# Comparative leaf and stem anatomy refute the involvement of *Senecio tropaeolifolius* (Asteraceae) as parent of *Senecio × kleiniiformis*

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**Abstract:** *Senecio × kleiniiformis* Suess. is a historically hypothesized hybrid species between *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin with a bifacial leaf blade and an unknown species with unifacial leaves. However, nuclear ribosomal ITS data suggest a close relationship between *S. × kleiniiformis* and *Senecio tropaeolifolius* MacOwan ex F.Muell., which also has a bifacial leaf blade. *Senecio × kleiniiformis* and *S. tropaeolifolius* have several traits in common, such as leaf venation pattern, presence of sclerenchyma strands in the stem, and the occurrence of clustered stomatal complexes in leaves. However, the similarity between *S. × kleiniiformis* and *B. articulatum* is higher. They share the same patterns of leaf blade anatomy, epidermal characters, the same leaf morphological series along the annual shoot, and most of the stem anatomical traits. Whereas leaves of the three investigated species have lateral lobes, only the lateral lobes of *S. × kleiniiformis* and *B. articulatum* are homologous to each other based on their development. Anatomical and morphological data obtained in the present study indicate that *B. articulatum* is more likely the bifacial-leaved parental species of *S. × kleiniiformis* than *S. tropaeolifolius*.

**Keywords:** Leaf anatomy, Leaf venation, Nodal anatomy, Stem anatomy.

## Introduction

Anatomical data play a crucial role in the systematics of different plant groups. For example, features of bark anatomy as emphasized by the late Prof. Lotova are of taxonomic importance in Pinopsida, Rosaceae, Fagaceae and others (Lotova, 1987; Lotova & Timonin, 1996, 2005; Lotova & Nilova, 2007).

Anatomical and morphological data combined with molecular data could be used for developing stable classifications of taxonomically complicated plant groups. This approach would contribute to improve the taxonomy of succulent representatives of the tribe *Senecioneae* (Cicuzza *et al.*, 2017).

*Senecio × kleiniiformis* Suess. is one of the enigmatic species of the tribe *Senecioneae*. Rowley (1994, 2002) assumed that it originated in culture as a hybrid between *S. articulatus* (Haw.) Sch. Bip. and a species with fleshy leaves. He made his assumption by the analogy of known hybrids of *S. articulatus*: *S. × peregrinus* (Kunk.) G.D.Rowley, a hybrid species between *S. articulatus* and *S. rowleyanus* H.Jacobsen, and the hybrid between *S. articulatus × S. hallianus* G.D.Rowley (Rowley, 1994). These hybrids possess large fleshy leaves with two large lateral lobes, and each lobe has a 'light window'. Rowley (1994) believed that hybrids gained the lateral leaf lobes from *S. articulatus*, while they inherited thickened leaf blades and the 'light window' from another parent. Leaf blades of *S. × kleiniiformis* typically also have two lateral lobes of the thickened leaf blade, but a light window is lacking. This combination of leaf characters suggests *S. × kleiniiformis* to be a hybrid between *S. articulatus* and any of the closely related species with a completely unifacial leaf blade.

Rowley (1994, 2002) hypothesized that the second parent species could be *S. talinoides* subsp. *cylindricus* (DC.) P.V.Heath, *S. ficoides* (L.) P.V.Heath or *S. repens* (L.) P.V.Heath. But this suggestion does not

fit well with the chromosome number analysis. The base chromosome number for *Senecio* species is n = 10 (Pelser *et al.*, 2007). According to the CCDB *S. × kleiniiformis* is a hexaploid with 2n = 60, whereas *S. articulatus* is a diploid with 2n = 20. The chromosome numbers of *S. talinoides* (2n = 20), *S. ficoides* (2n = 120), and *S. serpens* (2n = 100) correspond to diploid, dodecaploid, and decaploid, respectively (Rice *et al.*, 2015). Consequently, none of them can be considered as the second parent species. Lack of close relatedness between *S. talinoides*, *S. ficoides*, and *S. serpens* was also confirmed during the ITS sequencing (Ozerova *et al.*, 2017). Thus, the second parent species remains unknown.

ITS analysis changed the phylogenetic position of *S. articulatus*. It was segregated into the monotypic genus *Baculellum* as *B. articulatum* (L.f.) L.V.Ozerova & A.C.Timonin (Ozerova *et al.*, 2017). Other supposed parent species have been recognized as members of the genus *Curio* (Heath, 1997, 1999; Pelser *et al.*, 2007). Accordingly, *S. × kleiniiformis* could be regarded as an intergeneric hybrid. *Curio* and *Baculellum* seem to be closely related (Ozerova *et al.*, 2017).

ITS analysis also doubted the position of *S. articulatus* as a presumable parent species for the *S. × kleiniiformis*. It was shown that another species, *S. tropaeolifolius* MacOwan ex F. Muell. from a taxonomically distant group of succulent groundsels, is phylogenetically close to *S. × kleiniiformis* (Malenkova *et al.*, 2014). But *S. tropaeolifolius* is also a hexaploid with 2n = 60 chromosomes and is an inappropriate candidate to the parent species for the latter one. Since both *S. tropaeolifolius* and *S. × kleiniiformis* are polyploids, they might have several ITS sequences, and this can affect the accuracy of the phylogenetic tree. However, the relatedness between *S. tropaeolifolius* and *S. × kleiniiformis* remains unknown.

The present investigation is aimed to investigate the leaf anatomy of *Senecio × kleiniiformis* and its presumable parent species *B. articulatum* and *S. tropaeolifolius* and to determine which species is more likely to be one of the potentially parental species for the putative hybrid. Special attention was paid to the structure of the epidermis, leaf blade anatomy and venation, and nodal anatomy. The structure of the epidermis has long been used in the diagnostics and taxonomy of flowering plants (*e.g.*, Stace, 1966; Ullah, 2018). The nodal anatomy has usually been used in high-level systematics (Beck *et al.*, 1982), but it is also valuable for species taxonomy (Costea & DeMason, 2001). The leaf venation patterns are of importance for infrageneric and even infraspecific taxonomy (Grohar *et al.*, 2018; Carmo *et al.*, 2019). The leaf anatomy could be especially useful because it was successfully applied in a taxonomy of *Kleinia*, *Curio*, and *Caputia* (Cicuzza *et al.*, 2017), which were segregated from former succulent groundsels (Pelser *et al.*, 2007).

## Materials and Methods

Plant material was taken from living plants grown in the greenhouses of Tsitsin Main Botanical Garden of the Russian Academy of Science, Moscow, and fixed in 70% ethanol. Fixed mature leaves of the most typical morphology (5th or 6th leaf of the leaf series counted from the youngest distinguishable leaf on this year's annual shoots) were hand cut with a razor blade. Obtained transverse sections were kept in PBS (phosphate buffered saline, pH 7.0) (Sigma-Aldrich, Darmstadt, Germany), proceeded with 10 ng/ml Calcofluor White M2R/ Fluorescent Brightener 28 (Sigma-Aldrich, Saint Louis, USA), and washed twice with fresh PBS according to standard protocols (Yeung *et al.,* 2015). Fixed young leaves (the 3rd leaf of the leaf series counted from the youngest distinguishable leaf on this year's annual shoots) were dehydrated in an ethanol series and embedded in paraffin through xylene. Transverse microtome sections 10 μm thick were mounted on slides, deparaffinized, rehydrated, and stained with Krauter mixture (0.01% basic fuchsin (ReaHim, Moscow, Russia), 0.04% safranin (ReaHim, Moscow, Russia), and 0.15% astra blue (Sigma-Aldrich, Darmstadt, Germany) according to Krauter (1985) or with 1% toluidine blue (Sigma-Aldrich Saint Louis, USA) (Ruzin, 1999) and mounted in Euparal (Carl Roth, Karlsruhe, Germany). The preparations were examined under a light microscope (LM) Nikon Eclipse Ci (Nikon Corporation, Tokyo, Japan) with a module for epifluorescence detection and equipped with a digital camera Nikon digital sight DS-Vi1 (Nikon Corporation, Tokyo, Japan).

Leaf epidermal characters were investigated on paradermal leaf sections prepared with a hand-razor from frozen material (Barykina *et al.*, 2004). Preparations were stained with toluidine blue.

We investigated the early stages of the leaf development under a scanning electron microscope (SEM) to test the homology of the lateral lobes in the leaves of *Senecio* × *kleiniiformis* Suess. and *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin. For SEM examination, shoot apices were fixed in 70% ethanol, dehydrated through an ethanol and acetone series, critical-point dried with a HCP-2 (Hitachi Ltd., Tokyo, Japan) critical point dryer and mounted on stubs. Then samples were coated with Pd-Au using an IB-3 Ion Coater (Eiko Engineering Co., Tokyo, Japan) and examined under a Camscan-S2 scanning electron microscope (Cambridge Instruments, Cambridge, United Kingdom). The work was carried out at the Laboratory of Electron Microscopy, Lomonosov Moscow State University. Adobe Photoshop 2022 (Adobe) was used for image processing in order to adjust brightness, contrast and to use pseudocolors to highlight distinct morphological structures. For studies of nodal anatomy, young stems were sampled approximately in the region of the $4^{th}$–$7^{th}$ node, and samples of the oldest stems near the ground were also taken. The fixed material was hand cut with a razor blade. One part of the transverse sections was treated with phloroglucinol and HCl according to a standard protocol (Barykina *et al.*, 2004), and the other one was processed with Calcofluor White M2R/ Fluorescent Brightener 28 (Sigma-Aldrich) in the same way as described above for mature leaves.

Voucher specimens were deposited at the Syreishchikov Herbarium of Lomonosov Moscow State University (MW): *Baculellum articulatum* (MW0975113), *Senecio* × *kleiniiformis* (MW0975112) and *S. tropaeolifolius* (MW0975114).

## Results

## Leaf anatomy and morphology

*Baculellum articulatum* is a deciduous stem succulent with thick articulate stems and thin bifacial leaves with a short leaf base, a petiole, and a leaf blade (Fig. 1a). Leaf shape significantly changes along the annual shoot (Fig. 2a). The first leaves of the annual shoot are ovate or triangular without distinguishable lateral lobes. Upwards, the leaves gradually become trilobed with a large middle lobe and smaller lateral ones. The uppermost leaves of the annual shoots can develop lateral lobes with a narrow petiole-like basal part and a broader blade-like part. The margins of such uppermost leaves are often involuted (Fig. 1a; 2a).

