Wulfenia **23** (2016): 80–97

Wulfenia
Mitteilungen des
Kärntner Botanikzentrums
Klagenfurt

Anatomical characteristics of vegetative organs of selected members of *Coldenia* s.l. (Boraginaceae)

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Summary: The results of microscopic analysis of vegetative organs of *Coldenia procumbens* L., *Tiquilia canescens* (DC.) A.T. Richardson, *T. palmeri* (A. Gray) A.T. Richardson and *T. nuttallii* (Hook.) A.T. Richardson show many similar features of structure organization inspite of their taxonomic affiliation with different genera. Some specific anatomical features reflecting the ecology of habitats, the type of life form and systematic status stand out against them. Hygromesomorphic structure is characteristic of *Coldenia procumbens* and xeromorphic one is characteristic of *Tiquilia*. Great similarity in anatomical structure of shoot and root of annual *Tiquilia palmeri* and *T. nuttallii* confirms their close relationship. The dwarf shrub *T. canescens* is remarkable. In contrast to herbaceous representatives of this genus, it has revolutive leaf blades and a peculiar structure of palisades and phellem, which includes thin-walled cells and thick-walled lignified phelloids at the same time. Internal phloem at the border with pith and fields of interxylar phloem, which are so typical for annual species of *Tiquilia,* are absent in axial organs of *T. canescens* and *Coldenia procumbens* as well.

Keywords: Boraginaceae, Ehretioideae, *Coldenia*, *Tiquilia*, anatomy, assimilative leaves, stem, root

Engler & Prantl (1897) treated *Coldenia* L. (Ehretioideae, Boraginaceae) in 'Die natürlichen Pflanzenfamilien' in a broad sense including 12 species. Due to some features as the area of habitat, the character of plant life form and the structure of leaf blade and flower, two separate genera are distinguished nowadays: *Coldenia* L. and *Tiquilia* Pers. The first one is a monotypic genus from southern Asia and it is found here and there in north-eastern Africa, Madagascar and northern Australia. The second one includes 27 species which grow in tropical areas of the New World (North and South America) (Richardson 1977).

A great amount of information in literature about these two genera concerns their habitat, ecology, physiology, embryology, chromosome number, molecular genetics, morphology of vegetative and generative organs of some species (Humphrey 1932; Venkateswarlu & Atchutaramamurti 1955; Richardson 1977; Moore & Jansen 2006; Moore et al. 2006; Stevens 2012).

Microstructure of vegetative organs, especially roots of plants of different life forms, has been relatively insufficiently studied. The facts which are given on leaf and stem anatomy are often fragmentary, sometimes even mistaken and aren't confirmed by illustrations. Data on the comparative analysis of species are absent. In this regard, new additional knowledge of anatomy will allow not only to broaden the biological and morphological characteristics of this taxon, but to get to know the ways and mechanisms of structural adaptation and to help solving some systematic problems including the taxonomic status within Boraginaceae as well.

In this paper, the results of comparative anatomical analysis of leaf, stem and root in *Coldenia procumbens* L., *Tiquilia canescens* (DC.) A.T. Richardson, *T. palmeri* (A. Gray) A.T. Richardson and *T. nuttallii* (Hook.) A.T. Richardson are given.

Materials and methods

Plants of *Coldenia procumbens*, *Tiquilia canescens*, *T. palmeri* and *T. nuttallii* were obtained from herbarium collections of the Botanic Garden of Komarov Botanical Institute and of the Main Botanical Garden of the Russian Academy of Sciences.

Study of herbarium specimens, maceration of plant material, anatomical sections of leaf, stem and root, histochemical analysis of essential cell substances as well as the secondary products of metabolism were carried out according to the recommendations of 'Handbook of botanical microtechniques' (Barykina et al. 2004).

Anatomical sections were analyzed using light microscope Micromed-3. Images of the sections were obtained by means of light microscope Axioplan-2. Photographs were taken by the digital camera AxioCam MRc and processed using Adobe Photoshop.

Results

Coldenia procumbens L.

The thin leaf blade has oval lobes, middle and large lateral veins which are suddenly protruding at its bottom. The protrusion exceeds 2–3 times leaf blade thickness (Fig. 1 A). Smooth cuticle is distinctly noticed.

There are small, somewhat raised stomata (amphistomatic type) and very long, simple alive trichomes exceeding 1–3 times leaf thickness. The bulbously dilated base of trichomes is formed by an epidermal cell which can be distinguished by its large dimension (Fig. 1 B). There are more short glandular trichomes which have a 2–3-celled foot and a rounded head with brown contents. They are mainly on the abaxial leaf surface. Differentiated mesophyll includes one layer of narrow, relatively short palisades with slightly winding, anticlinal cell walls, large chloroplasts and 4–5 layers of spongy parenchyma. The ratio from palisade to spongy tissue is 45% in average. The length of palisades in different parts of the leaf blade is different, what causes the formation of a wavy adaxial surface.

In the midrib area, the mesophyll layer is interrupted and is replaced by collenchymatous tissue. The vein is one-bundled, rarely it consists of 2 closely drawn bundles of different size: near the main big bundle is the small one. The conductive tissues of midrib form a vigorous, horseshoe-like arch with almost completely connected brinks. The xylem (5–6 wide spiral tracheal elements in radial chain) predominates the phloem (small bundles of protophloem fibers). The large bundle is mostly bicollateral (Fig. 1 A), but in some cases, i.e. during the connection of phloem fields, it becomes amphivasal. Small bundles almost completely plunged in the mesophyll are amphicribral.

The stem of the orthotropic shoot is rounded in cross-section. The stems of the procumbent, lodged rooted shoot and peduncle as well are flat-bulging or oval (Fig. 2 A, B). Very long simple trichomes with traces of alive contents, papilla and glandular trichomes are numerous in the epidermis. Tannins are in the cavities of epidermal cells and in the cells of the subepidermal layer of the primary cortex, which provides their dark-brown colour. The large-cellular external part of the primary cortex is represented by lacunar-angular collenchyma with chloroplasts (Fig. 2 C). Cell size of the 7–8 layers of primary cortex is noticeably smaller by the stele. Vascular tissues form

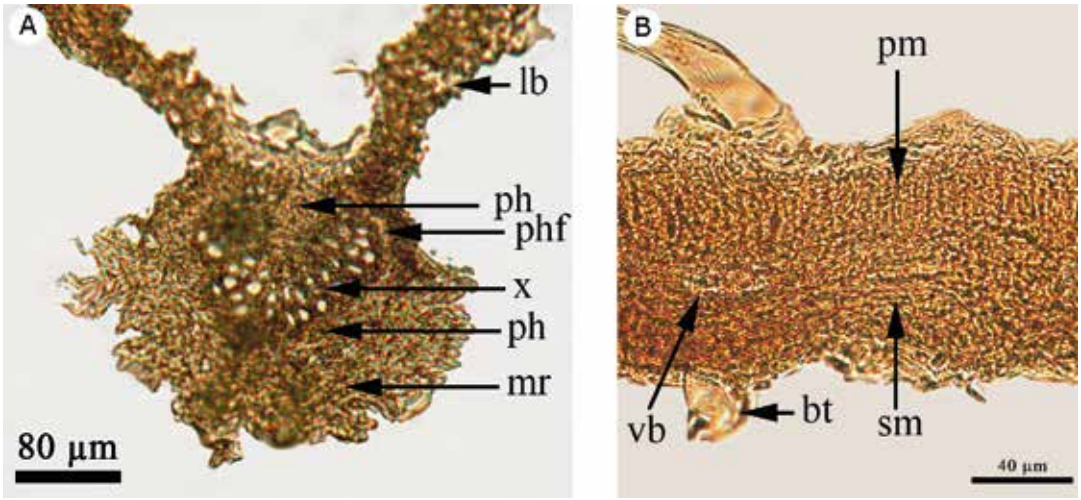

Figure 1. *Coldenia procumbens*. Leaf. A – anatomical structure of leaf in midrib zone; B – fragment of leaf blade cross-section. bt – base of simple trichome; lb – leaf blade; mr – midrib; ph – phloem; phf – phloem fibres; pm – palisade mesophyll; sm – spongy mesophyll; vb – vascular bundle; x – xylem.

a continuous cylinder (Fig. 2 D, E), which is crossed by relatively narrow pith rays. The vessels of the secondary xylem are settled down by radial bundles which include up to 8 elements. The phloem has distinctly little groups of thick-walled fibres. It can be distinguished by its brown colour. Sieve tubes have horizontal simple sieve plates. The parenchymal pith has a small-celled perimedullar zone, which is slightly similar to the external phloem due to its dark brown colour and structural elements. It can be easily separated from the large-cellular pith. In orthotropic shoots, most central cells of the pith are destroyed by the formation of a large air cavity due to fast growth of internodes.

