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RESEARCH PAPER

Stem and leaf structure of *Searsia erosa* (Thunb.) Moffett (Anacardiaceae) with systematic, ecological and ethnobotanical implications

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ABSTRACT

The anatomy of stems and leaves of the southern African evergreen shrub *Searsia erosa* (Anacardiaceae) was studied. This species shows the suite of typical traits of Anacardiaceae, such as the presence of secretory canals in the cortex, secondary phloem, wood rays and vascular bundle of leaf midrib, pericyclic fibres in nearly continuous bands, compound sieve plates on oblique walls, simple perforation plates, alternate intervessel pitting, and septate libriform fibres. Like other *Searsia* species, *S. erosa* has abundant sclereids in non-conducting secondary phloem, multicellular peltate glandular trichomes on leaf epidermis, short vessel elements and minute intervessel pits; two latter characters are thought to be diagnostic for this genus. Unlike Asian species of *Searsia*, *S. erosa* lacks marginal axial parenchyma as well as prismatic crystals in axial parenchyma and in libriform fibres, but it shows the helical thickenings on vessel walls. The presence of the last trait in a southern African species agrees with association of helical thickenings with the regions that experience water stress. The tangential expansion of secondary phloem in *S. erosa* and probably in other *Searsia* species is mainly performed by considerable increase in volume of its cells by their sclerification. Such a way of bark growth in girth is out of scope of bark anatomists. *Searsia erosa* is distinctive from other congeneric species in its hypostomatous leaves with abundant glandular trichomes on adaxial side. This condition demonstrates a labor division between the adaxial side with glandular trichomes that may contribute to leaf protection, and the stomata-bearing abaxial side providing the gas exchange. Unlike most members of Anacardiaceae, the secretory canals of *S. erosa* produce the oleoresin containing terpenoids (essential oils) and lipids, but lack polysaccharides. The abundance of glandular trichomes and secretory canals producing terpenoids is a presumable reason of the use of *S. erosa* in traditional medicine by the Basotho people.

Keywords: bark, wood, secondary phloem, sclereids, dilatation, hypostomaty, glandular trichomes, histochemistry, secretory canals, terpenoids, oleoresin, Basotho traditional medicine

РЕЗЮМЕ

Машимбайе Н.Н., Мотити А., Оскольский А.А. Анатомия стебля и листа *Searsia erosa* (Thunb) Moffett (Anacardiaceae) в связи с систематикой, экологией и традиционным использованием этого вида. Изучено анатомическое строение стебля и листа южноафриканского вечнозелёного кустарника *Searsia erosa* (Anacardiaceae). Для этого вида характерен набор признаков, типичных для других представителей семейства Anacardiaceae, таких как наличие секреторных каналов в кортексе, вторичной флоэме, лучах в древесине и в сосудистом пучке главной жилки листа, перициклические волокна в почти непрерывных полосах, сложные ситовидные пластинки на скошенных стенках, простые перфорационные пластинки, очередная межсосудистая поровость, септированные древесинные волокна. Подобно другим видам *Searsia*, *S. erosa* характеризуется обильной склерификацией непроводящей вторичной флоэмы, наличием многоклеточных пельтатных железистых трихом на эпидермисе листа, короткими члениками сосудов и мелкими межсосудистыми порами. Вероятно, два последних признака имеют диагностическое значение для рода. В отличие от азиатских видов *Searsia*, у *S. erosa* отсутствует маргинальная осевая паренхима, а также призматические кристаллы в клетках осевой паренхимы и в древесинных волокнах, однако имеются спиральные утолщения на стенках сосудов. Наличие последнего признака у южноафриканского вида согласуется с общей приуроченностью спиральных утолщений к регионам, испытывающим водный стресс. Тангентальное расширение луба у *S. erosa* и, по-видимому, у других видов *Searsia*, происходит главным образом за счет увеличения объема склерифицированных клеток. Этот способ увеличения периметра коры не привлекает достаточного внимания анатомов. *S. erosa* отличается от других изученных видов *Searsia* гипостоматными листьями с многочисленными железистыми трихомами на адаксиальной стороне. Такое строение связано с разделением функций между двумя сторонами листа: верхняя сторона, покрытая трихомами, обеспечивает защиту листа, в то время как нижняя сторона специализирована на газообмене. В отличие от большинства представителей Anacardiaceae, в секреторных каналах *S. erosa* образуются маслосмолы, в составе которых присутствуют терпеноиды (эфирные масла) и липиды, но отсутствуют полисахариды. Обилие железистых трихомов и секреторных каналов, продуцирующих терпеноиды, может быть причиной использования *S. erosa* в традиционной медицине басуто.

Ключевые слова: кора, древесина, вторичная флоэма, склереиды, дилатация, гипостомия, железистые трихомы, гистохимия, секреторные каналы, терпеноиды, традиционная медицина басуто

Searsia erosa (Thunb.) Moffett is an evergreen shrub or multi-stemmed tree up to about 4 m in height with compound trifoliolate leaves. This species is distributed in the Northern Cape and the Free State Provinces of South Africa, as well as in Lesotho (Moteetee & Van Wyk 2011). Leaves of *Searsia erosa* bear conspicuous sticky long and narrow leaflets, which are leathery in texture, and have a prominent midrib; its specific name 'erosa' is for the toothed leaf margins (Moffett 1994). These leaves have a strong turpentine resinous aroma when crushed, suggesting the presence of essential oils.

Searsia erosa is used in traditional medicine by the Basotho people for the treatment of a wide range of illnesses. These people are a southern African ethnic group found mostly in Lesotho and the Free State Province of South Africa, with a strong history with regard to the utilisation of medicinal plants (Moteetee et al. 2019). In both these countries, traditional medicine plays an important role in the health care and well-being of the rural population (Mugomeri et al. 2014, Shale et al. 1999, Buwa & Van Staden 2006). The therapeutic properties of *Searsia erosa* are not widely-known outside the Basotho culture due to the confined geographical range of this species.

