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Bark anatomy of chosenia (*Salix arbutifolia*, Salicaceae): origin of cuticular cracks and mechanism of cork abscission

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Abstract. The microscopic structure of the bark of chosenia (*Salix arbutifolia*) has been examined in detail. Its bark exhibits several anatomical traits characteristic of other *Salix* species, including the presence of phellem cells with thick lignified walls in the first periderm coupled with exclusively non-sclerified phellem cells in subsequent periderms. Detailed anatomical observations confirmed that these thick-walled cells belong to the periderm rather than to the multiple epidermis, as some authors have suggested. *S. arbutifolia* is similar to *S. cardiophylla*, its closest phylogenetic relative, in possessing an exceptionally thick cuticle on the epidermis of young twigs. This cuticle undergoes tangential expansion and thickening during the vertical contraction and horizontal stretching of epidermal cells. The openings observed on the cuticular surface of *S. arbutifolia* are expansion cracks (i.e., ruptures in the protective tissues subtended by newly formed periderm) rather than lenticels (i.e., transformed parts of the existing periderm). In the subsequent periderms of chosenia, the phellem is subdivided into an outer layer of thin-walled (phelloid) cells and an inner layer of densely packed cells with thicker walls. The outer phellem layers facilitate the separation and shedding of bark flakes, while the inner phellem layers protect the bark surface exposed after their abscission. Thus, the two-layered phellem enables the regular shedding of the outer regions of the rhytidome, contributing to the shaggy appearance of mature bark - a distinctive feature of *S. arbutifolia* compared to other *Salix* species.

The article contains 7 Figures, 2 Tables, 47 References.

Keywords: *Salix*, chosenia, bark, periderm, phellem, phelloid cells

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Анатомия коры чозении (*Salix arbutifolia*, Salicaceae): происхождение кутикулярных трещин и механизм опадения пробки

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Аннотация. Подробно изучена микроскопическая структура коры чозении (*Salix arbutifolia*). Ее кора обладает рядом анатомических особенностей, характерных и для других видов *Salix*, включая наличие клеток феллемы с утолщенными одревесневшими стенками в первой перидерме в сочетании с несклерифицированными клетками феллемы в последующих перидермах. Подробные анатомические наблюдения подтвердили, что эти толстостенные клетки относятся к перидерме, а не к многослойному эпидермису, как предполагали некоторые исследователи. *S. arbutifolia* похожа на *S. cardiophylla*, филогенетически самый близкий, тем, что имеет исключительно толстую кутикулу на эпидермисе молодых веточек. Эта кутикула подвергается тангенциальному расширению и утолщению во время вертикального сжатия и горизонтального растяжения клеток эпидермиса. Крапинки и рубцы, наблюдаемые на кутикулярной поверхности *S. arbutifolia*, являются вертикальными разрывами (т.е. просветы в защитных тканях, под которыми закладывается новая перидерма), а не чечевичками (т.е. трансформированными частями существующей перидермы). В последующих перидермах чозении феллема подразделяется на внешний слой тонкостенных (феллоидных) клеток и внутренний слой плотно упакованных клеток с более толстыми стенками. Наружные слои феллемы служат для отделения и сбрасывания чешуек коры, а внутренние защищают её обнажающуюся поверхность. Таким образом, двухслойная феллема обеспечивает регулярное опадание внешних участков ритидома, что приводит к появлению лохматой зрелой коры, отличительной черты *S. arbutifolia* по сравнению с другими видами *Salix*.

Ключевые слова: *Salix*, чозения, кора, перидерма, феллема, феллоидные клетки

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Introduction

Chosenia (*Salix arbutifolia* Pall.) is a tall tree commonly found on alluvial deposits in river valleys throughout Northeast Asia. This species is unique in the region for forming forests and maintaining its tree form even within the tundra zone north of the Arctic Circle [1]. The extensive range of chosenia extends from Lake Baikal and the Lena River to the Pacific coast of Asia, and from Korea and Northeast China to the Chukchi Peninsula.

S. arbutifolia is distinguished from other *Salix* species by the structure of its vegetative buds, as well as its wind pollination and floral traits, which include pendant catkins, short staminal filaments, and the absence of nectaries associated with this syndrome. Based on these differences, Nakai [2] classified *Chosenia* as a separate, monospecific genus. However, this generic segregation from *Salix* has not been supported by molecular evidence [3-6] and is accepted by most modern taxonomists [7-10].

While certain features of bark appearance are widely utilized in the systematics of willows (e.g., [7, 11, 12]), the diversity of bark microstructure within the genus *Salix*, as well as among other members of the Salicaceae family, remains underexplored. The bark anatomy of several *Salix* species has been investigated by various researchers [13-20]. Notably, the microstructure of the bark in *S. arbutifolia* has been examined in detail by Malychenko [21] and by Eremin and Kopanina [13]. However, several important aspects of the bark anatomy of this species remain unclear.