The main root is tetrarch to pentarch at the whole length. This is confirmed by rhizotaxis. *Coldenia procumbens* is a rachis-rooted annual with strongly branched, semi-rosette, monocarpic shoots. And it is characterized by a vigorous secondary thickening of the root stele (Fig. 3 A). Long and intensive cambium functioning is stimulated by growth and branching of the whole photophilous shoot. Especially the increase of secondary xylem is great, where wide tracheal elements predominate. Porous vessels with simple perforations (Fig. 3 C) are united, 2–6 each in horizontal (Fig. 3 B), rarely askew oriented groups. Secondary pith rays have 1–2 rows. In secondary xylem as well as in some rachis-rooted plants, e.g. *Consolida regalis* Gray (Ranunculaceae), several (3–4) growth layers can be clearly distinguished (Barykina 1992). The same can be observed in the secondary phloem. Groups of fibres with relatively weakly lignificated, thick cell walls are formed. They are arranged in 3–4 layers (Fig. 3 B–D). Such structure of root stele vasculature displays a cambium activity rhythm, which is connected with the consecutive formation of the first rosette leaves actively photosynthesizing and with the formation of elongated leafy metamers of main and lateral vegetative shoots and with paracladia with reproductive organs as well. Vigorous formation of secondary vascular and sclerenchyma elements which provide effective supporting function is important for the plant, especially during the phase of branched elongated flower shoot development. The growth of stele is accompanied by deformation of primary cortex cells, which die off early. The cork of pericyclic origin which includes several layers of thin-walled suberized cells forms a protective tissue in secondary thickened root.

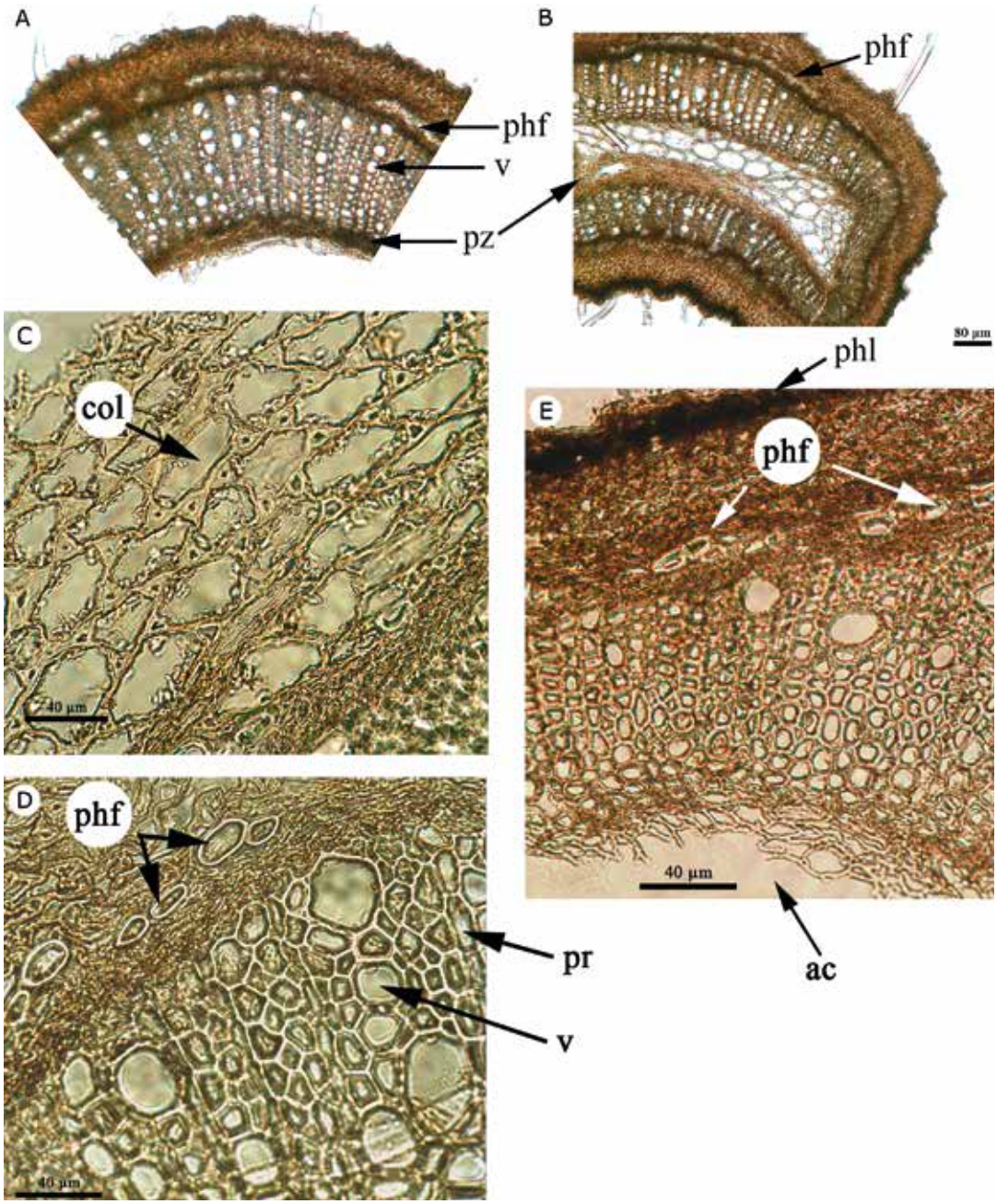

Figure 2. *Coldenia procumbens*. A–E – stem of annual shoot. ac – air cavity; col – collenchyma; phf – groups of fibres in phloem; phl – phellem; pr – pith ray; pz – perimedullar zone; v – vessels.

Tiquilia palmeri (A. Gray) A.T. Richardson

Small oval-oblong leaves with short petioles are pubescent abundantly by long, bristly, thick-walled, simple and glandular trichomes. Leaf blades are amphistomatic. Epidermal cells are small, thick-walled with straight anticlinal cell walls. The external adaxial and rarely the abaxial leaf surface is large-wavy (Fig. 4A). This fact is connected with the peculiarities of isopalisade mesophyll differentiation. Its cell length and number of cell layers change along the leaf blade