The quite distinguishable sub-unifacial petiole has a narrow 'light window' on the adaxial side (Fig. 1b). The petiole is rounded with a flattened or invaginated adaxial side in cross-section (Fig. 1b). The 3–4 layered homogeneous chlorenchyma is under the hypodermis on the abaxial side whereas the chlorophyll-free water-storage parenchyma adjoins the hypodermis of the adaxial side (Fig. 1c). This water storage tissue also occupies the inner part of the petiole (Fig. 1b, c). There are collateral vascular bundles with endoscopic xylem between the chlorenchyma and the water-storage parenchyma (Fig. 1c). The bundles are accompanied by schizogenous ducts located outside of them.

The leaf blade is bifacial except for its very tip and tips of lobes if present (Fig. 2a). The single layer of slightly flattened water-storage polygonal hypodermal cells is under the adaxial epidermis (Fig. 1d, e). This tissue lacks the chloroplasts, and it runs over the abaxial side for a short distance along the leaf edge. The hypodermis is absent on the abaxial side except for the leaf margins and zones of the large vascular bundles (Fig. 1d). Several (up to 4) layers of large, elongated in median plane cells lay under the adaxial hypodermis. The chlorenchyma is fuzzy differentiated into palisade and spongy tissue and usually consists of up to 5 layers (Fig. 1d). Collateral vascular bundles occupy the position at the boundary of the palisade-like and spongy tissues. Most of them are accompanied by schizogenous ducts on the abaxial side (Fig. 1d). The unifacial tips are rounded and filled by homogeneous mesophyll tissue hosting a single collateral bundle.

*Senecio tropaeolifolius* is a liana with peltate leaves consisting of a short leaf base, a long petiole, and a polygonal cleft leaf blade (Fig. 3a, g, f). The leaf blade has a short leaf tip hardly discernible from the

numerous main marginal teeth. Shorter, additional teeths are sometimes present between these main teeth.

The sub-unifacial petiole is semi-rounded with a flattened adaxial side (Fig. 3b). A single layer of the epidermis is underlaid by up to five layers of
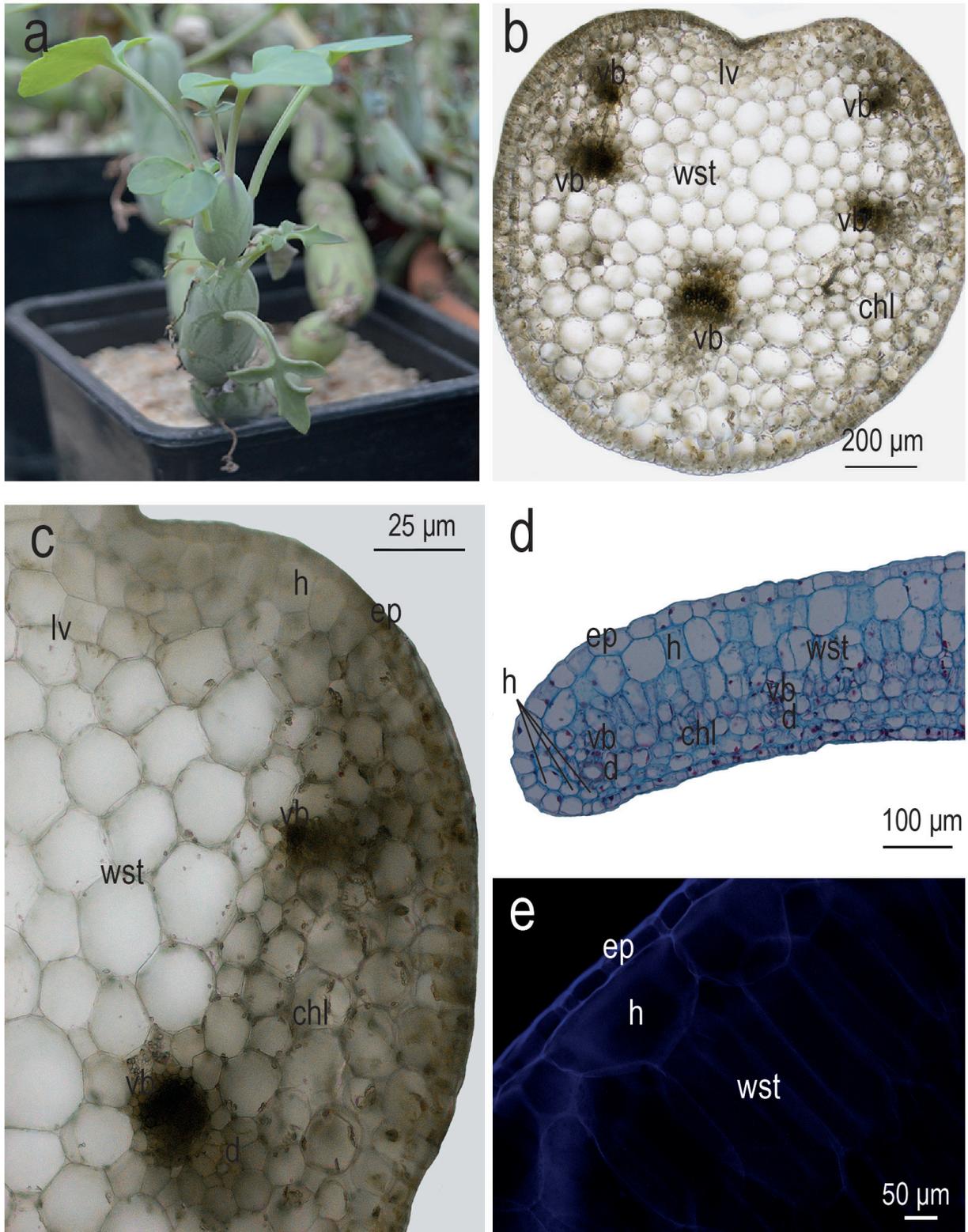


**Fig. 1**. *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin: **a**. Habit; **b**. Petiole cross-section; **c**. Petiole cross-section at higher magnification; **d**. Cross-section of margin of young leaf; **e**. Leaf blade cross-section (chl: chlorenchyma, d: schizogenous duct, ep: epidermis, h: hypodermis, lv: "light window", vb: vascular bundle, wst: water-storage tissue).

chlorenchyma (Fig. 3c). The chlorenchyma is also present at the adaxial side, and the petiole lacks a "light window" (Fig. 3b, c). The inner part of the petiole is filled by large, round water-storage parenchyma cells. Collateral vascular bundles with endoscopic xylem lie between the inner water-storage tissue and chlorenchyma (Fig. 3c). All vascular bundles are accompanied by a schizogenous duct from the outside (Fig. 3b).

The hypodermis in the leaf blade of *S. tropaeolifolius* is absent (Fig. 3d). The leaf blade chlorenchyma is clearly differentiated into palisade and spongy tissue (Fig 3d). Collateral vascular bundles accompanied by small schizogenous ducts from the abaxial side are between the palisade and spongy chlorenchyma tissues (Fig. 3e, f).

*Senecio × kleiniiformis* is an evergreen perennial leaf-succulent herb (Fig. 4a). The leaves consist of a short leaf base, a long petiole, and a leaf blade. The shape of the leaf blade changes significantly (Fig. 2b) in successive leaves on the annual shoot. The lowest leaves have flattened and rounded cochleariform (spoon-shaped) blades with small teeth. The upper leaves have scaphoid (boat-shaped) leaf blades. A series of intermediate forms is in between. Lateral teeth in this leaf series enlarge to become small lateral lobes (Fig. 2b).

The completely unifacial petiole of *S. × kleiniiformis* is cordate in cross-section (Fig. 4b). It is composed of epidermis, homogeneous chlorenchyma, and large water-storage parenchyma cells (Fig. 4c). Collateral vascular bundles with endoscopic xylem lie on the border between the water-storage inner parenchyma and chlorenchyma tissues. Each larger bundle is accompanied by an outer schizogenous duct (Fig. 4c).

The leaf blade is bifacial, but its adaxial surface narrows basipetally to be hidden in a narrow furrow of the petiole-to-leaf blade transitional zone (Fig. 5a-f). The furrow becomes deeper towards the leaf blade tip (Fig. 5a-c) and its bottom becomes wider and forms a kind of cavity covered by the leaf blade margins (Fig. 5d-e). At the distal end of the transitional zone, the margins diverge and release the adaxial side.
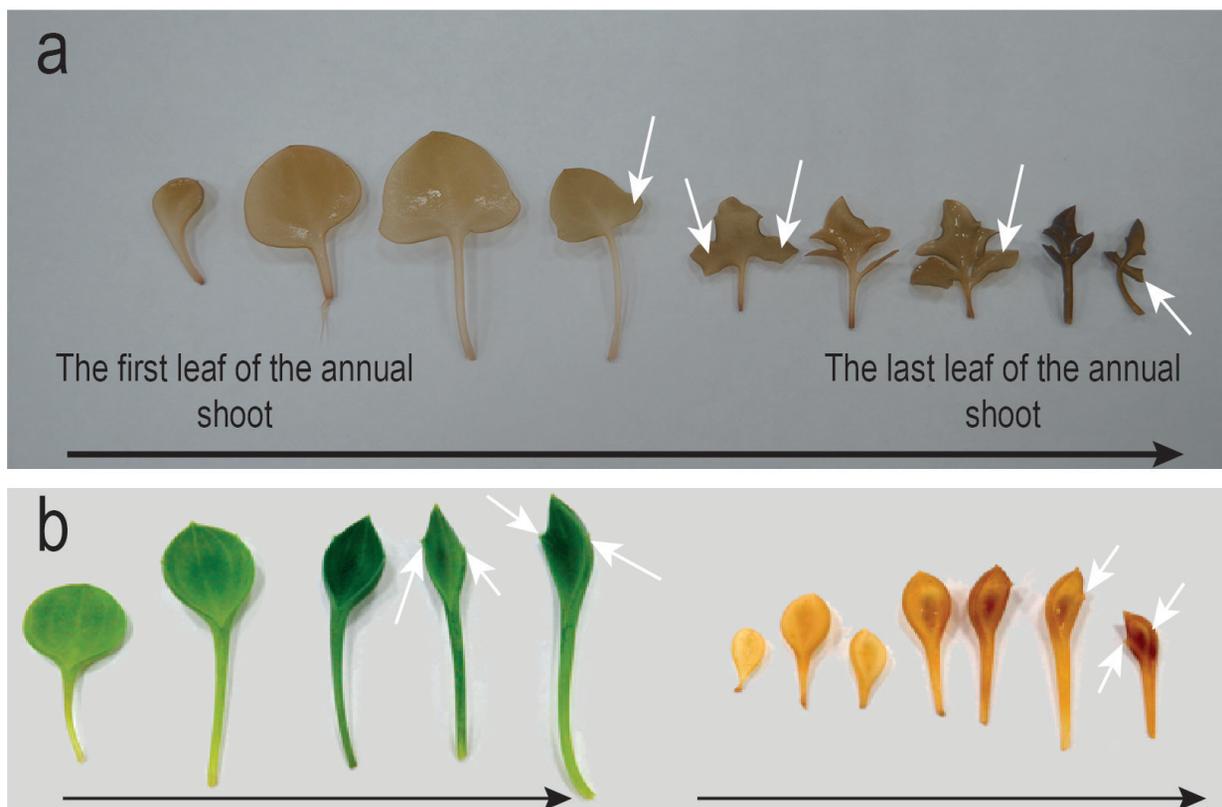