The genus *Searsia*, belonging to the sumac family Anacardiaceae has a disjunctive geographical range. The majority of its 120 species occur in Africa with the highest diversity in southern Africa, whereas three species are distributed along the eastern Himalayas in eastern Asia, and sparsely in NE India, Bhutan, SW China, and N Myanmar (Moffett 2007, Yang et al. 2016). Searsia has long been treated as part of the genus *Rhus* L., but was segregated from the latter by Barkley (1942) and Moffett (2007) on the basis of a distinctive combination of morphological traits (ternate leaves and a mesocarp adherent to the endocarp at maturity). The molecular phylogenetic analyses confirmed the monophyly of *Searsia* as well as its separated position from *Rhus* s.str. (Yi et al. 2007, Weeks et al. 2014, Yang et al. 2016).

The genus *Searsia* is very poorly explored by plant anatomists. The wood structure has been studied in two of the three Asian species of this genus namely, *S. parviflora* (Roxb.) F.A. Barkley (= *Rhus parvifolia* Roxb.) and *S. mysorensis* (G. Don) Moffett (= *Rhus mysorensis* G. Don), (Gupta & Agarwal 2008), and also in two African species *S. chirindensis* (Baker f.) Moffett (= *Rhus chirindensis* Baker f.) and *S. lancea* (L. f.) F.A. Barkley (= *Rhus lancea* L. f.) (Kromhout 1975). Bark structure of several southern African species, i.e. *S. batophylla* (Codd) Moffett (= *Rhus batophylla* Codd), *S. chirindensis* (= *Rhus chirindensis*), *S. gueinzii* (Sond.) F.A. Barkley (= *Rhus gueinzii* Sond.), *S. lancea* (= *Rhus lancea*), *S. leptodictya* (Diels) T.S. Ti, A.J. Mill. & J. Wen (= *Rhus leptodictya* Diels), *S. natalensis* (Bernh. ex Krauss) F.A. Barkley (= *Rhus natalensis* Bern. ex Krauss), *S. pendulina* (Jacq.) Moffett (= *Rhus pendulina* Jacq.), *S. pyroides* (Burch.) Moffett (= *Rhus pyroides* Burch.), *S. rehmanniana* (Engl.) Moffett (= *Rhus rehmanniana* Moffett), *S. undulata* (Jacq.) T.S. Ti, A.J. Mill. & J. Wen (= *Rhus undulata* Jacq.) has been described by Ramovha (1997). The leaf anatomical data have been reported by Jordaan & Kruger (1992) for *S. burchellii* (Sond. ex Engl.) Moffett (= *Rhus burchellii* Sond. ex Engl.). Apart from that, some data on structure of young stem and leaf of *S. glutinosa* (Hochst. ex A. Rich) Moffett has been reported by Madani & Farouk (2019). In the present study, we describe the wood, bark and leaf structure of *Searsia erosa* combined with histochemical examination of some substances in its tissues in order to elucidate the taxonomic value and ecological significance of selected traits within this plant lineage as well as the structural background of traditional uses of this plant.

The purpose of the present study is to investigate the anatomical structure of stems and leaves of *Searsia erosa*, the plant organs used in the Basotho medicine. It was carried out as a part of pharmacognostic and phytochemical research of this species in order to find a scientific rationale for its traditional use. At the same time, the data on structure of vegetative organs of *Searsia erosa* are of great interest for systematics and ecology of the genus *Searsia* F.A. Barkley.

MATERIAL AND METHODS

Wood, bark and leaf sample of *Searsia erosa* was collected by L. Moteetee and the second author at Cholotsa Hill, Lekokoaneng in Berea District, Lesotho. The branch tips with leaves as well as the fragments of stems of 3 cm in diameters covered by mature bark with prominent periderm were fixed in 70 % ethanol. Herbarium voucher (A. Moteetee and L. Moteetee # 34) is deposited in JRAU. Fresh materials for histochemical studies were collected from the same locality.

Transverse, radial, and tangential sections of bark and wood from mature stem portions as well as transverse sections of juvenile stems were made using a freezing microtome (Ernst Leiz GMBH, Wetzlar, Germany), stained with a 1:1 alcian blue/safranin mixture, and mounted in Euparal. Wood macerations were made using Jeffrey's solution (Johansen 1940). Descriptive terminology follows recommendations of the IAWA Committee (1989) for wood structure, and Angyalossy et al. (2016) for bark.

The leaf fragments were embedded in glycol methacrylate (GMA) according to a modification of the Feder & O'Brien (1968) method. Transverse sections of about 1 μm thick were cut by using a Porter Blum MT-1 ultramicrotome and stained with Schiff – toluidine blue method before being mounted in Entellan.

Fresh leaves, branch tips and portions of mature bark, without fixation, were used for histochemical analysis. Free hand cross sections from stems, bark, and from the middle portion of leaflets were subjected to the following reagents/tests: Vanilin-HCl (Gardner 1975) and ferric chloride (Johansen 1940) for tannins, Nadi reagent (David & Carde 1964) for essential oils and other terpenoids; ruthenium red (Retamales & Scharaschkin 2014) for non-cellulosic polysaccharides (including mucilage, pectins, gums etc.), and Sudan IV (Johansen 1940) for lipids (including suberin). For each procedure employed on the samples, control samples, slides without any treatment (blanks), were also mounted.

Light microscopic observations were performed with Olympus CX41 microscope; digital images and measurements were taken with an Olympus Stream Essentials 2.3.3 program.

The wood structure and the surface of dried leaves were investigated by scanning electron microscopy (SEM,

TESCAN, soft – VegaTS) at the The Central Analytical Facility of the Faculty of Science, University of Johannesburg (Spectrum). Samples for SEM observations were mounted on aluminum stubs with double-sided carbon tape and coated with gold.