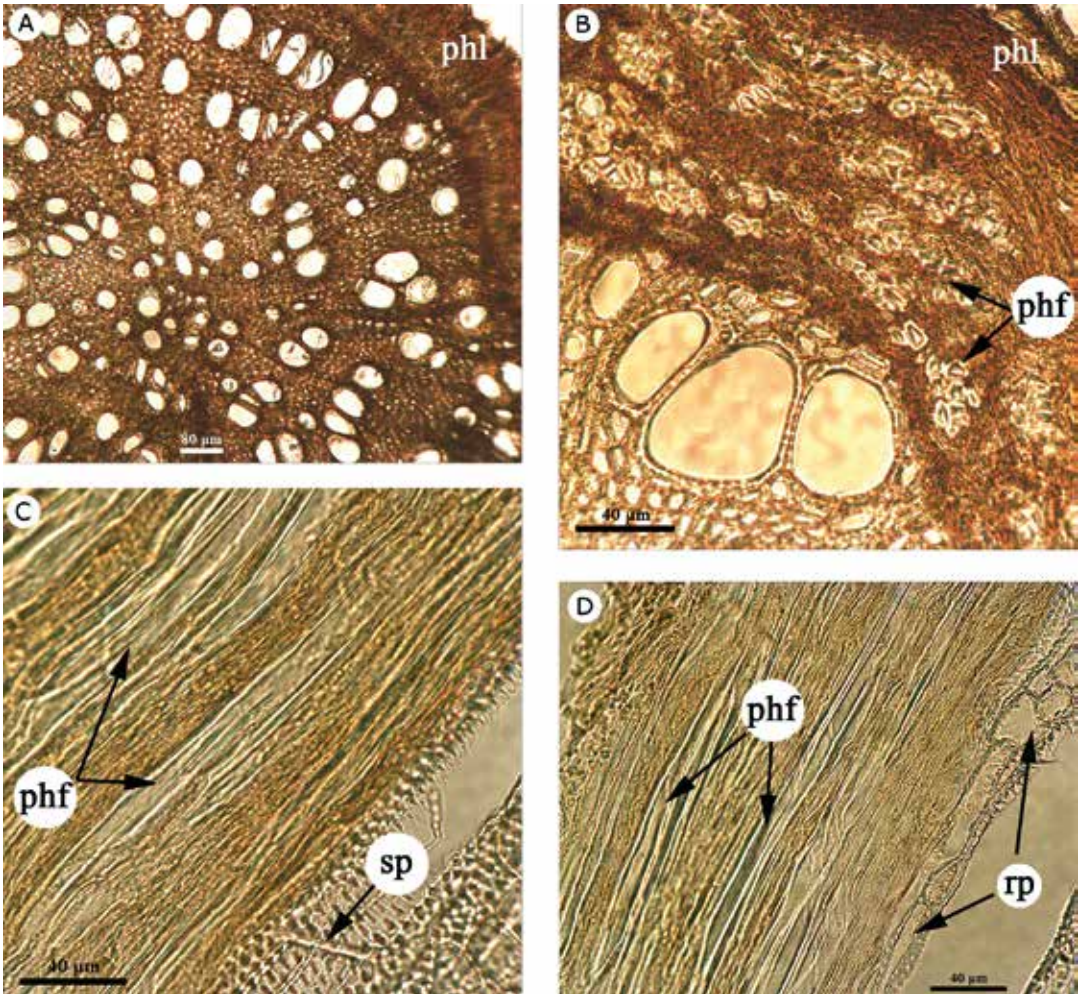

Figure 3. *Coldenia procumbens*. Root. A – cross-section, illustrating 4 rings of secondary xylem; B – fragment of cross-section on the border of xylem and phloem; C, D – tangential sections of the same zone. phf – phloem fibres; phl – phellem; rp – ray parenchyma; sp – simple perforation of porous vessel.

below the vascular bundles and between them (2 layers of very long palisades alternate with 1 layer of short ones). It is also connected with the development of a very large middle bundle and 5–6 lateral bundles which are somewhat deepened on the adaxial surface and strongly projected on the abaxial side. This ridge may exceed the thickness of the leaf blade 3–4 times. In the area of large veins, 6–7 layers of mesophyll are replaced by short palisades on the adaxial side (Fig. 4 B) and by compact collenchymatous tissue (angular type with chloroplasts) on the abaxial side (Fig. 4 B–D). The middle part of leaf blade is lighter because of parenchyma sheaths of vascular bundles and a less number of chloroplasts in palisades compared to the subepidermal layer (Fig. 4 A). Vascular tissues of large bundles are settled down in wide arc (Fig. 4 D) and form 1, rarely 2, closely drawn bicollateral bundles of different size. Relatively thin veins are completely plunged into mesophyll and usually are like amphicribral bundles.

Leaf petiole as well as leaf blade is rounded, densely pubescent with long simple and many short glandular trichomes. It has one large bicollateral bundle which is similar to the bundle of the middle vein of the leaf blade. The node is one-lacunar, the leaf trace has one bundle.

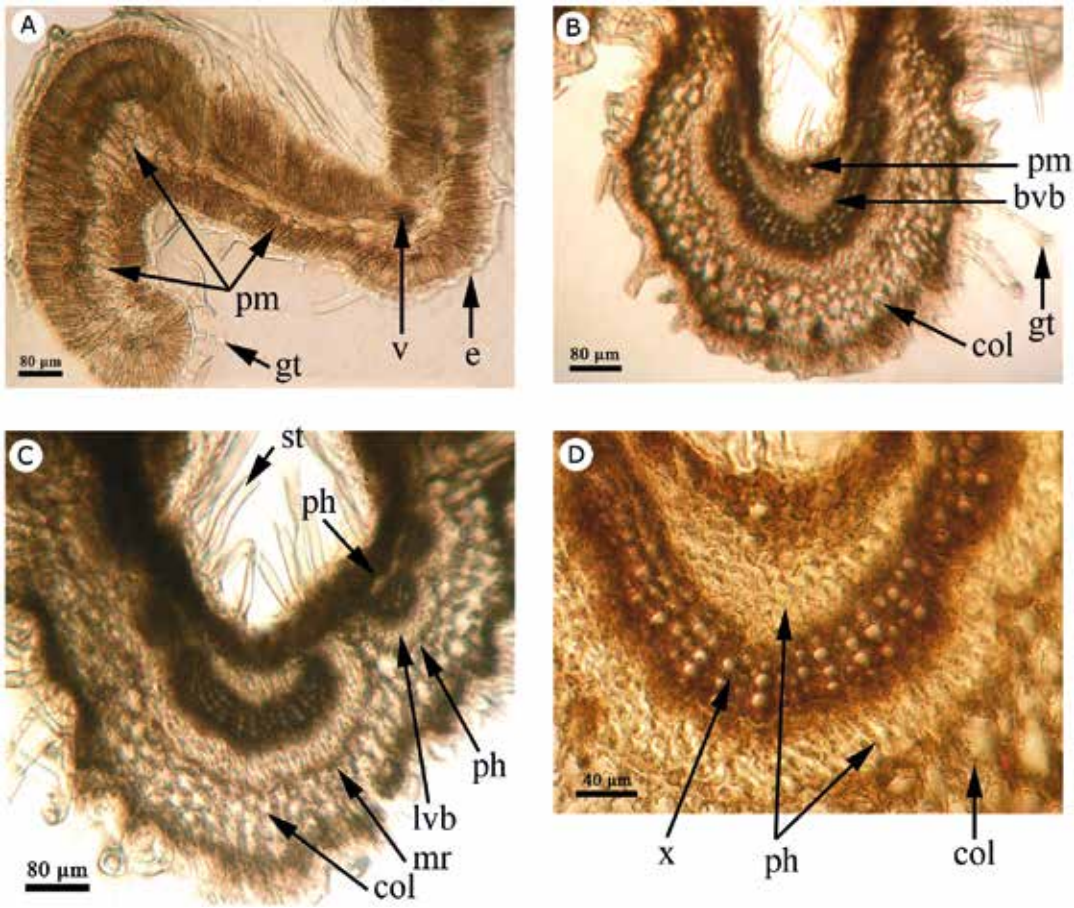

Figure 4. *Tiquilia palmeri*. Cross-sections of leaf blade: A – near the leaf edge, the waviness of the adaxial surface is evident; B, C – midrib zone; D – bicollateral vascular bundle. bvb – bicollateral vascular bundle; col – collenchyma; e – epidermis; gt – glandular trichome; lvb – lateral vascular bundle; mr – midrib; ph – phloem; pm – palisade mesophyll; st – simple trichome; v – vein; x – xylem.

Stem of young vegetative shoot near the apex and peduncle are of similar anatomical structure. They are rounded in cross-section (Fig. 5A, B), have a smooth cuticle and thick-walled, bristly, simple and glandular trichomes as well. The latter ones have stem, which is formed by several cells, and a unicellular head (Fig. 5A, D). One to two layers of primary cortex which adjoin the small thick-walled epidermal cells are brown-coloured because of tannins. Thin-walled cortical parenchyma cells with small intercellular ducts and the cells of an inner starch-containing layer of the cortex (parenchyma sheath) are in the heart of stem (Fig. 5A). Secondary phloem and xylem are arranged in an almost continuous ring. There are up to 7 tracheal elements in a radial xylem chain. Porous and ladder-shaped vessels with simple perforations predominate. Wide pith rays which separate them are often sclerified. Small pith is rounded-triangular with 3(4) fields of small-celled internal phloem. They border on large cells of basic parenchyma.

