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Heteroblastic leaf development on the generative shoots of some dicotyledons

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Summary: The results of developmental and structural studies on three leaf formations of generative shoots of *Nandina*, *Mahonia* and *Helleborus* species of different eco-geographical origin from native area and introduced ones are outlined here. The development of three leaf formations (cataphylls, assimilative leaves, bracts) from different parts of leaf primordia, in our view, is the base of leaf heterogeneity. The results of comparative studies of developmental and anatomical structures show that homological formations (cataphylls and assimilative leaf bases) of shrubs and herbaceous forms are characterized by their considerable similarity. More vigorous development of leaf bases are due to their multifunctionality. Amphistomacy and the presence of homogeneous mesophyll are distinctive features of cataphylls and bracts, unlike assimilative leaf laminas. Cataphylls of herbaceous forms lack many special defensive features which are characteristic of many woody ones. The defensive function of cataphylls is supplied by anatomical features of the epidermal complex and by the presence of anthocyans and tannin substances as well. Great anatomical similarity of bracts and sepals are revealed. It confirms the possibility of bracteous origin of the calyx in Berberidaceae and Ranunculaceae.

Keywords: Berberidaceae, Ranunculaceae, *Nandina*, *Mahonia*, *Helleborus*, cataphylls, bracts, assimilative leaves, heteroblastic development, anatomy

The leaf heterogeneity of the generative shoot is a widely extended phenomenon in woody and herbaceous dicotyledons with buds with scales.

At first it was described by Schimper (1836) as the occurrence of three leaf formations: cataphyll (cataphyllum), foliage leaf (folium medianum), hypsophyll (hypsophyllum). Cataphylls are situated at the shoot base and usually protect axillary buds. They have a wide base, simple outlines and many of them lack stipules and lamina. Usually they are 'squamae gemmae'. Foliage leaves are characterized by the greatest morphological differentiation. Hypsophylls develop in the reproductive zone of the shoot and usually subtend axillary flowers (bracts) or inflorescence branches. They are underdeveloped and weakly dissected sessile leaves with a narrow base of different color, often green or similar to the color of axile flowers. In this case their role of secondary attractants cannot be excluded.

The heteroblastic leaf development of the individual shoot was studied from different views. Many investigators noticed a common morphological nature of all three leaf categories. The evidence of this common nature are transitional forms between different leaf categories, the native remetamorphosis of bud scales of the lammas shoots (rami Johanni) into small green leaves with underdeveloped base in *Quercus*, *Fagus*, *Fraxinus*, *Picea* (Wretschko 1864; Serebryakov 1950) and experimental data concerning the voluntary change of leaf primordia morphogenesis (Klebs 1903; Goebel 1923). Under certain conditions Klebs (1903) was not only successful in mutual transformations of different leaf forms, but he was also able to change arbitrarily the trend of morphogenesis of the whole generative shoot. He demonstrated the sprouting of a just formed inflorescence apex of *Veronica chamaedrys* L. into a photophilous shoot. Its first leaves kept size, shape, nature of attachment (sessile), leaf arrangement (spiral) and villosity (glandular) as well as primordia of axillary flowers, which are distinctive to bracts. The next leaves were larger, short-petioled and decussate with axillary vegetative buds. So they were typical 'folia medianae'.

The development and anatomy of bud scales in many woody and shrubby specimens of different species have been studied in detail and to a relatively lesser degree also few herbaceous plants with buds with scales (Sacher 1955; Efimova 1959; Vasilevskaya & Shilova 1960; Barykina & Chubatova 1985; Zelinskaya & Lotova 1987; Barykina & Churikova 1989).

In the course of biomorphological reflection, cataphylls are usually compared with laminas of 'folium medianum', with noting a particular more rapid morpho- and histogenesis. Tissues of cataphylls develop quickly. They don't attain the same extent of differentiation which is typical of assimilative leaves (Brick 1914; Foster 1931, 1937; Cross 1938; Vasilevskaya & Shilova 1960) and undergo insignificant ontogenetic changes. Defensive functions of bud scales (from evaporation, sun rays burns, abrupt fluctuations of temperature, withering) are ensured by the development of a thick cuticle, sometimes a periderm on the abaxial side and air cavities, glandular trichomes, which excrete balsam, resinous and sticky substances on the adaxial side and by the presence of anthocyans as well (Efimova 1959). Frost resistance of bud covering cells is increased by the presence of phenolic compounds, including tannin substances (Zelinskaya & Lotova 1987). Anatomical features of cataphylls may vary widely in different species according to taxonomical origin of plants and also according to their eco-geographical origin. So according to Shilova (1974), fine cells of epidermis and high villosity of closely adjoined bud scales are peculiar to more xerophilous hawthorn (*Crataegus turkestanica*) unlike the mesophyllous one (*C. sanguinea*).

There is a relatively small amount of data on macro- and microstructure of bracts. In particular it is noted that their form varies from tiny scales to leaf-shaped structures, from deciduous to always preserved ones, from alternate to verticillate leaf arrangement (Eames 1964). According to Goebel (1923), their small size is determined due to an excessively early and quick development of axile structures. Microstructure of bracts is often considered to be an index of species characteristics (Alimukhamedova 1979) and of the direction of adaptation to the soil-climatic conditions. So, Nigmanova (1979) showed that features of succulence in bracts appeared under the influence of chloride and sulfate salts considering *Salsola orientalis* S. G. Gmel. as example. During the growth on the less salted gypsoferous soil features of scleromorphosis appear.

The heteroblastic leaf development of the individual shoot is considered as an apical meristem reaction to the changes of its physiological status (Allsopp 1964), which is determined by the influence of some exogenous (temperature, day length, water and nutritious substances supplying) and endogenous factors (hormonal level, in particular, the level of gibberellinous substances (Feldman & Cutter 1970), etc.).

The proposal about control action of carbohydrate nutrition to the heteroblastic leaf formation was announced by Goebel (1908).

However, all published data on leaf heterogeneity of the annual shoot don't answer the question: what are the main reasons of structural transformations which are the basis of their heteroblastic development? According to Goethe (1790), one shouldn't study the nature's creatures when they are already formed, but must hang on to their origin. Therefore, it would be expedient to study the real situation of appearance, structure and development of leaf primordia. Eichler (1861) showed that the primordial leaf differentiates into two parts: the basic part (Blattgrund), which gives rise to the base of leaf and scale leaves, and the upper one (Oberblatt), from which the lamina and petiole develop. Thus, another comparative approach to the structure-functional assessment of different leaf formations, as used by the majority of researchers, is needed. It would be more logical, in our view, to compare the cataphyll's anatomy with leaf base and bract microstructure with its lamina, because leaf types are the result of the realization of the developmental programme of either the entire leaf primordium or only of parts with further individual morphogenesis.

No doubt, studying the regularities of macro- and microstructure development of leaves during the shoot morphogenesis of more representatives of different taxonomical groups is important for knowledge of fundamental processes in plant.