RESULTS

Bark anatomy

The epidermis on young stems is composed of a single layer of more or less isodiametric rectangular (occasionally cone- or dome-like) cells with thin inner walls and thicker outer walls covered by thin cuticle (Fig. 1A, B). The majority of epidermal cells contain dark tannin deposits (positive vanilin-HCl and ferric chloride tests). Scarce peltate glandular trichomes were found on stem epidermis.

The cortex is very narrow (up to 3–5(8) cells in width), composed of 2–3 layers of lamellar collenchyma and 1–5 layers of parenchyma (Fig. 1A). The cells of cortical tissues are of 10–20 μm in tangential size. Brown tannin deposits occur in cortical collenchyma cells. No crystals found in the cells of cortical collenchyma and parenchyma. Pericyclic fibres thick-walled (commonly with lamellar gelatinous walls), in broad (5–7 cells in width) arcs near secretory canals forming nearly continuous band interrupted only by narrow (2–4-seriate) medullary rays. Secretory canals are in the phloem parts of conductive bundles. The canal lumina are commonly 40–65 μm in tangential size (Fig. 1A, B). Their epithelial cells and the contents of canal lumina show positive reactions with Nadi reagent (violet staining) and with Sudan IV suggesting the presence of terpenoids and lipids, and negative reactions with ruthenium red indicating the lack of polysaccharides.

Mature bark non-peeling, brittle, with shallow fissured surface. The initiation of first-formed periderm is in the sub-epidermal layer of cells (Fig. 1B). Phellem is composed of 4–7 layers of isodiametric to somewhat radially-flattened or radially-elongated thin-walled cells. The phelloderm comprises 8–12 layers of radially flattened, thin- to moderately thick-walled cells, occasionally also very thick-walled cells (Fig. 1D). Some phelloderm cells contain brown tannin deposits (positive vanilin-HCl and ferric chloride tests). No crystalliferous cells were found in periderm. Subsequent periderms were not found (Fig. 1D, E).

Sieve tubes members 11–19 μm wide, and 424 μm (277–606 μm) long. Sieve tubes are in radial groups or clusters of 4–9 (Fig. 1G), while sieve plates are composed of 2–4 sieve areas located on vertical or slightly oblique cross walls (Fig. 1C). Axial parenchyma associated with conductive elements in strands of 4–10 cells, which are 10–22 μm in tangential size. No crystalliferous cells found in conducting secondary phloem. Axial secretory canals are scattered throughout the secondary phloem (Fig. 1D). They are lined by a single layer of 20–60 μm in tangential size. 2–4-seriate parenchyma sheaths are present near the secretory canals. Epithelial cells and the contents of lumina of secretory canals show positive reactions with Nadi reagent (violet staining) and with Sudan IV suggesting the presence of terpenoids and lipids, and negative reactions with ruthenium red indicating the lack of polysaccharides.

The transition from conducting to non-conducting secondary phloem is sharp, marked by partial obliteration of sieve tubes, tangential stretching of secretory canals, and sclerification of some axial parenchyma cells (Fig. 1D). Sclereids are isodiametric, tangentially stretched and/or vertically elongated cells of 40–65 μm in tangential size, with thick to very thick (occasionally lamellate) walls, arranged into large clusters, which are extended in the outer regions of non-conductive secondary phloem (Fig. 1F, G). Large prismatic crystals occur in some sclereids as well as in axial parenchyma strands adjacent to the sclereid clusters. Non-sclerified axial parenchyma cells in collapsed secondary phloem are mostly non-dilated, occasionally tangentially stretched and, rarely, in short tangential strands of 2–3 cells.

Secondary phloem rays are uniseriate and 2–3 seriate (Fig. 1H), composed of square and upright cells (the latter are mostly in uniseriate portions). Radial secretory canals not found. Some rays in non-conducting secondary phloem show weak dilatation by tangential stretching of rays cells, but the majority of rays are non-dilated (Fig. 1D, I). Sclerified cells occur in the rays of collapsed secondary phloem. Prismatic crystals rarely occur in ray cells (Fig. 1I).

Wood anatomy

Growth rings are distinct, marked by 3–6 rows of radially flattened fibres (Fig. 2A). Wood is diffuse-porous, occasionally semi-ring-porous. The vessels are rounded in outline, medium in diameter (average tangential size 70.2 μm, range 36–96 μm) and few in number (average frequency 38.5 per mm^2). Vessels are solitary and in radial multiples or in small clusters of 2–9. Vessel elements are 260 μm (143–355 μm) long. Perforation plates are simple (Fig. 2C, D). Intervessel pitting alternate, pitts minute, 2–4 μm in vertical size, circular to oval in shape, with rounded borders (Fig. 2B, E) and slit-like apertures in narrow (occasionally coalescent) grooves (Fig. 2F). Spherical warts occur in chambers of some intervessel pits (Fig. 2E). Vessel-ray pits are larger than intervessel pits, which are oval to horizontally elongated (scalariform) in outline, simple with reduced borders (Fig. 2B). Helical thickenings occur on the wall of some vessel elements (Fig. 2D). No tyloses were found.

Fibres are libriform, septate, thin- to thick-walled, with small simple pits on radial walls (Fig. 2B, C). Fibre length is 545 μm (356–778 μm).

Axial parenchyma is scanty paratracheal, in solitary or an incomplete sheet around the vessels, with 3–6 cells per strand. No crystals found in axial parenchyma cells.

Rays 4.9–7.3 per mm, uni- and 2–3 seriate, occasionally 4–5-seriate, with radial canals (Fig. 2B). Ray height up to 0.65 mm. All rays are heterogeneous, composed of procumbent, square and upright cells mixed throughout (Fig. 2C). Radial canals in few wide rays of 5-seriate. Prismatic crystals in non-chambered upright and square ray cells (Fig. 2C).