The shaggy appearance of mature bark, characterized by detached strips of rhytidome, is regarded by Skvortsov [11] as a distinguishing feature of *S. arbutifolia*, which is rare among most willows, with the exception of *Salix triandra* and a few related species. Anatomical data from other plants exhibiting peeling bark [22-25] suggest that this bark architecture involves the presence of regular separation layers - specifically, layers of fragile tissues that facilitate the regular abscission of the outer portions of the bark. These layers are typically associated with protective layers of dense (usually sclerified) tissues that cover the surfaces exposed after the outer bark is shed. Malychenko's [21] observations indicate that the distribution of separation and protective tissues in mature bark can vary among different *Salix* species: while the rhytidome strips in chosenia peel along

a few outermost cell rows of phellems in subsequent periderms, in *S. triandra*, they are abscised along the collapsed phloem. However, Malychenko [21] has not described the structural differences between the cells forming the separation layer and the protective layer in the phellem of chosenia in sufficient detail.

Also, the available data on the structure of young shoots of chosenia [13, 21] indicate the presence of a prominent thick solid layer (approximately 15-20 μm) covering the surface of its twigs. Malychenko [21] suggested that this covering layer consists of the thicker outer walls of epidermal cells, while Eremin and Kopanina [13] consider it to be a cuticle. Further anatomical examination is necessary to clarify its nature.

The presence of small diamond-like spots has been observed on the surface of chosenia twigs (e.g., [1]). These spots, typically found on willow twigs, are commonly referred to as lenticels (e.g., [26]). However, it is challenging to accept that lenticels, which are transformed portions of the periderm [27-29], can be located on a surface covered by epidermis. Therefore, the morphological nature and origin of lenticel-like spots on the twig bark of chosenia and other *Salix* species require further clarification.

Although the bark is literally in front of the eyes of any botanist, its appearance attracts very little attention from plant researchers. Consequently, the relationships between the macro- and microstructure of bark remain poorly understood. To date, Whitmore has made the most significant contributions to this field [30-33]. He identified seven macromorphological types of bark along with associated anatomical characteristics. Unfortunately, this classification is challenging to apply due to the lack of sufficient illustrations in his publications.

Recently, Shtein et al. [24] proposed a classification of four architectural bark types, referred to as stretched, exfoliating, furrowed, and peeling barks, which can be identified by their macroscopic traits. These types represent four possible combinations of binary states based on two features: (1) the ability or inability of the outermost layers of bark to maintain continuity during tangential expansion, and (2) the presence or absence of separation layers. This classification raises further questions regarding the mechanisms of bark expansion and abscission, as these processes significantly influence the appearance of the bark.

This study aims to elucidate the relationships between macroscopic appearance and microstructure of the bark of chosenia (*S. arbutifolia*) through a detailed anatomical examination. Additionally, we seek to evaluate the significance of certain bark traits for the systematics and diagnostics of the boreal Salicaceae.

Material and methods

The bark samples collected from the twigs, branches, and trunks of *S. arbutifolia* were gathered by the second author during the 2023 field season in the mainland of the Kamchatka Territory, specifically in the Olyutorsky district (Table 1). These samples, along with herbarium vouchers, are deposited at the Komarov Botanical Institute in St. Petersburg, Russia.

From each tree, portions of twigs and branches of varying ages (Fig. 1), as well as pieces of mature bark have been collected from the trunks at approximately 30 cm above the ground, were gathered and preserved in 70% alcohol.

Table 1

Information on samples and locations of their collection

Sample number	Locality	Geographic coordinates	Habitat	Tree height H and maximum diameter D_{max}
KS6_23	NE of Achaivayam village, Apuka river valley	61°00'46"N, 170°31'14"E	Poplar and chosenia open forests on pebble deposits	H = 4 m, D_{max} = 9.2 cm
KS14_23	S of Srednie Pakhachi village, Pakhacha river valley	60°49'25"N, 169°03'54"E	Chosenia forest with sparse herb layer	H = 8 m, D_{max} = 12 cm
KS23_23	S of Srednie Pakhachi village, Pakhacha river valley	60°49'25"N, 169°03'54"E	Chosenia forest with sparse herb layer	H = 6.5 m, D_{max} = 8 cm
KS24_23	S of Srednie Pakhachi village, Pakhacha river valley	60°49'25"N, 169°03'54"E	Chosenia forest with sparse herb layer	H = 7 m, D_{max} = 10.5 cm

Note. KS = Konstantin Skvortsov, abbreviation of collector and his reference number.

Additionally, photographs of the bark surface from different sections of these trees were taken in the field. For a comparative study of bark morphology, we also examined the chosenia trees cultivated in the Dendrarium of the Komarov Botanical Institute.