Taking into account the considerations mentioned above, we conducted the comparative morpho-anatomical analysis of different leaf formations of annual generative shoots at the evergreen subtropical shrubs *Nandina domestica* Thunb. and *Mahonia japonica* (Thunb.) DC. (Berberidaceae) and at the four *Helleborus* species *H. caucasicus* A. Br., *H. niger* L., *H. foetidus* L. and *H. purpurascens* Waldst. & Kit. (Ranunculaceae) differing by the rhythm of seasonal development and by the ecology of their natural sites.

Materials and methods

Plants of *Nandina domestica, Mahonia japonica* and *Helleborus* species were obtained from the live collections of Nikita Botanical Garden, Sukhumi Botanical Garden and Botanical Garden of Moscow State University and they were collected in nature in Ukraine and Georgia, respectively. The ecological influence on the anatomical structure of the three leaf formations of studied species is relatively insignificant and has a mainly quantitative character.

It is worth mentioning that cultivated plants reveal some sort of smoothing of some structural characteristics, in contrast to those developed in natural habitats. Anatomical sections of three leaf formations were analyzed using light microscope Micromed-3. Images of the sections were taken with light microscope Axioplan-2 Imaging equipped with digital camera AxioCam MRc and processed with Adobe Photoshop.

Histochemical analysis of essential cell substances as well as the secondary products of metabolism was carried out according to the recommendations of the 'handbook of botanical microtechniques' (Barykina et al. 2004).

Regarding our previous investigations of *Nandina domestica* (Barykina & Chubatova 1985) which have shown a close similarity of microstructure of bracts and the leaves of perianth, an anatomical analysis of hypsophylls and sepals was also applied.

Results

Nandina domestica

The monocarpic, monocyclic shoot includes 2–3 bud scales and 7–9 large three times odd-pinnate, longpetioled leaves with a wide base as well. The inflorescence is terminal, bracteose, multiflowered, with peduncle 20–40 cm in length (Fig. 1A).

Bud scales have a well remarkable wavy cuticle. Epidermal cells are thick-walled with small papillas on the adaxial side and unitary stomata, which are slightly submerged into epidermis,

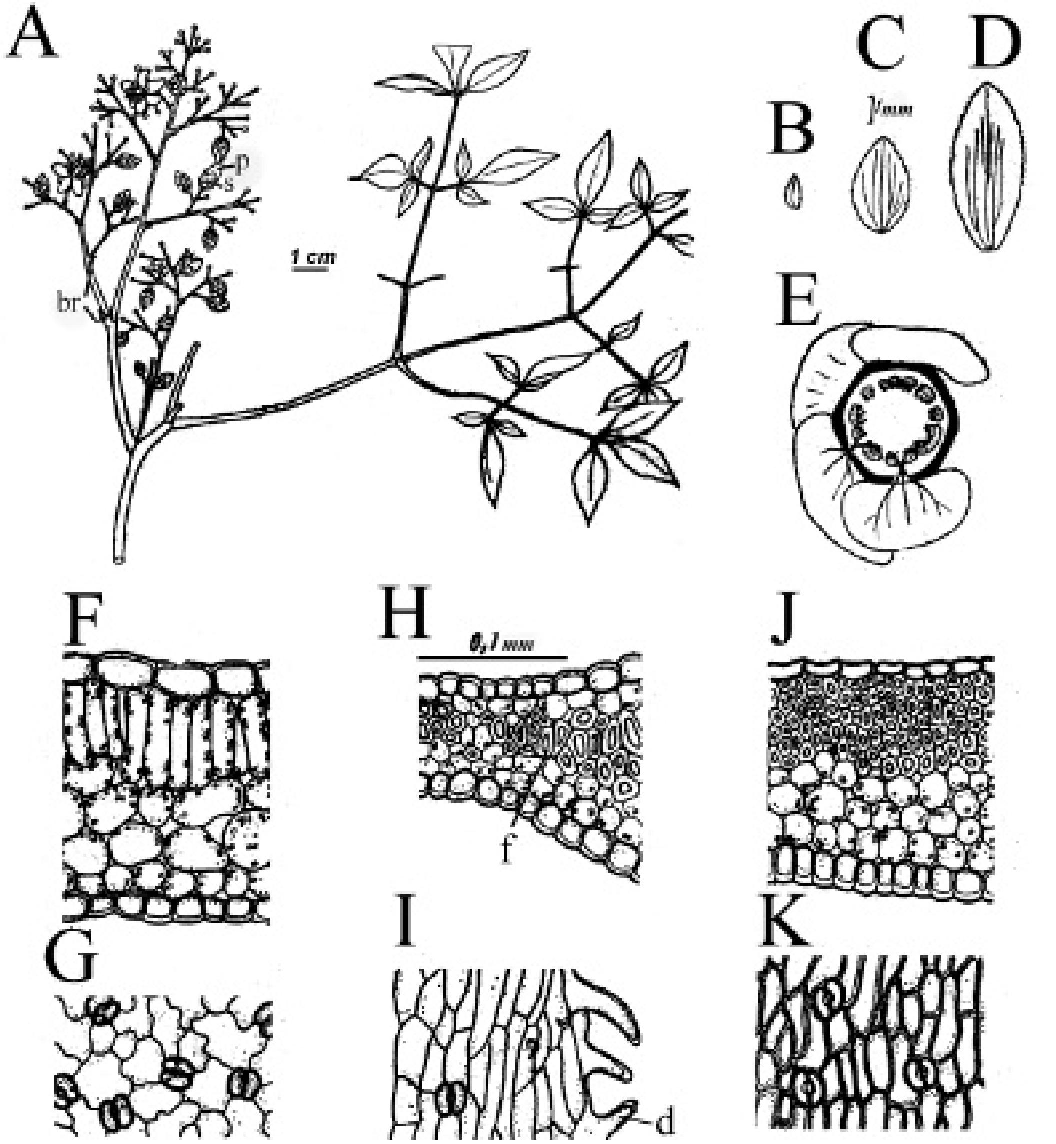

Figure 1. *Nandina domestica*. A – closed bracteous panicle with upper assimilative leaf; B – bract; C, D – sepals; E – scheme of their innervation. Anatomical structure. F, G – leaf lamina; H, I – bract; J, K, – sepal (F, H, J – cross-section; G, I, K – lower epidermis). *br* – bract; *d* – denticle; *f* – fibers; *p* - petals; *s* – sepal.

and tannin substances on the abaxial side. There are small starch grains and groups of sclereids in the homogeneous 20–25-layered mesophyll. Up to 26 vascular bundles are located near the lower bud scale side in the form of a wide arc. Slightly ribbed surface of bud scales is due to the largest vascular bundles (Fig. 2A, B). They are open, with relatively low cambial activity (average number of secondary xylem elements in radial chains is 3–4). Phloem has large bundles of alive protophloem fibres. The second arc of small vascular bundles may differentiate on the adaxial side of the largest bud scales (Fig. 2C).

The base of foliage leaf has little reduced scale leaves and relatively thin edges, which almost entirely envelope the stem (Fig. 2A, D). Leaf base has the same anatomical structure as the bud scales, but it is more vigorous and includes more than 30 layers of a homogeneous mesophyll.

