RESEARCH PAPER

Development of the leaf forerunner tip (Vorläuferspitze) in *Curio* affinities (Asteraceae: *Senecioneae*), a structure unique to dicots

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ABSTRACT

Forerunner tip, known also as Vorläuferspitze (Germ.), is a distinct usually unifacial part of a leaf which outraces all other its definitive constituents during leaf development and plays a central role in the modern models of leaf development in monocots. It is supposed to be absent in eudicots, but recent data indicate that at least genus *Curio* is an exception. Unlike monocots, the forerunner tips in this genus become unifacial without activity of the adaxial meristem. The scrutinized morphological and anatomical investigation revealed that precocious differentiation and unifacial structure which are widely used in identifying the forerunner tip are necessary but insufficient criteria of the structure concerned. Two additional criteria for recognition of the forerunner tip are proposed, viz. the forerunner tip takes significant part of the upper leaf zone immediately after the completion of its development and it remains distinguishable at all subsequent stages of leaf morphogenesis.

Keywords: forerunner tip, Vorläuferspitze, leaf development, unifacial, upper leaf zone, lower leaf zone, *Curio*

РЕЗЮМЕ

Федотов А.П., Озерова Л.В., Тимонин А.К. Развитие предшественника верхушки листа (Vorläuferspitze), уникальной структуры для двудольных, у представителей *Curio* (Asteraceae: *Senecioneae*). Предшественник верхушки листа (Vorläuferspitze на нем.) – это отдельная, обычно унифациальная часть листа, которая опережает все другие части листа в развитии и играет центральную роль в современных моделях развития листа у однодольных. Предполагается, что он отсутствует у двудольных, за исключением, как показывают последние данные, рода *Curio*. В отличие от однодольных, предшественник верхушки листа у этого рода становится унифациальным без активности адаксиальной меристемы. Проведённое тщательное морфологическое и анатомическое исследование показало, что критерии раннего заложения и унифациальности, которые широко используются для выявления предшественника верхушки листа, являются необходимыми, но недостаточными для выявления рассматриваемой структуры. Мы предлагаем два дополнительных критерия для выявления Vorläuferspitze: сразу после завершения развития Vorläuferspitze занимает значительную часть верхней зоны листа, кроме того, он остается различимым на всех последующих стадиях морфогенеза листа.

Ключевые слова: предшественник верхушки листа, Vorläuferspitze, развитие листьев, унифациальность, верхняя зона листа, нижняя зона листа, *Curio*

The forerunner tip alias Vorläuferspitze (Germ.) is a distinct part of the leaf that arises first during leaf morphogenesis (Troll 1939, Kaplan 1973). It protects the bud (Raciborsky 1900, Troll 1939) and it is also substantial in the development of monocot leaves (Troll 1939, Bharathan 1996, Rudall & Buzgo 2002).

According to the Troll's model of leaf morphogenesis (Troll 1939, Kaplan 1973, Conklin et al. 2018), an initially bifacial leaf primordium first divides into the lower leaf zone (LLZ) alias Unterblatt (Germ.) and upper leaf zone (ULZ) alias Oberblatt (Germ.). The forerunner tip develops from the ULZ at the next stage of leaf development. The structure concerned usually becomes unifacial (Roth 1949, Kumar et al. 1984) due to the activity of the adaxial rounding meristem alias Rundungsmeristem (Germ.) (Hagemann 1970).

The monocots are characterized by a variety of leaf morphogenesis resulting in either bifacial or unifacial leaves (Conklin et al. 2018, Crang et al. 2018). Unifacial

leaves differ from the bifacial ones in the absence of the adaxial side. Therefore, the most appropriate criterion for distinguishing between these morphological types of leaves is the absence/presence of the morphological margin delimiting the two sides of the leaf (Napp-Zinn 1973). In addition to these morphological types of leaves, an intermediate subunifacial type has also been recognized. It is characterized by highly reduced very narrow but quite perceptible adaxial side and discernible morphological margins of the leaf blade (Hagemann 1970, Hillson 1979).

Rather many monocots have the invariably unifacial forerunner tips in their leaves (Troll 1939, Knoll 1948). Either the entire ULZ or only some part of it differentiates into the forerunner tip, which can only be ascertained from a detailed investigation of leaf development (Bloedel & Hirsch 1979, Tillich 1998).

On the contrary, the only leaf development pathway is described in dicots (Bharathan 1996, Rudall & Buzgo 2002). All eudicots are thought to have bifacial leaves, which

develop differently from the monocots's ones and have no forerunner tips (Bharathan 1996).

This idea is based primarily on the fact that when recognizing the two main morphological types of leaves, the structure of their leaf blades is mainly considered. However, the criterion for the presence of a morphological margin is applicable to any part of the leaf (Eberwein 2019). Thus, unifacial petioles have been described in different dicots taxa (Franck 1976) and unifacial rachis is known in some species of Apiaceae (Charlton 1992). Even the unifacial and subunifacial leaf blades are described in the families Aizoaceae, Asteraceae, Crassulaceae (Timonin & Ozerova 1993, Melo-de-Pinna et al. 2016). So, morphogenesis of the dicots' leaves might be as diverse as those of the monocots' leaves. Accordingly, the concept of the absence of forerunner tips in eudicots may be incorrect (Knoll 1948), at least because the leaf tips of all studied species of the genus *Curio* (Asteraceae) were identified as the forerunner tips (Ozerova & Timonin 2009), although not convincing enough.