Fig. 1. The bark appearance at different stages of development: A-B Twigs under a stereomicroscope. A - current year's twig. Scale = 1 mm, B - 1-year-old twig. Scale = 1 mm (samples from Dendrarium), C-E The photographs were taken during field work, C - age-related changes in the periderm between 3- and 4-year-old twigs. Scale = 5 mm (KC24-23), D - Lanceolate to diamond-like shape cracks on thin trunk. Scale = 8 mm (KC14-23), E - the bark surface of a mature trunk (KC24-23)

Transverse and longitudinal (radial and tangential) sections of the bark were prepared using a freezing microtome (Ernst Leitz GMBH, Wetzlar, Germany). Unstained sections were mounted in glycerol and examined under a light microscope. Other sections were stained with a mixture of alcian blue/safranin (1 : 1) and subsequently mounted in Canada balsam. The maceration of secondary phloem was done in Jeffrey's solution for 24 hours prior to mounting the macerated material in glycerol.

Sections were examined using an Olympus CX-43 and BX-51 microscope (Olympus, Japan). Suberin and lignin were assessed for their autofluorescence [34-36] using the Olympus BX-51 microscope equipped with a fluorescence filter cube for ultraviolet excitation (wideband) U-MWU2. Additionally, histochemical tests were conducted using Sudan IV [37] for suberin and phloroglucinol/hydrochloric acid [38] for lignin on hand-made and freezing microtome bark sections.

Digital images and measurements of anatomical samples were captured using an Olympus DP74 digital camera (Olympus Corp., Japan) with cellSens Standard 4.1 software (Olympus, Japan). The bark macrostructure of young stems was examined under a Zeiss Stemi 2000 CS stereomicroscope, also equipped with the Olympus DP74 digital camera.

Results

The surfaces of twigs and young stems (up to approximately 2 cm in diameter) are typically smooth and usually glabrous; however, pubescence with simple hairs may occasionally be noticeable near the bud attachments (Figs. 1, A, 1, B). Their coloration ranges from green to yellow, with red to brown areas, and in some instances, they may be entirely red. The young twigs and stems often exhibit a glossy appearance, but in certain cases, a bluish-white coating (presumably wax) may develop on the stem surface. On thicker stems (greater than 2 mm in diameter), lanceolate to diamond-shaped pale brown superficial cracks, approximately 0.5 mm in width, are present. These cracks are sometimes arranged in vertical or slightly diagonal series.

The epidermis is uniseriate and primarily composed of upright rectangular cells measuring 5-13 μm in tangential size and 8-33 μm in radial size on current-year twigs (Figs. 2, A, 2, B). On two-year-old twigs, the epidermis consists mainly of square cells, which range from 6-26 μm in tangential size and 9-19 μm in radial size. In contrast, three-year-old and older twigs exhibit predominantly dome-like, cone-like, and flattened cells, with tangential sizes of 9-27 μm and radial sizes of 4-19 μm. Notably, the radial size of epidermal cells tends to decrease in older twigs, while their horizontal size increases with age (Figs. 2, C, 2, D and Fig. 3). The epidermis is covered by a prominent cuticle, which measures 7-23 μm in thickness on annual twigs and can reach up to 27 μm on older twigs (Table 2, Fig. 4). The thickness of the cuticle generally increases with age (Table 2). In unstained sections, a saturated yellow outer layer of the cuticle and a pale yellowish inner layer, with a gradual transition between them, are discernible. In stained sections, the colors range from saturated yellow to pale crimson. In the cuticle of older twigs, light dome- or cone-like portions