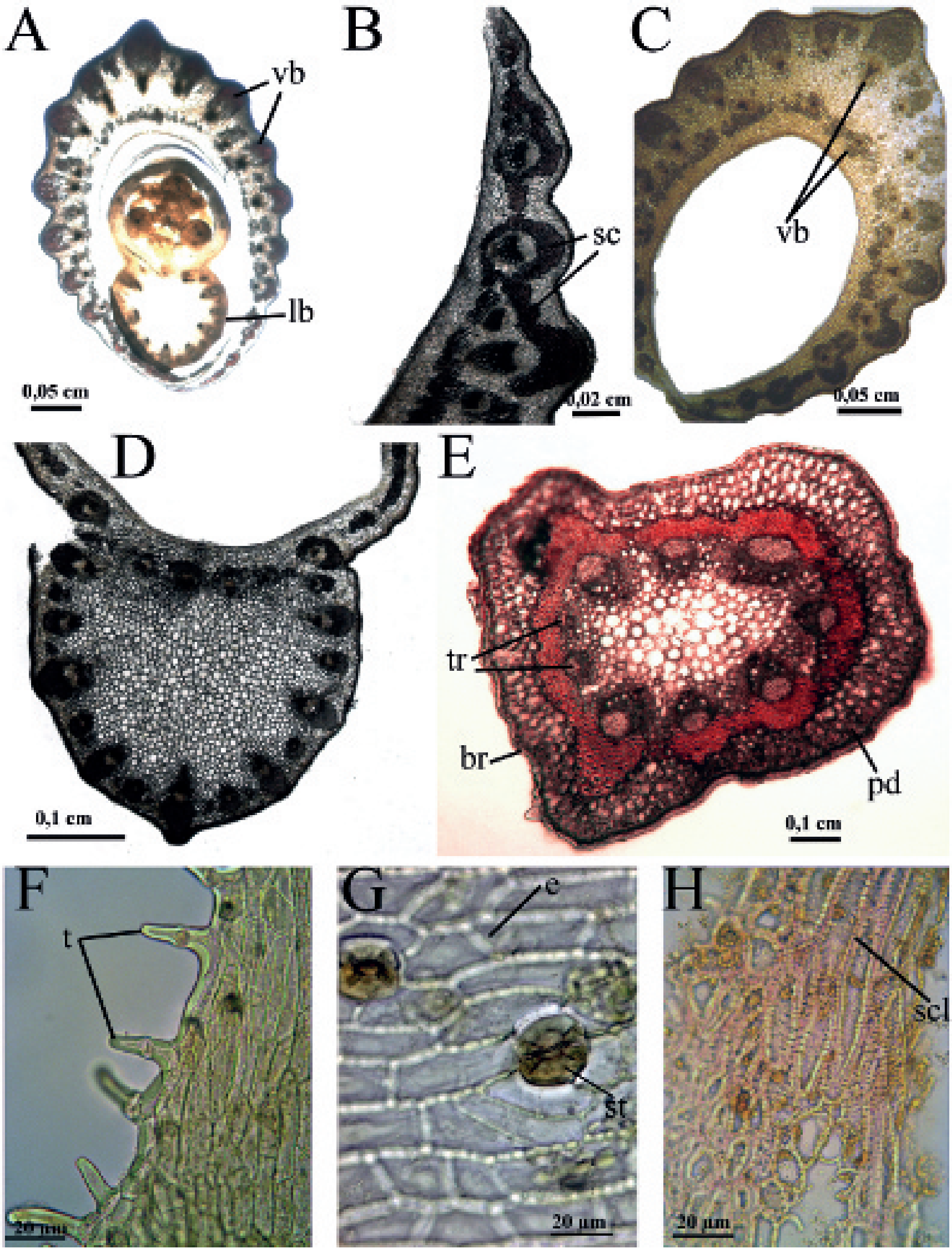

Figure 2. *Nandina domestica.* Anatomical structure: A, B, C – bud scales; D – base of assimilative leaf; E, F, G, H – bracts (E – cross section of peduncle near the node). *br* – bract; *e* – thick-walled epidermal cells; *lb* – leaf base; *sc* – sclereids; *st* – stoma; *t* – trichomes; *tr* – trace; *vb* – vascular bundle.

Thick-walled epidermal cells at the abaxial side are filled up with tannin substances and 2–3 subepidermal layers contain anthocyan. There are several stomata among the epidermal cells. 20–25 large vascular bundles protrude from the surface of the leaf base. They have vigorous caps of lignified protophloem fibres with a narrow central cavity. These caps exceed the size of vascular tissue. Cell groups in interbundle zone undergo sclerification, too. An inner arc of smaller vascular bundles appears at the level of scale leaf attachment. Xylem is directed inside the organ. Compared with bud scales, the cambial activity is more intensive there (there are up to 16 vigorous elements in the radial chains of secondary xylem). The vascular bundles in thin outside sections of the leaf base are small and often they are not well distinguished among surrounding bundles of sclerenchyma.

Skinny dorsoventral leaf laminas (Fig. 1F, G) are hypostomatic (the average number of stomata is 360/mm^2). Two exterior layers of mesophyll are closely packed palisades. The midrib is 1–3-bundled. It runs out noticeably from the lower leaf surface. Considerable durability of leaf lamina is due to epidermal cells with strongly thickened, cutinized cell walls, to a well developed cuticle, to mechanical bundle sheaths of vascular bundles and to bundles of thick walled alive sclerenchyma fibres which are arranged at edges.

Bracts are small, scale-like, narrow lanceolate with a thick cuticle (Fig. 1B). Epidermal cells are narrow, stretched along the bract, with straight or weakly waved anticlinal and noticeably thickened external tangential walls. The latter form thick-walled denticles and trichomes at the edges (Figs 1I; 2F). There are stomata on the both sides of bracts (Fig. 2G). Mesophyll is 6–7-layered, palisades are absent. The rigidity of bracts is due to a multilayered sclerenchyma, which differentiates under the upper epidermis. It is represented by alive fibres and sclereids (Figs 1H; 2H), which have thick, quickly lignified cell walls and lack chloroplasts. They contain sugars and numerous grains of assimilative starch. 2–3 subepidermal layers at abaxial side are represented by loosely joined, rounded, thin-walled cells with a great number of chloroplasts.

Sepals have a quite similar anatomical structure, but they are bigger and thicker (Fig. 1C, D, J, K). They have a more differentiated vascular system. The nodes of bracts and sepals are unilacunar, trace is with one, rarely two vascular bundles (Figs 1E; 2E).

Mahonia japonica

Anatomical analysis of three leaf formations of the East Asian species *M. japonica* revealed a number of taxonomical features which differ from *N. domestica*.