Thus, there is only fragmentary information about the diversity of leaf morphogenesis in dicotyledons and even more fragmentary information about the presence and morphogenesis of the forerunner tips in this taxon. Considering that the forerunner tip is of importance in the development of monocots' leaves, this imbalance of data precludes a meaningful comparison of leaf morphogenesis in these two groups of plants. The present investigation of the forerunner tip development in South African succulent species from the genera *Curio* (Asteraceae) and *Baculellum* (Asteraceae) which have diverse morphological leaf types, is aimed to fill this gap to provide a solid basis for comparing leaf morphogenesis patterns in eudicots and monocots.

MATERIAL AND METHODS

For the present investigation, we have chosen species whose diverse leaves cover all known leaf types in genus *Curio*. *Curio muirii* (L. Bolus) van Jaarsv. with entire obovate leaves is the only representative of the genus with bifacial leaves. Two species with unifacial leaves, *C. citriformis* (G.D. Rowley) P.V. Heath and *C. talinoides* (DC.) P.V. Heath, and three species with subunifacial leaves, *C. hallianus* (G.D. Rowley) P.V. Heath, *C. herreanus* (Dinter) P.V. Heath, and *C. rowleyanus* (H. Jacobsen) P.V. Heath, were used in the study, as they were reported earlier to have the forerunner tips (Ozerova & Timonin 2009). Unifacial lobed leaves of *Baculellum arcticulatum* (L. f.) L.V. Ozerova & A.C. Timonin were additionally investigated because they were once considered the original leaf type for *Curio* species (Timonin & Ozerova 1993, Fedotov et al. 2016).

The material was obtained from the collection of the living plants of the Tsitsin Main Botanical Garden of the Russian Academy of Science, Moscow. Terminal shoot buds were fixed either in 70 % ethanol (for morphological investigation) (Barykina et al. 2004) or in FAA overnight with post-fixation in 70 % ethanol (for anatomical investigation) (Ruzin 1999).

The fixed material for the morphological investigation was dehydrated in a series of alcohol solutions of growing concentrations (80 %, 90 %, two times 96 %, two times 100 %) and impregnated with acetone through series ethanol: acetone 1:1, acetone 100 % and acetone 100 %. Samples were critical point-dried at HCP-2 (Hitachi) facility and fastened on stubs. The shoot tips thus prepared were coated with Pd–Au at the IB-3 Ion Coater (EIKO) and examined under scanning electron microscope Camscan-S2 (Cambridge Instruments) at the accelerating voltage 20 kV (SEI mode). This part of the work was carried out at the Laboratory of electron microscopy (Lomonosov Moscow State University).

Fixed material for anatomical investigation was dehydrated in an ethanol series (80 %, 90 %, two times 96 %, two times 100 %), transferred to xylene through the series of ethanol : xylene solutions (3:1; 1:1; 1:3) and embedded in paraffin. 10 μm thick serial transverse sections were made on rotary microtome Microm HM355S (Thermo Fisher Scientific) and dried for 2 days at 37°C in drying chamber. The sections were deparaffinized according to the standard procedure (Barykina 2004). Some sections were stained with combination of 1 % aqueous Safranin O and 0.1 % (in 3 % acetic acid) Alcian Blue solutions. The other slides were stained with Sharman's stain (Sharman 1943) modified as follows: 5–6 min in 0.2 % Toluidine Blue in 0.2 % Sodium Tetraborate between the steps of Safranin O and Orange G-Tannic Acid staining (Jernstedt et al. 1992). Automated slide stainer Varistain Gemini ES (Thermo Fisher Scientific) was used for slide preparation. The stained slides were examined under transmission light microscope AxioPlan (Carl Zeiss) or under slide scanner VS120 (Olympus).

RESULTS

In all species concerned, the leaves develop in a typical angiosperm way from the initiation to the differentiation of the lower (LLZ) and upper (ULZ) zones of the primordium, which remains completely bifacial throughout this period of development.

Bifacial leaves

Having formed, the upper leaf zone of *B. articulatum* begins to lengthen due to the apical growth. As a result, a leaf axis arises. The leaf primordium becomes subulate (Fig. 1A). The primordium is totally meristematic at this stage, and it is impossible to distinguish individual meristems in it. As the leaf axis develops, the distal part of the ULZ turns into a terminal radially symmetrical structure (Fig. 1A,B).

Upon completion of the formation of the leaf axis, the apical growth of the leaf primordium is replaced by its intercalary growth. At this developmental stage, there is only the small distal part of the ULZ which is radially symmetrical. It smoothly passes into the leaf blade, which has a clearly defined morphological margin. The terminal radially symmetrical part without distinguishable morphological margins is approximately 50 μm long, while the total length of the ULZ is about 400 μm at this stage of leaf development (Fig. 1C). Dimension of the radially symmetrical part remains unchanged at later stage of development when the lobes of the leaf blade begin to form.