Fig. 2. **a**. Acropetal series of *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin leaves from the annual shoot; **b**. Two acropetal series of *Senecio × kleiniiformis* Suess. leaves from the annual shoot (Black arrows show the direction from the first leaf of the annual shoot to the last. White arrows point to lateral lobes).
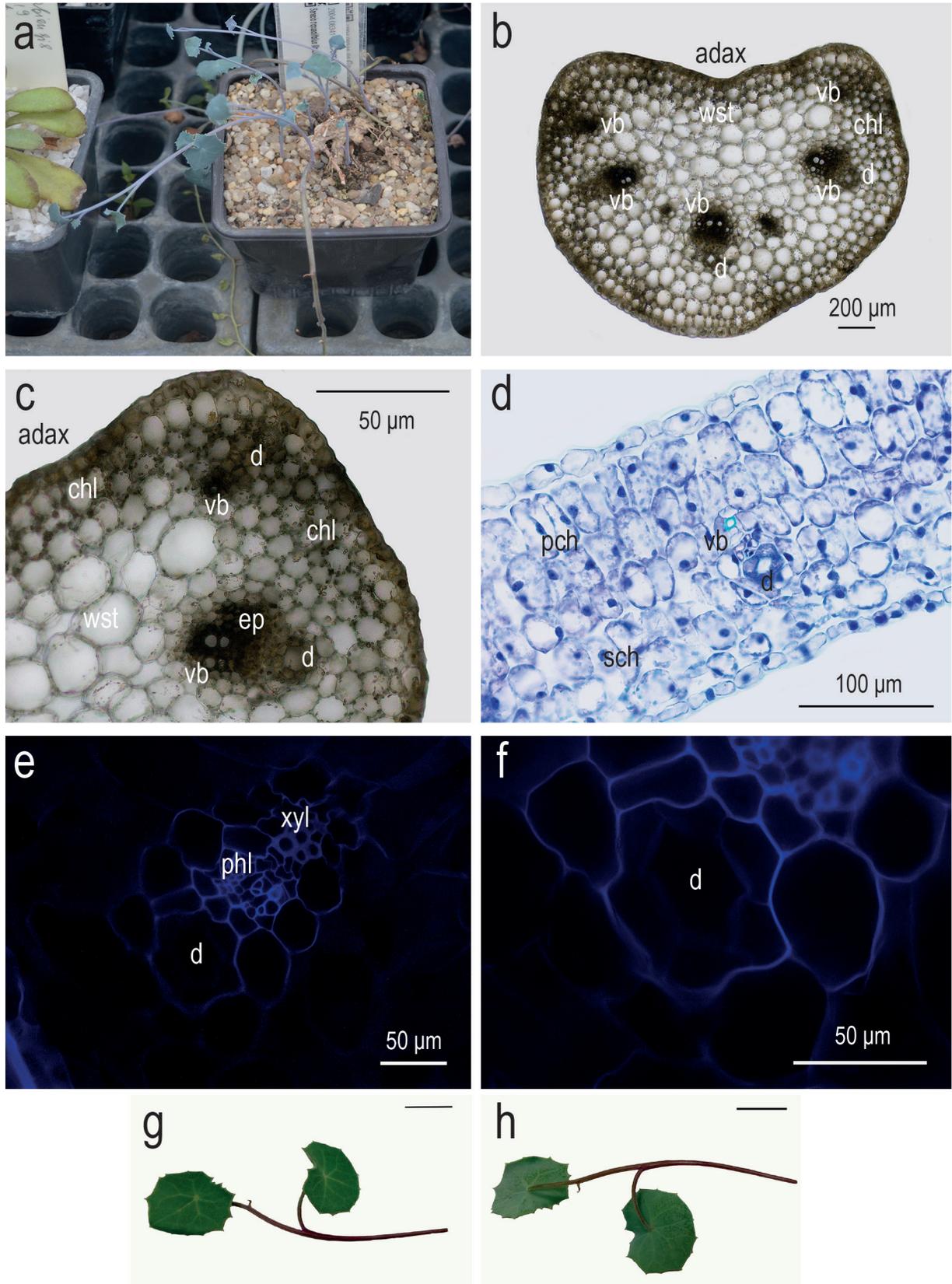
**Fig. 3**. *Senecio tropaeolifolius* MacOwan ex F.Muell.: **a**. Habit; **b-c**. Petiole, cross-section; **d**. Young leaf blade, cross-section; **e-f**. Mature leaf blade, cross-section; **g-h**. Leaves appearance from the adaxial and abaxial side respectively (adax, adaxial side; chl, chlorenchyma; d, schizogenous duct; pch, palisade chlorenchyma; phl, phloem; sch, spongy chlorenchyma; vb, vascular bundle; wst, water-storage tissue; xyl, xylem).

On the adaxial side of the leaf blade, the epidermis is underlaid by a single layer of nearly polygonal water-storage cells which lack chloroplasts (Fig. 4d, e). At the leaf margins, this tissue is also present abaxially on short distances (Fig. 4d). A hypodermis is absent from the abaxial side except for the margins and zones of large vascular bundles (Fig. 4d, f). Six to seven water-storage cells elongated in the median plane lay under the adaxial hypodermis. The water storage cells surround a chlorenchyma tissue consisting of ovate cells. The chlorenchyma is penetrated by collateral vascular bundles
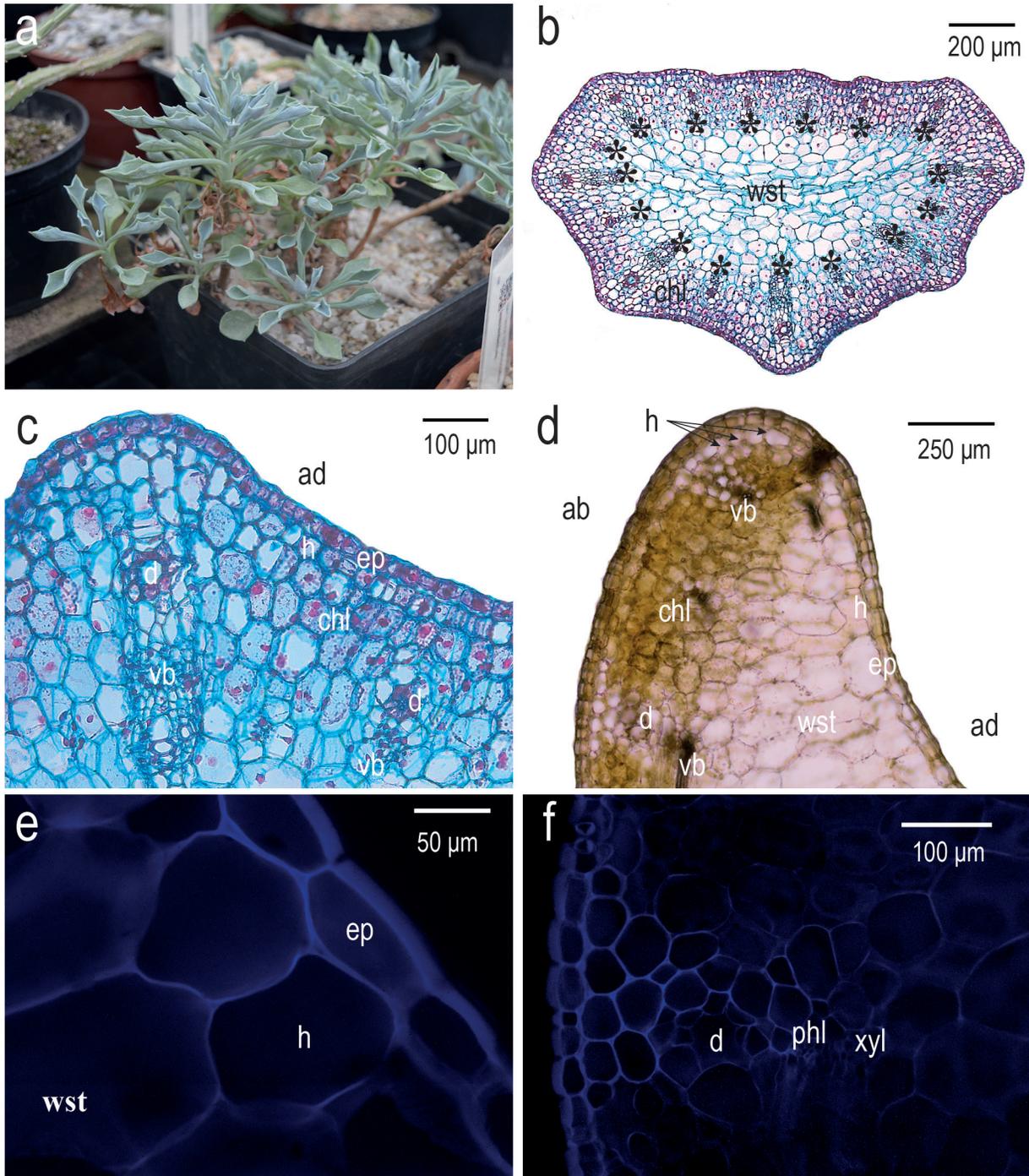


**Fig. 4**. *Senecio × kleiniiformis* Suess.: **a**. Habit; **b-c**. Young petiole, cross-section; **d**. Mature leaf blade, cross-section; **e**. Mature leaf blade cross-section, adaxial side; **f**. Mature leaf blade cross-section, abaxial side (ab, abaxial side; ad, adaxial side; chl, chlorenchyma; d, schizogenous duct; ep, epidermis; h, hypodermis; phl, phloem; vb, vascular bundle; wst, water-storage tissue; xyl, xylem).

accompanied by schizogenous ducts from the abaxial side (Fig. 4f). The leaf tip is rounded in cross-section and lacks the chlorenchyma.