Age structural changes are noticed in internodes of the middle part of the photophilous shoot. They are connected with more intensive cambium activity, shoot elongation and strengthening of stabilizing function as well. Originally thin-walled cells of primary cortex transformate into an angular collenchyma (Fig. 5C). The layer of fibrous sclereids, single or united in small groups,

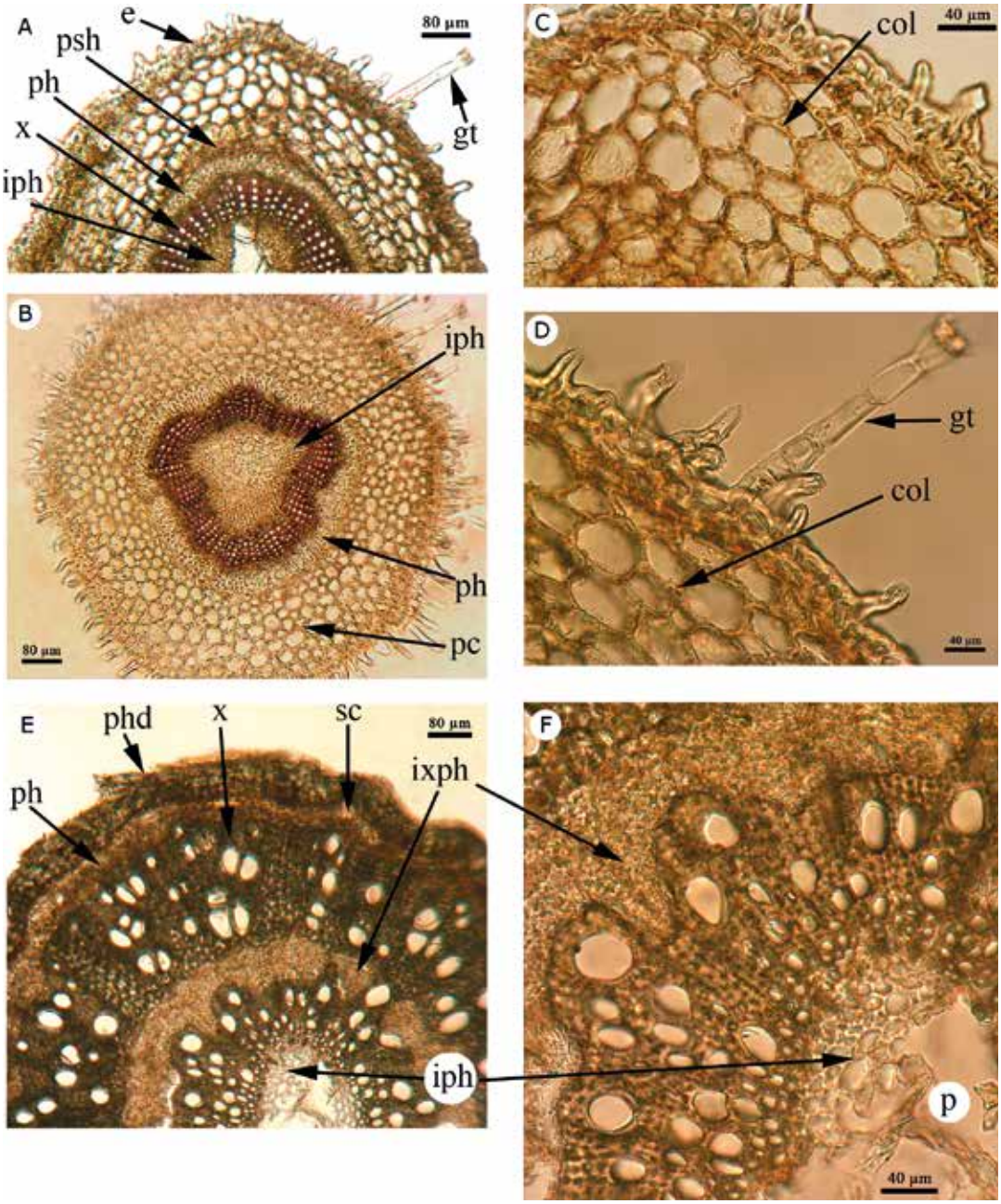

Figure 5. *Tiquilia palmeri*. Cross-sections of stem and their separate fragments on early (A–D) and later (E, F) stages of vascular cambium activity, accompanied by interxylar phloem and phellogen with the formation of its derivatives as well. col – collenchyma; e – epidermis; gt – glandular trichome; iph – internal phloem; ixph – interxylar phloem; p – pith; pc – primary cortex; ph – phloem; phd – phelloderm; psh – parenchyma sheath; sc – sclereids; x – xylem.

(Fig. 5 E) with lightly lignified cell walls appears in the phloem. Secondary xylem substantially spreads in volume. Small fields of interxylary phloem are found there. Pith and the fields of inner primary phloem are destroyed. The stem becomes hollow.

Basal metamers of monocarpic shoot undergo more essential anatomical transformations. They are due to intensity of vascular cambium activity which is accompanied by abnormal secondary

growth and formation of phellogen in the inner layer of the primary cortex. Phellogen produces periderm. In this part of shoot, 3–4 layers of homogenous phellem are noticed (Fig. 5 E). They cut off the cortex and phelloderm layer which contains tannins. Secondary xylem predominates in the stele. Interxylary phloem is plunged into its field in the form of almost continuous horizontal strip (Fig. 5 E, F). According to its elements structure, 'inclusive phloem' (Fig. 6 C, G, E) doesn't differ from phloem on the periphery of stele.

The diarch root is secondary thickened, with 5 layers of phellem of pericyclic origin (Fig. 6 A, B). Its cell walls are lignified and suberized and the cavities contain tannins. Secondary phloem has small groups of fibrous sclereids. Wide xylem vessels (3–4) are united in tangential bundles. As well as in the stem base, interxylary phloem is well noticed (Fig. 6 A–E). It forms small fields and undergoes sclerification in the older part of the root.

Tiquilia nuttallii (Hook.) A.T. Richardson

The leaf surface is abundantly covered by relatively short, alive, simple, awl-like and glandular trichomes. The first ones are thick-walled with narrow cavity which is considerably widen at its base and the latter ones differ by their globular form, thin-walled cells and protoplasmic contents (Fig. 7 A). Glandular trichomes consist of 4–5 cells. The adaxial surface of the leaf blade is fine wavy. Numerous ridges are between vascular bundles on leaf cross-section. Their development is connected with the formation of more long palisades there. Large rounded cells of the bases of simple trichomes, smaller cells of glandular trichomes as well as slightly plunged stomata are distinctly noticed among isodiametric covering cells on both sides of the leaf.

Four to five layers of isopalisade mesophyll are penetrated by numerous (20 and more) vascular bundles with parenchymal sheaths. The middle one-bundled vein (Fig. 7 B) and large lateral veins slightly protrude from the abaxial leaf surface. The ridge is of almost the same size as the leaf blade thickness. It is slightly flattened at the bottom. In the middle vein area, the palisade mesophyll is replaced by 2–3 layers of brown-coloured parenchyma containing tannins. The vascular bundle is bicollateral. A small bundle of phloem is in the deepening of the xylem arc. Small bundles which are plunged into the mesophyll are amphicribral or collateral.

The stem of annual shoot is rounded, slightly wavy and hollow (Fig. 7 C). The epidermal complex includes small supporting, isodiametric cells with thick, straight, anticlinal cell walls, stomata with slightly plunged guard cells, numerous 1-, rarely 2-celled awl-like, long, simple and shorter glandular trichomes with one-celled head. 5–7 relatively wide layers of the primary cortex consist of large cells and look like collenchymatous tissue with small intercellular ducts on periphery (Fig. 7 D). Inner starch-containing layer of cortex (parenchyma sheath) is noticed distinctly. Secondary phloem is arranged in a narrow layer. Single or small groups of relatively large sclereids with slightly thickened lignified cell walls join in it. Secondary xylem which looks like an almost continuous unit predominates the stele. Narrow pith rays consist of 1–2 rows. Wide vessels are mainly single or they join in radial bundles (2–5 vessels) (Fig. 7 E). The annual growth includes 25–28 elements. Several fields of inner primary phloem adjoin from inside to primary xylem (Fig. 7 F). In the middle internodes, 3–4 fields of intraxylary phloem which consist of small, non-lignified cells are wedged into the secondary xylem near the cambial zone. At the end of the vegetation period, tangential divisions of cork cells adjoining the external phloem are noticed in basal metamers of the annual shoot. This leads to the formation of phellogen and later phellem with thin-walled cells there like in the shoot of *T. palmeri*.