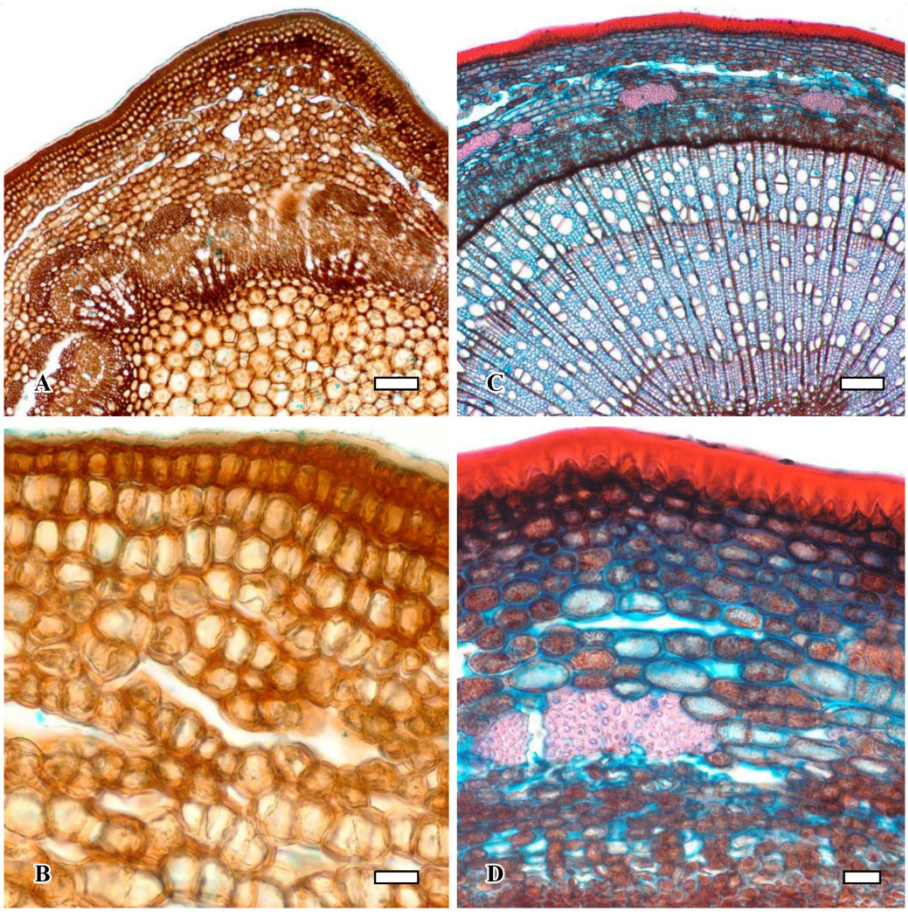

Fig. 2. A-D Transverse section, A-B current year's twig, cuticle and epidermis: A - scale = 100 μm, B - scale = 20 μm, C-D 4-years-old twig with thick cuticle: C - scale = 100 μm, D - scale = 20 μm

of the inner layer can be distinguished over the adjacent epidermal cells. The cuticle and epidermis are interrupted by expansion cracks that occur with an increase in stem circumference (Figs. 5, B, 5, C). Stomata are not present in the epidermis.

The cortex consists of 10 to 15 layers of isodiametric parenchyma cells. Vertically elongated cells, which could be attributed to cortical collenchyma, were not observed. The outer cortical region contains 4 to 7 layers of smaller parenchyma cells, measuring 8 to 20 μm in tangential diameter (average 14.5 μm), without prominent intercellular spaces. In contrast, the deeper part of the cortex is composed of larger cells, ranging from 15 to 42 μm in tangential diameter (average 21.6 μm), which are loosely arranged and feature prominent intercellular spaces (Fig. 2, A). The dilatation of the cortex is influenced by the expansion and anticlinal divisions of parenchyma cells, forming strands of 2 to 8 cells,

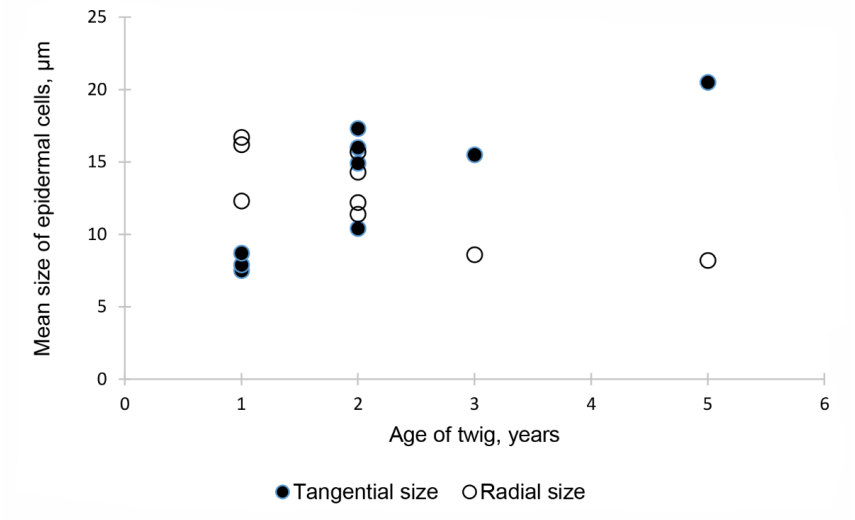

Fig. 3. Age transformations of epidermal cell size and cuticle thickness in *S. arbutifolia.* Average tangential (dots) and radial (circles) sizes of epidermal cells plotted against the twig age

as well as by the enlargement of intracellular spaces (Fig. 2, C). Druses occasionally occur in dilated cortical parenchyma cells, while sclereids were not observed.

Table 2

Size of epidermal cells and cuticle thickness on the twigs of different age and diameter

Sample. Age of twig	Twig diameter, mm	Cuticle thickness, µm mean/min-max	Size of epidermal cells, µm mean/min-max	
			Tangential size	Radial (vertical) size
KC6-23				
1 year	2.1-2.3	9.4/7.3-11.2	7.5/4.9-10.9	16.2/13.6-19.3
2 year	4.7-4.8	16.2/13.6-19.3	14.9/10.2-23.3	11.4/8.7-14.1
KC14-23				
1 year	1.8-2.2	18.7/14.6-22.6	7.9/5.5-11.3	12.3/9.3-16.7
2 years	4.5	20.1/17.0-23.7	16.0/9.5-26.6	15.7/11.6-21.6
KC23-23				
1 year	1.5-1.6	15.7/13.2-18.1	8.7/5.4-13.3	16.7/7.8-33.0
3 years	2.4-2.6	20.2/16.7-27.2	15.5/9.0-26.6	8.6/4.3-12.5
5 years	6.4-6.5	22.5/14.5-26.5	20.5/12.5-27.0	8.2/4.8-13.9
KC24-23				
2 years	1.6	14.7/12.3-17.1	10.4/6.0-15.0	14.3/10.4-18.8
3 years	2.3-2.8	23.2/17.2-26.2	17.3/11.1-24.9	12.2/8.5-16.9

