PLANT BIOSYSTEMS - AN INTERNATIONAL JOURNAL DEALING WITH ALL ASPECTS OF PLANT BIOLOGY
https://doi.org/10.1080/11263504.2021.1887954

Histological description of *Saxifraga paniculata* leaves with special focus on structures that release $CaCO_3$

Santiago Michavila[a,b], Antonio Encina[a], Carlos Frey[a,b] and Rafael Álvarez[b]

[a]Department of Ingeniería y Ciencias Agrarias, área de Fisiología Vegetal, Universidad de León, Leon, Spain; [b]Department of Biología Molecular, área de Biología Celular, Universidad de León, Leon, Spain Q4 Q4

ABSTRACT

Saxifraga paniculata is a subalpine succulent perennial plant arranged in a rosette that is usually found in shallow soil among limestone rocks. Stereoscopic, light and scanning electron microscopy were used to describe the anatomical structure of *S. paniculata* leaves, paying special attention to structures related to $CaCO_3$ (calcium carbonate) release. Anomocytic stomata are unevenly distributed on each leaf face, being absent in the lower third. The basal leaf margin presents translucent pluricellular trichomes of variable length and width. Towards the apical margin, trichomes become teeth. Both trichomes and teeth are completely covered with whitish $CaCO_3$ crystals. Each tooth has a circular cavity connected to a single hydathode through pores. Clearing treatment revealed camptodromous leaf venation. Anatomical structure shows a bifacial cross-section with spongy mesophyll cells at basal part, becoming heterogeneous at the apex with palisade mesophyll on the adaxial face. Hydathodes are epithematic and connected to outer cavities via two kidney-shaped guard cells showing large substomatal cavity. The epithem is surrounded by a thickened sheath and is formed of highly packed elongated cells with interspersed tracheary elements. $CaCO_3$ deposits consist of microscopic crystals with varying geometries, of which the rhombus is the basic unit.

ARTICLE HISTORY
Received 26 May 2020
Accepted 19 January 2021

KEYWORDS
Saxifraga paniculata Mill; leaf histology; hydathodes; epithem; camptodromus venation pattern; anomocytic stoma; calcium carbonate ($CaCO_3$) and limestome rocks

Introduction

Saxifraga is the largest and most complex genus in the Saxifragaceae family (Tkach et al. 2015b). The number of species in the genus ranges from 440 to approximately 640, species depending on the author (Vargas 2000; Deng et al. 2015; Tkach et al. 2015b). Saxifrages form a complex group which is probably a monophyletic group, based on molecular phylogenies (Prieto et al. 2013; Tkach et al. 2015a). They are mostly distributed throughout the Holarctic, from temperate to polar zones and are particularly abundant in montane and alpine habitats (Tkach et al. 2015b, Ebersbach et al. 2017). Saxifrages are found on different types of soil, with species ranging from calcifuge to strict calcicole (Conti et al. 1999; Ebersbach et al. 2017).

Saxifrages are small perennial, biennial or annual herbaceous plants. Many morphological features have been used to better understand the classification of the genus, notable among which are leaf morphology and disposition in general, and leaf pattern of venation in particular. Three venation patterns can be distinguished in *Saxifraga*: palinactinodromous, acrodromous and camptodromous (Zhang et al. 2015) and this leaf trait is particularly complex and variable in saxifrages due to the high selective pressure acting on this feature (Roth-Nebelsick et al. 2001). More recently, studies have explored the diversity and complexity of hydathodes (Ebersbach et al. 2017; Wightman et al. 2017, 2018). These may be active (epidermal) or passive (epithemal), depending on water loss via the guttation process (Singh 2013). Further knowledge of these two leaf traits will help to better understand the systematics of the genus. Q1

Saxifraga paniculata Mill. is a member of S. sect. *Ligulatae* subsect. *Euaizoonia* (Conti et al. 1999; Tkach et al. 2015b) and is of particular interest in this respect. This species is patchily distributed throughout the European mountains and is present to a lesser extent in Greenland and northern North America (Reisch et al. 2003). The fragmentation of its distribution area probably occurred in the Pleistocene (Conti et al. 1999). It is preferably subalpine, it can be found from 200 m above sea level, occupying habitats with very extreme weather and soil conditions (Reisch et al. 2003; Wezel 2007). Despite having succulent leaves and inhabiting in subalpine and rocky areas, *S. paniculata* has an obligatory C3 metabolism (Codignola et al. 1990) and high resistance to photoinhibition caused by cold (Hacker and Neuner 2006). This latter feature is important because the plants are usually

CONTACT Rafael Álvarez ralvn@unileon.es Department of Biología Molecular, área de Biología Celular, Universidad de León, Leon, Spain.
Supplemental data for this article is available online at https://doi.org/10.1080/11263504.2021.1887954.

covered by snow in winter (Beato Bergua et al. 2019). Although *S. paniculata* is not a strict calcicole, it is usually found in shallow soil among limestone rocks (Conti et al. 1999; Vargas Gómez 2003; Wezel 2007). Limestone is easily solubilised in water, causing erosion and generating cracks through which water drains (karstification process). Consequently, limestone does not host much shade-generating vegetation, and therefore, the temperature rises considerably when it is exposed to the sun.