Leaf anatomy

Leaf blade is dorsiventral, 230–320 μm thick (Fig. 3A). Epidermis in mostly uniseriate, but some cells of abaxial epidermis undergo periclinal divisions forming small bi- and triseriate patches. Epidermal cells with evenly thin walls, with cuticle of ca. 1 μm thick on their outer wall,

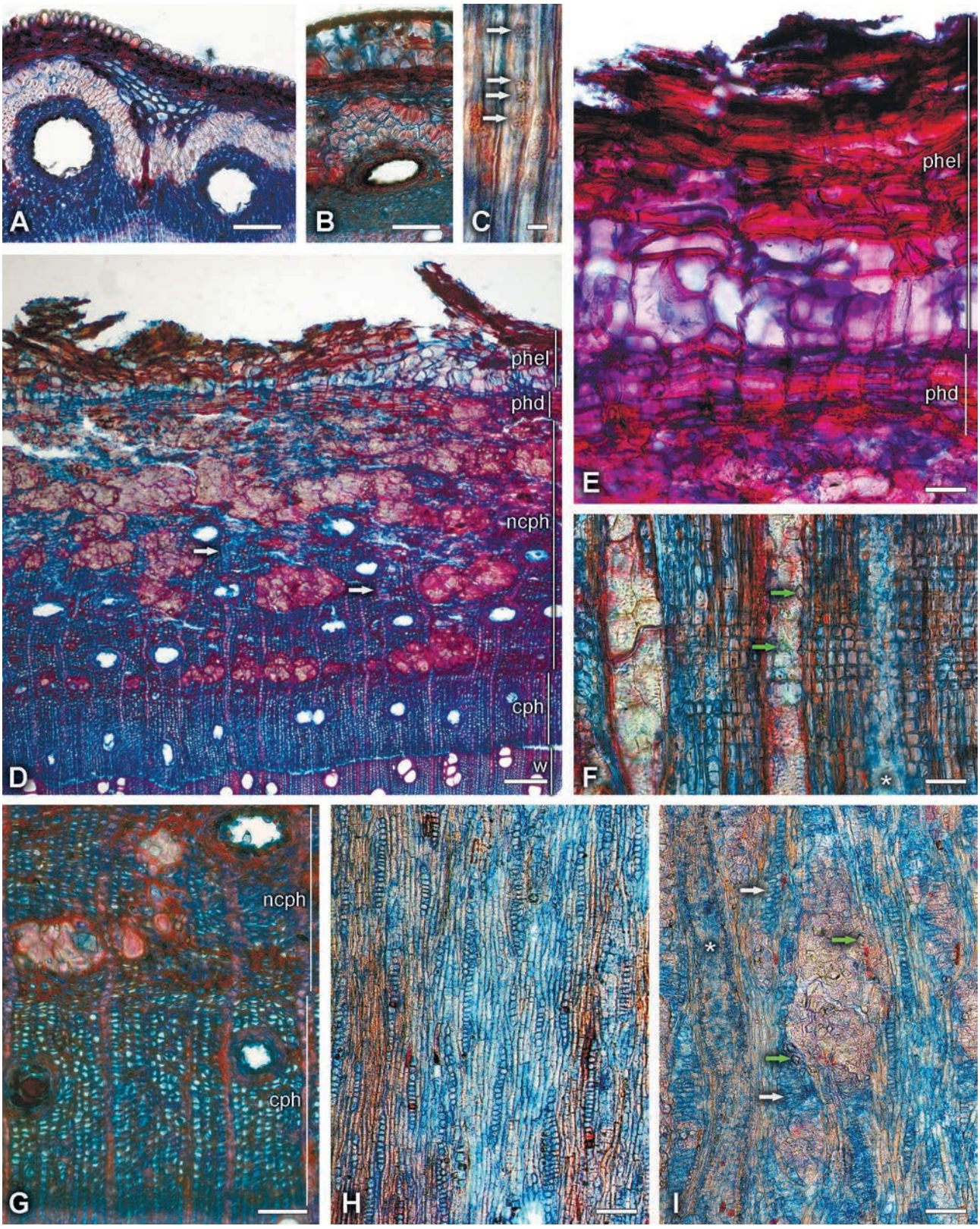

Figure 1 Structure of juvenile (A–B) and mature (C–I) bark of *Searsia erosa*, light microscopy: (A) epidermis with peltate trichome, cortex composed of lamellar collenchyma and parenchyma, pericyclic fibres in broad arcs forming nearly continuous band interrupted only by narrow medullary ray, secretory canals in primary phloem, light microscopy, transverse section (TS); (B) young periderm located in subepidermal position (TS); (C) compound sieve plates with 3 or 4 sieve areas (arrowheads), radial longitudinal section (RLS); (D) wood (w) and mature bark subdivided into homogenous conducting secondary phloem (cph) with secretory canals, non-conducting secondary phloem (ncph) with abundant thick-walled sclereids, dilated secretory canals and rays (arrowheads), and periderm with phellem (phel) and phelloderm (phd) (TS); (E) mature periderm with phellem (phel) and phelloderm (phd) (LM, TS); (F) secondary phloem rays made mostly of square cells, groups of thick-walled sclereids, prismatic crystals in sclereids, dilated secretory canal (asterisk) (RLS); (G) conducting secondary phloem (cph) and inner portion of non-conducting secondary phloem (ncph) (LM; TS); (H) 1-3-seriate phloem rays in the boundary zone between conducting secondary phloem and non-conducting one, tangential longitudinal section (TLS); (I) non-dilated and dilated (white arrowheads) rays with partially sclerified ray cells in non-conducting secondary phloem, prismatic crystals in ray cells (green arrowheads), groups of thick-walled sclereids, dilated secretory canal (asterisk) (TLS). Scale bars = 200 μm (D), 100 μm (G, H, I), 50 μm (A, B, E, F), 10 μm (C)

occasionally contain dark tannin deposits (positive vanilin-HCl and ferric chloride tests). Cells of adaxial epidermis are rounded, isodiametric to flattened in transverse sections, 12–16 μm in tangential size, with straight to slightly curved anticlinal walls, bearing cuticle with striate microsculpture (Fig. 3C, E). Cells of abaxial epidermis rounded to polygonal, isodiametric to somewhat upright in transverse section, 7–14 μm in tangential size, with curved to wavy anticlinal walls, bearing smooth cuticle (Fig. 3D, F). The palisade mesophyll consists of one or two layers of long upright cells (height/width ratio 6 to 13) (up to 7). The spongy mesophyll is in 3–5 layers of mostly upright (occasionally isodiametric) cells with large intercellular spaces in-between (Fig. 3A, B).