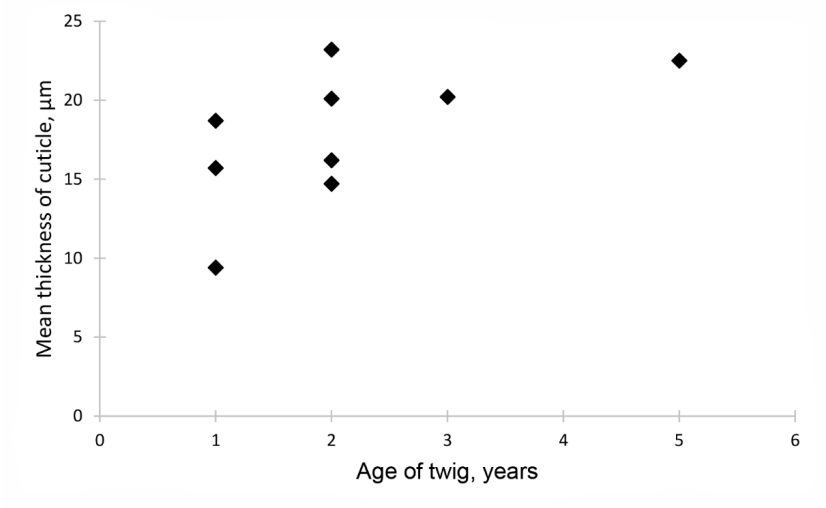

Fig. 4. Average thickness of cuticle plotted against the twig age

Protophloem fibers with thin walls (Fig. 2, A) are arranged in a nearly continuous 3-4-seriate band in 1-2-year-old twigs, while in older twigs, they are thick-walled and found in separated clusters of 4 to 50 or more fibers (Fig. 2, D).

The bark on branches and thin trunks (approximately 3 to 5 cm in diameter) is green or greenish-brown, sometimes pale brown, exhibiting a smooth to rugose texture (Figs. 1, C, 1, D). It features shallow vertically elongated fissures measuring 1-3 mm, lanceolate to diamondshaped sometimes with a darker center. The margins of the cracs occasionally reddish. Lenticels are absent. In some instances, structures resembling lenticels were observed; however, upon closer examination, they were identified as cracks with a darker center. The bark on thicker branches and trunks (greater than approximately 5-7 cm in diameter) varies from brownish to gray, displaying a rugose texture with V-shaped, narrow vertical fissures (less than 1 cm wide). These fissures form flattened ridges approximately 1-3 cm in width and can extend 5-50 cm or more in length when still in tight contact with the underlying layers. In the superficial layers, these

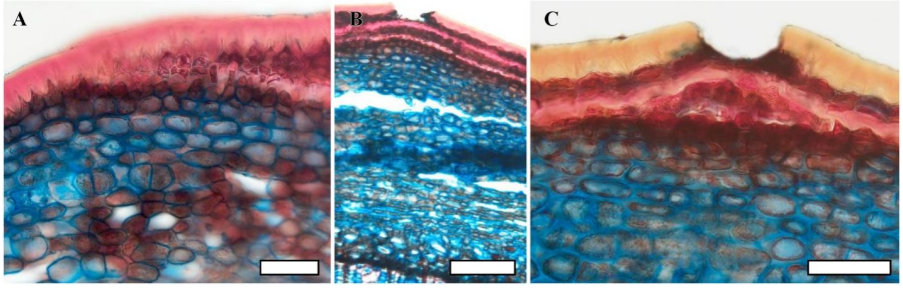

Fig. 5. Transverse section (KC24-23), A - epidermis and first periderm initiation in 3-years-old twig Scale = 50 µm, B-C epidermis with expansion crack, stratified phellem, dilated cortex in 5-years-old twig. B - Scale = 100 µm, C - Scale = 50 µm

ridges develop horizontal cracks and become detached, resulting in shaggy, curved, ribbon-like flakes measuring 5-15 cm in length (Fig. 1, E).

The first periderm is primarily initiated in the subepidermal layer of cortical parenchyma, with some development occurring in the epidermis, in 3-year-old twigs (Fig. 5, A). This periderm persists beneath the epidermis even in more mature bark, up to 6-7 years of age. The phellem typically consists of a single layer of dome-shaped cells, measuring 10-30 μm in height, with thick (6-18 μm) sclerified outer walls, and 1-3 layers of thin-walled, flattened cells, measuring 3-12 μm in height. Occasionally, the phellem is stratified, featuring 2 to 8 layers of thick-walled cells interspersed with zones of thin-walled cells (Fig. 5, B). The phelloderm comprises one or two rows of thin-walled, flattened cells, measuring 10-20 μm in height.