S. paniculata is a perennial, rhizomatous species with spatulate leaves arranged in a rosette measuring 2–5 cm in diameter. A floral stem of up to 35 cm high emerges from the centre of leaf rosettes. The whitish cream flowers with small reddish spots are arranged in an umbelliform panicle. The leaves (10–30 mm × 4–6 mm) have a dentate margin and are covered by a whitish sandy crust of $CaCO_3$ (calcium carbonate) crystals (Vargas Gómez 2003) which is particularly prominent at marginal teeth, where small lumps of $CaCO_3$ are formed over the hydathodes. Hydathodes are modified stomata used for gas exchange, intake of elements such as phosphate (Setoguchi et al. 1989; Nagai et al. 2013), or the elimination of fluids with substances dissolved in them by gutting (Singh 2013). In *S. paniculata* in particular, large amounts of $CaCO_3$ are released through the hydathodes by these means. In the lime soils where this species normally occurs, hydathodes help to regulate its internal concentration of Ca^{2+}. The accumulation of $CaCO_3$ can also serve as a defence mechanism, as Ca^{2+} mineralisation protects some plants against herbivores (Bauer et al. 2011).

The histological characteristics of *S. paniculata* leaves and their hydathodes have not yet been fully described, as the only study identified to date does not describe it in detail (Andrei and Paraschivoiu 2008). The same is true for the venation pattern, which has been defined but not described (Zhang et al. 2015). Therefore, the aim of this article is to describe leaf morphology, paying particular attention to the venation pattern and hydathodes.

Q2 Material and method

Plant material

Leaf rosettes of *Saxifraga paniculata* Mill. were collected from the wild between March (before flowering) and August (after flowering) at Redipuertas (43.0037°N, −5.2810°W) and Valdejeta (42.5550°N, −5.2437°W), León (Spain). Samples were taken in locations situated between 1300 and 1500 m above sea level, facing north-east. Plants were growing in limestone crevices and small ledges resulting from sedimentary deposition of limestone washings. All samples were fixed *in situ*, in formalin-acetic acid-alcohol (FAA).

Stereoscopic microscopy

Intact fixed leaves were immersed in 2 M hydrochloric acid (HCl) for 12 h at room temperature to remove calcium carbonate (clarifying treatment). To clear the leaves (clearing treatment), two previous methods (Vasco et al. 2014; Zhang et al. 2015) were modified. Intact fixed leaves were treated with 10% (w/v) sodium hydroxide solution for 2 weeks, then washed with distilled water and imbibed in 5% (v/v) sodium hypochlorite bleaching solution for at least 30 min. Bleached leaves were washed with distilled water and dehydrated in ascending grades of ethanol. Then, they were stained with Safranin and washed with 95° ethanol acidified with 37% (v/v) HCl for 30 min before being imbibed for 10 min in absolute ethanol.

Fresh, clarified and cleared leaves were observed with a stereoscopic microscope (Nikon SMZ 1500) and photographed using a Nikon D4 full-frame camera and NIS-Elements F.3.2 software.

Light microscopy

Following Álvarez et al. (2009), samples were dehydrated in an increasing ethanol series and embedded in paraplast to create different blocks, using isoamyl acetate as intermediate liquid. Each block was then sectioned using a rotary paraffin microtome to perform serial 12 µm thick cuts and sections were deposited on slides. Once these had been dewaxed, routine staining was performed using Safranine-Fast Green. After dehydration, sections were mounted permanently on microscope slides using Entellan as mounting medium.

Some sections were permanently mounted without dyeing, while others were stained with hematoxylin-eosin for cytological study. Slides were observed with a Nikon E600 under bright-field, epifluorescence and polarised light conditions.

Samples of leaf epidermis peels were obtained from leaves by dissection and placed on a microscope slide. Epidermis preparations were mounted with distilled water and stained with fuchsine. Slides were observed with a Nikon Eclipse-600 microscope under bright-field, epifluorescence and polarised light conditions. Photographs were taken with a Nikon digital camera (DXM 1200) using NIS-Elements F.3.2 software.

Scanning electron microscopy

Fixed leaf fragments were passed through an increasing alcohol series, gold scattered and observed using a Jeol JSM-6480LV SEM.

Results

Leaf description

Leaves in rosette appear almost horizontal when hydrated, closing together under water stress. All leaves are sessile, with outer leaves being older and larger than inner leaves (Figure 1A). Leaves are oblanceolate, pink coloured in junction zone and changing from light greenish white in basal area to intense green in apical part (Figure 1B), with similar but paler colouring on the abaxial face (Figure 1C). Basal leaf margins exhibit thin trichomes, which become increasingly large teeth pointing towards the leaf apex along the distal margin. $CaCO_3$ deposits are present on both faces, increasing in density towards the margins, especially on apical teeth of