Midrib (Fig. 3B) contains single large collateral vascular bundle accompanied with 4–5- seriate zone of annular to angular collenchyma on its adaxial side, and with 2–3-seriate zone of parenchyma on its abaxial side. Prismatic crystals occur in some collenchyma cells. The epidermis covering midrib on both its sides is distinctive from it from adjacent portions of lamina in having dome-like to bottle-like cells with thick outer walls. The vascular bundle in midrib consists of tangentially extended phloem and xylem zones, extended biseriate layer of sclereids on abaxial side, and a cluster of sclereids on adaxial side. Two large secretory canals (20–45 μm in diameter) found in phloem. Their epithelial cells and the contents of canal lumina show positive reactions with Nadi reagent (violet staining) and with Sudan IV suggesting the presence of terpenoids and lipids, and negative reactions with ruthenium red indicating the lack of polysaccharides. The vascular bundle is sheathed by one or two layers of parenchymatous cells with dark tannin deposits (positive vanillin-HCl and ferric chloride tests). The tannins also found in solitary parenchyma cells located in phloem and xylem.

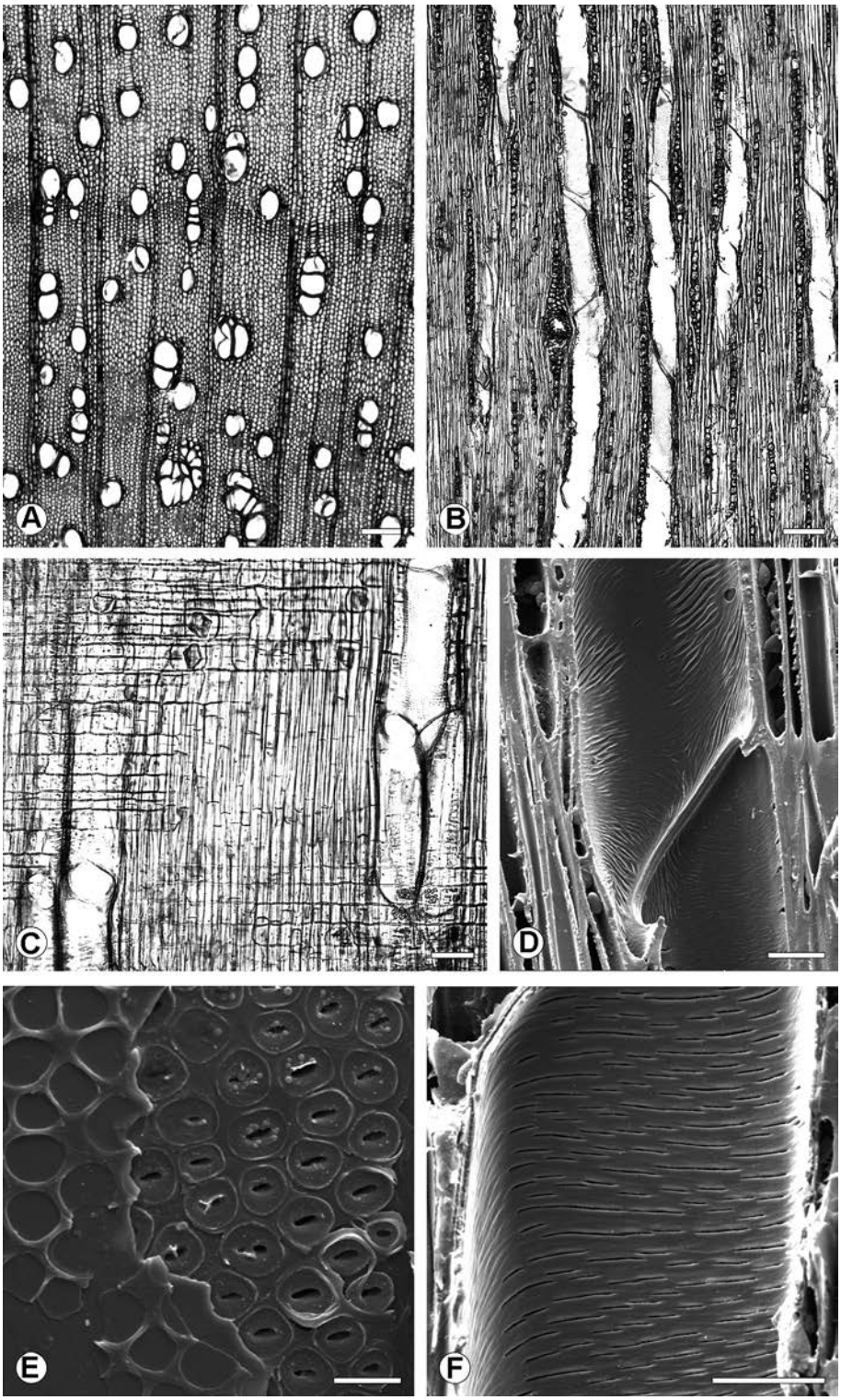

Figure 2 Wood structure of *Searsia erosa*: (A) distinct growth ring, vessels solitary, and radial multiples and clusters of 2–9 (LM, TS); (B) alternate intervessel pitting, uni- and biseriate rays, radial secretory canal (LM, TLS); (C) vessel elements with simple perforation plates, septate fibers, oval to scalariform vessel-ray pits with reduced borders, septate libriform fibers, procumbent, square and upright ray cells mixed throughout, prismatic crystals in non-chambered ray cells (LM, RLS); (D) simple perforation plate, helical thickenings on vessel wall, scanning electron microscopy (SEM) (RLS); (E) minute intervessel pits in alternate arrangement, spherical warts in chambers of some pits (SEM, TLS); (F) solitary and coalescent narrow grooves with apertures of intervessel pits on inner vessel wall (SEM, TLS). Scale bars = 100 μm (A, B), 50 μm (C), 20 μm (D, F), 5 μm (E).