Subsequent periderms are initiated as short or long arches in the deeper layers of the cortex or secondary phloem. The phellem consists of 8 to 15 layers of thin-walled cells that exhibit no signs of sclerification, while the phelloderm is typically uniseriate, although it may be poorly defined. The outer zone of the phellem is composed of 2 to 4 layers of more or less isodiametric phelloid cells with non-suberized walls, whereas the inner zone consists of 6 to 12 layers of radially flattened cells with suberized walls (Figs. 6, A, 6, B). No crystals were detected.

Rhytidome is reticulate, featuring 2 to 4 (or more; the exact number is difficult to determine due to the brittle and crumbling nature of the structure) zones of secondary phloem or cortex, which are separated by successive periderms (Fig. 6, C). The abscised flakes of outer bark are shed along the outer phellem zone, composed of phelloid cells.

Secondary phloem comprises bands of conductive elements interspersed with axial parenchyma, which consists of 8 to 20 tangential layers. These are alternated with bands of phloem fibers associated with crystalliferous axial parenchyma, containing 4 to 8 tangential layers (Fig. 7, A). The transition from conducting to non-conducting secondary phloem is gradual. The obliteration of the sieve tubes occurs within 9 to 15 tangential bands that contain these elements.

In conducting secondary phloem, the sieve tube members measure 30-48 μm in tangential diameter, with lengths ranging from 140 to 434 μm (average 268 μm). The sieve plates can be simple or compound, featuring 4-10 sieve areas located on oblique cross walls. Axial parenchyma is organized in strands of 6 to 18 cells, exhibiting a brown content that stains red-brown with safranin and alcian blue. Phloem fibers are relatively long, measuring between 474 and 1360 μm (average 874 μm) in length. Their walls are thick and double-layered. Crystalliferous cells, associated with the fibers, contain prismatic crystals (Fig. 7, A). Secondary phloem rays are uniseriate (Fig. 7, B) and are primarily composed of procumbent cells, with rare square cells observed in radial sections.

Dilation of the non-conducting secondary phloem occurs due to tangential stretching of both axial parenchyma and phloem rays (Fig. 6, C). Anticlinal divisions of ray cells give rise to 2- and 3-seriate rays, which are found in the outermost regions of the dilated phloem cells. Starch grains are abundant in the

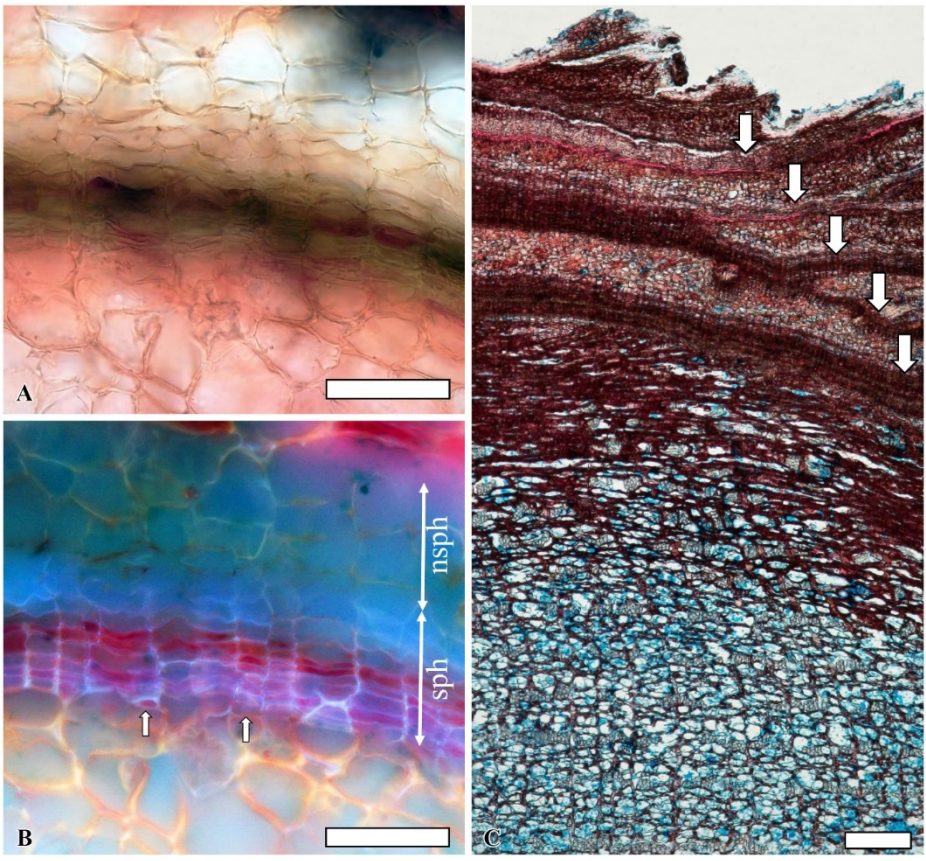

Fig. 6. A-B Subsequent periderm. Scale = 50 μm (fresh samples from the Dendrarium of the Komarov Botanical institute). A - usual light, B - fluorescence. In outer zone of phellem cells with non-suberized walls (nsph), in inner with suberized walls (sph). Blue glow (autofluorescence) indicates presents of suberin in the cell walls. Small arrows pointed to phellogen, phelloderm on this photo is not visible. C - rhytidome, non-conducting secondary phloem. Scale = 200 μm (KC14-23)

dilated ray cells. Druses are present in the radial and axial parenchyma of the dilated regions of the phloem. Sclereids are absent.