When the leaf blade initiates, the histogenesis begins in the topmost part of the ULZ. The dermal and ground
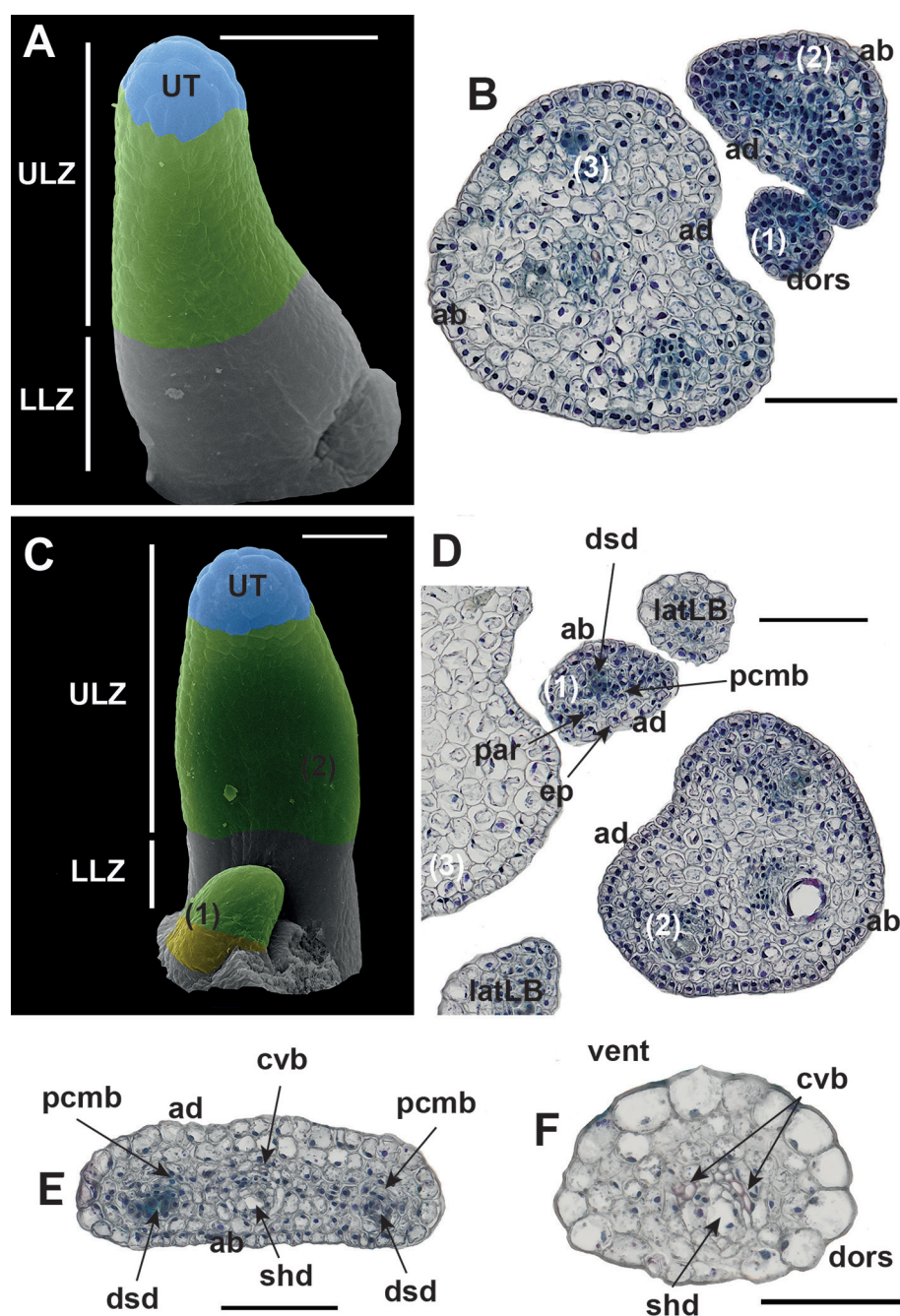
Figure 1 A–F – *Baculellum articulatum* (L. f.) L.V. Ozerova & A.C. Timonin. A – leaf primordium during the apical growth; B – cross-section through the unifacial part of leaf primordium at stage of two leaf zones (1) and various zones of older leaf primordia; C – leaf primordium at the stage of intercalary growth; D – cross-section through the ULZ of the leaf primordium (1) and various zones of older leaf primordia; E – cross-section through the bifacial differentiated part of ULZ; F – cross-section through the differentiated unifacial part of ULZ. ab – abaxial side; ad – adaxial side; cvb – collateral vascular bundle; dors – dorsal side; dsd – developing schizogenous duct; ep – epidermis; latLB – lateral lobe; LLZ – lower leaf zone; par – parenchyma; pcmb – procambium strand; shd – schizogenous duct; ULZ – upper leaf zone; UT – unifacial tip; vent – ventral side. Numbers in parentheses indicate leaf primordia in order from youngest to oldest. Scale bars: A – F: 100 μm.

strand is accompanied by the schizogenous duct on the dorsal side (Fig. 1D). Three collateral vascular bundles are plainly visible in the distal part of the ULZ after the lateral lobes of the leaf blade have initiated (Fig. 1E). These vascular bundles acropetally merge into one collateral vascular bundle in the part of ULZ which has clearly discernible morphological margins (Fig. 1F). The accompanying schizogenous ducts also merge into one, located dorsally to the sole distalmost vascular bundle. The sole vascular bundle ends blindly at about 40 μm from the leaf top, its accompanying schizogenous duct also ends blindly about 60 μm from the leaf top.

