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Leaf development in *Curio articulatus* (L. f.) P.V. Heath (Asteraceae–Senecioneae)

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Summary: The leaf initiates as paraboloid-shaped primordium without any signs of ad- and abaxial sides. Distinct wider Unterblatt and narrower Oberblatt usually do not develop in the primordium. It grows longer and changes into bifacial formation nearly throughout but the very tip. The latter one remains unifacial and morphogenetically inactive. This tip is incorrectly considered homologous to the Vorläuferspitzen of other *Curio* species. The lamina generally develops in a typical way, but unifacial tips form on the developing lamina lobes. The lamina lobe tips are quite similar to the leaf tip. Short-functioning adaxial and cross-leaf meristems successively arise in the developing petiole. Activity of the latter one causes the basal lamina lobes to turn to the median plane. It also results in relative narrowing of the adaxial side and subunifacial petiole. Such a petiole is not homologous to the subunifacial terete laminas of other *Curio* species.

Keywords: leaf primordium, faciality, unifacial, bifacial, Unterblatt, Oberblatt, Vorläuferspitze, cross-leaf meristem, adaxial meristem, lamina, petiole, phyllode

Leaf dorsiventral polarity is currently investigated in mutants of a few model species. These mutants show only some symptoms that barely resemble unifacial leaves, because neither model species nor their close affinities have naturally unifacial leaves. Species of the genus *Curio* P.V. Heath segregated from *Senecio* L. are very diverse in development of the adaxial side of their leaves. The latter vary from typical dorsiventral bifacial petiolate to subunifacial, to unifacial, to secondary bifacial (Timonin & Ozerova 1993a, b). That is why the genus *Curio* is a very convenient natural model for studying dorsiventral polarization of the primordial leaf. *Curio articulatus* has bifacial petiolate leaves (Fig. 1) most similar to the majority of *Senecio* species. Such leaves were regarded as the original leaf type in the genus *Curio* which had given rise to different leaves of deviating dorsiventral polarity (Timonin & Ozerova 1993a). Therefore, the leaves of this species are of paramount importance for understanding core principles of diversification of leaf faciality in this genus.

The definitive leaves of *C. articulatus* were previously scrutinized anatomically (Thoday & Woodhead 1932), but their genesis remains unexplored. However, recognized genesis of the leaf is indispensable to realize leaf morphology, its evolutionary paths and causes (Roth 1957). Therefore, not only definitive structure of the presumably ancestral leaf, but also leaf genesis are necessary to effectually investigate causes and laws of deviating dorsiventral polarity of leaves in different *Curio* species. The present study is aimed to yield information to fill obvious gaps in our knowledge about genesis of leaves in *C. articulatus*.

Materials and methods

Tips of growing shoots were taken from the plants grown in the greenhouse at Tsitsin Main Botanical Garden of Russian Academy of Science, Moscow, and fixed in 70% ethanol.

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Figure 1. Leafy shoots of *C. articulatus*.

Morphological transformations of developing leaves were examined by scanning electron microscopy (SEM). The fixed shoot tips were dehydrated in a series ethanol 90%, ethanol 96%, ethanol 100%, ethanol : acetone 1:1, acetone 100% and acetone 100%, critical point-dried at HCP-2 (Hitachi) facility and fastened on stubs. The tips thus prepared were coated with Pd-Au mixture at the IB-3 Ion Coater (EIKO) and examined under scanning electron microscope Camscan-S2 (Cambridge Instruments). The work was carried out at Electron microscopy laboratory of Lomonosov Moscow State University.

Leaf histogenesis was investigated on serial cross-sections of the fixed shoot tips. The latter were dehydrated in a series ethanol 90%, ethanol 96%, ethanol 100%, ethanol 100%, ethanol : xylol 3:1, ethanol : xylol 1:1, ethanol : xylol 1:3, xylol 100% and xylol 100% and embedded in paraffin wax (Barykina et al. 2004). The microtome slices (10 µm thick) were mounted on slides, dewaxed with xylol and rehydrated with a series ethanol 100%, ethanol 100%, ethanol 90%, distilled water. Rehydrated slices were successively stained with Delafield's Hematoxylin and Carbolic Fuchsin, dehydrated and embedded in Canada Balm according to Barykina et al. (2004). The preparations were examined under light microscope (LM) Nikon eclipse Ci equipped with digital camera Nikon digital sight DS-Vi1.