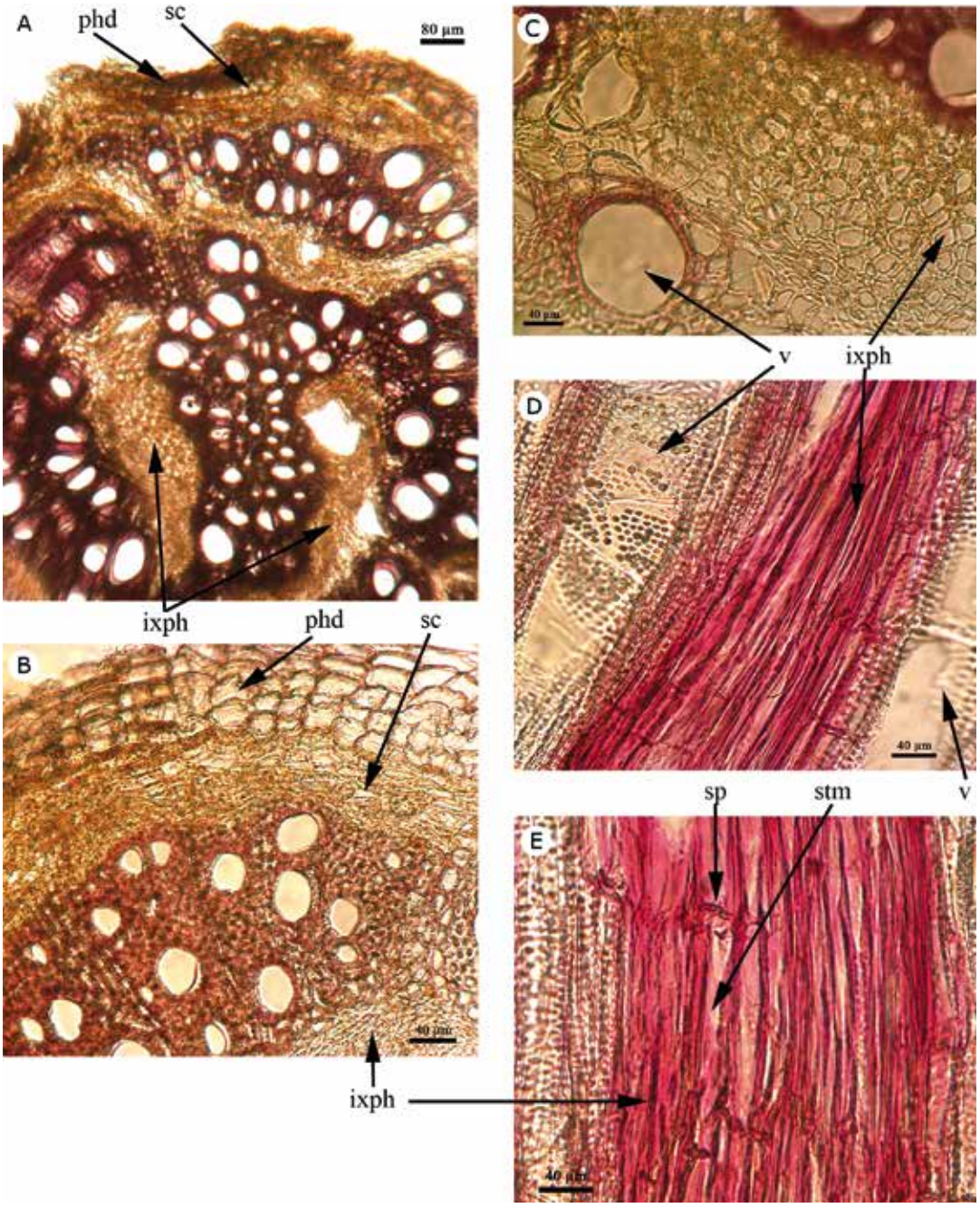

Figure 6. *Tiquilia palmeri*. A–C – parts of root cross-sections; D, E – longitudinal sections of secondary xylem with bundles of secondary interxylary phloem. ixph – interxylary phloem; phd – phelloderm; sc – sclereids; sp – sieve plate; stm – sieve tube member; v – vessel.

The root is diarch. Phellem is homogenous. Secondary phloem forms a narrow layer. In comparison to the sieve elements, bundles of larger sclereids with relatively thin lignified cell walls are differentiated there. Strongly sclerified secondary xylem predominates. Single or joined two by two wide vessels are dispersed across the whole cross-section. Several fields of interxylary phloem (Fig. 7 G) are noticed close to the cambium.

Tiquilia canescens (DC.) A.T. Richardson

Tiquilia canescens is distinct from other studied species of *Tiquilia* from the New World. Leaves are distinctly xeromorphic. Leaf blades are relatively small, oval, lanceolate or inversely egg-shaped, sometimes decurrent to the peduncle, with roundly delated edges which turn down (revolutive type). There are 1–2 more vigorous alive trichoms which are surrounded by several relatively large epidermal cells. Within one individual the adaxial surface may be flat or frequently slightly wavy because of local alteration of more or less long palisades there (Fig. 8 A). Middle and some large lateral veins protrude at the bottom (Fig. 8 B). The leaf blades are densily pubescent, especially on the abaxial side (Fig. 8 C). Pubescence consists of long, bristly, alive, one-celled trichomes which are surrounded by a rosette of epidermal cells and fewer short (2–3-celled) glandular trichomes. Thick-walled epidermal cells have straight anticlinal cell walls (Fig. 8 D). Stomata which are not plunged in but slightly raised above covering cells are noticed on both leaf surfaces. Thus, the leaves are amphistomatic, but not hypostomatic, according to Humphrey (1932). Stomata cavity is small. Stomata density in upper and lower epidermis fluctuates from 146–160 per 1 mm².

Mesophyll consists of 3–6 layers of identical palisades. Densely settled palisades are narrow and very long on the adaxial side. This layer occupies about ½ thickness of the leaf blade and reaches parenchymal sheaths of numerous vascular bundles. The number of mesophyll layers may increase up to 2 above the middle vein. Palisades are shorter there as well as on the abaxial side. Anticlinal cell walls of all palisades are wavy. Thin pleats in the form of a girdle are well noticed across cells. They are tinged with pink by ruthenium (Fig. 8 E). Palisade mesophyll interrupts only on the adaxial side in the area of the middle vein and is replaced by a large-cellular parenchyma with very small intercellular ducts. The bundles of parenchyma (parenchymal sheaths of vascular bundles) are distinctly visible along the numerous lateral vascular bundles (22 and more in the cross-section) which are absolutely plunged into the mesophyll. Sometimes this zone wrongly is mixed up with the spongy mesophyll.

Middle vein has one, rarely two bundles (Fig. 8 B). Small-celled tissue which is similar to external phloem differentiates in the deepening of the xylem arc and rather above it. It's characteristic of bicollateral structure of vascular bundles. The thin leaf peduncle is 1.2–7.5 mm long. It is semicircular in cross-section (Fig. 8 F) and asymmetrical near decurrent parts of leaf base. It is hairy and has one large vascular bundle. The anatomical structure of this bundle is quite similar to the bundle of the middle vein of the leaf blade. Along the peduncle from leaf blade to the leaf base the sclerification of some external and internal phloem cells is observed. At the same time, the tendency towards transition from bicollateral to amphicribral vascular bundle structure is noticed. The node is one-lacunar, the leaf trace has one bundle.

The stem of annual shoots is felted by long simple and some glandular trichomes. The wide primary cortex has a parenchyma sheath (Fig. 9 A). Continuous stele is crossed by starch-containing, mainly one-rowed secondary pith rays. Pith is rounded-triangular in cross-section (Fig. 9 C) with several fields of small-celled tissue on the periphery. Unlike other species, secondary thickening of the stele happens early and it is accompanied by phellogen formation in internal layers of the primary cortex on the border with the parenchyma sheath (Fig. 9 B) and periderm formation as well. The differentiation of 4–5 phellem layers spreads acropetally from basal shoot metamers to apical ones. For all that, the external fields of died cork are cut off (Fig. 9 C, D).