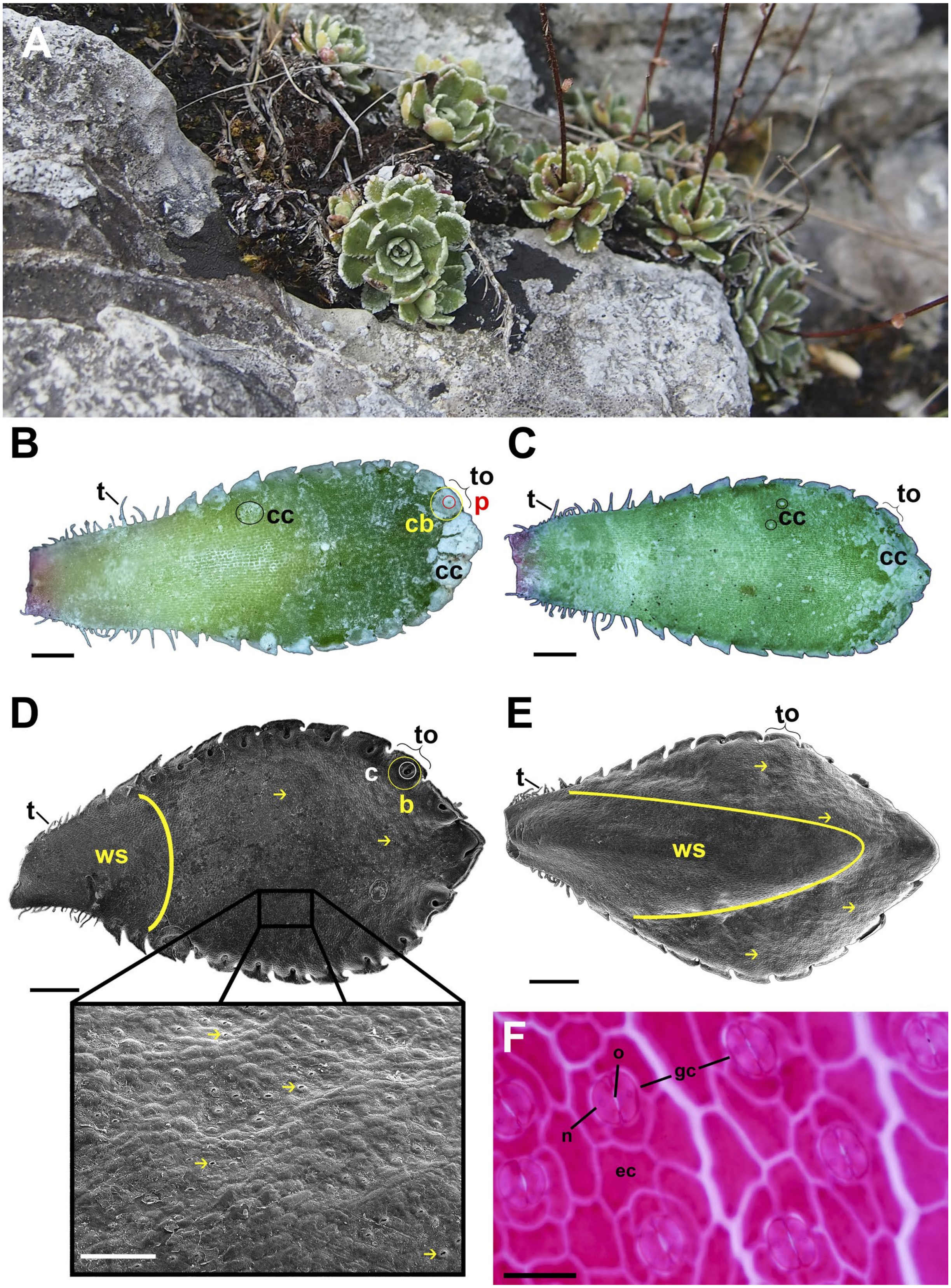

Figure 1. External appearance of *Saxifraga paniculata* leaves and stomata. (A) *S. paniculata* aspect and habitat. Macroscopic view of (B, D) adaxial and (C, E) abaxial face of *S. paniculata* leaves under (B, C) stereoscopic and (D, E) scanning electron microscopy. (F) Stomata structure. Abbreviations: (cc) calcium carbonate deposits, (c) cavity, (cb), calcium carbonate bulge, (ec) epidermal cell, (gc) guard cells, (o) ostiole, (p) pit, (t) trichome, (to) tooth, (b) (tooth bulb), (ws) stomata free area, (yellow arrow) stoma. All scales bars 1000 µm, except expanded rectangle (200 µm) and (F) 50 µm.

the adaxial face where they accumulate in form of a bulge ($CaCO_3$ bulge) with a central pit. Removal of calcium deposits revealed the presence of a cuticle except on marginal teeth, where small cavities are visible on the adaxial face inside a bulge (tooth bulge; Figure 1D) but not the abaxial face (Figure 1E).

S. paniculata leaves are amphistomatic, but stomata are distributed differently on each leaf face. Stomata are absent on the lower third of the adaxial face and distributed randomly on the upper two thirds (Figure 1D). Stomata are also absent on the proximal third of the abaxial face and in second third are only present in two lateral bands, widening until joining in the distal third (Figure 1E). Stomata are of anomocytic type, surrounded by irregular epidermal cells distinct from kidney-shaped form of guard cells (Figure 1F).

Trichomes and teeth

The basal third of the leaf margin is characterized by the presence of translucent pluricellular trichomes with two morphologies: (1) thin, needle-like-trichomes and (2) broad, beak-like-trichomes entirely covered with whitish crystals of CaCO3 (Figure 2A). Narrowed, conical and sharp trichomes near to base of the leaf are formed entirely by circular overlapping cells. Those trichomes found more distant from the base of the leaf are wider and triangular with more rectangular cells (Figure 2B). Towards the apical margin, trichomes become teeth characterized by the presence of a thick whitish crystal deposit showing a pit opening located in the center of the whitish deposit (Figure 2C). Mechanical removal of the whitish crust revealed a circular cavity deeper than the initially visible opening, and a marginal bugle also thinning towards the flat leaf surface (Figure 2D). In the tooth bulge and in cavity, cells are identical to other leaf epidermis cells; irregular, polygonal and swollen (see Supporting Information Figure 1). The external part of the tooth and cavity interior have elongated, flattened cells pointing towards the leaf apex. Cavities have thickened edges and an irregular bottom part mainly presenting one pore in smallest teeth (proximal) and two in the larger distal one (Figure 2E).

Observation of trichomes without $CaCO_3$ revealed that they have the same colour as the internal part of the leaf and tooth bulge. Towards the distal part, the tooth crest becomes more transparent as size increased (see Supporting Information Figure 2). At least one secondary vein reaches each cavity, but usually there are two (rarely three; Figure 2F). These veins come from a primary and central vein and branch out dichotomously but may reconnect with nearby veins. This vascular system distribution corresponds to the camptodromous venation pattern (Figure 2G).

