferruginea* appears to be a first record for the Fabaceae. Among dicotyledons, the occurrence of crystals in trichomes has been reported in Moraceae, Boraginaceae, Loasaceae, Ulmaceae and Cannabaceae (Evert, 2006). The presence of crystals only in *V. oroboides* subsp. *ferruginea* helps support the recognition of this infraspecific taxon (Van Wyk, 1986).

Metcalfe and Chalk (1950) reported that phellogen in *Virgilia* is initiated in the epidermis (an uncommon condition). However, in the samples we studied, the first phellogen is formed in the subepidermal cell layer or even deeper in the cortex. Therefore, *Virgilia*

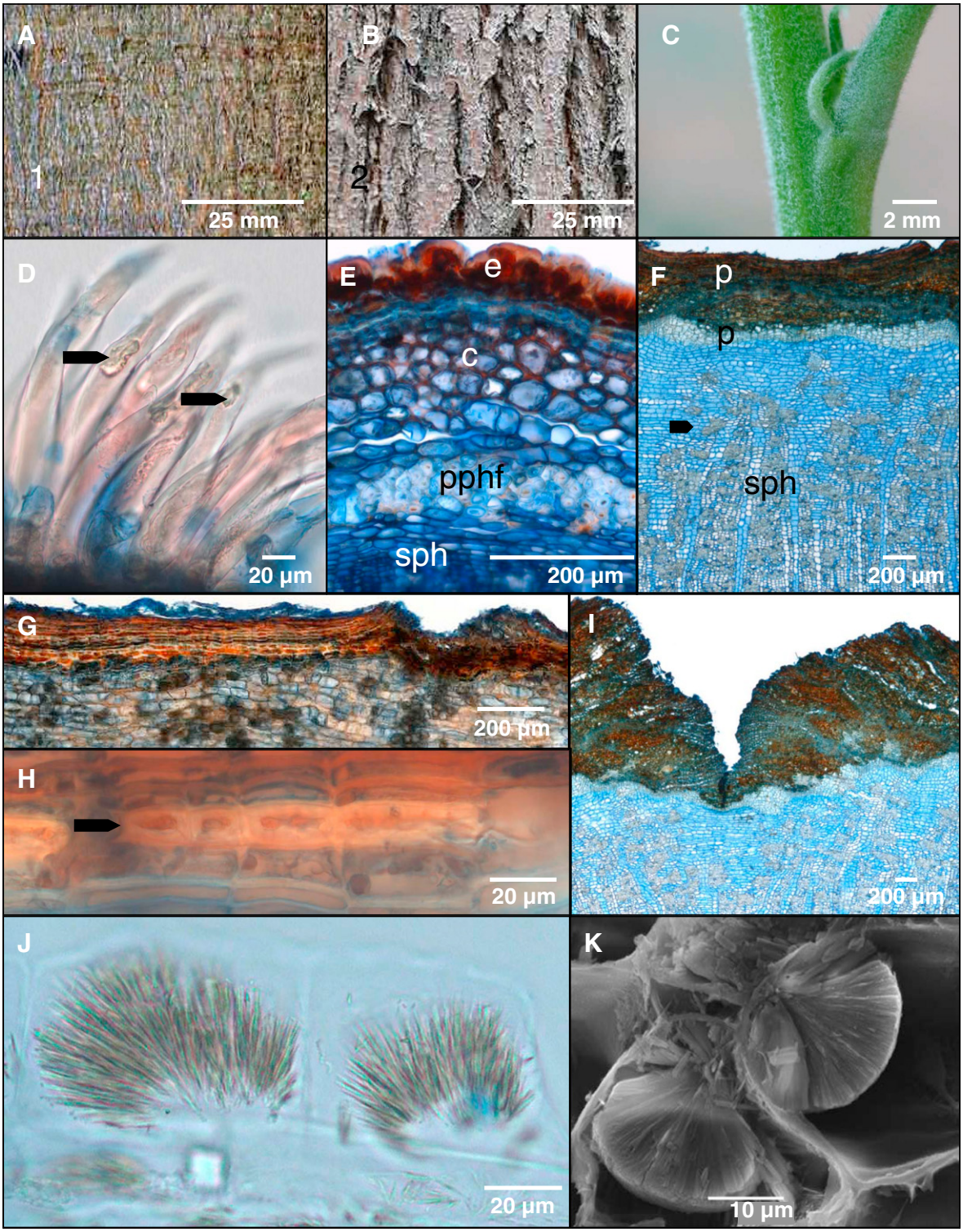

Fig. 1. Bark and stem morphology and anatomy of *Virgilia* species. A, Bark surface of *V. divaricata*; B, bark surface of *V. oroboides* subsp. *oroboides*; C, young shoot of *V. oroboides* subsp. *oroboides* showing stipules and white hairs (trichomes); D, trichomes on the epidermal surface of *V. oroboides* subsp. *ferruginea* (arrows indicate crystals); E, transverse section of young bark of *V. divaricata* (e = epidermal cells; c = cortex; pphf = primary phloem fibres; sph = secondary phloem); F, transverse section of mature bark of *V. divaricata* (p = periderm, sph = secondary phloem; arrow indicates sclerified parenchyma cells); G and H, transverse sections of the rhytidome of *V. divaricata* (arrow indicates sclerified periderm cells); I, transverse section of the rhytidome of *V. oroboides* subsp. *oroboides* (note the deep fissure and numerous layers of periderm); J, acicular crystals (in sheaf-like aggregates) in the cortical cells of *V. oroboides* subsp. *oroboides* (maceration; light microscope); K, acicular crystals in the cortical cells of *V. oroboides* subsp. *oroboides* (maceration; SEM).

seems to be similar to other dicotyledons (Metcalfe and Chalk, 1950; Junikka and Koek-Noorman, 2007; Kotina and Oskolski, 2010 etc.) examined to date.

A remarkable feature of the genus *Virgilia* is the common occurrence of aggregated acicular crystals in radial arrangement. These crystal aggregates probably correspond to the 'acicular crystals' reported by Metcalfe and Chalk (1950) for other members of Podalyrieae, viz. *Podalyria* Lam. and *Cyclopia* Vent. Further bark anatomical studies of this tribe should examine the taxonomic value of this feature.