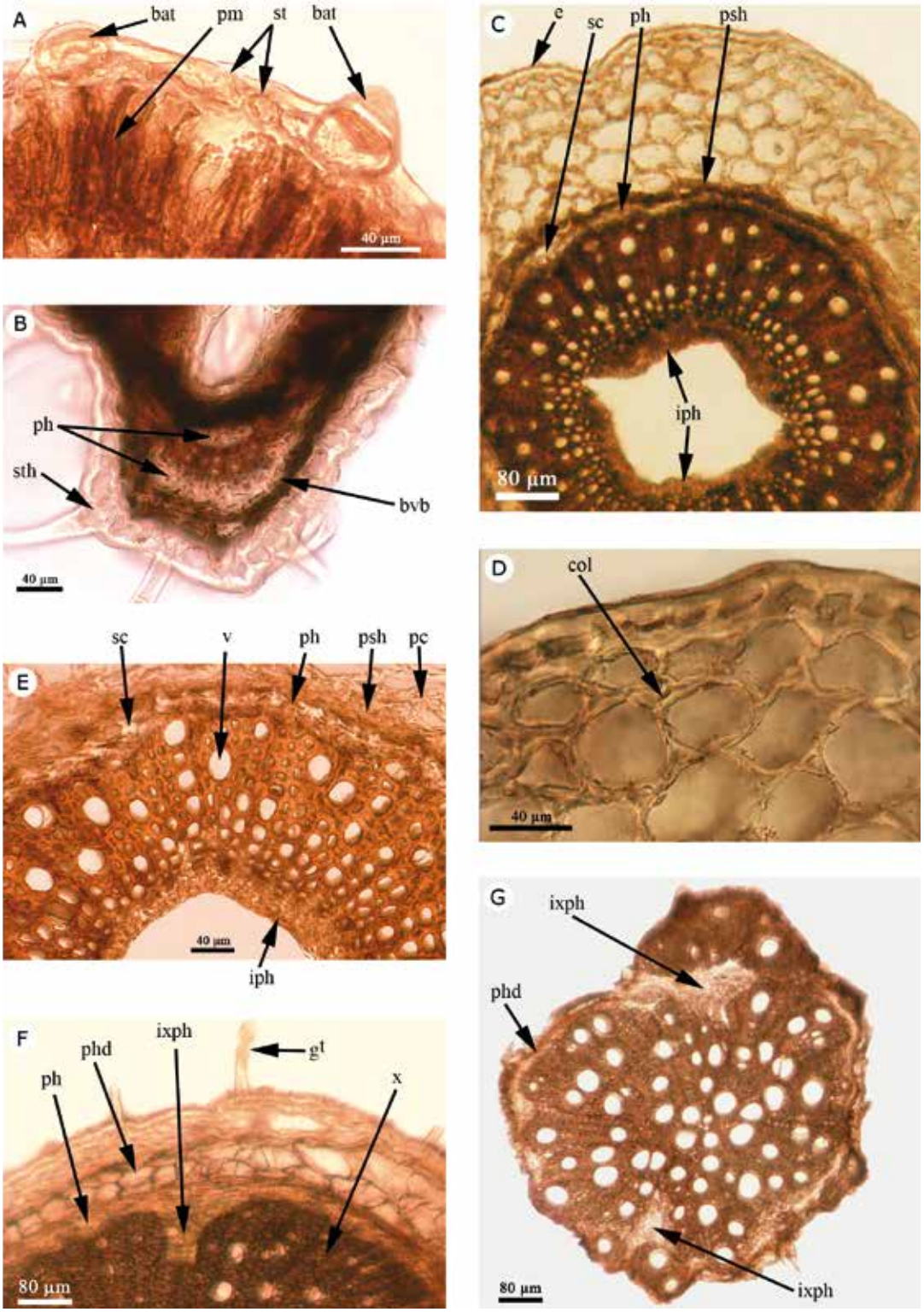

Figure 7. *Tiquilia nuttallii*. A, B – leaf; C–F – stem of annual shoot; G – root cross-section. bat – base of awl-like simple trichome; bvb – bicollateral vascular bundle; col – collenchyma; e – epidermis; gt – glandular trichome; iph – internal phloem; ixph – interxylar phloem; pc – primary cortex; ph – phloem; phd – phelloderm; pm – palisade mesophyll; psh – parenchyma sheath; sc – sclereids; st – stomata; sth – simple trichome; v – vessel; x – xylem.

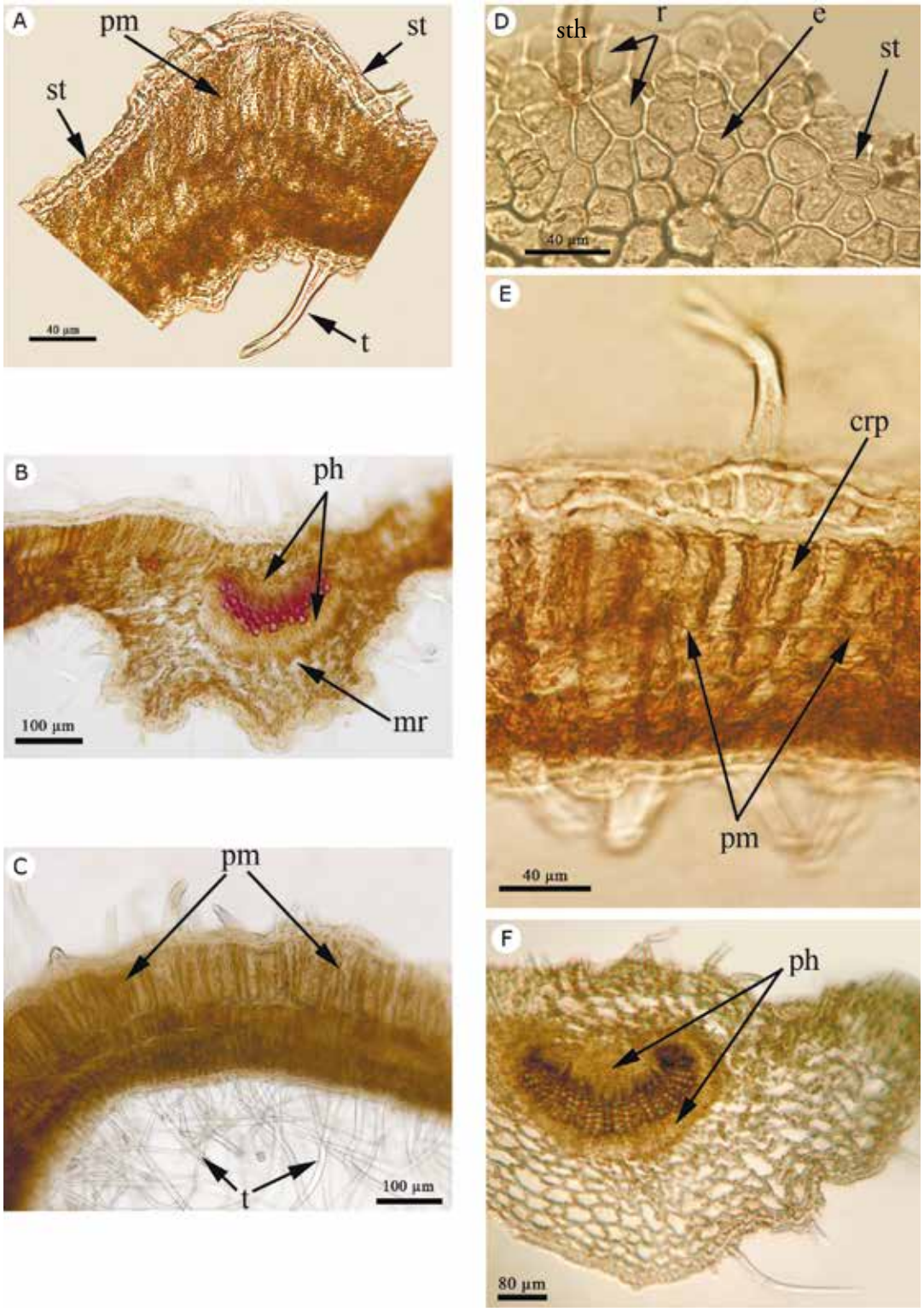

Figure 8. *Tiquilia canescens*. Leaf. A – wavy adaxial surface; B – leaf blade with midrib; C – leaf blade with numerous trichomes on abaxial surface; D – fragment of adaxial leaf surface with epidermal cells and stomata; E – mesophyll; F – petiole. crp – cross rugosity of palisades; e – epidermal cells; mr – midrib; ph – phloem; pm – palisade mesophyll; st – stomata; sth – simple trichome; r – rosette of cells at the base of simple trichome; t – trichome.