Secondary and tertiary vascular bundles collateral, with uniseriate sheaths of parenchyma cells occasionally containing tannins. Larger vascular bundles have also the adaxial

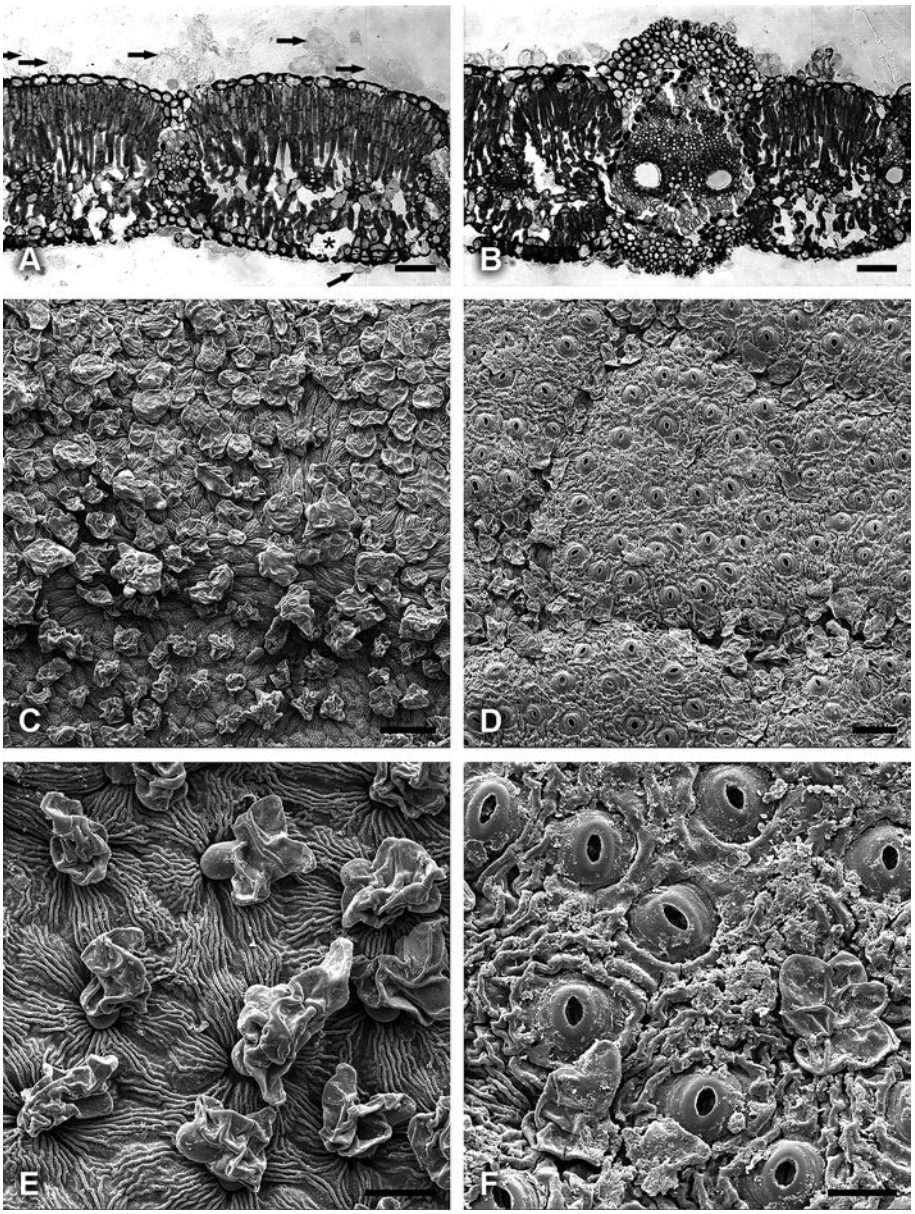

Figure 3 Leaf structure of *Searsia erosa*: (A) dorsiventral leaf lamina, abundant peltate trichomes on adaxial epidermis (black arrows), periclinal divisions in abaxial epidermal cells (arrowhead), stomatal chamber (asterisk) (LM, TS); (B) leaf midrib with single large collateral vascular bundle accompanied with zone of annular to angular collenchyma on its adaxial side, zone of parenchyma on its abaxial side, sheath of parenchyma cells with dark tannin deposits, epidermis on both sides dome-like to bottle-like cells with thick outer walls, and two large secretory canals in phloem part of vascular bundle (LM, TS); (C) adaxial epidermis with numerous peltate glandular trichomes and lacking stomata (SEM); (D) abaxial epidermis with numerous stomata and few peltate glandular trichomes confined to vein grooves (SEM); (E) cells of adaxial epidermis with straight to slightly curved anticlinal walls, cuticle with striate microsculpture (SEM); (F) cells of abaxial epidermis with curved to wavy anticlinal walls, anomocytic stomata (SEM). Scale bars = 50 μm (A, B, C, D), 20 μm (E, F)

and abaxial sclereid clusters, and their sheaths are associated with adaxial and abaxial extensions. Secretory canals lack in secondary and tertiary vascular bundles (Fig. 3A).

Stomata are anomocytic, scattered only on abaxial epidermis (325–338 per mm^2), situated mostly at the level of the outer epidermal cell wall (Fig. 3A, D, F).