Leaf histology and hydathode internal organisation

The cross-section of the basal part of this bifacial leaf is flattened on the adaxial face and convex towards the centre of the abaxial face (Figure 3A,B). Uniseriate epidermis is formed by big flattened cubic cells in adaxial face and small cubic cells on abaxial face, covered by a thick cuticle. Trichomes are formed by a variable number of elongated epidermal cells. The internal structure of the leaf consists of a homogeneous mesophyll with rounded parenchyma cells (spongy mesophyll). There is a primary vascular bundle that is visible at the leaf centre, with branching secondary bundles, which do not reach trichomes. All bundles are surrounded by a thickened sheath. Some thickened sheath and epidermal cells contain polyphenolic-type compounds as can be seen with orange (Figure 3A) or garnet color (Figure 3B).

Mesophyll becomes heterogeneous and dorsiventral towards apex in which rounded parenchyma cells are gradually replaced by palisade parenchyma on the adaxial (palisade mesophyll), and on the abaxial face near the tooth crest. (Figure 3C). Mesophyll and cuticle cells contain polyphenolic compounds (Figure 3C). Stomata are at the level of the cuticle and slightly above the epidermis with small substomatal cavities. Inside marginal tooth, secondary vascular bundles reach the leaf margin with hydathodes (Figure 3C). Hydathodes are surrounded by a thickened sheath and their internal organization is formed by a core of xylem cells communicating with the epithem that opens into an external cavity pointing towards the adaxial face of the leaf (Figure 3C). The external cavity is lined by thickened epidermal cells similar to a thick trichome (tooth crest) (Figure 3C).

A more detailed inspection revealed that epithem is formed by highly packed, thin-walled, elongated cells showing a prominent nucleus (Figure 3D). Among epithem cells, interspersed tracheary elements can be distinguished. The epithem is connected with an external cavity through an ostiole encircled by two kidney-shape guard cells, similar to stomatal guard cells, but larger. Between the epithem and hydathode pore a substomatal chamber exists, a little larger than in stomata (Figure 3D).

The cells present birefringence in trichomes and teeth. In the trichomes, birefringence appeared in form of elongated ribs (Figure 3E). Adaxial thickening teeth polarized the light in a way similar to trichomes. Whitish deposits on cavities are also highly birefringent (Figure 3F) $CaCO_3$ is deposited on the leaf surface in the form of thin plates (Figure 3G), between which the stomata can be observed (Figure 3H). These deposits are formed by microscopic crystals with varying geometries, with the rhombus as the basic unit (Figure 3I).

Discussion

According to Gornall (1987), $CaCO_3$ deposits endow leaves with a frosty appearance, and this has prompted the common name for the S. sect. *Ligulatae* of the 'silver' or 'encrusted' saxifrages (Conti et al. 1999). $CaCO_3$ density increases towards margins and apex, where the largest $CaCO_3$ deposits are found, probably in response to very active transpiration zones, such as hydathodes in teeth or stomata in apex. There are between 12 and 28 teeth on each leaf (Vargas Gómez 2003), and $CaCO_3$ accumulates on all of them. The genus *Saxifraga* has one of the most varied stomata distribution patterns of all the Saxifragaceae (Metcalfe and Chalk 1950). As with other *Saxifraga* (Andrei and Paraschivoiu 2008), and alpine genera such as *Sempervivum* (Codignola et al. 1990),