The early stages of leaf development in *C. muirii* are similar to those in *B. articulatum*. Having passed two zone stage, triangular leaf primordium grows predominantly apically. It completely consists of meristematic tissue. Individual meristems are therefore indiscernible in it (Fig. 2A,B). At this stage of development, the distal part of the ULZ becomes radial-symmetric in cross section.

The basipetal differentiation of tissues begins in the distal part of the ULZ after the leaf axis has formed and the intercalary growth of leaf primordium has replaced its apical growth. The differentiated distal part of ULZ is often thicker than its basalmost part at this stage of leaf development (Fig. 2C). By this time, the radially symmetric distalmost part of ULZ accounts for about a third of its length. This radially symmetrical tip remains clearly visible at all subsequent stages of leaf morphogenesis (Fig. 2D).

Three principal collateral vascular bundles develop in the ULZ which has distinguishable morphological margins except for its distal part (Fig. 2F). Schizogenous ducts develop along each of them on the dorsal side. Vascular bundles fuse into one as they enter the tip of the leaf (Fig. 2E). Schizogenous ducts are merge into one, which is located dorsally of single collateral vascular bundle. The latter
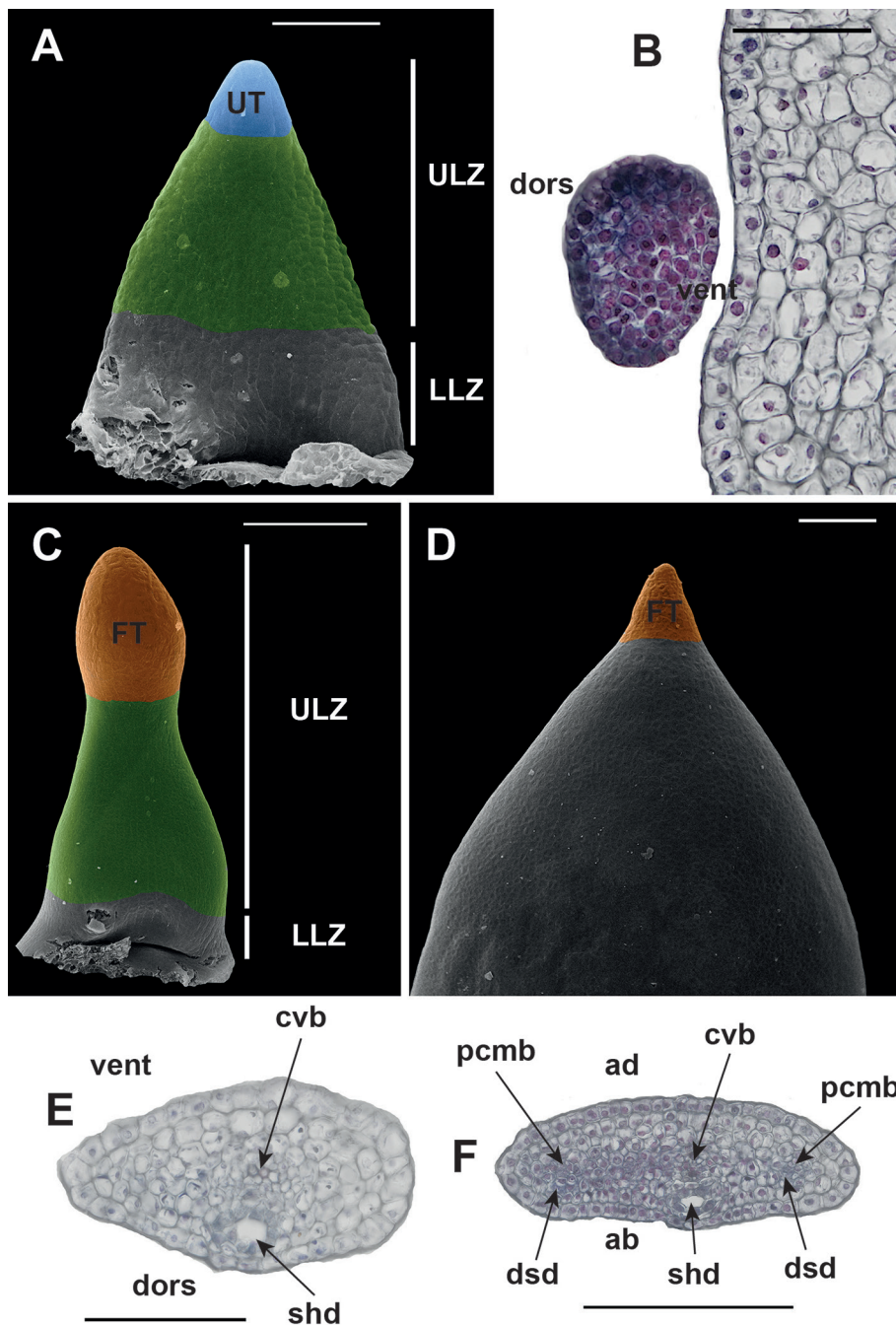
tissues differentiate basipetally. The epidermis in the radially symmetrical terminal part of the ULZ clearly differs in cell size from the protoderm of the more basal parts of the leaf. The stomata are in the epidermis of the radially symmetrical part of the ULZ. On the contrary, the procambium differentiates acropetally, and each principal procambium