Results

The apical dome is slightly convex to slightly concave (Fig. 2A–C). The leaf initiates as paraboloid-shaped projection on the apex edge (Fig. 2A). It is usually round in cross-section (Fig. 3A), but sometimes flattened in the median plane (Fig. 3B). The leaf primordium entirely consists of embryonic tissue at this developmental stage (Fig. 3A, B). The primordia typically grow apically to become peg-shaped (Fig. 2 B, C), but very few ones differentiate into a tangentially

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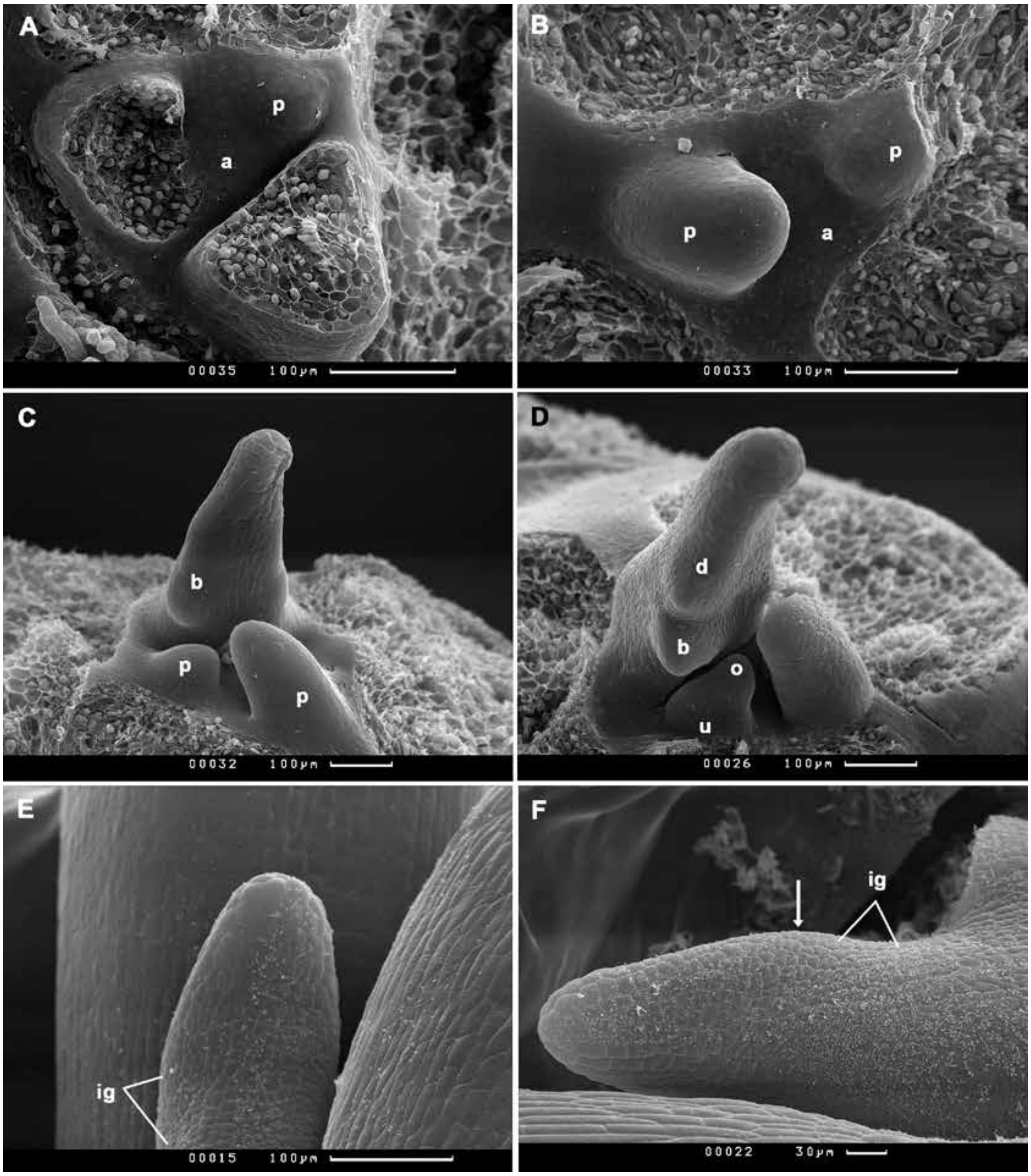

Figure 2. Morphology of developing leaf, SEM. A – initiated leaf primordium; B – apically growing leaf primordium (on the left); C – growing leaf primordia and lamina initiation; D – primordium of Unterblatt and Oberblatt, peg-shaped primordium and initiated lamina lobes; E – intercalary elongating primordium; F – initiated lamina. a – apex; b – lamina basal lobe; d – lamina distal lobe; ig – intercalary growth zone; o – Oberblatt; p – leaf primordium; u – Unterblatt; arrow – initiated lamina.

wider Unterblatt and a narrower Oberblatt (Fig. 2 D). The few cells long tip of the peg-shaped primordium becomes morphogenetically inactive in a rather short period of time; its cells grow larger and develop large vacuoles (Fig. 3 C, D). Thereafter, the apical growth of the primordium is replaced by the intercalary elongation distant from the primordium base (Fig. 2 E). The growing primordium increasingly thickens from the inactive tip to the base and changes its cross-section form from tangentially flattened (Fig. 3 E) to semi-circular (Fig. 3 F) and to triangular (Fig. 4 A).