Discussion

The suite of bark features observed in *S. arbutifolia*, including epidermis with a prominent cuticle, clusters of long secondary phloem fibers arranged in a tangential pattern, abundant crystalliferous axial parenchyma associated with these clusters, compound sieve plates, uniseriate phloem rays, and the formation of rhytidome, is also characteristic of other species of *Salix* and *Populus* studied to date [13-15]. However, the available data on the bark anatomy of other members of the Salicaceae family [39, 40] are insufficient for assessing the taxonomic significance of our findings in the broader context of the entire family.

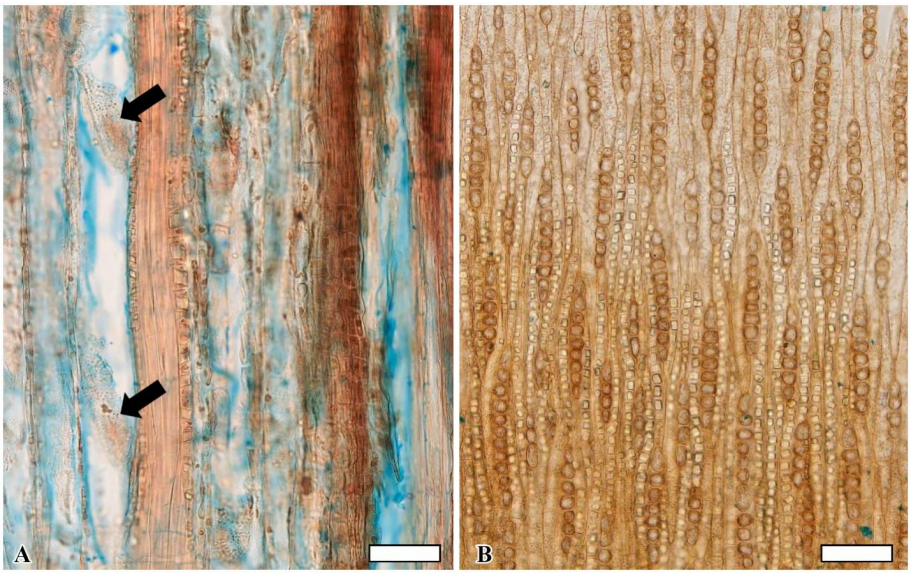

Fig. 7. Secondary phloem: A - radial section, sieve tube with sieve plates (arrows), crystalliferous cells associated with fibers. Scale = 50 μm (KC23-23), B - tangential section, uniseriate rays and crystalliferous cells with prismatic crystals. Scale = 100 μm (KC6-23)

Our observations confirm the presence of an exceptionally thick cuticle (up to 27 μm in thickness) on the epidermis of young twigs in *S. arbutifolia* [13] while the epidermal cells exhibit uniformly thickened walls, contrary to Malychenko's [21] suggestions. Although other *Salix* species also possess a prominent cuticle, its thickness is generally about half that of *S. arbutifolia*. Notably, a similarly thick cuticle (approximately 20 μm) has been reported in *S. cardiophylla*, whose bark Eremin and Kopanina [13] described under the name *Toisusu urbaniana*. This species, native to Japan and Sakhalin, shares similarities with chosenia in both habit and ecological strategy and is considered as the closest relative to *S. arbutifolia* based on molecular phylogenetic data [41]. The presence of a very thick cuticle in both species further supports their close relationship. It is highly likely that this structure plays a role in protecting the bark of young twigs from the formation of ice crystals during the frost season [42, 43]. Such anti-icing protection is especially crucial for *S. arbutifolia* as it is the only tree that extends far north of the Arctic Circle [1].

The cuticle on the twigs of *S. arbutifolia* remains continuous while undergoing tangential expansion over a period of at least five years. Our observations and quantitative data (Table 2) suggest that cuticle thickness also increases significantly as the epidermal cells experience vertical contraction due to tangential stretching. This process is believed to begin with the partial separation of epidermal cells in their upper regions, which is accompanied by the deposition of cuticular outgrowths along the radial cell walls. Consequently, cavities form over the epidermal cells on the lower surface of the cuticle. The cuticular material is then deposited into these cavities by the cells during their vertical contraction. This scenario is supported by the observed pattern of older cuticle, which

features prominent lighter dome- or cone-like portions over the stretched epidermal cells. After the obliteration of the epidermis observed on the five-year-old stems, fragments of cuticle can persist on the cork surface for an extended period (Fig. 5).