Figure 2 A–F – *Curio muirii* (L.Bolus) van Jaarsv.; A – leaf primordium during the apical growth; B – cross-section through the ULZ of the leaf primordium at the stage of two zones; C – leaf primordium at the stage of the intercalary growth; D – forerunner tip at later stages of development; E – cross-section through the forerunner tip; F – cross-section through the developing leaf blade. ab – abaxial side; ad – adaxial side; cvb – collateral vascular bundle; dors – dorsal side; dsd – developing schizogenous duct; FT – forerunner tip; LLZ – lower leaf zone; pcmb – procambium strand; shd - schizogenous duct; ULZ – upper leaf zone; UT – unifacial tip; vent – ventral side. Scale bars: E - 50 μm; A, B, F – 100 μm; C, D – 300 μm

Thereafter, the leaf primordia grow apically and become subulate, while the whole ULZ becomes radially symmetric in cross section (Fig. 3A). At this stage of development, the whole primordium consists of meristematic tissue, in which the adaxial meristem is undetectable.

The distal part of the ULZ begins to lose meristematic activity and differentiate basipetally when the leaf primordium reaches about 250 μm in *C. citriformis* and 150 μm in *C. talinoides*. The distinct feature of *C. citriformis* is the large semispherical epidermal cells in ULZ tip (Fig. 3B). In *C. talinoides*, the epidermal cells of the ULZ tip differ slightly in shape from the cells of the epidermis of the rest ULZ (Fig. 3C). In both species, stomata sometimes develop in the radially symmetrical tip. The loss of meristematic activity of the distal part coincides with the transition to intercalary growth.

The leaf primordium becomes needle-like at the stage of intercalary growth and remains so further on (Fig. 3D). It is impossible to determine a clear boundary between the radially symmetric tip and the rest ULZ. There are three principal collateral vascular bundles in the distal part of the ULZ, which fuse into one collateral vascular bundle towards the tip of the leaf primordium (Fig. 3E). Schizogenous ducts are located dorsally to each principal vascular bundle; they also fuse into one located dorsally to the single vascular bundle. The vascular bundle in the most distal part of the ULZ does not reach the tip of the primordium about 70 μm in *C. citriformis* and 30 μm in *C. talinoides*. The vascular bundles are characterized by a relatively large amount of xylem and a few phloem elements.

A subepidermal adaxial rounding meristem develops at the base of the ULZ during the intercalary growth of the leaf primordium. It forms characteristic rows of cells resulting from periclinal cell divisions (Fig. 3F). Further thickening of the developing leaf blade results from adaxial meristem activity.

enters the radially symmetrical part of the ULZ and blindly ends at a distance of about 160 μm from the tip of leaf primordium, while the schizogenous duct ends at a distance of about 150 μm from the tip.

Unifacial leaves

Leaf primordia of *C. citriformis* and *C. talinoides* develop in a similar fashion up to differentiation of two zones.

Subunifacial leaves

The subunifacial leaves of *C. hallianus*, *C. herreanus* and *C. rowleyanus* typically develop the ULZ and LLZ whose differentiation is followed by the apical growth of the ULZ to result in the formation of the leaf axis (Fig. 4A). The latter is approximately 300 μm in length *C. hallianus* and *C. herreanus*, and about 500 μm in *C. rowleyanus*. The distal part of the ULZ becomes radially symmetrical in cross-section (Fig. 4B). The whole ULZ consists of meristematic tissue during the apical growth of the leaf primordium.

The apical growth of the leaf primordium is replaced by intercalary growth after the formation of the leaf axis. Radially symmetric apical part of the ULZ differentiates basipetally concurrently with the intercalary growing of the leaf primordium. This part occupies about a third of the total length of the ULZ (Fig. 4D) and is clearly distinguishable at all subsequent stages of leaf development. The epidermis, parenchyma, procambium (it differentiates acropetally) and dorsal schizogenous duct are differentiated there (Fig. 4C). There are rare stomata in the epidermis of this structure. Three principal collateral vascular bundles are formed in the lower part of ULZ during the intercalary growth of the leaf (Fig. 4E). They fuse into one toward the radially symmetric tip of the leaf primordium (Fig. 4F). This vascular bundle is characterized by a relatively large amount of xylem and a small amount of phloem. It does not reach the leaf tip of about 50–80 μm. Each principal vascular bundle is accompanied by the schizogenous duct on the dorsal side. The duct develops simultaneously with the differentiation of the procambium it matches. An adaxial meristem is at the base of the rounded part of the ULZ, where leaf blade originates (Fig. 4B).