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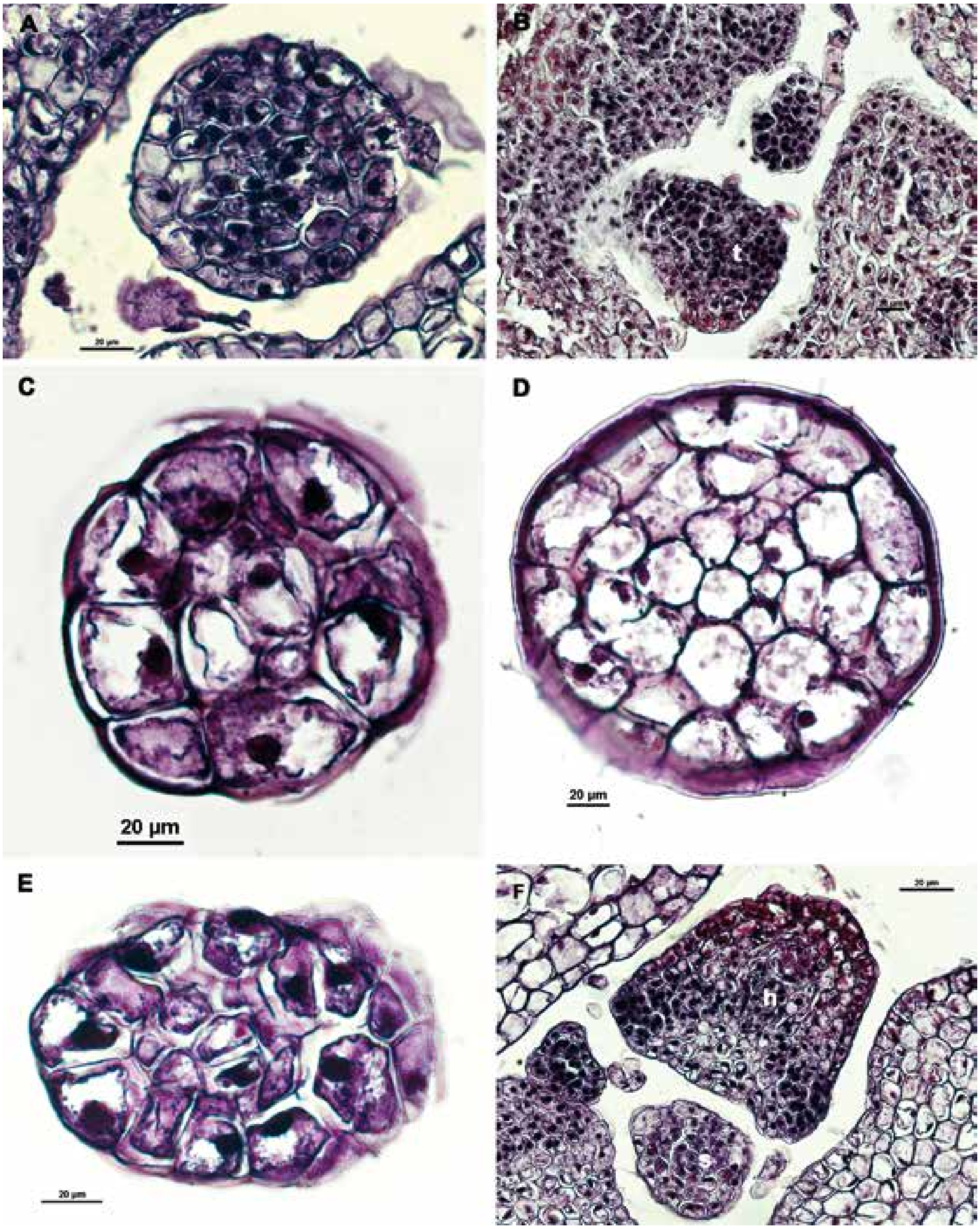

Figure 3. Leaf histogenesis, cross-sections, LM. A – leaf primordium of embryonic tissue; B – elliptic and triangular primordia; C – partially differentiated tissue of the primordium tip; D – differentiated tissue of the primordium tip; E – tangentially flattened primordium; F – semi-circular and heart-shaped primordia. h – heart-shaped primordium; s – semi-circular primordium; t – triangular primordium.

Figure 4. Leaf anatomy and histogenesis, cross-sections, LM. A – triangular primordium; B – heart-shaped primordium; C – initiating leaf margins; D – growing lamina margin; E – growing lamina; F – definitive lamina; G – inactive leaf tip with vascular bundle. c – corpus-like tissue; abl – abaxial cell layers; adl – adaxial cell layers; bd – vascular bundle; ch – chlorenchyma; d – ether-oil duct; m – initial cell of the marginal meristem; ml – middle cell layers; sm – initial cell of the submarginal meristem; t – tunic-like tissue; w – water-storage tissue.