The initial periderm of *S. arbutifolia* is distinctly different from its subsequent periderms due to the presence of one or a few rows of cells with thick-walled, lignified walls in its phellem. Kurczyńska [16, cited from: 17] identifies this feature as characteristic of the genus *Salix*. Available data confirm that this trait is present in all willows [13-15], but it is also observed in certain poplars, such as *Populus davidiana* [13].

It should be noted that Kurczyńska [16, cited from 17] interpreted these layers of thick-walled cells as a multiple epidermis rather than as periderm. However, Słupianek et al. [17] did not find any traits of a multiple epidermis in *Salix polaris*, and our data confirm this finding. As we observed in *S. arbutifolia*, the bark on its twigs is covered by a uniseriate epidermis from the very beginning of their formation, while the typical initiation of periderm occurs only in the third year. The phellogen is commonly initiated in the subepidermal cell layer, which is not collinear with the epidermal cells. Thick-walled cells located beneath the epidermis are aligned in radial files with other cells derived from the phellogen. A similar mode of periderm formation has been observed by Słupianek et al. [17] in *S. polaris*. Therefore, we propose that the outer region of the bark on the twigs of *S. arbutifolia*, and likely other *Salix* species, consists of a uniseriate epidermis and the first periderm rather than a multiple epidermis, as suggested by Kurczyńska [16, cited from 17].

Tiny diamond-like light dots appearing on the cuticular surface of the young bark of *S. arbutifolia* as well as other *Salix* species are commonly identified as lenticels (e.g., [21, 26]). However, our observations suggest that these structures are not lenticels but expansion cracks, as defined by Schneider [44], which are ruptures in protective tissues supported by newly formed periderms. Schneider [44] has described their formation in detail for the bark of the lemon tree (*Citrus* x *limon*). Such mechanism of young bark dilatation undoubtedly occurs in many other taxa, yet these structures are often confused with lenticels. Unlike the expansion cracks, lenticels are the structures of exclusively peridermal origin, specifically the portions of a persistent periderm exhibiting a particular type of phellogen activity [27-29]. While the filling tissue in lenticels is a modified phellem, the expansion cracks may contain non-peridermal tissue, such as cortical tissue. It is possible that the lenses of periderm formed under expansion cracks on young twigs of *S. arbutifolia* can develop into true lenticels found on the bark of older (7-year-old) stems. Further investigation on the relationship between expansion cracks and lenticels during bark dilatation would be of great interest to the field of plant anatomy.

Our observations confirm that Malychenko's data [21] indicated the flakes of the outer rhytidome in *S. arbutifolia* shed from the outermost layer of phellem in subsequent periderms. We found that the cells in this layer possess thin, non-suberized walls, while other phellem cells contain suberin in their walls. Clearly, the suberized cell walls in the inner phellem layer provide protection to the bark

surface exposed after abscission. Therefore, this two-layered phellem can be regarded as a microstructural feature that facilitates the regular abscission of flakes on the mature bark of *S. arbutifolia*. The presence of two layers in phellem is also corroborated by the microphotographs of Eremin and Kopanina [13] for chosenia, although it has not been reported for other species within the Salicaceae family.

The phellem, serving as a separation layer, is the most common characteristic among taxa with peeling bark. This pattern has been observed, for instance, in several species of *Eucalyptus* L'Hér. [45], *Melaleuca* L. [46, 47], *Lonicera* L. [13, 15], *Buddleja* [22, 23], *Cliffortia*, and *Polylepis* [25]. In most of these taxa, however, the sclerified secondary phloem functions as a protective layer, whereas in *Polylepis*, there are no solid protective layers at all. In contrast, in *S. arbutifolia*, we found a well-defined separation layer and a protective layer located within the phellem. This is an uncommon condition that has not yet been reported in other taxa.

Conclusion

S. arbutifolia shares several bark characteristics with other species of *Salix* and *Populus*, including clusters of long secondary phloem fibers arranged in a tangential pattern, crystalliferous axial parenchyma associated with these clusters, compound sieve plates, uniseriate phloem rays, and the formation of rhytidome. However, *S. arbutifolia* is distinctive from most *Salix* species due to its exceptionally thick cuticle. This conspicuous cuticle has also been reported in *S. cardiophylla*, suggesting a close phylogenetic relationship between these two species. The openings observed on the cuticular surface of *S. arbutifolia* are expansion cracks (i.e., ruptures in the protective tissues supported by newly formed periderms) rather than lenticels (i.e., transformed parts of the existing periderm). The first periderm of *S. arbutifolia*, unlike subsequent periderms, consists of thick-walled phellem cells. This layer of thick-walled cells should not be classified as a multiple epidermis, as some authors have suggested. The two-layered phellem, with the outer layer composed of non-suberized thin-walled cells, is a microstructural feature that facilitates the regular abscission of flakes on mature bark. Additionally, there is a well-defined separation layer and a protective layer within the phellem. This condition is uncommon and has not yet been reported in other taxa.

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