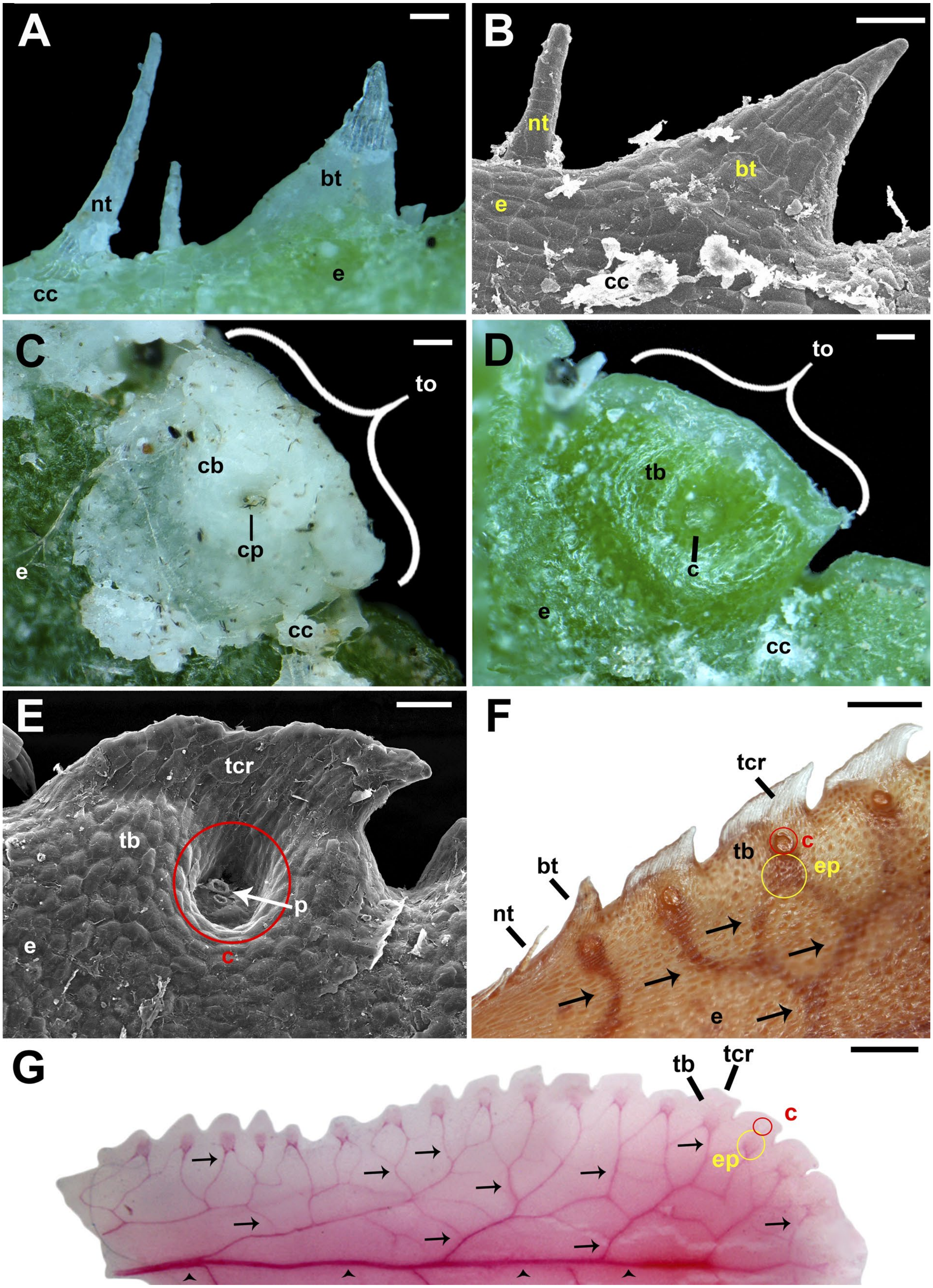

Figure 2. External appearance of *Saxifraga paniculata* trichomes and hydathodes with and without $CaCO_3$, trichomes and teeth with clarify treatment and vascular pattern with clear treatment. (A, B) Structure of leaves trichomes, (C–E) theet and (F–G) venation pattern of *S. paniculata* leaves. Photographs were taken under (A, C, D, F, E) stereoscopic and (B, E) scanning electron microscopy. (E) Clarifyed leaf and (G) cleared leaf venation pattern under stereoscopic microscopy. Abbreviations: (bt) beat-like-trichomes, (c) cavity, (cc) calcium carbonate, (cb) calcium carbonate bulb, (cp) calcium carbonate pit, (e) epidermis, (ep) epithem, (nt) needle-like trichomes, (p) pore, (to) tooth, (tb) tooth bulb, (tcr) tooth crest, (arrow) secondary nerve and (arrowhead) primary nerve. All scale bars 100 µm, except (F) 500 µm and (G) 1000 µm.