## Venation

In *Baculellum articulatum*, three leaf traces enter the petiole (Fig. 11a). Each lateral vascular bundle branches in the petiole therefore, five vascular bundles enter the leaf blade: the midrib (M), a pair of lateral bundles (L1 and L1') and their paired derivatives L2, L2' (Fig. 6a). The venation pattern of the leaf blade is campylodromous, according to Hickey's classification (1979). The outermost vascular bundles (L2, L2') run along the leaf margin throughout the entire length of the blade as the encircling marginal vascular bundles (left and right ones). The midrib branches to produce several small and medium-size vascular bundles that fuse with the midrib in its distal part or with the encircling marginal vascular bundles. The lateral vascular bundles L1, L1' innervate the lateral lobes of the leaf blade. They smoothly turn to the lateral teeth after entering the leaf blade and merge with the encircling marginal vascular bundle in the distal part of the lobes. L1, L1' branch less frequently than the midrib. Their derivatives are arch-recurved toward the tip of the lateral teeth and fuse with an encircling marginal vascular bundle.

Three leaf traces enter the petiole of *Senecio tropaeolifolius* (Fig. 11b). The two lateral vascular bundles branch twice therein. Thus, seven vascular bundles innervate the leaf blade: the midrib (M), a pair of large laterals (L1 and L1') and two pairs of medium-sized vascular bundles (L2/L2'and L3/L3') (Fig. 6b). The venation is campylodromous, according to Hickey's system (1979), due to the form of a recurved, arched pattern of the lateral veins. The pair of vascular bundles (L3/L3') located near the basal part of the leaf blade form the encircling vascular bundle running along the entire margin. The midrib branches six times and runs directly to the leaf tip. Its derivatives never merge with the midrib in its distal part but incorporate with encircling vascular bundles or other derivatives. The L1/L1' vascular bundles innervate the marginal teeth in the distal and lateral parts of the leaf blade, whereas the L2/L2' ones innervate the teeth in its basal part. All vascular

bundles branch to innervate several leaf teeth and also produce smaller branches merging each other, or the encircling vascular bundles.

*Senecio* × *kleiniiformis* demonstrates a kind of perfect basal acrodromous venation (Fig. 6c). Five vascular bundles innervate the leaf base (Fig. 11c). In the petiole, lateral vascular bundles branch frequently, and 15–20 vascular bundles can innervate the leaf blade in total (Fig. 4b). However, only seven vascular bundles remain large: the midrib (M), a pair of lateral bundles (L1/L1') and two more pairs of medium-sized vascular bundles (L2/L2'and L3/L3') (Fig. 6c). The outermost pair of large vascular bundles in the basal part of the leaf blade runs along the leaf margin throughout its entire length as encircling vascular bundles. The midrib branches four times to produce several small and medium-sized vascular bundles, which fuse with the midrib in its distal part or with each other. The L1/L1' vascular bundles run through the leaf blade toward its tip. The L2/L2' vascular bundles innervate the lateral teeth and fuse there with the encircling vascular bundles. The smaller vascular bundles merge with other vascular bundles or run through the leaf blade to the leaf tip.

## Epidermal characters

All three investigated species have anisocytic stomatal complexes and lack any trichomes on the leaf epidermis (Figs. 7, 8).

The leaf blade of *Baculellum atriculatum* is hypostomatous (Fig. 7a, b). The adaxial epidermis consists of extended pavement cells with deeply curved cell walls (Fig. 7a). Pavement cells of the abaxial epidermis have a more irregular shape and are larger than those of the adaxial epidermis (Fig. 7b, c). The stomata are usually surrounded by three mesogenous subsidiary cells (Fig. 7b). The last formed subsidiary cell sometimes gives rise to a satellite meristemoid that can go through several divisions and become a guard mother cell (Fig. 7c).

The leaves of *Senecio tropaeolifolius* are amphistomatous (Fig. 7d, e). The pavement cells of the adaxial epidermis have an irregular form and slightly curved cell walls (Fig. 7d). The stomata complexes have a pair of mesogenous subsidiaries and a single

In some cases, fascicular and interfascicular cambia in concert form a half ring of one or several layers of parenchyma tissue which then lignifies. During secondary thickening, the primary phloem elements become significantly wrinkled. In younger stems, the protophloem elements can be lignified.

The nodes of *S. × kleiniiformis* are 3-lacunar. The stele is of the closed type (Fig. 11c). The lateral leaf traces are distant from the median leaf trace by 2 or 3
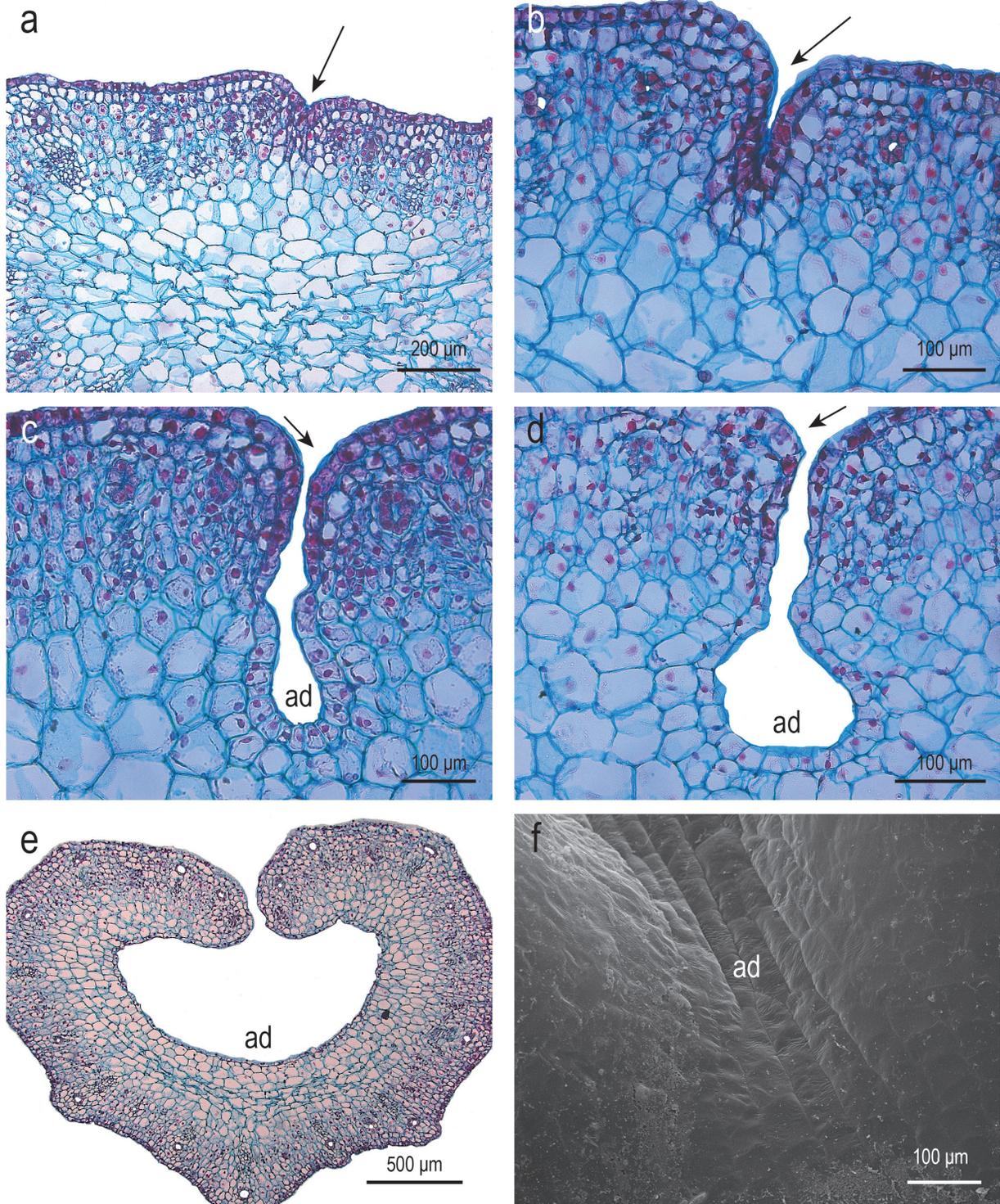


**Fig. 5**. *Senecio × kleiniiformis* Suess.: Leaf blade transitional zone: **a**. Proximal part of the transitional zone with small tissue depression; **b**. More distal part of the transitional zone; **c**. Appearance of the adaxial side; **d**. The expanded bottom of the furrow; **e**. The distal part of the transitional zone; **f**. Initiating furrow (ad, adaxial side; arrow marks furrow).

perigeneous neighbouring cell. Some stomata are solitary and separated from neighbouring stomata by 4–5 pavement cells, whereas others are clustered (Fig. 7d). The pavement cells of the abaxial epidermis have the same shape as the adaxial pavement cells (Fig. 7e, f). Most stomata are surrounded by cells that are indistinguishable from the pavement cells. However, there are stomata with clearly defined 1–2 mesogenous subsidiaries organized in an ordinary anisocytic type (Fig. 7e).

In *Senecio* × *kleiniiformis*, the pavement cells of both adaxial and abaxial epidermises of the amphistomatous (Fig. 8a, b) leaves have irregular shapes and smooth cell walls (Fig. 8a, c). The stomata on both sides have three, infrequently four, mesogenous subsidiaries (Fig. 8c, d). Abaxial stomata are distributed evenly, whereas adaxial stomata are frequently clustered and contiguous (Fig. 8a, b).

## Leaf development

We investigated early stages of leaf development to figure out the morphological nature of the lateral leaf lobes and teeth. Young leaves of the *Senecio tropaeolifolius* and *S.* × *kleiniiformis* go through the same stages of development as *Baculellum articulatum*. However, they differ in leaf blade development during differentiation of the main morphological leaf parts.

In *B. articulatum* lateral leaf lobes appear as a pair of small bulges at the leaf blade margins at the beginning of the leaf blade initiation. By that moment developing leaf is approximately 300 μm long. The second pair of lateral lobes may appear distally later during the leaf blade differentiation before the intercalary growth begins. During the following development, lateral lobes elongate by the intercalary growth and stay rather radial in cross-section.