V. divaricata and *V. oroboides* subsp. *ferruginea* are distinct from *V. oroboides* subsp *oroboides* also in the patterns of phellem sclerification. The sclerified phellem cells are one or two cells thick (Fig. 1H) and are arranged in more or less continuous layers (Fig. 1G) in *V. divaricata* and *V. oroboboides* subsp. *ferruginea*. As a result, the phellem layer causes the periderm to flake off regularly (i.e. to easily break free along the phellem layers). In contrast, the phellem in *V. oroboides* subsp. *oroboides* occurs as short discontinuous layers, often only a few cells wide (in transverse view), so that the periderm layers persist to form a thick and corky rhytidome (Fig. 1I). The phellem sclerification patterns therefore explain the macroscopic differences observed in the mature bark. This is important not only for the taxonomy of the genus (Van Wyk, 1986) but also for understanding the ecological and adaptive significance of thick bark in *V. oroboides* subsp. *oroboides*.

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References

Evert, R.F., 2006. Esau's Plant Anatomy: Meristems, Cells and Tissue of the Plant Body: Their Structure, Function and Development, third ed. John Wiley and Sons, New Jersey 607.
Greinwald, R., Veen, G., Van Wyk, B.-E., Czygan, F.-C., Witte, L., 1989. Distribution and taxonomic significance of alkaloids in the genus *Virgilia*. Biochemical Systematics and Ecology 17 (3), 231–238.
Jansen, S., Choat, B., Vinckier, S., Lens, F., Schols, P., Smets, E., 2004. Intervascular pit membranes with a torus in the wood of *Ulmus* (*Ulmaceae*) and related genera. New Phytologist 163, 51–59.
Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill, New York.
Junikka, L., 1994. Survey of English macroscopic bark terminology. IAWA Journal 15, 3–45.
Junikka, L., Koek-Noorman, J., 2007. Anatomical structure of barks in neotropical genera of Annonaceae. Annales Botanici Fennici 44, 79–132.
Kotina, E., Oskolski, A.A., 2010. Survey of the bark anatomy of Araliaceae and related taxa. Plant Diversity and Evolution 128 (3–4), 455–489.
Metcalfe, C.R., Chalk, L., 1950. Anatomy of the Dicotyledons, Vol. I. Clarendon Press, Oxford.
Trockenbrodt, M., 1990. Survey and discussion of the terminology used in bark anatomy. IAWA Bulletin 11, 141–166.
Van der Bank, H., Van Wyk, B.-E., Van der Bank, M., 1996a. Genetic variation within and geographical relationships between four natural populations of *Virgilia oroboides* (Tribe Podalyrieae: Fabaceae). Biochemical Systematics and Ecology 24, 135–143.
Van der Bank, M., Van Wyk, B.-E., Van der Bank, H., 1996b. Speciation in *Virgilia*: allopatric divergence followed by introgression? Plant Systematics and Evolution 201, 57–73.
Van Wyk, B.-E., 1986. A revision of *Virgilia* (Fabaceae — Podalyrieae). South African Journal of Botany 52, 347–353.