Peltate glandular trichomes consist of small basal cell (8–12 μm in tangential size) sunken between larger epidermal cells, spherical stalk cells (10–14 μm in diameter) and 4–8 head cells (Fig. 3A). Abundant trichomes (422–438 per mm^2) evenly scattered on adaxial epidermis (Fig. 3C), whereas fewer ones (112–122 per mm^2) are confined to vein grooves on abaxial epidermis (Fig. 3D). The walls of basal and stalk cells of these trichomes are suberized (positive Sudan IV test). The head cells contain essential oils indicated by blue staining with Nadi reagent.

DISCUSSION

A suite of bark features (including pericyclic fibres in nearly continuous bands, the presence of secretory canals in the cortex and secondary phloem, compound sieve plates on oblique walls, axial parenchyma of secondary phloem in conspicuous tangential bands, and the occurrence of prismatic crystals in axial parenchyma cells) found in *Searsia erosa*, is typical for other genera of Anacardiaceae whose bark structure has been explored to date (Metcalfe & Chalk 1950, Zahur 1959, Ellis 1974, Roth 1981, Ramovha 1997, Schweingruber et al. 2011, 2019, Eremin & Kopanina 2012, Crivellaro & Schweingruber 2013, Madani & Farouk 2019). Among seven African species of *Searsia* examined by Ramovha (1997), *S. erosa* shows the greatest similarity to *S. rehmanniana* in its bark structure sharing the lack of dilatation meristem, the presence of sclereids in non-conducting secondary phloem, and the absence of tangential bands of sclereids in phelloderm, but it is distinctive from the latter species in the presence of prismatic crystals in sclereids.

Typical pattern of dilatation, such as tangential stretching of thin-walled axial parenchyma and ray cells, is poorly expressed in non-conducting secondary phloem in *Searsia erosa*. Ramovha (1997) even described this condition as "the absence of dilatation tissue", that is considered by him as a taxonomically important trait for the genera *Searsia* and *Smodingium*. He also noted that "the secretory ducts are the sole site of dilatation in these two genera" (Ramovha 1997, p. 93). Our observations on *Searsia erosa* suggest, however, that the tangential expansion of outer regions of its bark is mainly performed by the considerable increase in volume of axial parenchyma cells in the course of their sclerification. This

effect must also be significant in other *Searsia* species as well as in *Smodingium argutum* sharing abundant sclerenchyma in their non-conducting secondary phloem (Ramovha 1997). The predominance of the considerable increase in the cell walls thickness over the dilatation of thin-walled cells occurs also in mature bark of other trees having abundant sclerenchyma, as e.g. in some *Populus* species (Eremin & Kopanina 2012). Such a way of bark transformation in the course of its growth in girth is, however, out of scope of bark anatomists; it has not been mentioned even in the recent comprehensive review of microscopic bark features (Angyalossy et al. 2016). Comparative assessment of the contributions of sclerification and dilatation into tangential expansion of bark in different plant taxa would be of great interest for plant anatomy.

Such suite of wood traits as exclusively simple perforation plates, alternate intervessel pitting, septate libriform fibers, paratracheal axial parenchyma, heterocellular rays occasionally containing radial secretory canals, and the presence of prismatic crystals in ray cells, found in *Searsia erosa*, is typical of most genera of the family Anacardiaceae examined to date (Meltcalfe & Chalk 1950, Mitchell & Daly 2015, Dong & Baas 1993, Gupta & Agarwal 2008, Crivellaro & Schweingruber 2013, InsideWood, 2004–onwards). At the same time, the species under study shows shorter vessel elements (average length 260 μm) and smaller intervessel pits (2–4 μm in vertical size) than the majority of Anacardiaceae members. Short vessel elements and minute intervessel pits has also been reported, however, in *Searsia parviflora* and *S. mysorensis*, two of three Asian species of this genus (Gupta & Agarwal 2008). The similarity between *S. erosa* and these two species confirms close affinity between African and eastern Asian lineages of *Searsia* revealed by molecular phylogenetics (Yang et al. 2016). Our data also suggest that the combination of those quantitative wood traits can be diagnostic for the genus *Searsia*, supporting its segregation from *Rhus* (Moffett 2007), the genus showing longer vessel elements and large intervessel pits (Dong & Baas 1993, Gupta & Agarwal 2008). A comprehensive wood anatomical study of African *Searsia* species is required, however, to test diagnostic value of these traits.

Unlike Asian species of *Searsia*, however, *Searsia erosa* lacks marginal axial parenchyma as well as prismatic crystals in axial parenchyma and in fibers, but it shows the helical thickenings in vessel elements. The last trait is found in several lineages of Anacardiaceae, i.e. in the clade comprising *Rhus*, *Toxicodendron*, *Cotinus*, *Pistacia* and *Haplorhus*; in *Schinus* and *Lithraea* as well as in *Tapirira* and also in *Smodingium* (InsideWood 2004–onwards, Weeks et al. 2014, Muellner-Riehl et al. 2016, Silva-Luz et al. 2019). Our data showed that helical thickenings are gained also in the African *Searsia*. Among modern angiosperms, this feature is most common in woods of temperate regions, but is rare in the tropics (Wheeler et al. 2007). As noted by Carlquist (2001), helical thickenings are associated with regions that experience water stress created by drought or freezing. He suggested that this feature might diminish in some way the danger of cavitation, aid in refilling of vessels, or increasing vessel wall strength. The occurrence of helical thickenings in wood of a species from southern Africa coupled with its lack in its relatives from tropical Asia is in good agreement with this ecological trend. A comprehensive wood anatomical study of African *Searsia* species is required, however, to test this hypothesis and to evaluate diagnostic value of their wood traits as well.