The margins of young leaves of *S. tropaeolifolius* are characterized by an uneven growth from the beginning of leaf blade initiation. It results in the formation of leaf blade outgrowths that remain flattened in the transverse plane, contrary to the round cross-section leaf lobes of the *B. articulatum*. Thus, they are lateral teeth and none of them can be described as a lateral lobe. The first pair of teeth arise in the proximal part of the upper leaf zone at the beginning of leaf blade initiation (Fig. 9c), and all teeth of the mature leaf can be observed soon after the beginning of the leaf intercalary growth (Fig. 9d).

In *S.* × *kleiniiformis*, the leaf blade initiation is detectable by the emergence of the small adaxial depression in the median plane of the upper leaf zone (Fig. 9e). At this stage, the developing leaf is approximately 600 μm long and has neither clear distinguishable lateral lobes nor their primordia. The lateral lobes appear after the beginning of the intercalary growth later in the leaf development. They enlarge due to the intercalary growth of their base (Fig. 9f) and are radial in cross-section. Notably, their development is not simultaneous and one of them may lag in development.

## Stem and nodal anatomy

In *Baculellum articulatum*, the succulent fleshy stem (Fig. 10a) has a 1-layered epidermis (Fig. 10b).
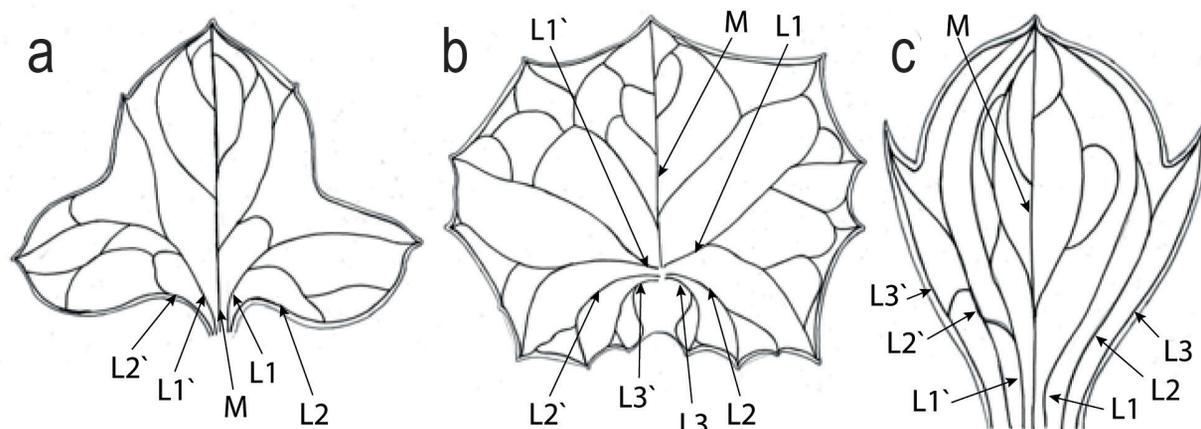


**Fig. 6**. Venation patterns: **a**. *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin; **b**. *Senecio tropaeolifolius* MacOwan ex F.Muell.; and **c**. *Senecio* × *kleiniiformis* Suess. (Only largest vascular bundles are depicted).

One of the vascular axial bundles which is opposite to the median leaf trace branches and restores the number of axial vascular bundles of the stele.

The stem of *Senecio × kleiniiformis* has a 1-layered epidermis (Fig. 13b). The outer cortex consists of 2–4 subepidermal layers of angular collenchyma and the inner of multilayered chlorenchyma (Fig. 13b). Each of the 20–25 collateral vascular bundles of the typical eustele is accompanied by an outer schizogenous duct (Fig. 13e, f). Old shoots show limited secondary thickening (Fig. 13c–f). There are recognizable fascicular and interfascicular cambia.
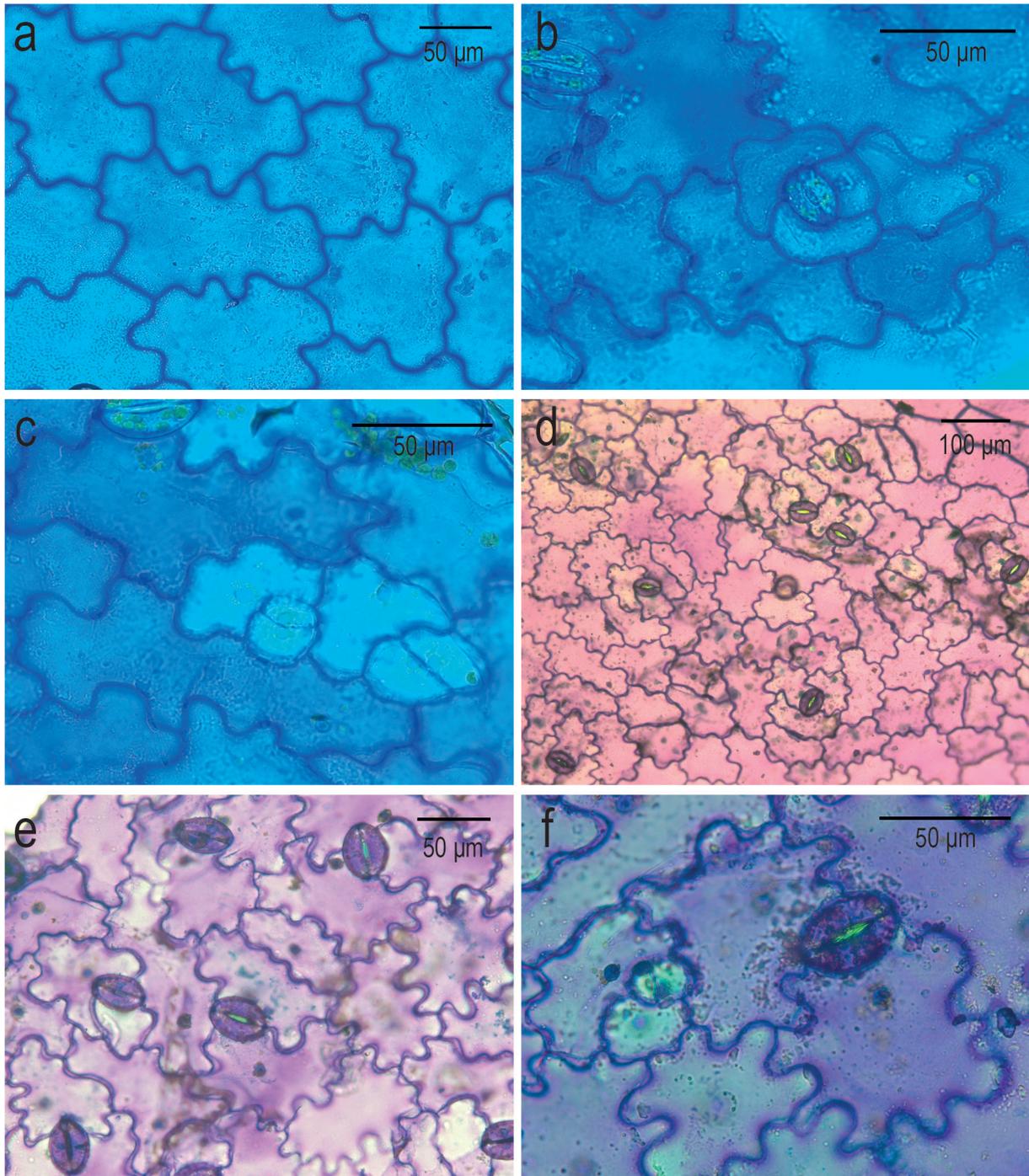


**Fig. 7**. Epidermal characters of *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin and *Senecio tropaeolifolius* MacOwan ex F.Muell.: **a**. Adaxial epidermis of *B. articulatum*; **b**. Abaxial epidermis of *B. articulatum*; **c**. Formation of the anisocytic stomatal complex in *B. articulatum*; **d**. Adaxial epidermis of S. tropaeolifolius; **e**. Abaxial epidermis of *S. tropaeolifolius*; **f**. Stomatal complex on the abaxial surface of *S. tropaeolifolius*.

The periphery of the cortex is occupied by 2–3 subepidermal layers of rounded and slightly flattened cells of angular or annular collenchyma (Fig. 10b). It is underlaid by multilayered chlorenchyma of isodiametric cells. The pericycle and endoderm are indiscernible. The conductive system is a typical eustele of over 25 collateral vascular bundles accompanied by outer schizogenous ducts (Fig. 10a, c). Older stems undergo limited secondary thickening by means of both fascicular and interfascicular cambia (Fig. 10d). Primary phloem cells are partly wrinkled during secondary thickening, but outer non-conductive protophloem elements persist during this process, although there are no lignified elements there. The wide pith consists of large parenchyma cells.

The nodes of *B. articulatum* are 3-lacunar (Fig. 11a). The median leaf trace emerges from the stele just in the median plane of the leaf it supplies; therefore, this leaf trace goes directly into the leaf (Fig. 11a).

The lateral leaf traces leave the stele at a distance of up to 4 or 5 vascular bundles; they go round the stele to enter the leaf. The lateral leaf traces never branch in the cortex. The vascular bundles are organized in a closed type system according to Beck *et al.* (1982). The adjacent axial vascular bundles branch off and form an anastomosis above the lacuna.