Bud scales are relatively narrow and include 14–22 layers of a homogeneous mesophyll and they are hypostomatic. They have up to 14 vascular bundles which form one arc. The latter one is displaced to the abaxial side. The arc is separated from the low epidermis by 4–5 layers of densely joined small cells of chlorenchyma. Sclerenchyma elements differentiate above the phloem of all vascular bundles and partly on the side of the xylem. Groups of mesophyll cells adjoining the upper epidermis undergo sclerification, too. Small air cavities occur between them. Epidermal cells on both sides are filled up with tannin substances.

The leaf base is flat-domed (Fig. 3A), its thickness is more than 100 cells in transverse section. The edge parts run out and consist of sclerenchyma, each containing one round collateral bundle. Besides them there is a ring of large, stretched in radial direction open vascular bundles (up to 30). Wide bundles of sclerenchyma fibres with thick lignified cell walls are situated above vascular bundles. There are thick-walled cells of homogeneous parenchyma on the outside, adjoining the subepidermal hypodermis. They contain tannin substances and are covered by a mighty layer of cuticle on the outside.

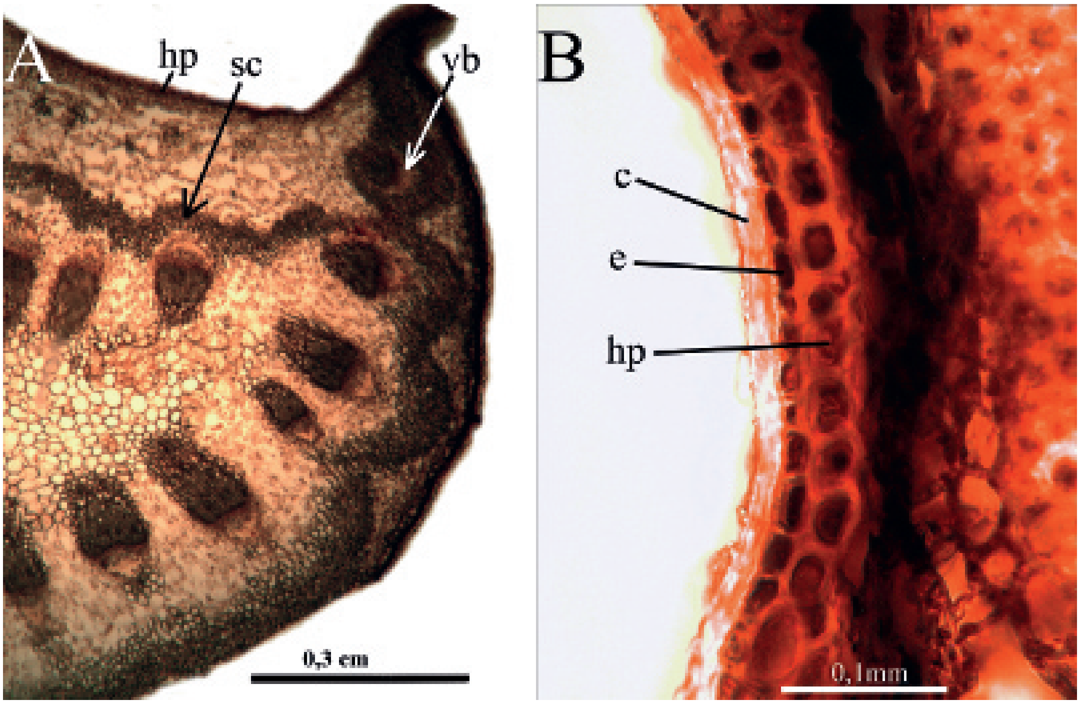

Figure 3. *Mahonia japonica.* A – the base of assimilative leaf; B – abaxial side of bract. *c* – cuticle; *e* – epidermis; *hp* – hypodermis of alive fibers; *sc* – sclerenchyma.

Supporting tissues undergo a considerable development in the lamina. Besides mechanical bundle sheaths of vascular bundles and sclerenchyma bundles situated on the brink of the lamina, a double-sided subepidermal hypodermis differentiates (Fig. 4A, B, C). Under the upper epidermis (Fig. 4E) it is represented by 1–2 layers of closely adjoined alive cells with strongly thickened walls of fibres. They are directed along the organ (Fig. 4F). The lower hypodermis is of transitional to spongy mesophyll type (Fig. 4G). Its cells are loosely arranged and form large intercellular cavities above stomata. They have cell outlines in transverse sections similar to mesophyll cells. But they differ by their thick walls, unitary chloroplasts and the presence of tannin substances as well. Well developed cuticle and thick-walled epidermis provide the durability of leaf lamina. The epidermal cells are partly transformed into sclereids. (Fig. 4C, E).

Bracts are skinny, flat-trenched, with homogeneous (up to 8–9 layers) mesophyll with air cavities on the adaxial side and they are hypostomatic. 3–11 vascular bundles are complete within the mesophyll. Thick-walled epidermal cells (Fig. 3B) are stretched along the bract on both sides. They have almost right or winding anticlinal walls, tannin substances and numerous lipid drops of different size. Subepidermal layers of hypodermis are clearly distinct. It is represented by thick-walled fibres on the adaxial side and by groups of sclereids on the abaxial one.

Helleborus caucasicus, *H. foetidus*, *H. niger* and *H. purpurascens*

Helleborus caucasicus was taken as model species. Its monocarpical shoot is dicyclic, semi-rosette. The median leaves are preceeded by 2–3 large, scale-like leaves. 1–2 bottom leaves are winter-green, with long petioles and wide leaf sheaths. Their laminas are skinny, 5–11-segmented. The elongated part of the generative shoot is weakly leaved. 3–4 small summer-green, superious leaves, which are inferior in their size to the rosette ones, are trisected. Leaves near the perianth