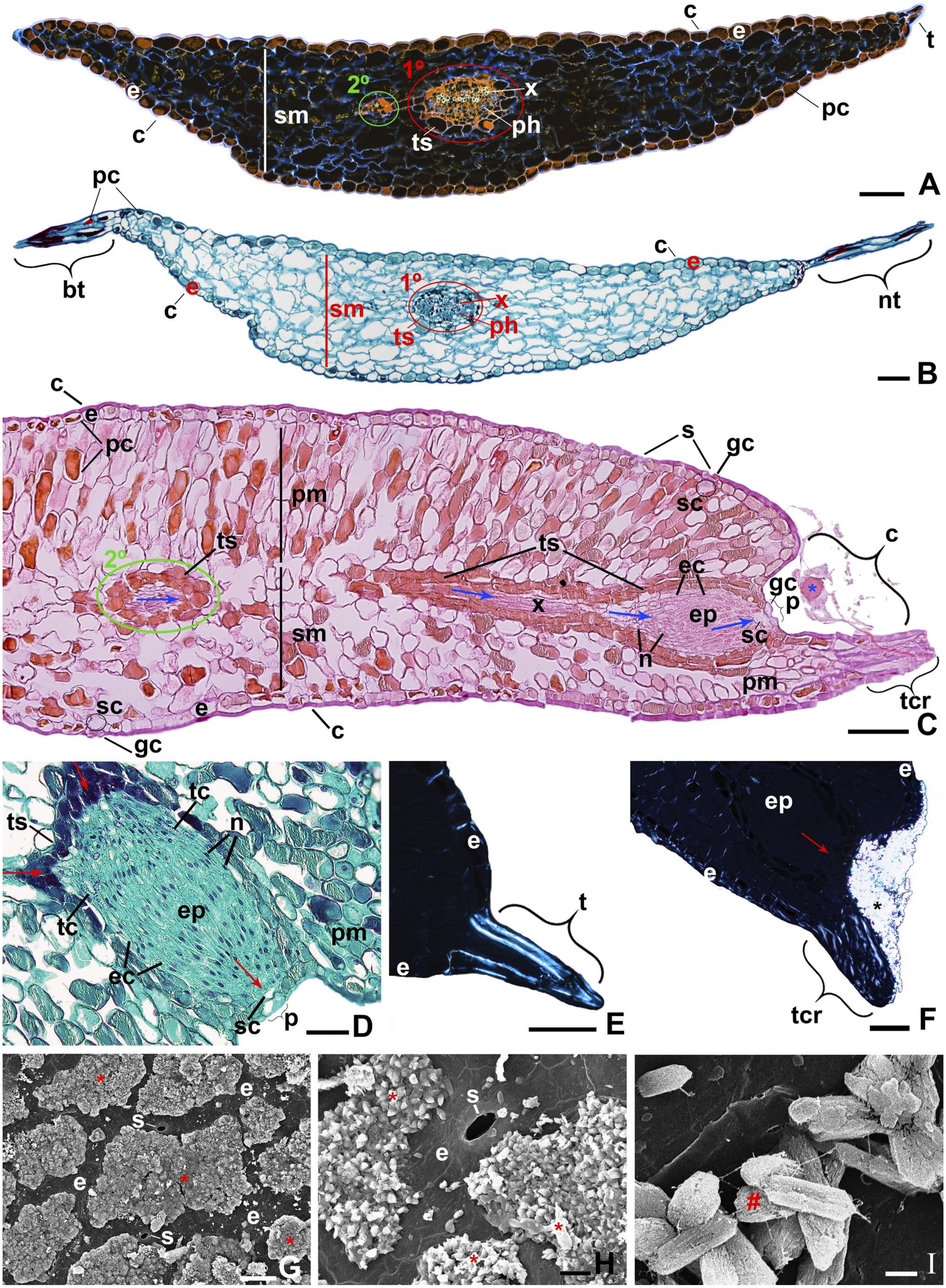

Figure 3. Anatomical structure of *Saxifraga paniculata* leaves with special attention to trichomes and hydathodes, external aspect of $CaCO_3$ crusts and morphology of its crystals. (A, B) leaves and (C–F) hydathodes. (G–E) $CaCO_3$ deposits on *S. paniculata* leaf surface. Abbreviations: (bt) beak-liketrichomes, (c) cuticle, (c) cavity, (e) epidermis, (ec) epithem cell, (ep) epithem, (gc) guard cells, (n) nucleus, (nt) needle-like-trichomes, (p) pore, (pc) polyphenolic compounds, (ph) phloem, (pm) palisade mesophyll, (s) stoma, (sc) substomatal cavities, (sm) spongy mesophyll, (t) trichome, (tc) traqueid cell, (tcr) tooth crest, (ts) thickened sheath, (x) xylem, (1°) primary nervous, (2°) secondary nerves, (blue and red arrows) guttation flux, (*) calcium carbonate, (#) calcium carbonate crystals. Scale bars: (A, B, C, E and F) 100 µm (D and G) 50 µm (H) 10 µm and (I) 2 µm.

S. paniculata is amphistomatic. Stomatal density is greater on the abaxial than adaxial face, and in general is higher than in *Sempervivum* (Codignola et al. 1990). Contrary to a previous report by Andrei and Paraschivoiu (2008), stomata in *S. paniculata* are not tetracytic. This stoma has four subsidiary cells around guard cells (Lawson and Matthews 2009), but in *S. paniculata* the cells surrounding the guards are similar to those on the rest of the epidermis and are therefore anomocytic. Guard cells are kidney-shaped, as previously shown (Lawson and Matthews 2009).

Needle-like trichomes closest to the base are non-glandular and uniseriate, as in *Saxifraga biflora* All., while beak-like trichomes are intermediate and non-glandular, as in *Saxifraga stribrnyi* (Velen.) Podpera (Gornall 1986). Trichomes thicken towards the apex until they become prominent teeth, as in other Saxifragaceae genera (Stern 1974). Teeth have a marginal crest pointing towards the tip and an internal part formed by a bulge and cavity that constitute the exterior part of the hydathode. The teeth crests are transparent and may serve to divert light to the nearby parenchyma, as in *Saxifraga scardica* Griseb. (Wightman et al. 2018). The $CaCO_3$ crystals that accumulate in the tooth cavity following $CaCO_3$ release through the pore may intensify this diverted light phenomenon through the Kerker effect (Barhom et al. 2019). Ca^{2+} and HCO^{3-} are transported to hydathodes through the xylem by transpiration (White 2001) and are stored in the epithem until transpiration is reduced or inhibited. This increases the hydrostatic pressure called root or exudation pressure, until the guard cells open without resistance due to the accumulated force (Singh 2013; Cerutti et al. 2019) (see epithem description below). Outside, the water evaporates and the $CaCO_3$ crystallises, forming a crust over the opening and hindering the gutting process (Takeda et al. 1991). It is probable that the stomata also release $CaCO_3$, but in lesser amounts than the hydathodes, and consequently the entire leaf surface is covered by a carbonate calcium crust.

Leaf venation is an important character because it is related to the transport properties and/or mechanical stability of leaves (Roth-Nebelsick et al. 2001); it is also an important morphological trait for taxonomy (Walls 2011). *S. paniculata* shows camptodromous venation (Zhang et al. 2015), but this has not been described in any previous study, although venation patterns shed useful light on the anatomy of a genus. All *Saxifraga* species with this venation pattern have entire leaves with marginal hydathodes, where the pinnate secondary veins end (Zhang et al. 2015).

The dorsiventral leaf of *S. paniculata* (Codignola et al. 1990; Andrei and Paraschivoiu 2008) is typical of *Saxifraga* species, but there are also reports of centric leaves in the genus (Metcalfe and Chalk 1950). The epidermis is uniseriate on both faces (Codignola et al. 1990) in contrast to some other species of the family where the epidermis, mainly the upper is multiseriate (Stern 1974). The cuticle is thickened as in *Saxifraga cochlearis* Rchb. and *S.scardica* (Wightman et al. 2017, 2018). Epidermal and mesophyll cells accumulate polyphenol content, especially those at the base of trichomes or in their elongated cells. These characteristics are very common in Saxifragaceae (Metcalfe and Chalk 1950).

In the basal region, leaf mesophyll with loosely packaged, unorganized, rounded parenchyma cells is non-photosynthetic (spongy mesophyll). This is replaced by palisade mesophyll in the distal part of the leaf (Codignola et al. 1990; Andrei and Paraschivoiu 2008), as the green colour of the leaf intensifies, a characteristic that is not ubiquitous in the *Saxifraga* genus (Metcalfe and Chalk 1950).

As previously reported, vascular bundles are surrounded by a thickened sheath (Andrei and Paraschivoiu 2008; Codignola et al. 1990). Vascular bundles end in the hydathodes, as in the rest of the species of this family (Metcalfe and Chalk 1950).