*Senecio tropaeolifolius* has a rather thin stem (Fig. 12a) with a 1-layered epidermis (Fig. 12b). The outermost layer of the cortex is angular collenchymatous (Fig. 12b). The space between collenchyma and stele is occupied by ovate cells of chlorenchyma tissue. The eustele consists of up to 14 vascular bundles. Each vascular bundle is accompanied by a schizogenous duct from the outer side. Some vascular bundles have a strand of protophloem fibers. In that case, bundles with and without fibers alternate through 1 or 2 bundles. There is a distinct fascicular cambium in old shoots, while the interfascicular cambium is undistinguishable. During secondary thickening,
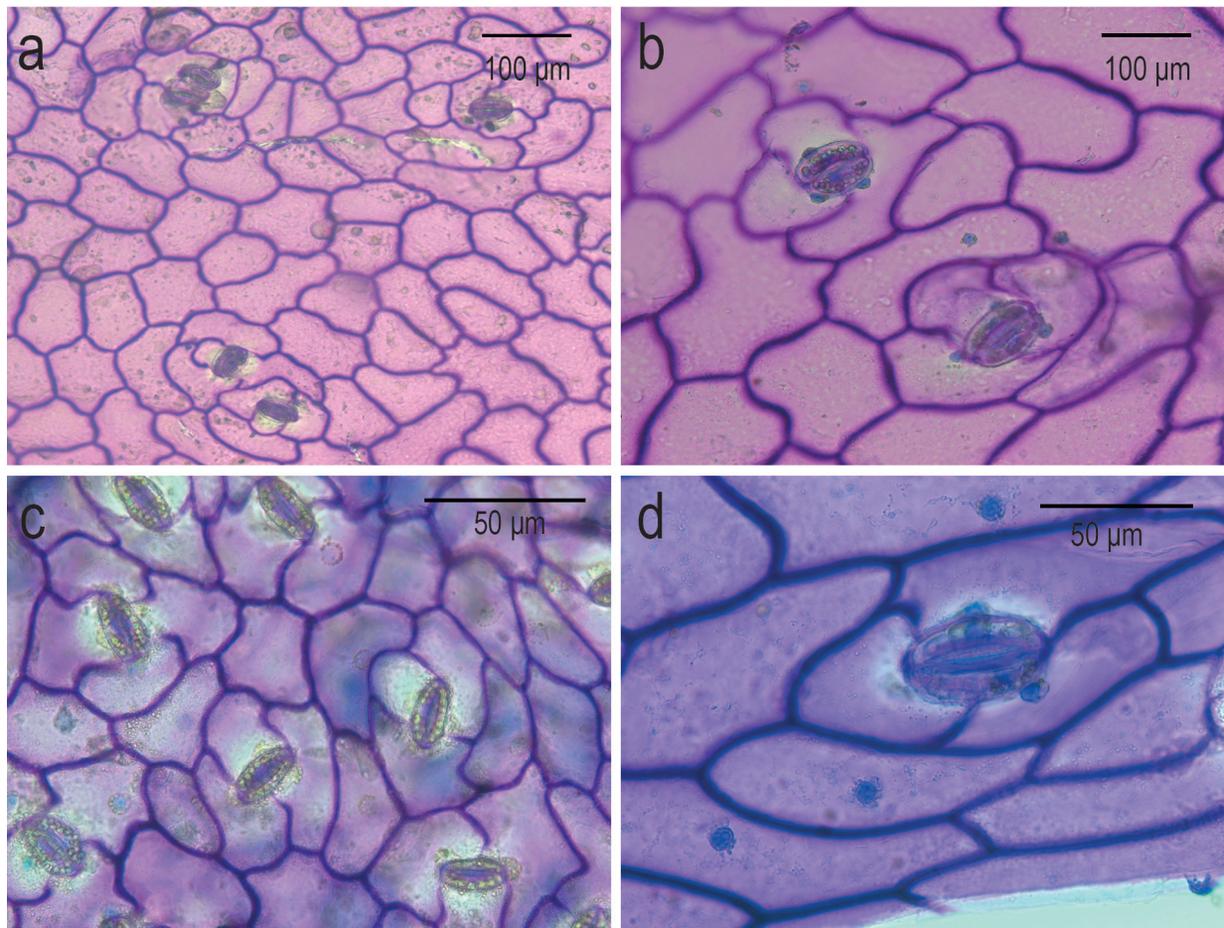


**Fig. 8**. Epidermal characters of *Senecio* × *kleiniiformis* Suess.; **a**. Adaxial epidermis. **b**. Abaxial epidermis. **c**. Structure of the stomatal complex on the adaxial side. **d**. Structure of the stomatal complex on the abaxial side.

the primary phloem elements hardly wrinkled or are wrinkled to a very small degree. The pith consists of large parenchyma cells (Fig. 12a).

The nodes of *S. tropaeolifolius* are 3-lacunar (11b). The stele is organized in an open type (Beck *et al.*, 1982). The lateral leaf traces branch from the axial vascular bundles at the base of the node through 1 or 2 vascular bundles from the median leaf trace (Fig. 11b). Both median and lateral leaf traces do not branch in the cortex and run directly to the leaf base.
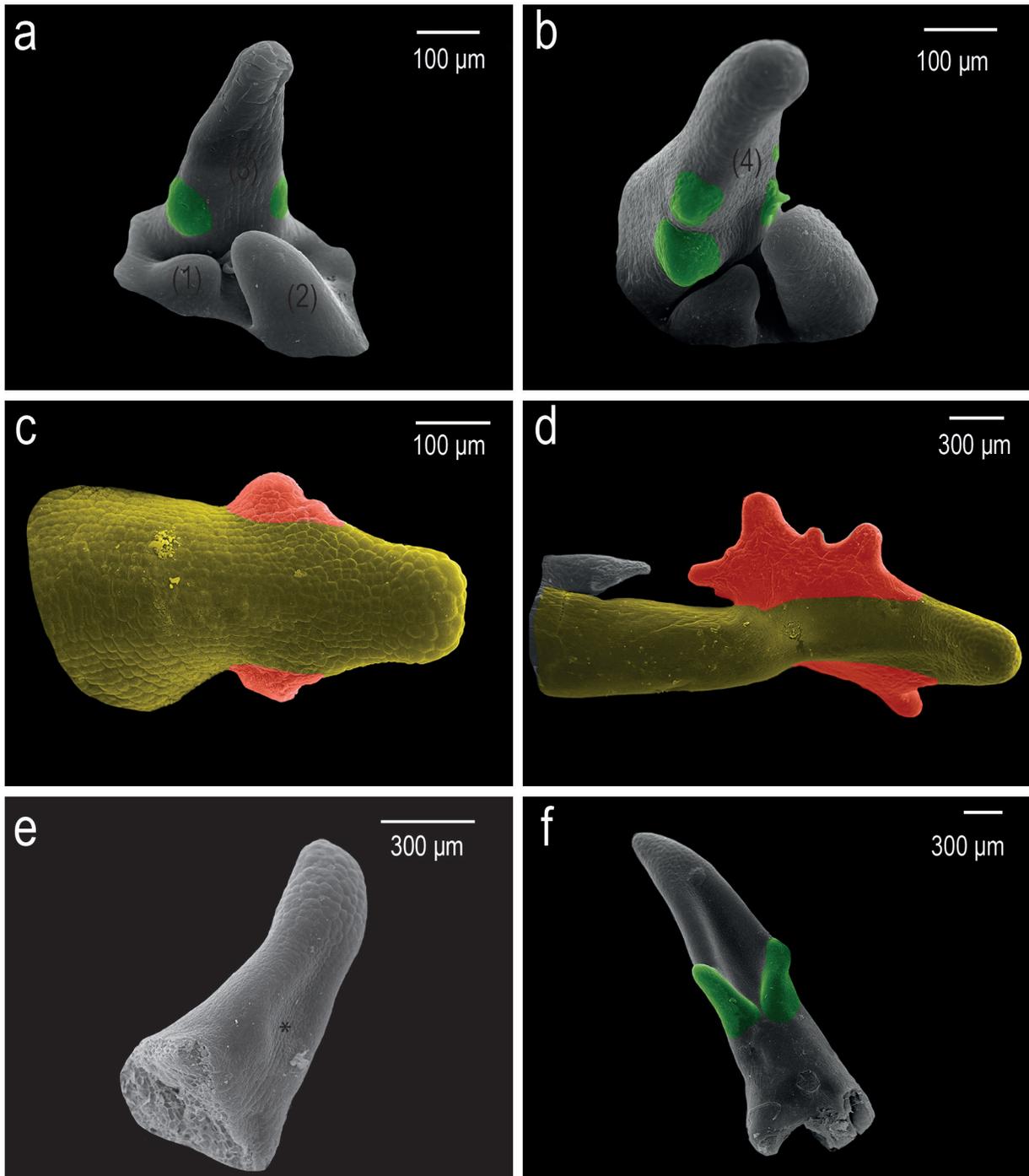


**Fig. 9**. Early stages of the leaf development in: **a, b**. *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin; **c, d**. *Senecio tropaeolifolius* O.Hoffm.;  **e, f**. *Senecio × kleiniiformis* Suess.(Numbers in brackets define the sequence of young leaf emergence. The youngest leaf has the lowest number. The green pseudo color indicates primordia of lateral leaf blade lobes. The red pseudo color indicates zones of lateral (marginal) growth of the leaf blade. The yellow pseudo color denotes the leaf axis. Asterisk indicate the adaxial depression).

axial vascular bundles. The lateral leaf traces branch once in the cortex before entering the leaf base. The neighbouring  axial vascular bundles form an anastomosis above the lacuna.

## Discussion

In both *Senecio* × *kleiniiformis* and *Baculellum articulatum*, the morphology of the leaf blades changes significantly during the development of the annual shoot (Fig. 2a, b), and there is the same tendency in such transformations. The first leaves of the annual shoots have flat, rounded leaf blades without lateral lobes, then through the series of transitional forms they get their final morphological form with well-developed lateral lobes (Fig. 2a, b; Fig. 4a). No such morphological-developmental variability of the leaf blades is observed in *Senecio tropaeolifolius* along the annual shoots (Fig. 3a) and is unknown for other *Curio* alliance species. The same

pattern of the morphological changes of the leaf blades can be inherited by *S.* × *kleiniiformis* from the *B. articulatum*.