►

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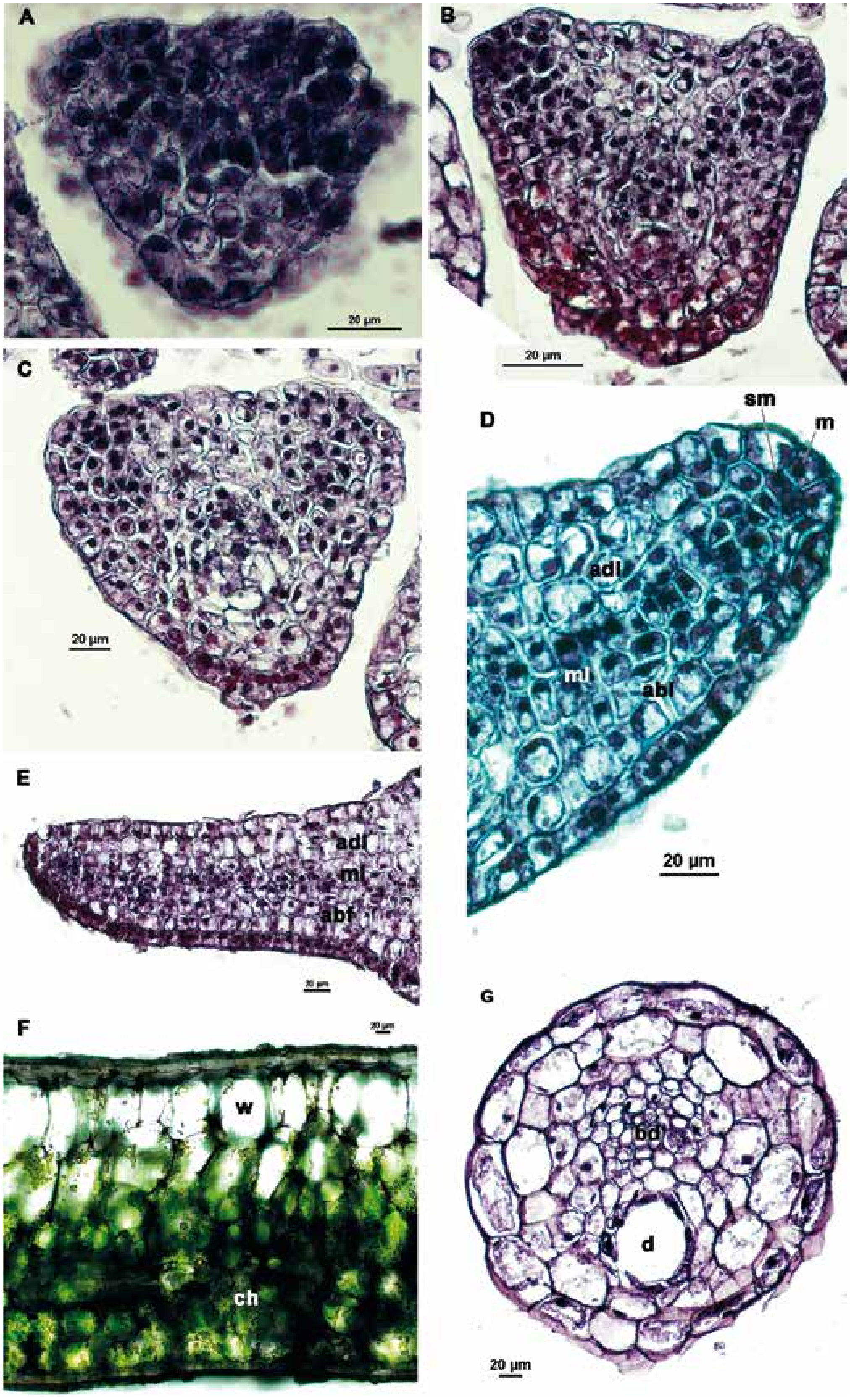