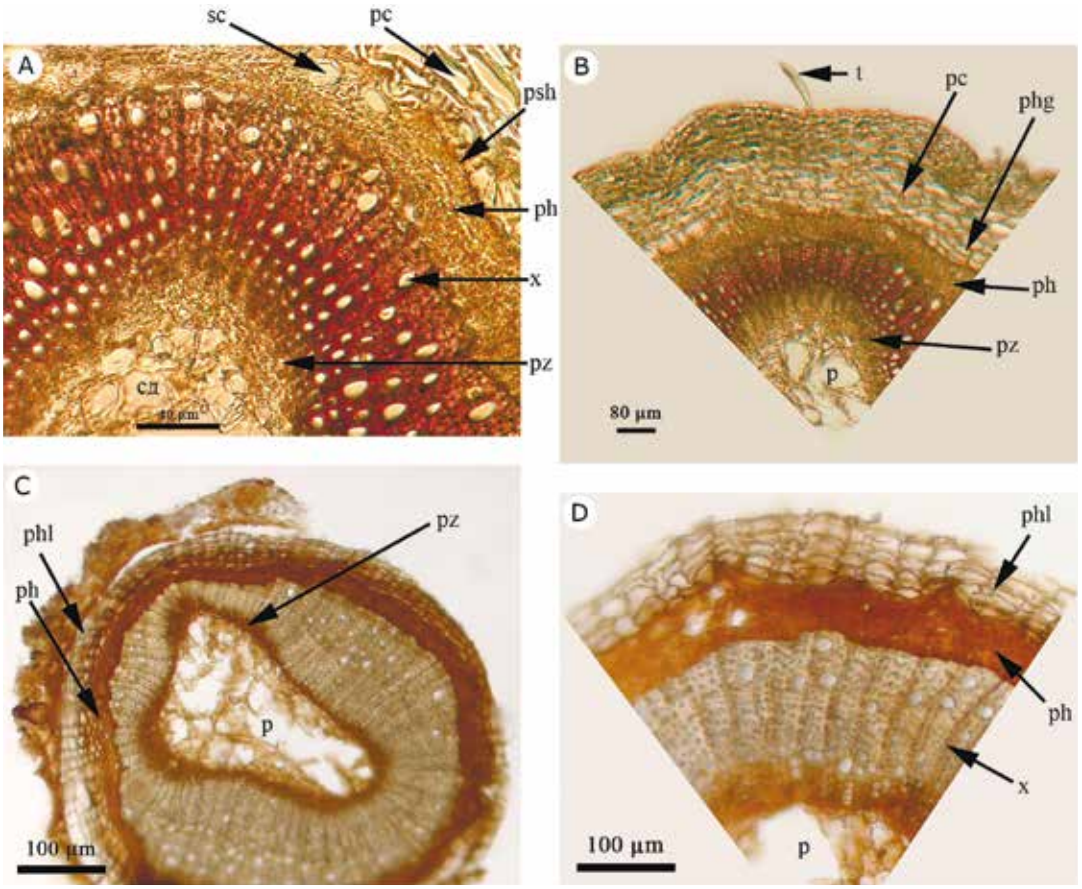

Figure 9. *Tiquilia canescens*. A–D – Stem of annual shoot. p – pith; pc – primary cortex; ph – phloem; phg – phellogen; phl – phellem; psh – parenchyma sheath; pz – perimedullar zone; sc – sclereids; t – trichome; x – xylem.

According to Humphrey (1932), the shoots of *T. canescens* remain for several years (15 and more). The number of phellem layers in a 5–6 years old stem with only 2–3 mm in diameter may grow up to 14–16. Cell walls and cell cavities of some cells are naturally tinged dark brown because of tannins. For all that, the layers of cells with relatively thin cell walls alternate with thick-walled brown phelloids (Fig. 10 A–C). In some specimens, the traces of scaly cork formation were noticed. Phellem spreads out in a narrow collenchymatous zone of secondary phloem which is of phellodermal origin. Secondary phloem is arranged in an almost continuous wide stripe with small air cavities on its periphery, groups of slightly lignified sclereids and sieve elements with simple sieve plates as well. Cambial zone consists of 3–4 layers. Secondary diffused ring-porous xylem (wood) is noticed by distinctly discerned annual growth boarders (Fig. 10 C, D). Short members of both wide and narrow porous vessels with simple perforations are there (Fig. 10 E). The former ones are frequently united (2–4) in horizontal groups (Fig. 10 A, C, D), which are separated from each other by one-rowed pith rays. Relatively narrow, thick-walled tracheidal elements with numerous bordered pits, axial (mainly terminal) (Fig. 10 D) as well as radial parenchyma are visible there. The central part of pith undergoes lysis with the formation of a small air cavity.

Thus, all peculiarities of anatomical structure of *T. canescens* axial organs are testified to their wood organization. Its features reveal early stages of plant morphogenesis. The plant is a typical subshrub or shrub.

Macrostructure and partly microstructure of *T. canescens* root system were described in detail by Humphrey (1932). It is allorhizous. The anatomy of roots which early undergo secondary thickening is similar to the anatomy of the stem.

Discussion

The results of comparative anatomical analysis of vegetative organs of 4 different species showed that *Coldenia procumbens* may be classified as annual tropical light hygromesophyte according to its anatomical features. It mainly grows on frequently flooded river banks and on withered rice fields (Humphrey 1932). The complex of leaf adaptive features includes: large, thin-walled, water-bearing epidermal cells; thin cuticle; few stomata on adaxial and abaxial leaf sides hardly protruding the covering cells and the presence of very long, thin-walled, alive, simple and glandular trichomes as well. Many of above-mentioned characters direct to an increase of water-returning. It doesn't eliminate the role of glandular trichomes of *C. procumbens* as simple hydatodes. Differentiated mesophyll (1 layer of palisades and 4–5 layers of spongy mesophyll), large bicollateral bundle with strongly differentiated xylem in middle vein, which is roughly projected at the bottom, are typical of the leaf blade.

In the stem of orthotropic and procumbent shoots in the zone of large-cellular primary cortex, the collenchyma is lacunar-angular. Its subepidermal layer, epidermis and secondary phloem are distinguished by their dark brown colour caused by tannins. Secondary vascular tissues form an almost continuous stele which is crossed by 1–2-rowed pith rays. Phloem has small groups of fibres. Secondary xylem is predominant. At the border with large-cellular, quickly destroyed pith, a small-celled zone is distinguished. It is similar to the external phloem in colour and structure. However, this similarity needs more careful histological investigations on living material. The stems of extending shoots are hollow, their centre is occupied by a large air cavity.

Main tap root has peculiar stele vasculature. It has up to 4 stages of growth of secondary phloem and xylem in its basal part. As it was mentioned in some other tap-rooted annuals (Barykina 1992), this fact is closely connected with the peculiarities of morphogenesis of semi-rosette, strongly branched, monocarpic shoots. Vigorous development of secondary vascular and sclerenchyma elements including phloem fibres is significant for plants which grows on wet and relatively soft substrate prior to the reproductive phase of its development. Homogenous, relatively thin-walled phellem of pericyclic origin has a protective function in the root.

Studied herbal and woody species of *Tiquilia* grow in dry regions of the New World with insufficient water-supply and intensive illumination. They are typical desert xerophytes. Small leaves with small transpiration surface, a thick layer of cuticle, thick-walled epidermal cells, dense pubescence (it defends the plant from large water losses, partly reflects sun rays and prevents overheating), great amount of tannins, isopalisade mesophyll and an intensive development of vascular system are characteristic of them. The features of xeromorphic and heliomorphic structures are noticed in the microstructure of leaf and stem as well. Annual, prolong-vegetated *Tiquilia palmeri* and *T. nuttallii* from section *Tiquiliopsis* Gray. subgenus *Tiquilia* are confirm this fact. At the same time, common features characteristic of these species are: dense pubescence not only on the leaf blade, but also on petioles and internodes, small size of cells, polygonal form of epidermal cells with thick tangential and relatively thin straight anticlinal cell walls, density of isopalisade mesophyll and small internal transpiration surface. Formation of sclereids in