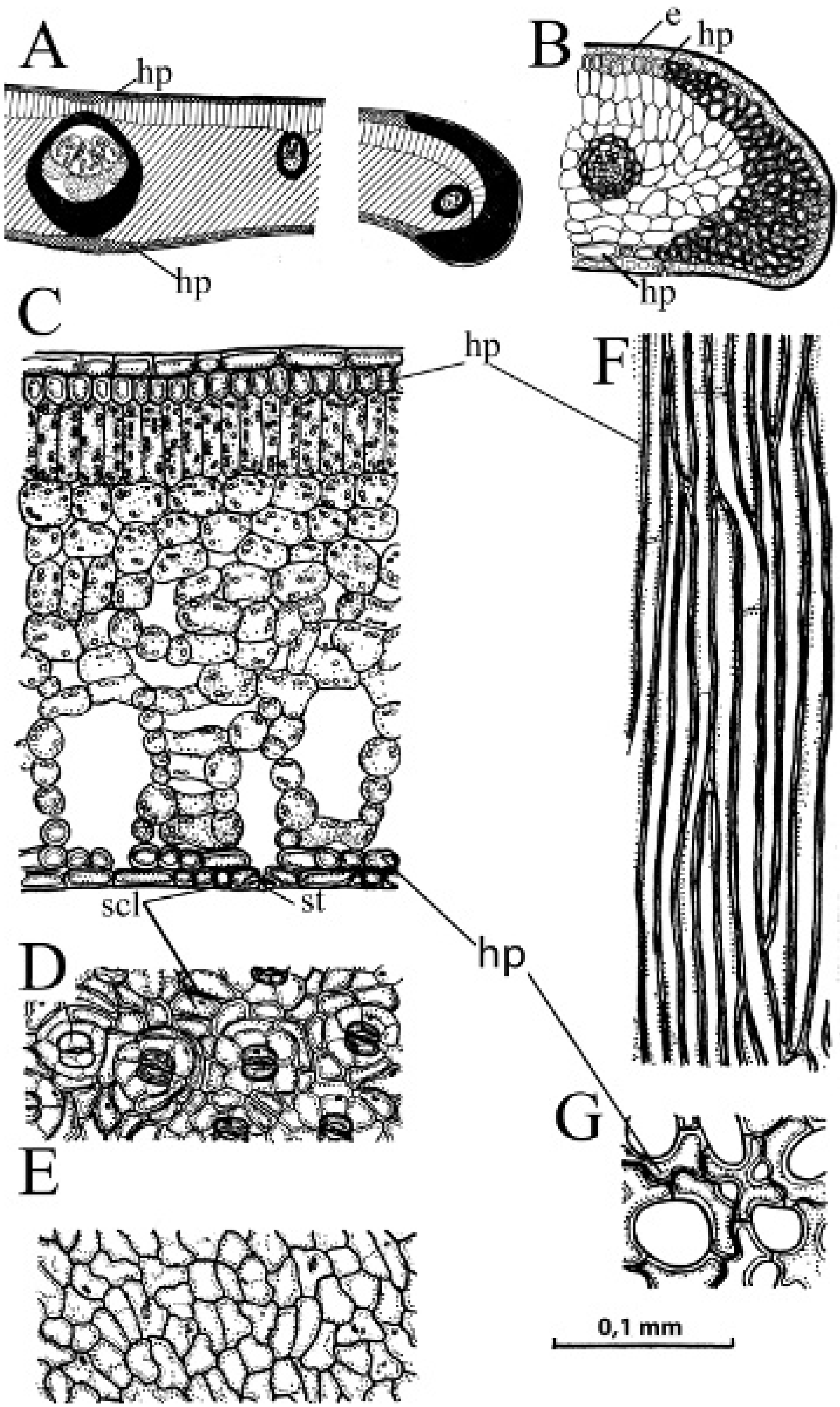

Figure 4. *Mahonia japonica.* A – scheme of cross section of leaf lamina; B – leaf edge; C – leaf lamina; D, E – low and upper epidermis; F, G – cells of hypodermis. *e* – epidermis; *hp* – hypodermis; *scl* – sclereids; *st* – stoma.

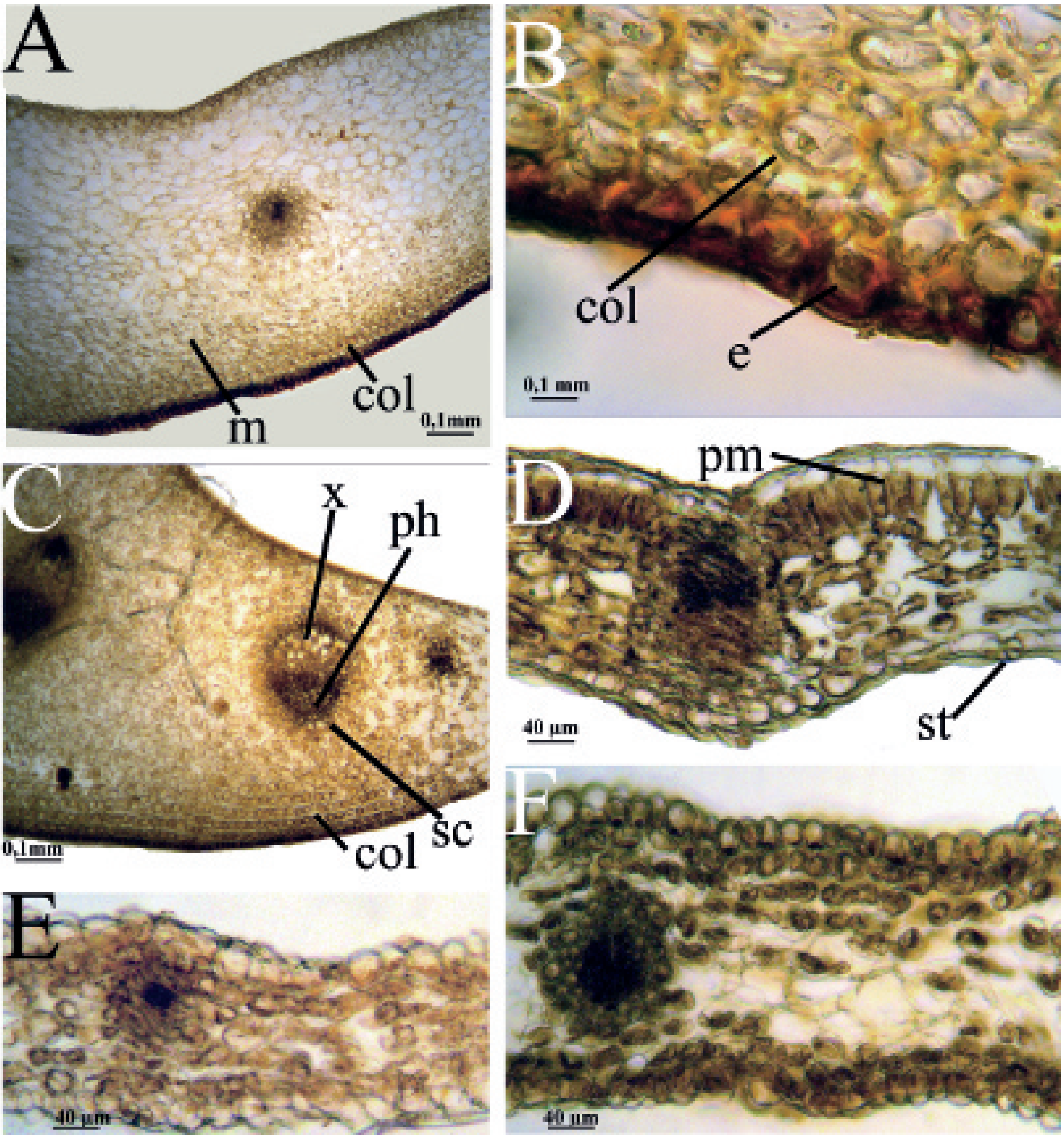

Figure 5. *Helleborus caucasicus*. Anatomical structure of different leaf formations. A, B – bud scale; C – the base and D – lamina of assimilative leaf; E – bract; F – sepal. *e* – epidermis; *col* – collenchyma; *m* – homogeneous mesophyll; *ph* – phloem; *pm* – palisade mesophyll; *sc* - sclerenchyma; *st* – stoma; *x* – xylem.

are entire. They are drawn together closely near the apex and act as bracts. Uniflorous peduncles often develop from their axial buds. The perianth is double-numbered. 5 petal-like, yellowish-green or greenish-white, scaly-arranged sepals retain with fruits. Petals look like tubular nectaries.