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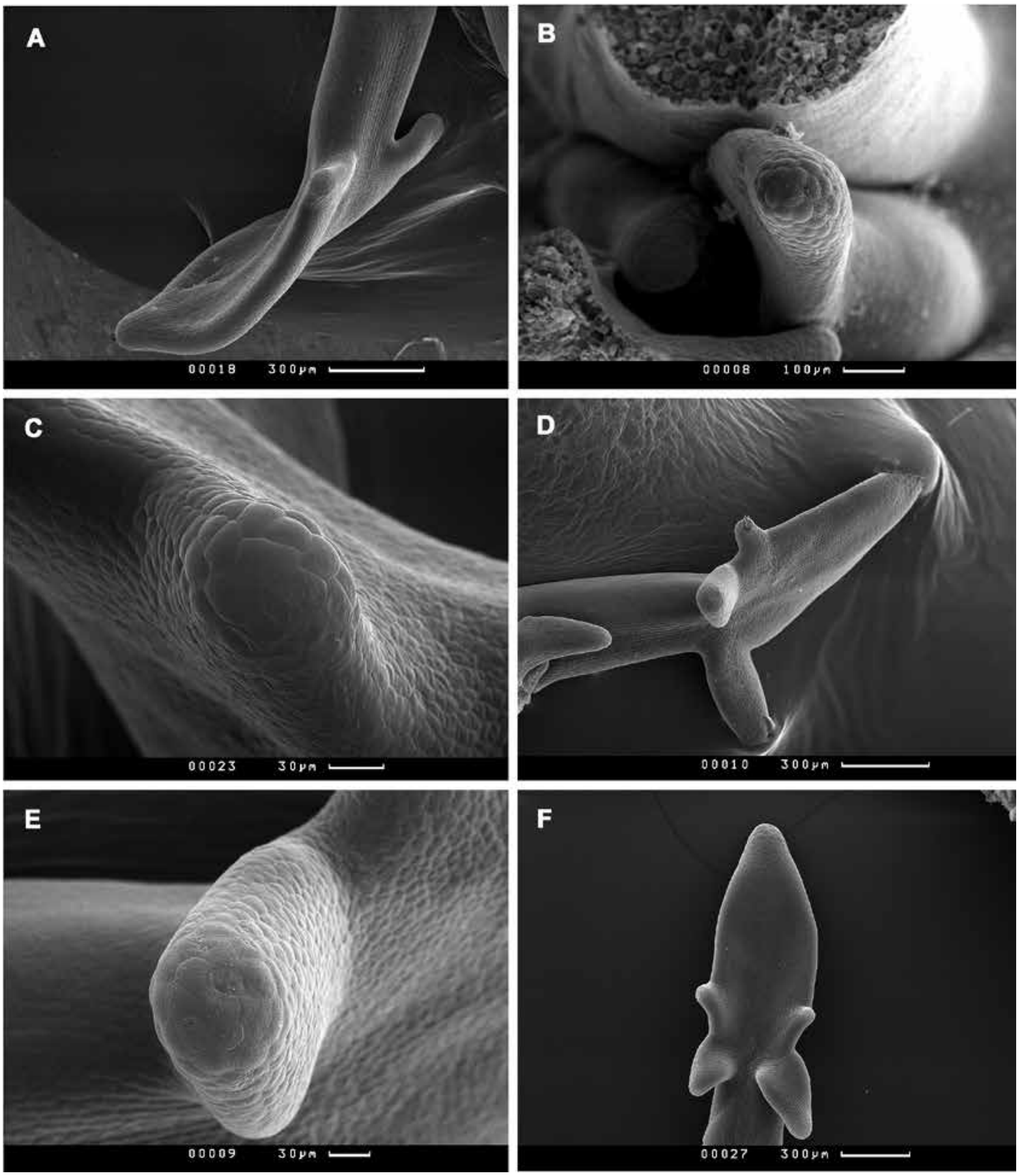

Figure 5. Developing lamina, SEM. A – leaf with one (basal) pair of lobes; B – inactive leaf tip; C – lamina lobe, initial position; D – inward turned lamina basal lobes; E – tip of the lamina lobe; F – inward turned basal and distal lobes of lamina.

The basal part of the primordium (prospective petiole and midrib base) even becomes heart-shaped in cross-section (Fig. 4 B). Easily identifiable margins resultantly appear in the leaf primordium. The latter one still consists of embryonic tissue throughout, but the inactive tip. The thickening growth is accordingly diffuse.

The lamina initiates just below the middle of the primordium at the distal edge of the intercalary elongation zone (Fig. 2 F). Paired projections developing into the lamina basal lobes initiate at first, though asynchronously (Fig. 2 C). They are usually followed by the paired primordia of