DISCUSSION

In all investigated species, the distal part of the ULZ becomes radially symmetrical in cross-section and loses the
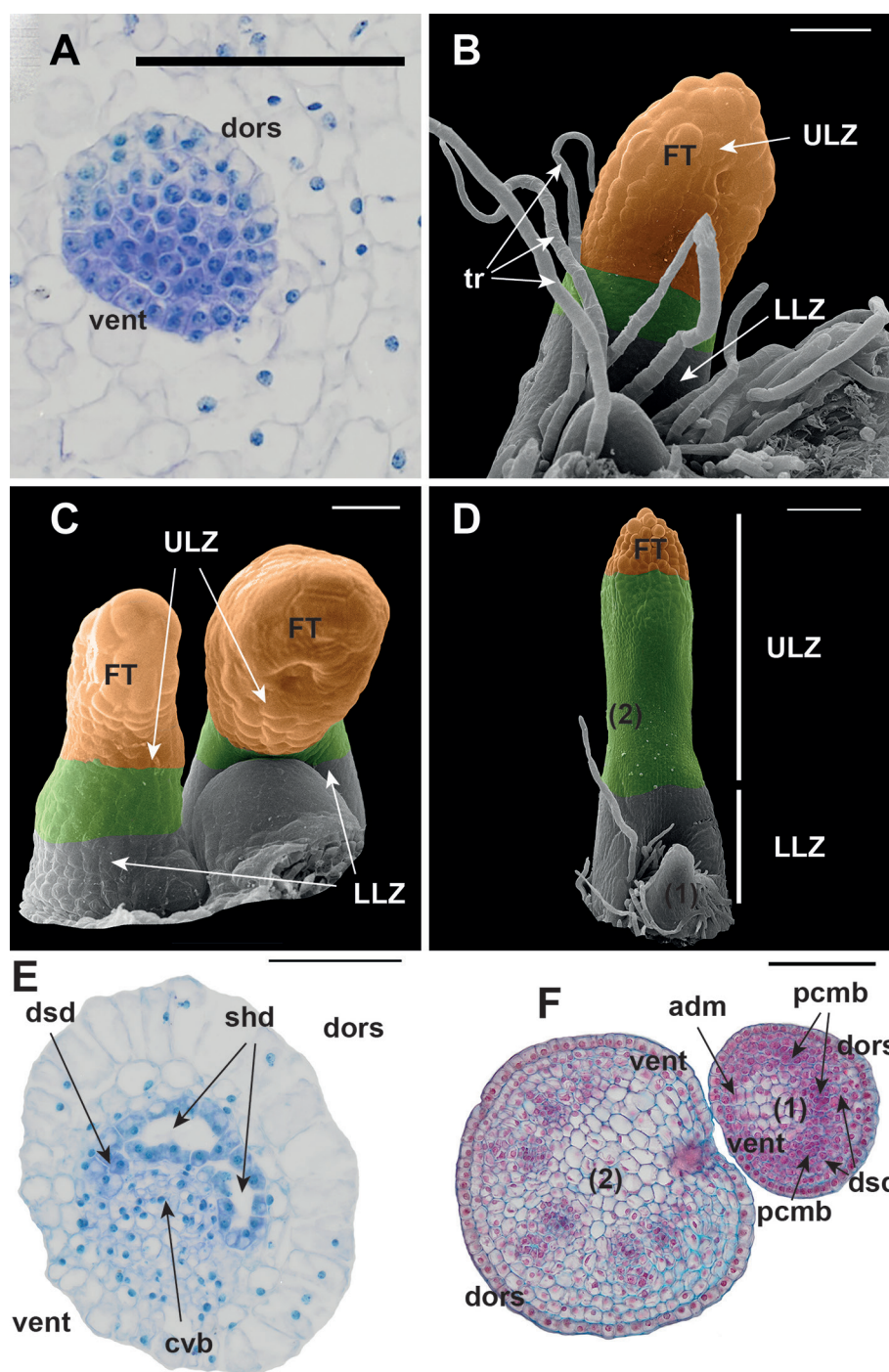

Figure 3 A, B, D, E – *Curio citriformis* (G.D.Rowley) P.V. Heath; C, F – *C. talinoides* (DC.) P.V. Heath. A – cross-section through the ULZ of the leaf primordium at the stage of two zones; B, C – leaf primordia at the beginning of the intercalary growth stage; D – leaf primordium at the stage of the intercalary growth; E – cross-section through the forerunner tip; F – cross-section through the developing leaf blade of leaf primordium (1) and older primordium. adm – adaxial meristem; cvb – collateral vascular bundle; dors – dorsal side; dsd – developing schizogenous duct; FT – forerunner tip; LLZ – lower leaf zone; pcmb – procambium strand; shd - schizogenous duct; tr – trichomes; ULZ – upper leaf zone; vent – ventral side. Numbers in parentheses indicate leaf primordia in order from youngest to oldest. Scale bars: C – 50 μm; A, B, D – F: 100 μm