The leaf blade anatomy also supports the hypothesis that *B. articulatum* is one of the presumable parent species of *S.* × *kleiniiformis*: Both species share the same anatomical organization type of the leaf blade (Figs. 1d, 4d). In both species, there is a single layer of the adaxial hypodermis which goes over the abaxial side in the leaf margins, water-storage tissue and chlorenchyma is different just in the number of layers. Thus, the anatomical structure of the *S.* × *kleiniiformis* leaf blade can hypothetically be obtained through the hyperplasia of the *B. articulatum* leaf blade. Hyperplasia probably can be a result of polyploidization. *S. tropeolifolius* completely lack an adaxial hypodermis and specialized water-storage tissue (Fig. 3d). Other members of the *Curio*
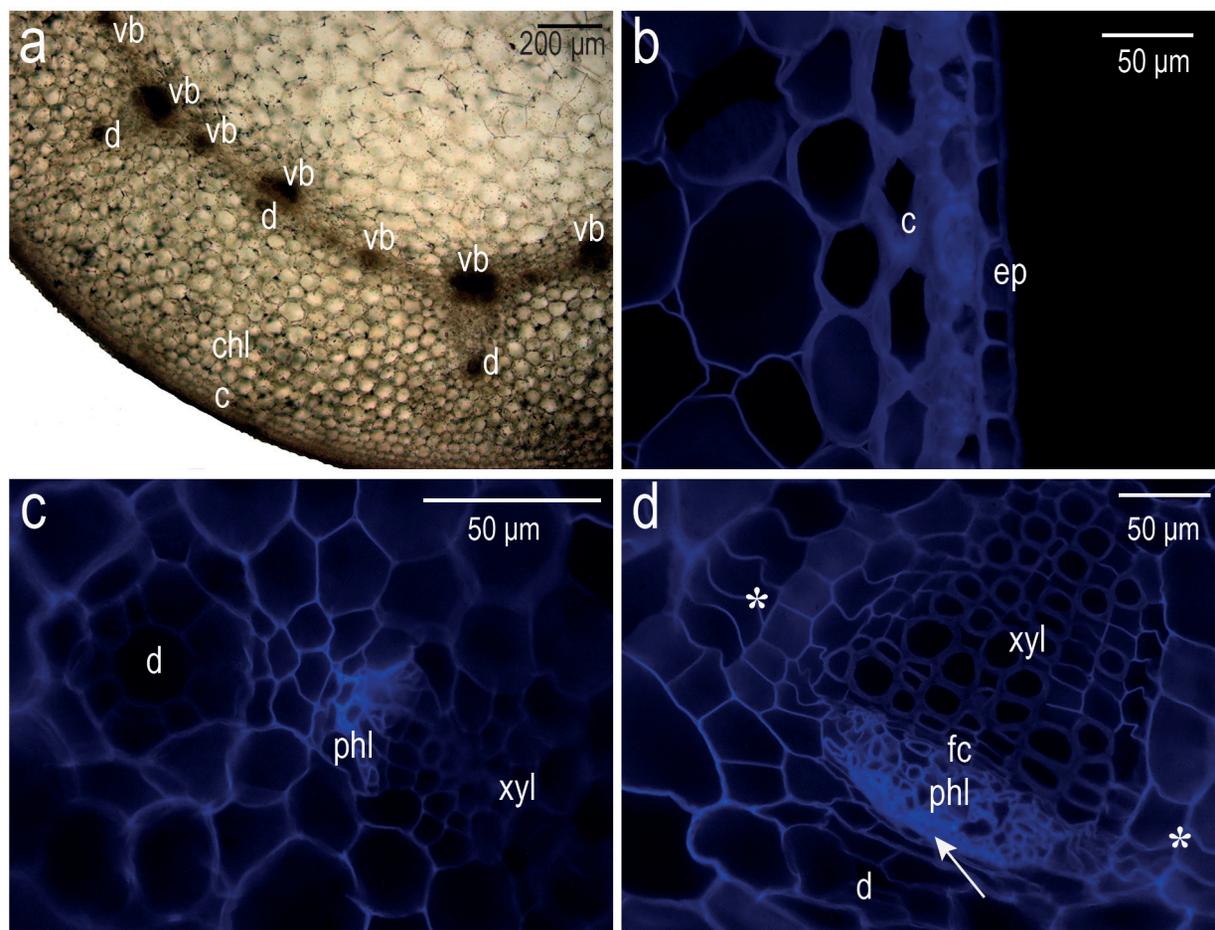


**Fig. 10**. Stem anatomy of *Baculellum articulatum* (f.) L.V.Ozerova & A.C.Timonin, transverse sections: **a**. General anatomy; **b**. Collenchyma at the cortex periphery; **c**. Axial vascular bundle in the young part of the shoot; **d**. Axial vascular bundle in the old part of the shoot (c, collenchyma; chl, chlorenchyma; d, schizogenous duct; ep, epidermis; phl, phloem; vcb, vascular cambium; vb, vascular bundle; xyl, xylem. Asterisk marks intervascular cambium. Arrow marks wrinkled protophloem tissue).
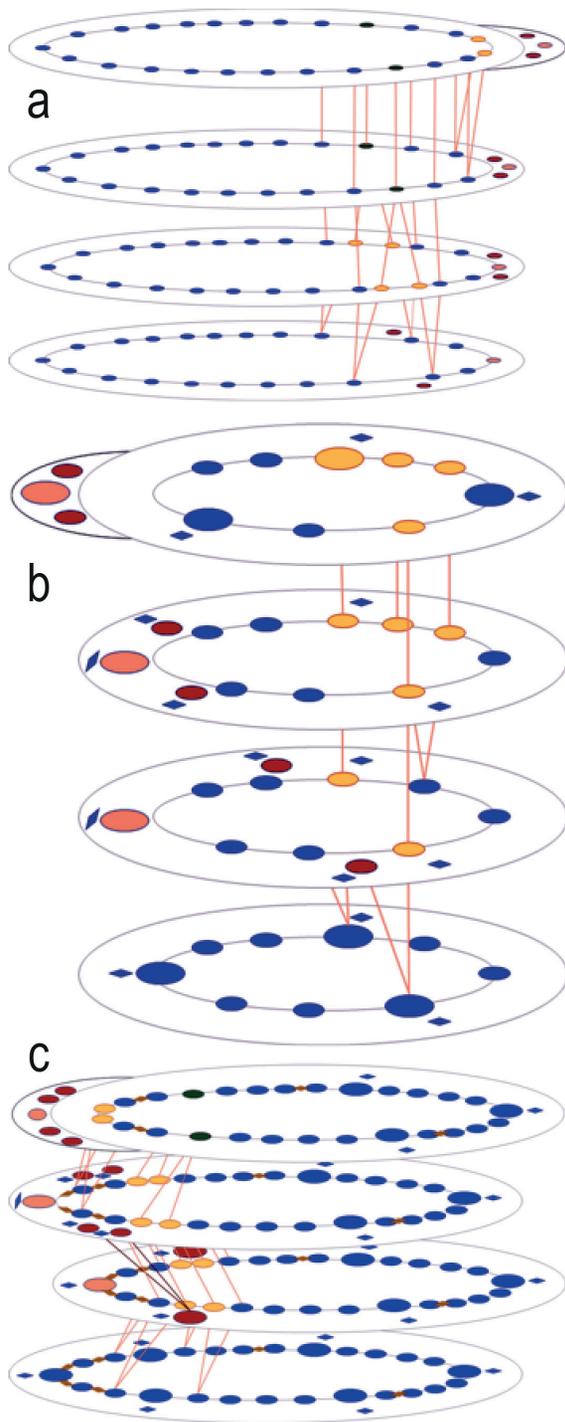
**Fig. 11. a**. Schematic images of the node structure of *Baculellum articulatum* (L.f.) L.V.Ozerova & A.C.Timonin; **b**. *Senecio tropaeolifolius* MacOwan ex F.Muell.; **c**. *Senecio × kleiniiformis* Suess. (Circles represent vascular bundles. The size of the circles reflects the size of the vascular bundles. Blue circles correspond to the axial vascular bundles of the stele; yellow circles with red edging correspond to axial vascular bundles appearing after branching of any vascular bundle in the stele; red circles with blue edging correspond to lateral leaf traces; beige circles correspond to the median leaf trace. Blue rhombuses correspond to the sclerenchyma stretches; golden rhombuses correspond to the anastomoses between vascular bundles).

alliance predominantly have cylindrical, unifacial or subunifacial leaves with distinct types of anatomical organization (Melo-de-Pinna *et al.*, 2016).

Both *B. articulatum* and *S. tropaeolifolius* have the sub-unifacial petiole with the "light window" representing the reduced adaxial side (Fig. 1d, 4b). However, *S.× kleiniiformis* has a completely unifacial petiole and the adaxial side of the leaf appears on the border of the petiole and the leaf blade (Fig. 4b). Thus, polarity of the petiole is an inappropriate anatomical character for revealing of the relatedness between *S. × kleiniiformis* and *B. articulatum* or *S. tropaeolifolius*.

The pavement epidermal cells of *S. × kleiniiformis* (Fig. 8) are similar to neither *B. articulatum* (Fig. 7a-c) nor *S. tropaeolifolius* (Fig. 7d-f). They could correspond to another still unknown parent species. Nevertheless, both *S. tropaeolifolius* and *S. × kleiniiformis* have amphistomatous leaf blades with often clustered stomata in the adaxial epidermis (Fig. 7d, 8a). However, the stomata in some clusters are contiguous in *S. × kleiniiformis* whereas they are separated at least by one cell in *S. tropaeolifolius*. *S. tropaeolifolius* has more complex stomatal complexes with mesogenous and perigenous subsidiary cells in the adaxial epidermis (Fig. 7d), while *S. × kleiniiformis* has ordinary anisocytic stomatal complexes with 3 mesogenous cells (Fig. 8b) just like the stomatal complexes in *B. articulatum*. Thereby, the epidermal characters of *S. × kleiniiformis* show closer relationship of this species with *B. articulatum*.

The lateral lobes of the leaf blade of *S.× kleiniiformis* and *B. articulatum* are similar in prolonged intercalary growth and both are radial in cross-section (Fig. 9a-b, e-f). However, lobes in *S.× kleiniiformis* develop at the later development stage 5, when in *B. articulatum* they arise at the stage 4, though in the same position in both species. *S. tropaeolifolius* lack distinguishable lobes (Fig. 9c, d). Only small lateral teeth are formed at the leaf margins with no signs of durable intercalate growth. Therefore, the leaf blade lobes of *S. × kleiniiformis* and *B. articulatum* are likely to be homologous. But the shift in time of their origin indicates heterochrony.

The three investigated species are similar in presence of angular collenchyma at the periphery of the

cortex (Fig. 10b; 12b, 13b). Secondary thickening was observed in all three species, but interfascicular cambium was revealed in *S. × kleiniiformis* and *B. articulatum*, but not in *S. tropaeolifolius*. The primary phloem elements become wrinkled during secondary thickening (Fig. 10d; 13d, f) in both species, while not in *S. tropaeolifolius* (Fig. 12d). This would support the idea of a closer relationships between *S. × kleiniiformis* and *S. tropaeolifolius*. Another common feature of *S. × kleiniiformis* and *B. articulatum* is a closed-type vasculature of the stem (Fig. 11a, c). *S. tropaeolifolius* vascular system is a the open-type (Fig. 11b). Lignified elements were found in the stems of *S. × kleiniiformis* and *S. tropaeolifolius* (Fig. 12c; 13e). However, the *S. tripoeolifolius* has only lignified protophloem fibers, while *S. × kleiniiformis* has lignified protophloem fibers only in young shoots and mostly lignified parenchymatous derivatives of the cambium in the aged stems. These two species have evidently different patterns of tissue lignification. Branching of the lateral leaf traces (Fig. 11c) in the stem cortex is a unique character of *S. × kleiniiformis* that is not presented in *S. tropaeolifolius* or *B. articulatum*.