A detailed description of hydathode anatomy in saxifrages would provide a useful tool for comparing species within the genus (Wightman et al. 2017). Epithem structure in *S. paniculata* is similar to that found in *S. cochlearis* (Wightman et al. 2017): the epithem occupies most of the hydathode, with vascular bundle cells scattered at the proximal end. Consequently, the hydathode can be classified as epithematic (Singh 2013). The hydathode anatomy of *S. paniculata* described here agrees with previous findings (Andrei and Paraschivoiu 2008). Their general function is to filter xylem sap (Singh 2013; Cerutti et al. 2019), but in *S. paniculata* they can also synthesise and store phytoferritin (Perrin 1970). Between epithem and pore a chamber exists (Singh 2013) that is bigger than the substomatal one. The guard cells surrounding the pore are similar to those found in stomata but larger and more kidney shaped. In contrast to stomata guard cells, those forming the hydathode pore have lost the capacity to change turgor upon stimuli such as light. In the case of hydathodes, pore opening is driven by an increase in root pressure, and they can therefore be classified as passive hydathodes (Singh 2013). As in *S. scardica* (Wightman et al. 2018), the mesophyll cells closest to the base of the hydathode are rectangular, resembling those of the chlorophyll parenchyma. These cells may receive more light intensity thanks to the probable diversion of light by teeth crests and $CaCO_3$ crystals in the cavity.

Teeth in *S. paniculata* increase in parallel with an increase in distance from the base, a characteristic that has been observed in other recently studied saxifrages (Wightman et al. 2017, 2018), and in most other species in the family (Stern 1974). Unlike *S. cochlearis*, which normally has two (rarely three) pores in the hydathode, one of which atrophies towards the apex (Wightman et al. 2017), *S. paniculata* has one main pore and two others towards the apex. Cells in the crest are similar to those of trichomes and both show birefringence. The crystals that accumulate in teeth cavities also polarise light because $CaCO_3$ shows birefringence (Herne et al. 2019).

$CaCO_3$ can solidify in a non-crystalline form (amorphous) or in anhydrous crystalline polymorphs (Cölfen 2003). In this study, *S. paniculata* leaves accumulated both amorphous solids and polymorphous crystals of $CaCO_3$. Foliar crystals are common in *Saxifraga* and other calcicole Saxifragaceae species (Gornall 1987). Three anhydrous crystalline carbonate calcium polymorphs can be found: calcite, aragonite and vaterite (Cölfen 2003). It is probable that the foliar $CaCO_3$

crystals described here correspond to calcite, as previously reported for *S. paniculata* and related species, such as *S. scardica* and *S. cochlearis* using Raman microscopy (Wightman et al. 2017, 2018). In contrast to cystoliths, where $CaCO_3$ is rapidly transformed into calcite (Setoguchi et al. 1989), external $CaCO_3$ crystallisation requires an element (organic or not) that acts as a nucleation point (Turner and Jones 2005). Organic compounds typically cause crystallisation into rhombic, rhombohedral or scalenorhombohedral forms (Cölfen 2003; Bosak and Newman 2005). In agreement with this, most of $CaCO_3$ crystals described here correspond to the rhombic system.

Conclusions

This description evidences that *S. paniculata* stomata are anomocytic and although present on both surfaces, they are not distributed in the same way. Foliar trichomes and teeth have also been described. The teeth have one hydathode, which has been described in detail, and this is connected to vascular bundles. The vascular system presents camptodromous venation. The $CaCO_3$ crust consists of rhomboidal crystals.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We thank the Universidad de León (Spain) for providing the funds for performing this study.

ORCID

Santiago Michavila http://orcid.org/0000-0001-6691-2270
Antonio Encina http://orcid.org/0000-0002-1559-1136
Carlos Frey http://orcid.org/0000-0002-0369-5536

References

Andrei M, Paraschivoiu RM. 2008. Anatomical researches on the overground vegetative organs of *Saxifraga mutata* L. subsp *demissa* (Schott & Kotschy) D.A. webb and *Saxifraga paniculata* Miller. Analele Stiint Ale Univ 'Al. I. Cuza' Iasi LIV.54:5–14.

Barhom H, Machnev AA, Noskov RE, Goncharenko A, Gurvitz EA, Timin AS, Shkoldin VA, Koniakhin SV, Koval OY, Zyuzin MV, et al. 2019. Biological Kerker effect boosts light collection efficiency in plants. Nano Lett. 19(10):7062–7071.

Bauer P, Elbaum R, Weiss IM. 2011. Calcium and silicon mineralization in land plants: transport, structure and function. Plant Sci. 180(6):746–756.

Beato Bergua S, Poblete Piedrabuena MÁ, Marino Alfonso JL. 2019. Snow avalanches, land use changes, and atmospheric warming in landscape dynamics of the Atlantic mid-mountains (Cantabrian Range. NW Spain). Appl Geogr. 107:38–50.

Bin DJ, Drew BT, Mavrodiev EV, Gitzendanner MA, Soltis PS, Soltis DE. 2015. Phylogeny, divergence times, and historical biogeography of the angiosperm family Saxifragaceae. Mol Phylogenet E. 83:86–98.

Bosak T, Newman DK. 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. J Sediment Res. 75(2):190–199.

Cerutti A, Jauneau A, Laufs P, Leonhardt N, Schattat MH, Berthomé R, Routaboul J-M, Noël LD. 2019. Mangroves in the leaves: anatomy, physiology, and immunity of epithemal hydathodes. Annu Rev Phytopathol. 57:91–116.

Codignola A, Fieschi M, Maffei M, Fusconi A. 1990. Leaf anatomy and photosynthetic characteristics of succulent alpine plants growing at high elevations. Nord J Bot. 10(1):49–56.

Cölfen H. 2003. Precipitation of carbonates: recent progress in controlled production of comples shapes. Curr Opin Colloid Interface Sci. 8(1):23–31.