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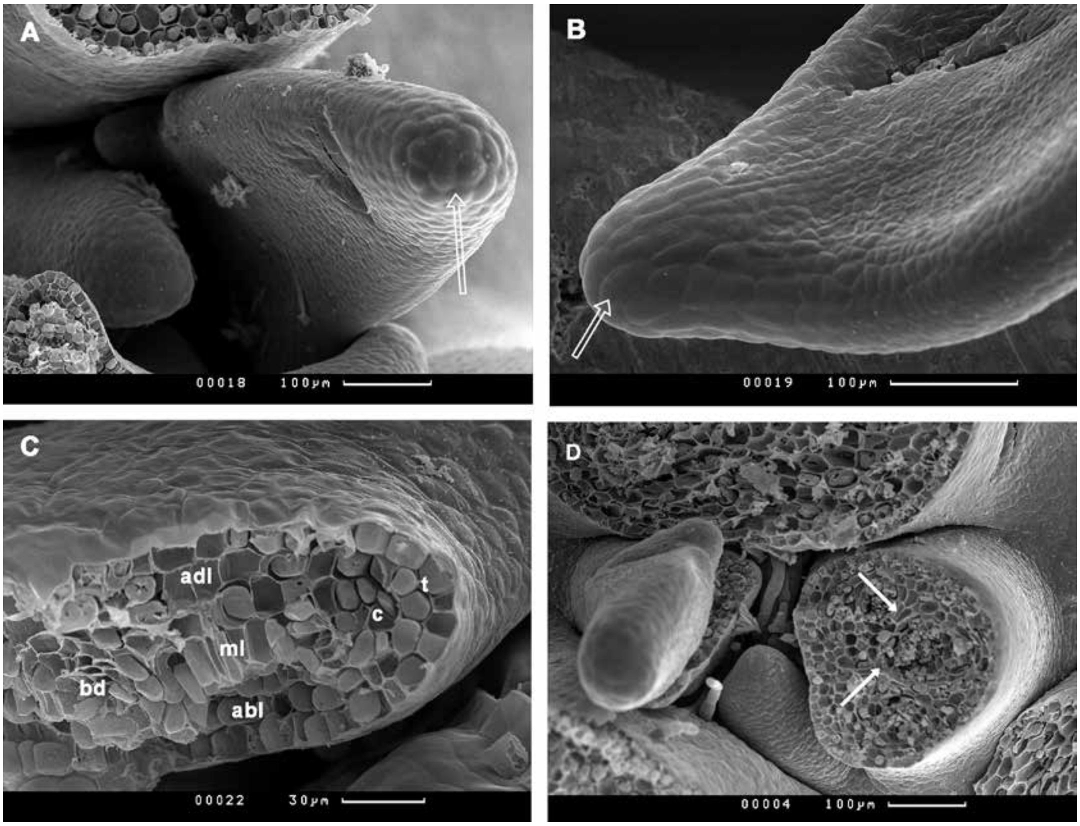

Figure 6. Leaf tip and leaf histogenesis, SEM. A – acropetally differentiating lamina and inactive leaf tip; B – inactive leaf tip and lamina margins, from the abaxial side; C – margin of the developing lamina; D – cross-leaf meristem in the developing petiole. abl – abaxial cell layers; adl – adaxial cell layers; bd – vascular bundle; ml – middle cell layer; c – corpus-like tissue; t – tunic-like tissue; cored arrow – leaf tip; solid arrow – cross-leaf meristem.

the lamina distal lobes (Fig. 2 D), but the latter are absent in some leaves (Fig. 5 A). Initiation of the basal lobes changes the peg-shaped primordium into prospective petiole and midrib. The intercalary elongation zone is in the distalmost part of the petiole and in the very base of the midrib. Developing lamina lobes are initially in the leaf blade plane (Figs 2 C, D; 5 B, C), but they become oblique with leaf development (Fig. 5 D, E), especially the basal ones (Fig. 5 F). Their basiscopic edges are convergent on the adaxial side of the midrib (Fig. 5 F).

The distal entire lamina acropetally progresses from the acroscopic edge of the distal lobes (if present) up to the inactive tip of the leaf (Fig. 6 A), but the tip *per se* is not affected by the lamina development. The leaf margins acropetally decrease and disappear completely near the inactive leaf tip (Figs 5 B; 6 B). Similar morphogenetically inactive tips develop on the lamina lobes; leaf margins also disappear near these tips (Fig. 5 C, E).

The margin of the developing lamina is initially roundish; initial cells of its marginal and submarginal meristems are undetectable (Fig. 4 B). The margin is sometimes similar to the shoot apex with its 2-layered tunica covering the corpus (Figs 4 C; 6 C). Later, the margin becomes sharper and the initial cells become identifiable (Fig. 4 D). Those of the marginal meristem divide anticlinally to form protodermis. The initial cells of the submarginal meristem divide three-directionally. Their adaxial derivatives divide periclinally to give rise to 2 adaxial cell layers