morphological margin. The sign of dorsiventral polarity of this structure is the collateral vascular bundle with ventral xylem and dorsal phloem and schizogenous duct dorsally to the vascular bundle (Fig. 1F, 2E, 3E, 4E). These characters could indicate the bifacial structure of the distal part of ULZ, since such a mutual arrangement of the conducting tissues in the bundle is considered characteristic of the
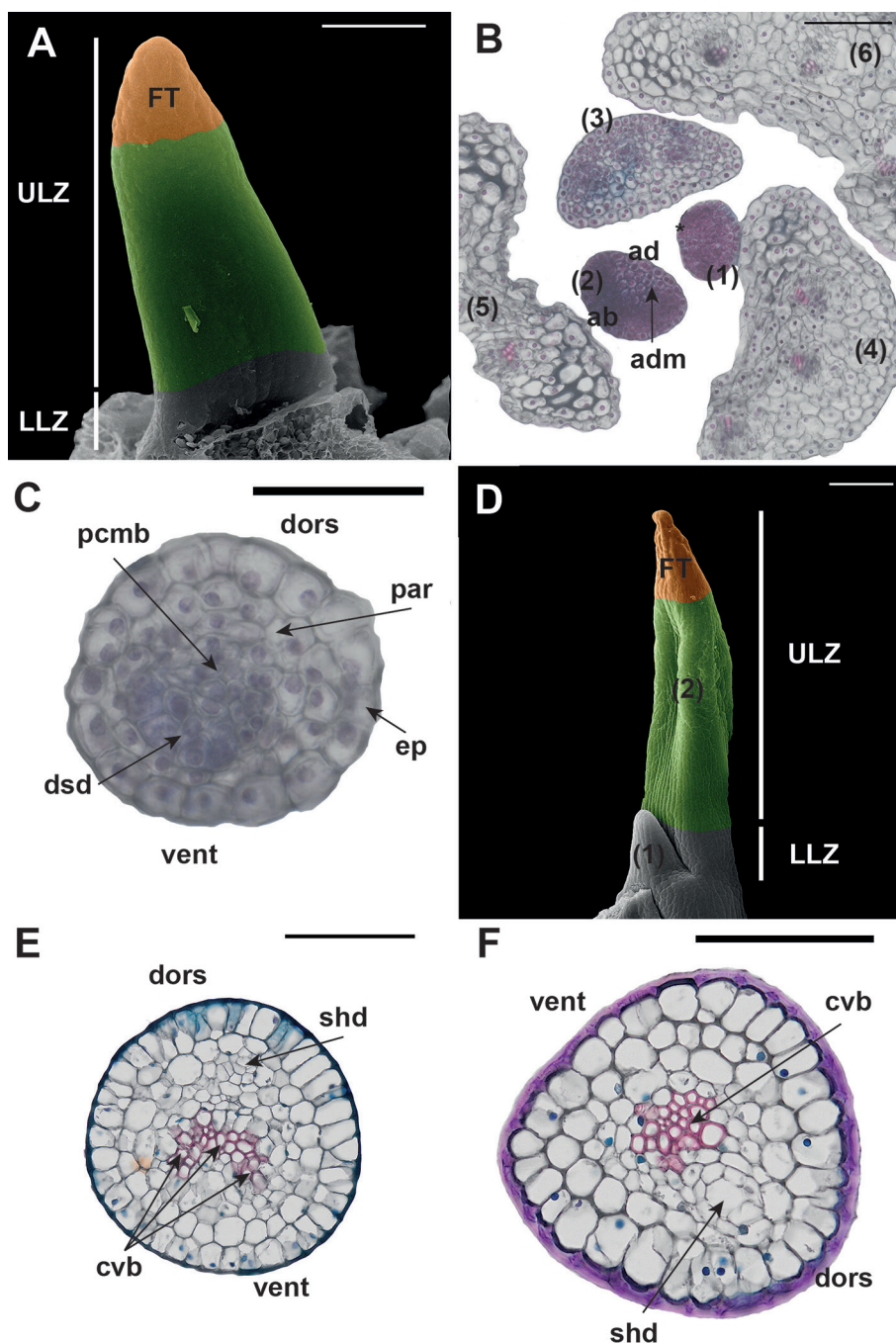
Figure 4 A, B, C – *Curio hallianus* (G.D. Rowley) P.V. Heath; D, E, F – *C. herreanus* (Dinter) P.V. Heath. A – leaf primordium during the apical growth; B – cross-section through the distal part of the leaf primordium (1), differentiating leaf blade of leaf primordium (2) and older leaf primordia; C – cross-section through the differentiating forerunner tip; D – leaf primordium at the stage of intercalary growth; E, F – cross-section through the forerunner tips. ab – abaxial side; ad – adaxial side; adm – adaxial meristem; cvb – collateral vascular bundle; dors – dorsal side; dsd – developing schizogenous duct; ep – epidermis; FT – forerunner tip; LLZ – lower leaf zone; pcmb – procambium strand; par – parenchyma; shd - schizogenous duct; ULZ – upper leaf zone; vent – ventral side. Numbers in parentheses indicate leaf primordia in order from youngest to oldest. asterics (*) indicates ventral side. Scale bars: C – 25 μm; E, F – 50 μm; A, B, D – 100 μm

criterion for leaf faciality, and priority should be given to the Napp-Zinn's (1973) morphological criterion for leaf faciality.

Following this criterion, we conclude that the apical part of the ULZ indeed becomes unifacial in all species studied.