The bud scales have a thick large-pleated cuticle and epicuticular wax. On each side there are alive trichomes and stomata among the thick-walled epidermal cells. Stoma density is on average 26–35/mm^2. The mesophyll is homogeneous (Fig. 5A), 26-layered with chloroplasts and assimilative starch grains. The peripherical layers are formed by lacunular-angular collenchyma (Fig. 5B). Epidermis and subepidermal layers are brownish. The presence of tannin substances enhances the protection functions of scales. The number of vascular bundles is about 8–11 per scale. Secondary phloem is predominant.

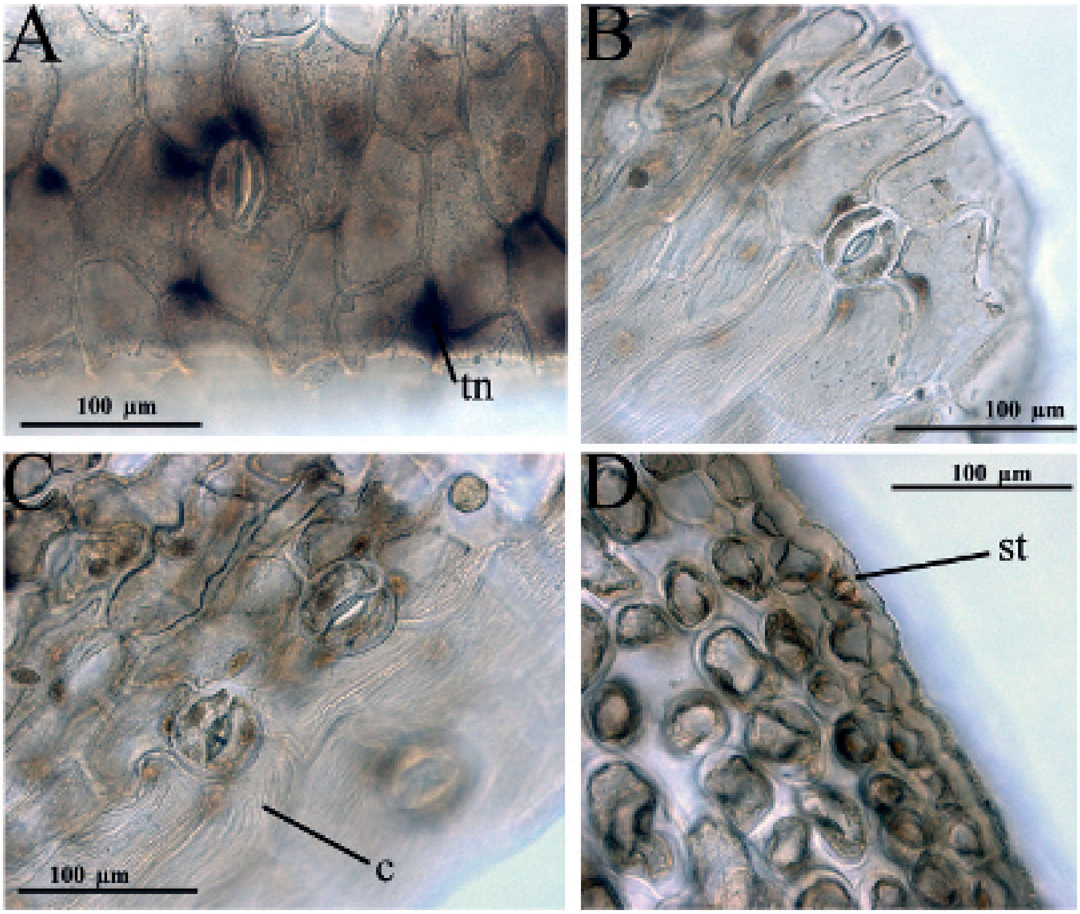

Figure 6. *Helleborus niger*. Epidermal cells. A – bud scale; B, C – bract (B – adaxial, C – abaxial side); D – longitudinal section of lower epidermis and subepidermal layer of bud scale. *c* – pleated cuticle; *st* – stoma; *tn* – tannin substances.

The rosette leaf base (Fig. 5C) is homologous to scale structure and it completely repeats its histological structure, but it is larger and includes about 54 cell layers. 3–9 external layers are similar to the collenchymatous tissue. The homogeneous mesophyll is represented by round cells with a system of small intercellulars. The stored starch accumulates near the 16–17, collateral, open vascular bundles. Mainly the secondary phloem is formative. It is strengthened by large bundles of thick-walled, lignificated protophloem fibres.

The rosette leaves and the very first superior leaves have the same structure (Fig. 5D). But they are thinner because of decrease of spongy mesophyll layers (from 9–10 to 6–7). The leaf blades are hypostomatic. Stomata up to 123/mm^2. The upper and the lower epidermis have few glandular trichomes, which are absent in overwintering leaves.

The leaf-shaped bracts (Fig. 5E) are represented by a growing apical zone of leaf primordia and their reduced basal part. They have an intermediate structure between the apical assimilative leaves and sepals (Fig. 5F). In contrast to the former, they are thinner, with a weakly differentiated 5–6-layered mesophyll, amphistomatic, with glandular trichomes. The palisades are very short or not expressed, spongy mesophyll consists of lacinate, loosely arranged cells. In sepals, the mesophyll is homogeneous and 7–9-layered. Stomata are mainly in lower epidermis.

A similar anatomical structure with little quantitative changes in the distinctive features of the leaf formations can be found in two other winter-green species: *H. niger* L. and *H. foetidus* L. Their bud scales are relatively thick (from 9 to 40 cell layers in *H. niger*). Abundant wax and a large pleated cuticle, which completely covers the epidermal cells surface, strengthen the protective function of scales. There are alive club-shaped trichomes on the epidermis. Stomata differentiate on both sides of bud scales. The cell walls in the lower epidermis are soaked with tannin substances, which quite often accumulate in vacuoles (Fig. 6A). A 1–2-layered collenchyma differentiates on the adaxial side (Fig. 6D). There are chloroplasts and starch in the homogeneous mesophyll.

The broad leaf base includes up to 50 round starch-containing cells with a system of small intercellulars. Mechanical tissue is presented by a narrow subepidermal collenchyma layer.

The leaf-like bracts of *H. niger* remotely resemble assimilative leaf segments. They are amphistomatic (Fig. 6B, C). The midrib of the leaf is completely submerged into the 7–8-layered homogeneous mesophyll. Its blade-like cells contain numerous grains of assimilation starch. Mechanical tissues are absent. The defensive function is provided by thick-walled epidermal cells and a thick cuticle. On the contrary, the lower bracts of *H. foetidus* keep a certain structural similarity with the median leaf lamina (Fig. 7A, B), but they are thinner. Mesophyll is differentiated into loose palisade and spongy parenchyma. Epidermal cells have thick external cell walls and cuticle (Fig. 7C, D). Glandular trichomes on both sides, assimilation starch and lipid drops are typical of them. There are no collenchymatous tissues near the midrib. Bracts, which are directly adjoined to the sepals, demonstrate a considerable anatomical similarity with them (Fig. 7E, F).