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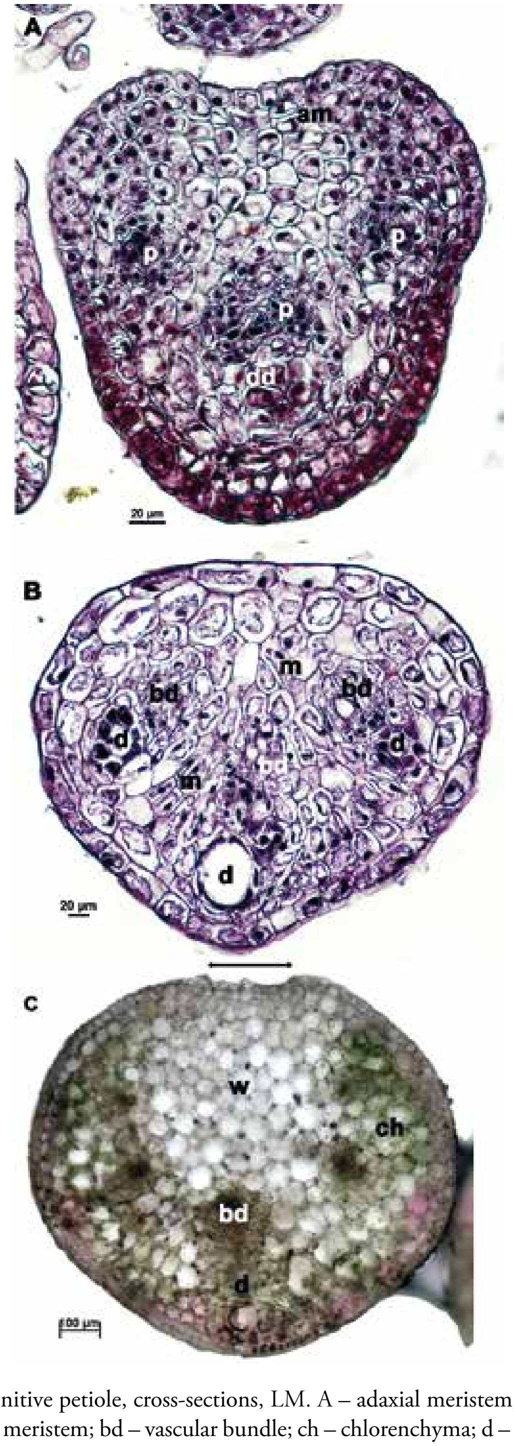

Figure 7. Developing and definitive petiole, cross-sections, LM. A – adaxial meristem; B – cross-leaf meristems; C – definitive petiole. am – adaxial meristem; bd – vascular bundle; ch – chlorenchyma; d – ether-oil duct; dd – developing ether-oil duct; m – cross-leaf meristem; p – procambial strand; w – water-storage tissue.

(Figs 4D; 6C). Their middle derivatives mostly divide anticlinally to form the (upper) middle cell layer. The abaxial derivatives divide periclinally into subepidermal and inner cells. The latter divide again tangentially to form (lower) middle cell layer (Figs 4D; 6C). The protoderm and mesophyll cells also divide anticlinally to widen the lamina.

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The cells of both adaxial layers first cease dividing. They grow up, become vacuolated (Fig. 4 E) and change into the constituents of subepidermal water-storage tissue. The cells of two abaxial layers cease dividing later to transform into cells of spongy chlorenchyma. The cells of the middle layers are the latest to cease dividing. Most of them divide only anticlinally and become palisade chlorenchyma cells (Fig. 4 E), but some cells divide in different directions to give rise to procambial strands (Fig. 4 D). The palisade and spongy chlorenchyma are easily recognizable in growing lamina (Fig. 4 D, E), but they hardly differ in the mature one (Fig. 4 F).

The embryonic tissue of the petiole and midrib mostly differentiates after developing of the procambial strands. These leaf constituents grow thicker by means of indistinctive adaxial meristem (Fig. 7 A). The median vascular bundle runs through nearly the complete leaf. It even develops in inactive leaf tip (Fig. 4 G) except for its very tip. Paired lateral vascular bundles differentiate in the petiole and basalmost midrib (Fig. 6 D). The cells between the provascular strands of median and lateral bundles divide in oblique cross-leaf plane to make the tissue they constitute similar to developing cambium (Figs 6 D; 7 B). These cell divisions are active but short. Nevertheless, they contribute to the petiole and midrib thickening. Some additional lateral bundles develop in the petiole during its thickening (Fig. 7 C).

Discussion

The leaf of *C. articulatus* drastically changes during its development. The leaf primordium just initiated has no visible signs of its bifacial structure (Figs 2 A, B; 3 A). Discernible boundary between the adaxial and abaxial sides appears later, when the primordium actively grows longer (Fig. 3 F). However, this boundary is absent in the very tip of the primordium (Figs 3 D; 5 B) which soon becomes morphogenetically inactive. There is a median vascular bundle with adaxial xylem and abaxial phloem in this tip. This bundle is embedded in uniform parenchyma (Fig. 3 D) which is equally dissimilar to any inner leaf tissue that could indicate adaxial and / or abaxial sides of the leaf. The epidermis of this tip also consists of uniform cells which clearly differ from both adaxial and abaxial epidermis of the lamina (Figs 5 B; 6 A, B). Thus, the vascular bundle is the only indicator of the conceivable bifacial structure of the leaf tip, whereas no other signs of bifacial structure of the leaf tip have been revealed.