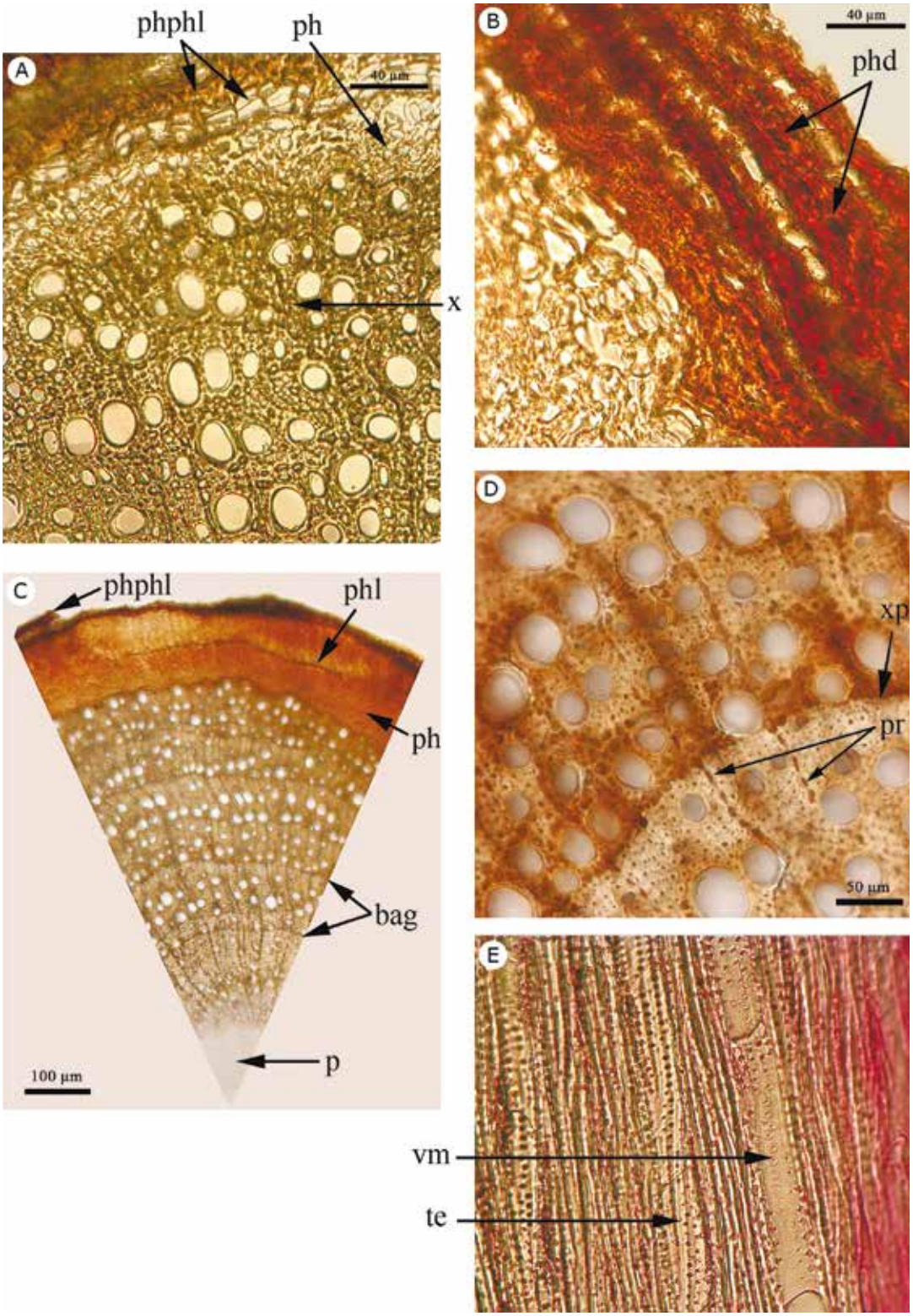

Figure 10. *Tiquilia canescens*. A–E – stem of perennial shoot. bag – the border of annual growth; p – pith; ph – phloem; phd – phelloids; phl – phellem; phphl – phelloid phellem; pr – pith rays; te – tracheal elements; vm – vessel member with simple perforation; x – xylem; xp – xylem parenchyma.

shoot and root phloem, development of almost continuous stele in stem, the presence of small-celled internal primary phloem at the border to large-celled pith (it looks like an amphiphloic siphonostele), breaking of normal vascular cambium activity in stem and root (it produces inwards separate fields of interxylary phloem. The structure, cell size of elements don't differ from the peripheral phloem), diarch type of roots, the presence of tannins in many tissues of vegetative organs are characteristic of them.

At the same time, some specific differences mainly of quantitative character are noticed. Adaxial leaf surface of *T. palmeri* is large wavy and *T. nuttallii* it is small wavy due to high density of its veins. The middle vein of leaf blade in *T. palmeri* consists of 1 or 2 vascular bundles (small vascular bundle is near the large one). It projects more strongly at the bottom. The ridge is semi-rounded and it exceeds blade thickness 3–4 times. In *T. nuttallii*, the vein is one-bundled. The ridge is slightly flattened and exceeds blade thickness only a little bit. Strengthening tissue in stem and root phloem in *T. palmeri* consists of relatively long fibrous sclereids. *T. nuttallii* has shorter macrosclereids. Secondary xylem in *T. palmeri* differs from *T. nuttallii* in larger fields of phloem which are included there. Stem pith is round-triangular in cross-section in *T. palmeri* and almost rounded in *T. nuttallii*.

It's impossible to pass over the fact of replacing the epidermis by cork in the lower sections of shoots in both annual species of *Tiquilia*. It may be regarded as concealed possibility of the plant to a more continuous life cycle (ontogenesis) or as tendency to formation of a subshrub or, at last, as retention (a retaining of ancestral wood form features at the base of shoot). The formation of a secondary protective tissue in annual *Coldenia procumbens* was not noted.

The dwarf-shrub *T. canescens* has some specific features of adaptive specialization together with many common structural features of the annual representatives of the genus. It is due to another life form, a prolonged life cycle and its systematic status (subgenus *Eddya*).

Thin leaf blades of small, oval, inversely egg-shaped leaves, sometimes decurrent to the petiole, have folded down roundly extended edges (revolutive type) and a flat or slightly wavy adaxial surface. In contrast to Humphrey (1932), they are amphistomatic, not hypostomatic. Long and relatively short mesophyll palisades have distinct, fine-wavy anticlinal cell walls and are traversed across cell cavities by thin pleats which can be tinged pink by ruthenium. Such structures of palisade mesophyll as well as apparently its adaptive significance aren't usual at all. Thus, in the 'Encyclopedia of Anatomy' (Meyer 1962) such type of assimilative tissue is not mentioned. More thorough and detailed microscopic investigations are needed. Apparently, this structure protects the cell protoplast from deformation during water loss and prolonged droughty periods. But another role of pleats, including gas transmission, cannot be excluded.

Unlike perennial representatives of *Tiquilia* from the New World, the secondary thickening of stele in the annual stem of *T. canescens* is accompanied by the formation of periderm on its border. It develops all over the circumference of stem and spreads acropetally from the base to upper internodes as completion of their primary elongation. At the end of vegetation, all metamers of annual shoots have phellem on their surface. It cuts off the died off primary cortex. The structure of phellem is heterogenous: The layers of light thin-walled cells with suberized cell walls alternate with the layers of naturally brown-coloured by tannins, thick-walled phelloids with lignified cell walls. As the result of such structure, phellem is shed from the surface by separated fragments. Stele includes small-celled phloem with groups of slightly lignified sclereids which are arranged

in an almost continuous wide stripe and strongly develops in secondary xylem, where the borders of annual growths can be distinctly noticed.

Thus, interxylary phloem which is characterictic of axial organs of *Tiquilia palmeri* and *T. nuttallii* is absent in *T. canescens*. According to Mullenders (1947), Mikesell (1979), Carlquist (1981, 2002) and Patil et al. (2011) it has been frequently revealed in representatives of different families of the tropical flora. There are no distinct features of a primary phloem presence in the perimedullar zone of the stem pith. These special features which are different from annual *Tiquilia* species are not characteristic of *Coldenia procumbens*. Engler & Prantl (1897) included it together with *C. canescens* in section *Eucoldenia* Benth. However, the similarity of some features doesn't always point to a relationship of taxa.

As a whole, the presence of many common anatomical features in the structure of vegetative organs are typical for all 4 studied species of *Coldenia* s.l.: well-pronounced pubescence of above-ground shoots, including simple and glandular trichomes, wavy adaxial leaf surface (expressed at different degrees) which is due to different palisade lengths, the presence of tannins, relatively slight differentiation of sclerenchyma, high level of conductive elements specialization, the presence of short porous vessel members with simple perforations in secondary xylem and sieve tubes with simple sieve plates in phloem, similar type of nodal structure (one-lacunar node, leaf trace with one bundle) which is characteristic of other representatives of subfamily Ehretioideae, in particular *Ehretia* species (Barykina 2012). Three-lacunar node with 3 bundles is characteristic of subfamily Cordioideae (Metcalfe & Chalk 1950).

The revealed differences in anatomical structure of vegetative organs of studied species correlate with different ecological conditions of their habitat, plant life form and systematic position. The results of microscopic analysis are adjusted with molecular data. In particular, in accordance to data of Moore & Jansen (2006), *Coldenia procumbens* is isolated. It belongs to the monotypic genus *Coldenia*. Three other *Tiquilia* species are members of two different subgenera: *Eddya* (*T. canescens*) and *Tiquilia* (*T. nuttallii* and *T. palmeri*).

Acknowledgements

The authors express their deep gratitude to Dr R. Kamelin, corresponding member of the Russian Academy of Sciences and to Dr A. Skvortsov, professor of biology, both former curators of herbaria at the Komarov Botanical Institute and at the Tsitsin Main Botanical Garden, for providing experimental materials.

This work was carried out in accordance to Government order for the Lomonosov Moscow State University (projects No. AAAA-A16-116021660045-2, No. AAAA-A16-116021660105-3).

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