Conti E, Soltis DE, Hardig TM, Schneider J. 1999. Phylogenetic relationships of the silver saxifrages (*Saxifraga*, Sect. *Ligulatae* Haworth): implications for the evolution of substrate specificity, life histories, and biogeography. Mol Phylogenet Evol. 13(3):536–555.

Ebersbach J, Schnitzler J, Favre A, Muellner-Riehl AN. 2017. Evolutionary radiations in the species-rich mountain genus *Saxifraga* L. BMC Evol Biol. 17:1–13.

Gornall RJ. 1986. Trichome anatomy and taxonomy of *Saxifraga* (Saxifragaceae). Nord J Bot. 6(3):257–275.

Gornall RJ. 1987. Foliar crystals in *Saxifraga* and segregate genera (Saxifragaceae). Nord J Bot. 7(3):233–238.

Hacker J, Neuner G. 2006. Photosynthetic capacity and PSII efficiency of the evergreen alpine cushion plant *Saxifraga paniculata* during winter at different altitudes. Arctic Antarct Alp Res. 38(2):198–205.2.0.CO;2]

Herne CM, Levey JR, McCausland TR. 2019. Polarization-dependent non-uniform rotation rates of rhombohedral calcite. Opt Express. 27(13):18445–18455.

Lawson T, Matthews J. 2009. Guard cell photosynthesis and stomatal function. New Phytol. 181(1):13–34.

Metcalfe CR, Chalk L. 1950. Anatomy of the dicotyledons. In: Leaves, stem, and wood in relation to taxonomy. Chapter 118, London: Oxford University Press; p. 553–557.

Nagai M, Ohnishi M, Uehara T, Yamagami M, Miura E, Kamakura M, Kitamura A, Sakaguchi SI, Sakamoto W, Shimmen T, et al. 2013. Ion gradients in xylem exudate and guttation fluid related to tissue ion levels along primary leaves of barley. Plant Cell Environ. 36(10):1826–1837.

Perrin A. 1970. Diversity of phytoferritin arrangement in epithem of hydathodes in *Taraxacum officinale* and in Saxifraga aizoon. Planta. 93(1):71–81.

Prieto JAF, Arjona JM, Sanna M, Pérez R, Cires E. 2013. Phylogeny and systematics of *Micranthes* (Saxifragaceae): an appraisal in European territories. J Plant Res. 126(5):605–611.

Reisch C, Poschlod P, Wingender R. 2003. Genetic variation of *Saxifraga paniculata* Mill. (Saxifragaceae): molecular evidence for glacial relict endemism in central Europe. Biol Linn Soc. 80(1):11–21.

Roth-Nebelsick A, Uhl D, Mosbrugger V, Kerp H. 2001. Evolution and function of leaf venation architecture: a review. Ann Bot. 87(5):553–566.

Setoguchi H, Okazaki M, Suga S. 1989. Calcification in higher plants with special reference to cystoliths. Orig Evol Mod Asp. 1839:409–418.

Singh S. 2013. Guttation: path, principles and functions. Aust J Bot. 61(7):497–515.

Stern WL. 1974. Comparative anatomy and systematics of woody Saxifragaceae. *Escallonia*. Botanical. 68(1):1–20.

Takeda F, Wisniewski ME, Glenn DM. 1991. Occlusion of water pores prevents guttation in older strawberry leaves. J Am Soc Hortic Sci. 116(6):1122–1125.

Tkach N, Röser M, Hoffmann MH. 2015a. Molecular phylogenetics, character evolution and systematics of the genus *Micranthes* (Saxifragaceae). Bot J Linn Soc. 178(1):47–66.

Tkach N, Röser M, Miehe G, Muellner-Riehl AN, Ebersbach J, Favre A, Hoffmann MH. 2015b. Molecular phylogenetics, morphology and a revised classification of the complex genus *Saxifraga* (Saxifragaceae). Taxon. 64(6):1159–1187.

Turner EC, Jones B. 2005. Microscopic calcite dendrites in cold-water tufa: implications for nucleation of micrite and cement. Sedimentology. 52(5):1043–1066.

Vargas P. 2000. A phylogenetic study of *Saxifraga* sect. *Saxifraga* (Saxifragaceae) based on nrDNA ITS sequences. Pl Syst Evol. 223(1–2):59–70.

Vargas Gómez P. 2003. Género *Saxifraga* [Genus Saxifraga]. In: Castroviejo S, Aedo C, Laínz M, Muñoz F, Nieto G, Paiva J, Benedí C, editors. Flora Ibérica [Iberian Flora]. Madrid: Real Jardín Botánico de Madrid; p. 185–186. Spanish.

Vasco A, Thadeo M, Conover M, Daly DC. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. Appl Plant Sci. 2(9):1400038.

Walls RL. 2011. Angiosperm leaf vein patterns are linked to leaf functions in a global-scale data set. Am J Bot. 98(2):244–253.

Wezel A. 2007. Changes between 1927 and 2004 and effect of rock climbing on occurrence of *Saxifraga paniculata* and *Draba aizoides*, two glacial relicts on limestone cliffs of the Swabian Jura, southern Germany. J Nat Conserv. 15(2):84–93.

White PJ. 2001. The pathways of calcium movement to the xylem. J Exp Bot. 52(358):891–899.

Wightman R, Wallis S, Aston P. 2017. Hydathode pit development in the alpine plant *Saxifraga cochlearis*. Flora Morphol Distrib Funct Ecol Plants. 233:99–108.

Wightman R, Wallis S, Aston P. 2018. Leaf margin organisation and the existence of vaterite-producing hydathodes in the alpine plant *Saxifraga scardica*. Flora Morphol Distrib Funct Ecol Plants. 241:27–34.

Zhang Z, Xia N, Gornall RJ. 2015. Leaf venation patterns in the genus *Saxifraga* (Saxifragaceae). Phytotaxa. 221(2):123–136.