The vasculature is inappropriate to be considered as main criterion of leaf faciality (Roth 1957). The presence of the leaf margins is thought to be the most reliable criterion of the bifacial structure (Napp-Zinn 1974). The leaf tip under consideration has no attribute of the leaf margins. The latter do not coalesce but completely disappear near the tip (Fig. 6 A, B). Therefore, the leaf tip of *C. articulatus* can be concluded with confidence to be unifacial. However, anatomical and morphological characteristics make it impossible to ascertain which side is absent. We believe that the adaxial side is absent, because the unquestionable adaxial side is absent in the unifacial Vorläuferspitzen of other *Curio* species (Timonin et al. 2006; Ozerova & Timonin 2009). Also, the adaxial side is underdeveloped in the petioles of *C. articulatus* (Fig. 7 C). If so, the adaxial side must be absent likewise in the lobe tips. Some special investigation of the expression of gene / genes that determine/s adaxial or abaxial side could elucidate the problem and unequivocally show which side is in the unifacial leaf tip of *C. articulatus*.

Development of the adaxial water-storage tissue shows special characteristics of the lamina histogenesis in *C. articulatus*. This histogenesis otherwise corresponds with one of the common types of lamina development of dicotyledons (Napp-Zinn 1973).

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The thickening of the petiole and basalmost midrib is initially diffuse. After development of provascular strands of the median and paired lateral bundles, the groups of actively dividing cells appear in between. More or less local groups of dividing cells in the developing leaf are usually considered meristems (Napp-Zinn 1973). Then, the groups in question can relevantly be regarded as specific interfascicular cross-leaf meristem. It occupies the abaxial part of the petiole and is absent in its adaxial part (Fig. 7 B). Thereof, it causes uneven tangential expansion of the petiole that does not affect its adaxial part. The adaxial side of the petiole retains its width, whereas the abaxial side grows wider. Relative 'narrowing' of the latter one resultantly takes place while petiole thickening. This 'narrowing' seems to shift basiscopic edges of the lamina lobes to the median plane and to make the lobes oblique. The adaxial meristem subsequently enhances widening of the abaxial side to result in subunifacial petiole (Figs 5 D, F; 7 C).

Only initial stages of leaf development in *C. articulatus* are quite similar to those in other species of *Curio*. Differentiation of wider Unterblatt and narrower Oberblatt is typical of *Curio* species, but it takes place in *C. articulatus* very rarely.

The leaf margins typically coalesce on the adaxial side near Unterblatt to Oberblatt transition in all investigated *Curio* species (Timonin et al. 2006; Ozerova & Timonin 2009) save *C. articulatus*. Therefore, the Oberblatt is unifacial and develops into manifest Vorläuferspitze. Different position of the intercalary growth zone in such a leaf primordium results in either unifacial or subunifacial terete leaves or their evolutionary derivatives (Timonin et al. 2006; Ozerova & Timonin 2009). The leaf primordium of *C. articulatus* is basically bifacial except for its tiny few cells long tip. Besides, the same unifacial tips terminate the lamina lobes (Fig. 5 C, E). These tips are certainly not homologous to the Vorläuferspitzen of other *Curio* species. Consequently, the leaf tip of *C. articulatus* is unlikely to be homologized to the Vorläuferspitze.

Thickening of the petiole in *C. articulatus* by means of activity of the adaxial meristem is quite similar to that of terete leaves in other *Curio* species (Hillson 1979; Ozerova & Timonin 2009). This similarity is consistent with the theory of phyllodic origin of the terete leaves in the genus *Curio* (Timonin & Ozerova 1993a). However, the petiole of *C. articulatus* takes only a basal third or a quarter of bifacial leaf primordium. If such a leaf transformed into phyllode, it would rather have lost most of its Oberblatt including its tiny unifacial tip, than have changed nearly complete Oberblatt into unifacial Vorläuferspitze. The typical Vorläuferspitze of *Curio* species should be compared to the complete Oberblatt of leaf primordium in *C. articulatus* (if recognized). The terete lamina of other *Curio* species should be regarded as evolutionary innovation. Initially unifacial leaf primordia of *C. articulatus* could be a precondition for its evolution to the unifacial Vorläuferspitze of other species.

Growth by means of the adaxial meristem seems to be the simplest way to make the terete formation thicker. Relative 'narrowing' of the adaxial side is likely a side-effect of such a growth. So this way is likely to be universal. The adaxial meristem in terete leaves of different *Curio* species must show a universal mode of thickening, but not their phyllode origin. The terete subunifacial leaves of *Curio* species could have evolved from the leaves of the type like in *C. articulatus* (Timonin & Ozerova 1993a). However, it is highly unlikely that this evolution was a reduction of the lamina and emergence of the phyllode.

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