Sharing most leaf traits with *Searsia burchellii* (Jordaan & Kruger 1992) and *S. glutinosa* (Madani & Farouk 2019), *S. erosa* is distinctive from both these species in its hypostomatous leaves (with stomata only on abaxial side) and very abundant glandular trichomes on adaxial side. Besides that, *S. burchellii* differs from *S. erosa* and *S. glutinosa* in common occurrence of tannins in its epidermal cells (Jordaan & Kruger 1992), whereas *S. glutinosa* is distinctive from two former species in having unicellular non-glandular trichomes along with multicellular glandular ones (Madani & Farouk 2019). Our data show, therefore, that the distribution of stomata between leaf sides can vary significantly within the genus *Searsia*. The amphistomaty (i.e. the presence of stomata on both leaf sides), the condition reported in *S. burchellii* and *S. glutinosa*, provides an advantage over hypostomaty in gas-exchange between mesophyll and atmosphere that allows for greater photosynthetic productivity (Drake et al. 2019). This feature is shown as adaptive for plants of open vegetation with high light environment (Jordan et al. 2014, Muir 2018). At the same time, the higher risk of desiccation due to increased transpiration surface, lower ability to buffer fluctuations of water potential while stomata are open, and greater susceptibility for entry of foliar pathogens through stomata in the upper epidermis are considered as the costs of amphistomatous leaf morphology (McKown et al. 2014, Drake et al. 2019). Due to these costs, the amphistomaty is a much less common condition within angiosperms than hypostomaty.

As all three species (*S. erosa*, *S. burchellii* and *S. glutinosa*) inhabit sunny and dry habitats, the difference in their stomatal distribution is unlikely related to the variations in light and water availability. More likely, it must be explained in terms of trade-off between carbon gain and leaf protection against fungal pathogens, herbivores or abiotic factors, as it has been shown for the intraspecific variation of leaf traits in *Populus trichocarpa* (McKown et al. 2014). Leaves of *S. erosa* demonstrate a prominent labour division between the abaxial side covered with abundant glandular trichomes that may contribute to resistance against insect pests, microbial pathogens, and/or atmospheric ozon stress (Wagner 1991, Li et al. 2018), and the stomata-bearing adaxial side providing the gas exchange between mesophyll and environment. *Searsia burchellii* and *S. glutinosa* have seemingly less protected leaves with better uptake of carbon dioxide than *S. erosa*. The scarcity of glandular trichomes in *S. burchellii* is thought to be partially compensated by the abundance of tannin deposits in its epidermal cells that may also be involved in protection against reactive oxygen species, UV radiation and/or herbivory (Constabel et al. 2014). A comparative eco-physiological study of *Searsia* species is required to assess the influence of different environmental factors on interspecific variation of their stomata patterning and quantity of glandular trichomes. As the essential oils from some individuals of *S. erosa* show high antimicrobial activity (unpublished results), the abundance of glandular trichomes is thought to be one of preconditions for its use in traditional medicine.

The occurrence of secretory canals in vegetative and reproductive organs is one of the most prominent features of the family Anacardiaceae (Metcalfe & Chalk 1950, Pell et al. 2011). Their secretion in the majority of studied taxa is characterized as gum-resin, as it consists of both lipophylic substances (terpenoids, occasionally also lipids), and hydrophilic ones (polysaccharides). The presence of gum-resins has been reported in the secretory canals in *Rhus glabra* L. (Fahn & Evert 1974), *Toxicodendron pubescens* Mill. (Vassilyev 2000), *Semecarpus anacardium* L. f. (Bhatt & Mohan Ram 1992), *Mangifera indica* L. (Joel & Fahn 1980), *Spondias dulcis* L. (Sant'Anna-Santo et al. 2006, Lacchia & Guerreiro 2009), *Tapirira guianensis* Aubl. (Lacchia & Guerreiro 2009), and in juvenile shoots of *Anacardium occidentale* L. (Nair et al. 1983). Unlike these taxa, the secretory canals in *Lannea coromandelica* presumably produce only gum, i.e. poysaccharide secretion (Venkaiah 1992), whereas *Spondias mombin* L. as well as flowers and fruits of *Anacardium humile* Hance ex Engl. share the resin secretions containing only lipophylic substances (De Vasconcelos et al. 2016, Lacchia & Guerreiro 2009). Our histochemical data suggest that the secretion of the canals found in cortex, secondary phloem and in leaf midribs of *Searsia erosa* also consists only of essential oils and lipids having no polysaccharide components. Such composition of secretion, that may be termed as oleoresin, is uncommon for the Anacardiaceae. The presence of numerous secretory canals producing the oleoresin can also make condition for traditional medicinal use of this plant species.

CONCLUSIONS

Searsea erosa shows overall similarity in structure of its stem and leaf to other *Searsia* examined to date. The comparison of our observations with other reported data on anatomy of this genus and other Anacardiaceae genera allow us to make the following conclusions:

1. The tangential expansion of outer regions of bark in *S. erosa* and probably in other *Searsia* species is mainly performed by the considerable increase in volume of axial parenchyma cells in the course of their sclerification. Such a way of bark transformation in the course of its growth in girth is out of scope of bark anatomists.

2. The combination of short vessel elements and minute intervessel pits can presumably be diagnostic for the genus *Searsia*.

3. The distribution of stomata between leaf sides (amphistomaty vs hypostomaty) as well as the abundace of glandular trichomes on the leaf epidermis can vary significantly within this genus *Searsia*. Leaves of *S. erosa* demonstrate a prominent labor division between the adaxial side covered with very abundant glandular trichomes that may contribute to resistance against insect pests, microbial pathogens, and/or atmospheric ozon stress, and the stomata-bearing abaxial side providing the gas exchange between mesophyll and environment.

4. Unlike most members of Anacaridaceae studied to date, the secretory canals in cortex, secondary phloem and vascular bundles of leaf midrib produce oleoresin containing terpenoids (essential oils and lipids). No polysaccharides found in their secretion.

5. The secretion of terpenoids in abundant glandular trichomes on leaves and in secretory canals found in cortex, secondary phloem and leaf midribs of *S. erosa* is a presumable reason of the use of this plant in traditional medicine of Basotho people.

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