## Conclusion

Our investigation has revealed that *Senecio × kleiniiformis* and *S. tropaeolifolius* are alike in some characteristics of the leaf epidermis and stem anatomy. They have amphistomatous leaf blades with clustered stomata on the adaxial side. The protophloem sclerenchyma is present in their stems. However, more characters are shared by *S. × kleiniiformis* and *Baculellum articulatum*. These characters are the leaf morphological series along the annual shoot, leaf blade and stem anatomy, and partly the epidermis characters. Both in *S. × kleiniiformis* and *B. articulatum*, leaves change from
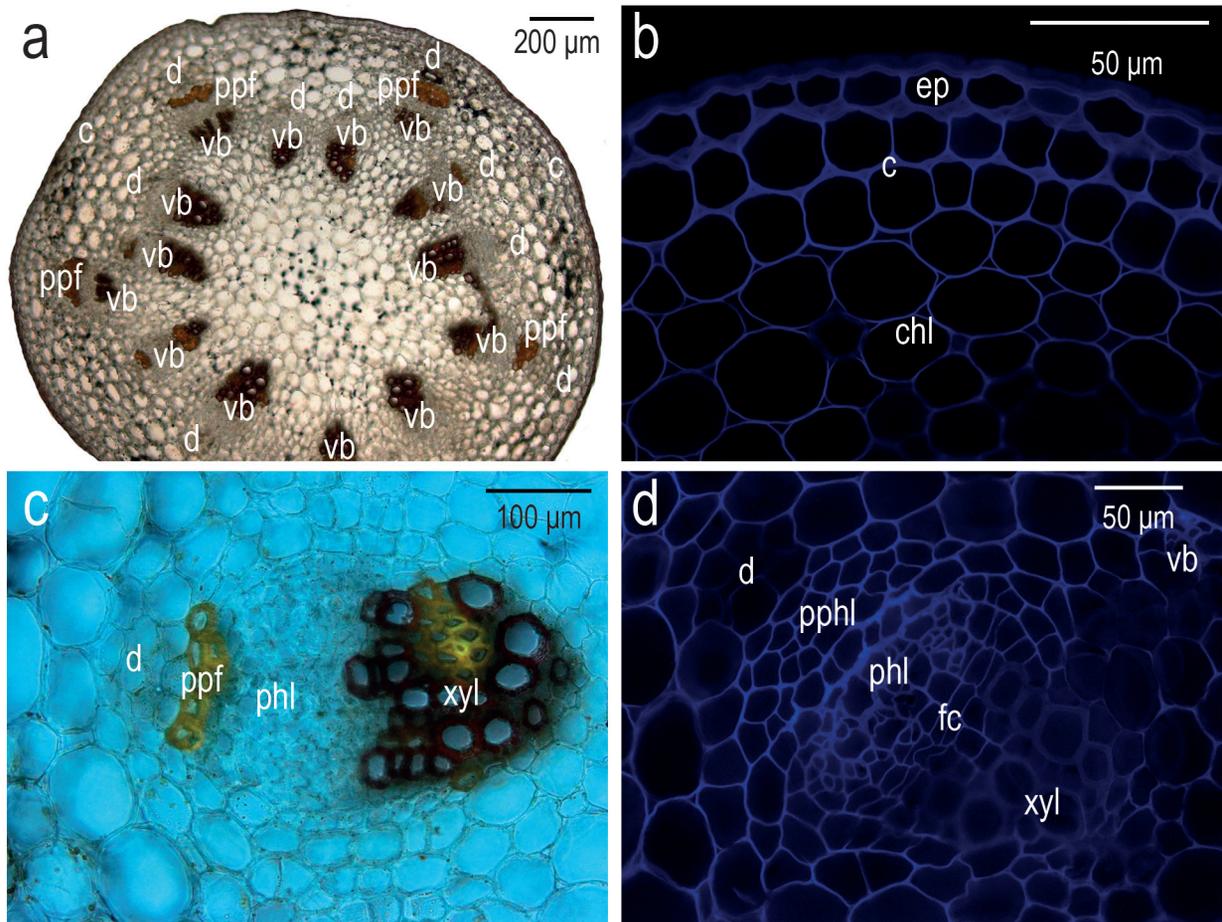


**Fig. 12**. Stem anatomy of *Senecio tropaeolifolius* MacOwan ex F.Muell., transverse sections: **a**. General anatomy; **b**. Collenchyma at the cortex periphery; **c**. Axial vascular bundle in the young stem; **d**. Axial vascular bundle in the mature stem (c, collenchyma; chl, chlorenchyma; d, schizogenous duct; ep, epidermis; phl, phloem; ppf, protophloem fibers; vcb, vascular cambium; vb, vascular bundle; xyl, xylem).

roundish to trilobed with acuminate lobes toward the tip of the annual shoot. Leaf blades in these species are characterized by the presence of several layers of water storage tissue on the adaxial side. *S. × kleiniiformis* and *B. articulatum* have similar stomata complexes. The stems of both species are similar in the closed-type vascular system. Thus, based on anatomical and morphological investigation, we refute the involvement of *S. tropaeolifolius* (Asteraceae) as a parent of *S. × kleiniiformis* and assume *B. articulatum* as a single putative parent species for *S. × kleiniiformis*. The contradictions between the
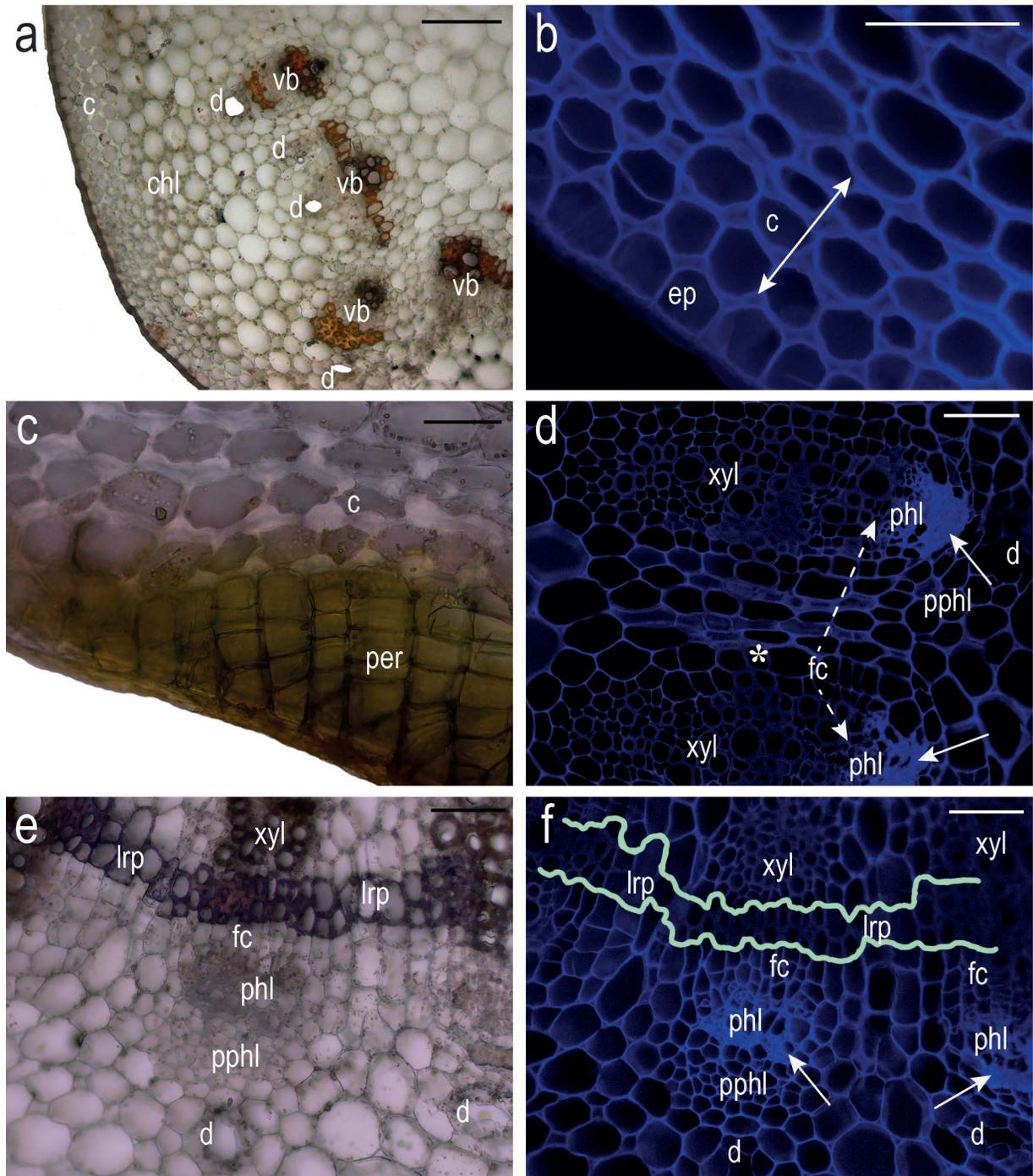


**Fig. 13**. Stem anatomy of *Senecio × kleiniiformis* Suess., transverse sections: **a**. General anatomy; **b**. Collenchyma at the cortex periphery; **c**. Periderm outside of the cortex; **d-f**. Axial vascular bundles in the mature stem (c, collenchyma; chl, chlorenchyma; d, schizogenous duct; ep, epidermis; per, periderm; lrp, lignified ray parenchyma; phl, phloem; pphl, protophloem elements; vcb, vascular cambium; vb, vascular bundle; xyl, xylem. Asterisk marks cell files which indicate the presence of intervascular cambium activity. Arrow marks wrinkled protophloem tissue. Light green lines denote boundaries of lignified parenchyma zone).

molecular data on the one hand (Malenkova *et al.*, 2014) and the data from the study of anatomy and morphology on the other hand can be explained by several reasons. Since the molecular phylogenetic study included species that are polyploids, this could interfere with the phylogenetic tree. However, the principle of anatomical and morphological trait inheritance in *Senecio* still remains unknown and observed similarities between *B. articulatum* and *S.* × *kleiniiformis* are a coincidence.

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