The leaf tips of the investigated species become unifacial at the end of the leaf axis formation, just before the apical growth of the leaf primordium is replaced by an intercalary one (Fig. 1A, 2C, 3B,C, 4A). At this developmental stage, the permanent tissues begin to differentiate basipetally in the ULZ, which fits with general ideas about leaf morphogenesis in angiosperms (Esau 1977). In *B. articulatum*, the apical part of the ULZ also becomes unifacial as in *Curio* species under consideration. However, this part occupies a relatively small part of the ULZ and becomes hardly distinguishable further on and it does not look like a distinct part of the leaf (Fig. 1A,C). Its unifacial structure could result from its terminal position and acropetally increasing suppression of the marginal growing of the leaf axis. Quite similar unifacial tips also terminate the lobes of the leaf blade (Fedotov et al. 2016). Therefore, we think that there is no genuine forerunner tip in *B. articulatum*. The opposite situation is observed in the investigated *Curio* species, whose apical part of the ULZ is noticeably larger and is distinguishable at all stages of leaf morphogenesis (Fig. 2C,D) (Ozerova & Timonin 2009).

All investigated species of the genus *Curio* sharply contrast with the *Baculellum articulatum* in that the unifacial apical part of their ULZs is much larger from the very beginning, namely, about a third of the ULZ in *C. muirii* and species with subunifacial leaves, and almost the entire ULZ in species with unifacial leaves at the stage of beginning intercalary growth. Besides, it remains clearly distinguishable at the morphological level at all subsequent developmental stages of a leaf (Fig. 2C,D, 3B,D, 4D). Therefore, it is worth being

bifacial leaf and similar dorsal schizogenous ducts are in the clearly bifacial leaf parts in *Curio* species. However, the vascular patterns of leaves are shown to correlate ambiguously with their faciality (Roth 1957, Kaplan 2001, Melo-de-Pinna et al. 2016). So, it is not a reliable indicator of leaf faciality. Therefore, the anatomical data cannot be used as a primary

considered a genuine forerunner tip as a distinct constituent of a leaf. Thus, all investigated species of the genus *Curio* indeed have the forerunner tips.

We believe that accustomed forerunner tip criteria of unifaciality and early differentiation (Troll 1939, Knoll 1948, Rudall & Buzgo 2002) are mandatory, but not sufficient. The tip of any leaf begins to differentiate first during leaf development. Moreover, not all forerunner tips are unifacial (Knoll 1948) whereas the unifacial leaf tip in *B. articulatum* should not be considered the forerunner tip.

Considering the above, we propose to supplement the traditional criteria for unifaciality with two more criteria, viz. when differentiating, the forerunner tip is a significant part of the ULZ and it remains quite discernible throughout subsequent leaf development.

Since the forerunner tip is a distinct part of the leaf, its delimitation from the developing leaf blade is an important issue. In investigated *Curio* species with unifacial and subunifacial leaves, the adaxial meristem develops in the leaf blade but not in the forerunner tip. Therefore, the adaxial meristem could be used as a marker to distinguish the forerunner tip and to delimit it from the leaf blade (Fig. 3A,F, 4B,C).

The *Curio* species with bifacial leaves have the adaxial meristem neither in the forerunner tip nor in the leaf blade. However, their forerunner tips are distinguishable in being unifacial in strike contrast with the bifacial leaf blade (Fig. 2C).

The development of the forerunner tip in *Curio* species drastically differs from that in monocots. The forerunner tip in *Curio* species becomes a unifacial structure at the stage of apical growth of the leaf primordium, when the whole ULZ consists of meristematic tissue and there is no distinct adaxial meristem (Fig. 2B, 3A, 4B). The forerunner tip in monocots becomes unifacial due to activity of the adaxial meristem just as their unifacial leaf blade (Troll 1939, Knoll 1948).

The unifacial and subunifacial leaves in *Curio* species are similar with the unifacial leaves in monocots in circular arrangement of the vascular bundles in cross section of the leaf blade. However, there is sole collateral vascular bundle in the forerunner tip in the investigated species of the genus *Curio* (Fig. 2E,F, 3E, 4E,F) contrary to the sole bicollateral vascular bundle in monocotyledonous species (Knoll 1948). *Curio* species have a single vascular bundle in the forerunner tip, which is divided into three at its base.

CONCLUSIONS

Forerunner tip is a distinct part of the leaf, which can be present not only in monocots, but also in some dicots. The presence of this structure can be confirmed only in careful morphological and anatomical investigation of leaf morphogenesis. The forerunner tip differs from ordinary leaf tip in that it differentiates as a distinct structure at the early stages of leaf development and remains distinguishable at all subsequent developmental stages. The forerunner tip is usually a unifacial structure. However, the unifaciality per se is not indicative for the leaf tip to be a genuine forerunner tip. The forerunner tips in species *Curio* are unique in that the adaxial meristem is not involved in their formation in any way. They arise due to the meristematic activity of the

whole ULZ during the leaf axis formation. The unifaciality and absence of the adaxial meristem (if it participates in the development of the leaf blade) are suitable markers for distinguishing forerunner tip from other parts of the leaf.

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