The summer-green *H. purpurascens* differs from winter-green species due to some of its anatomical features. The bud scales have 22–26 cell layers and are relatively thick (Fig. 8A). They have numerous alive club-shaped trichomes, a fine pleated cuticle, strongly thickened tangential external epidermal cell walls and contain anthocyans (Fig. 8B). Stomata differentiate on both sides of bud scales. Their density in the lower epidermis is twice as much in comparison to the winter-green species and to the base of median leaves as well. The latter differ by villosity, the greatest thickness (up to 76 layers of homogeneous mesophyll) and high-developed collenchyma-like tissue (Fig. 8C, D). Compared to winter-green species, 18–20 open vascular bundles include a larger number of relatively short tracheal elements. The cells of parenchyma bundle sheaths of vascular bundles as well as mesophyll cells contain grains of assimilative starch. The laminas of assimilative leaves (Fig. 8E, F) are densely pubescent, hypostomatic, with low palisade coefficient (22–25%). They have fine pleated cuticle and epicuticular wax. The midrib strongly runs out of adaxial side and includes 4–5 vascular bundles.

Non-dissected bracts adjoining the sepals are thin (5-layered), amphistomatic, with only few trichomes arranged mainly on the edge and weakly differentiated or homogeneous mesophyll (Fig. 8G). The sepals have the same structure: thick-walled epidermal cells, fine pleated cuticle. The subepidermal layer of homogeneous mesophyll on the upper side is notable for more closely united cells and for dark brownish colour. There are unitary starch grains in the parenchyma bundle sheaths of vascular bundles.

Discussion

To sum up the results of our investigations, it has to be noticed that the transition from one leaf formation to another is accompanied by some structure-functional changes of leaf primordia,

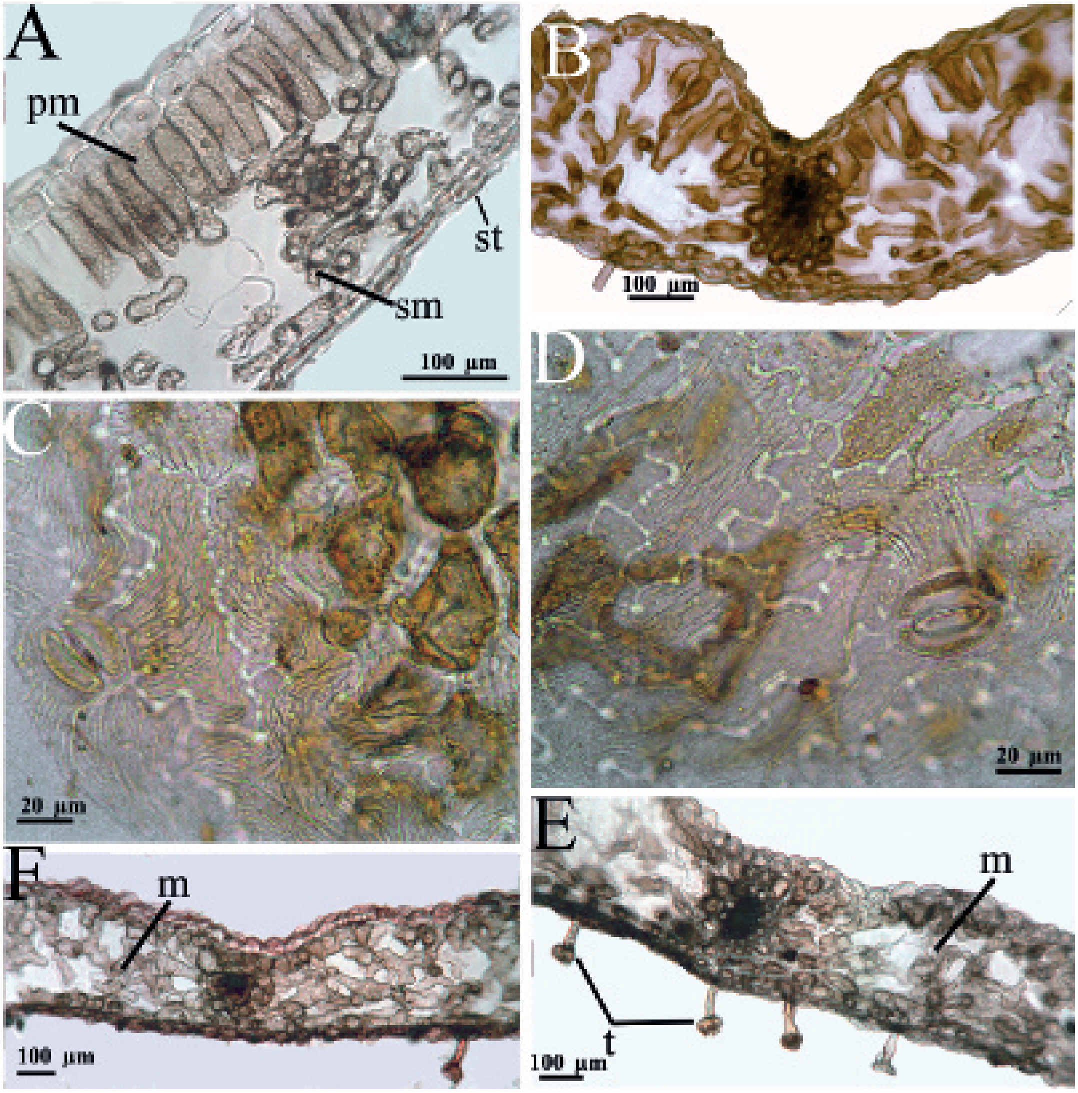

Figure 7. *Helleborus foetidus.* Anatomical structure of assimilative leaf (A), bracts (B, C, D, E) and sepal (F). *m* – homogeneous mesophyll; *pm* – palisade mesophyll; *sm* - spongy mesophyll; *st* – stoma; *t* – glandular trichome.

which consecutively appear along the shoot. The leaf heterogeneity is caused by their differentiation from different parts of leaf primordia with different meristematic activity, the early divergence in growth and forming patterns and the specific character of morphogenesis.

Well developed mechanical tissues which are represented by large bundles of alive protophloem fibres with strongly thickened lignified cell walls are typical of cataphylls of renewal buds of the evergreen subtropical shrubs *Nandina domestica* and *Mahonia japonica*. Separate groups of homogeneous mesophyll cells undergo sclerification, too. Thick-walled epidermal cells contain tannin substances. There are small air cavities in subepidermal layers of cataphylls. Cataphylls of buds of winter- and summer-green species of *Helleborus* situated near the soil surface are devoid of many special structural features for enduring unfavourable periods, which are characteristic of woody forms in torrid and especially temperate zones. The defensive features of the epidermal complex of bud scales of herbaceous perennials are determined by the presence of a thick cuticle,

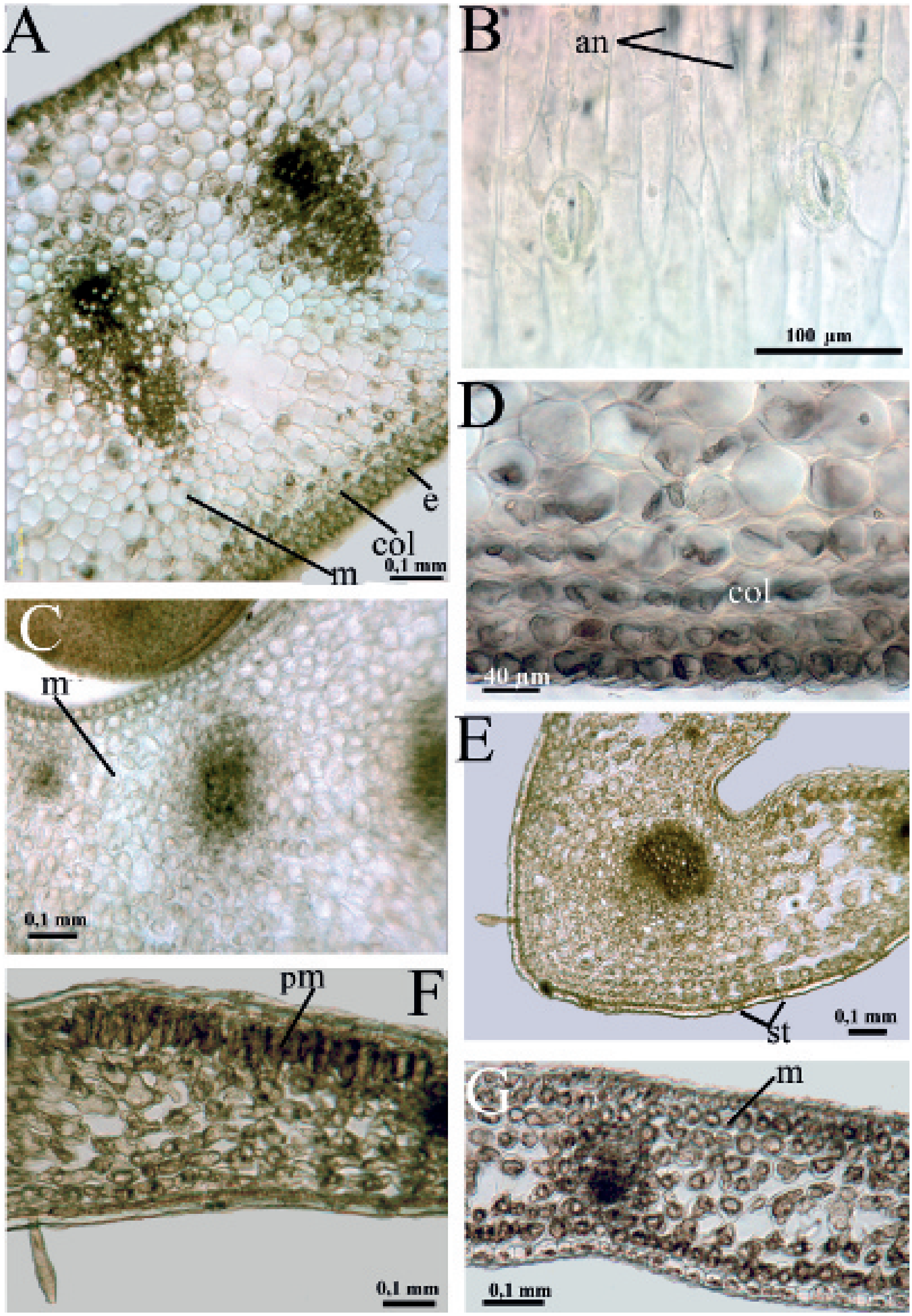

Figure 8. *Helleborus purpurascens.* Anatomical structure of bud scales (A, B), the base of assimilative leaf (C, D), leaf lamina (E – midrib zone, F – middle part), bract (G). *an* – anthocyans; *col* – collenchyma; *e* – epidermis; *m* – homogeneous mesophyll; *pm* – palisade mesophyll; *st* – stoma.

wax, trichomes, tannin substances and anthocyans. The amphistomaticy and the presence of assimilative starch in homogeneous mesophyll illustrate the active photosynthesis and gas-exchange in the latter. The thickness of bud scales varies depending on certain species. The winter-green *H. niger* has the thickest bud scales (42–44 cell layers) and the summer-green *H. purpurascens* has the thinest ones (up to 20 cell layers).

The differences in the microstructure of homologous structures, e.g. bud scales and the bases of median leaves, are connected with more prolonged existence, multifunctionality (the execution of defensive, supporting, transferring and storing functions) of the latter. This can be seen especially in evergreen shrubs which keep their leaves for 1.5–2 years. The leaf bases repeat the anatomical structure of bud scales, but they are larger and include more than 100 cells in cross section. The number of open vascular bundles is up to 30. They form 1–2 arcs or a circle and differ by intensive cambial activity and by large caps of protophloem fibers. The latter ones often exceed the vascular tissue size and run out of the leaf base surface in the form of small ribs. Large sclerenchyma bundles differentiate on the external side of all vascular bundles and separate groups of sclereids in the space between vascular bundles. In *Mahonia japonica* the subepidermal hypodermis has supporting function as well. Thick-walled epidermal cells often contain flavonoids and anthocyans. Parenchyma stores up mainly starch.

Scale-like bracts of *Nandina* and *Mahonia* noticeably differ from assimilative leaves. Xeromorphic features of anatomical structure (abundance of alive fibers and sclereids, thick-walled epidermal cells, well developed cuticle) are characteristic of the former ones. The leaf trace has one vascular bundle. The assimilative leaves are meso-xeromorphic. The leaf trace has many vascular bundles. The peculiarity of bract's anatomical structure of evergreen Berberidaceae species mentioned above is an important adaptation to the environment. It provides firm defence of reproductive structures from withering and low temperature influence.

Leaf-like bracts of herbaceous winter- and summer-green species of *Helleborus* poorly resemble assimilative leaf segments. They are not hypo-, but amphistomatic. The midrib is entirely submerged into homogeneous mesophyll with numerous assimilative starch grains and lipid drops. The supporting tissues are absent. The defensive function of bracts is provided by a large cuticle, thick-walled epidermal cells, trichomes, anthocyans (*H. purpurascens*) and tannin substances as well.

It is interesting to notice that both scale-like bracts, which are characteristic of *N. domestica*, and leaf-like bracts of *H. niger* and *H. purpurascens*, which closely adjoin the perianth, show a great structural similarity with sepals. This fact confirms one of the proposed ways of double perianth origin, namely the bracteous origin of the calyx in Berberidaceae and Ranunculaceae.

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