

Leaf blade anatomy of the rare Siberian flora species *Mertensia sibirica* (L.) G. Don fil. (Boraginaceae)

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The authors present the findings of a leaf blade anatomy study for the rare relict Siberian flora species *Mertensia sibirica* (L.) G. Don fil. (Boraginaceae). They collected samples for the study from natural habitats in Chita Region (Chikoy Range) and then planted them in the introduction area of the Siberian Botanic Garden (Tomsk) located in the southern taiga subzone of Western Siberia. The parameters of the photosynthetic and stomatal complex of *M. sibirica* were studied for the first time. It was found out that the rosette and cauline leaves of the species under study are hypostomatous, with an anomocytic stomatal complex. The epidermis is single-layer. On average, the adaxial epidermis has larger cells vs. abaxial epidermis. The leaf mesophyll is 242.90–369.90 μm thick, dorsiventral. The adaxial side of the leaf comprises glandular trichomes surrounded with pronounced rosettes of cells in the base part. The cauline leaf significantly differs from the rosette leaf in finer cells of its adaxial and abaxial epidermis (and, consequently, their larger number per 1 mm^2), while the adaxial epidermal cells are thicker, and in a larger number of stomata in the abaxial epidermis. The palisade mesophyll in the cauline leaf is more developed vs. the rosette leaf, while the cells are longer and the palisade/spongy mesophyll ratio is higher. The rosette leaves have a more developed system of vascular tissues vs. cauline ones, as they play the main role in providing plants with water and nutrients. The contribution of the cauline leaf palisade mesophyll to the photosynthetic potential of *M. sibirica* is higher vs. that of the rosette leaf (the ratio between palisade and spongy mesophyll is 0.45 vs. 0.36, respectively), which characterizes the cauline leaf as more heliophytic. The stomatal complex and mesophyll parameters under study are primarily characterized by low variance. As for dermal tissue parameters, medium variance is typical of the thickness and size of the abaxial and adaxial epidermal cells. Coefficients of variation for the cells of the upper mesophyll layer (CV=31.2–41.6%) and the number of stomata on the lower epidermis of the rosette leaf (CV=21.5%) demonstrate medium and high variance. A very high coefficient of variation (116.2–174.0) is registered for the adaxial epidermis parameter characterizing the density of trichomes per 1 mm^2 . The study results were used to develop an optimal *M. sibirica* cultivation regime under conditions of introduction in the southern taiga subzone of Western Siberia.

Keywords: *Mertensia sibirica*, leaf blade anatomy, rosette and cauline leaves, plant ecology, stomatal index, epidermis, mesophyll, trichomes.

Introduction

The *Mertensia* Roth genus (Boraginaceae) comprises about 45 species, 15 of which grow in extratropical Asia. In Eurasia, the genus is represented by the circumpolar *M. maritima* (L.) S.F. Gray species and Siberian species that remain in the refugia of Southern Siberian mountains (Nikiforova, 2014).

Mertensia sibirica (L.) G. Don fil. (Boraginaceae) is an endemic Eastern Siberian species that can be found in specific isolated locations. The north-western border of its habitat lies in the Lower Yenisei, where the species grows in the areas with the special microclimate of floating warm fogs. The south-eastern border of the habitat is the forest communities of the Chikoy Range located on the border between Chita Region and Buryatia. The species belongs to the *Mertensia* section, *Mertensia* subsection, *Sibiricae* M. Pop. series where species were formed under the influence of Atlantic climate rather than Pacific climate. This is a Pleistocene relict species that grows in relict taiga forests, grassy tundras, on meadows, along forest edges, pebble beaches, streams, and on moist mountains (Kozhevnikov, 1996; Nikiforova, 2008, 2014).

Flora of China (Zhu, 1995) describes this species for the Shanxi Province. The peculiar ecology of this species is connected with its high demand for moist substrates.

This species is listed in the regional Red Books in Siberia (Yamskikh & Pospelova, 2012; Ivanova & Verkhovina, 2010; Popova, 2017). This is an original ornamental spring and nectariferous plant that is good for creating groups under trees and shrubs and decorating watersides.

It is cultivated at the botanic gardens of Novosibirsk, Tomsk, Irkutsk and Yakutsk (Ivanova & Verkhozina, 2010; Kupriyanov & Banaev, 2017).

One of the basic strategies for preserving the genetic diversity of plants (ex situ) is cultivating plants as part of botanic gardens' collections. To successfully introduce rare species, it is necessary to study their biological features that reflect the interaction of organisms with environment and allow revealing their complex of structural adaptations. The most important structural and adaptive element of plants is the leaf blade, including the epidermis and stomatal complex.

There are limited literature data on the leaf anatomy of certain species within the *Mertensia* genus. For instance, E.V. Burkovskaya and Yu.A. Khrolenko (2015) studied the structure of the photosynthetic complex of *M. maritima* (L.) S. F. Gray from different natural populations in relation to their adaptation to the conditions of the Far Eastern coastal-marine floristic community. The authors detected both common leaf structure features (dorsiventral, amphistomous, anomocytic stomata, wax glands, single-layer epidermis) and specific features connected with geographic confinedness (leaf thickness, number of chloroplasts and mesophyll cells; size of guard cells; number of stomata per 1 mm² of the leaf). The authors believe that the detected variance can be connected with the presence of different chromosome races in this species. They describe that *M. maritima* has special elements – wax glands responsible for the glaucous color of leaf blades and protection of plants from the withering effect of salt water, wind and sun. Wax glands consist of elongated epidermal cells that radially converge to the base of wax chambers.

The formation of the *M. maritima* leaf blade mesostructure is influenced by the complicated living environment of this species with a vast habitat. Its optimal structure was formed primarily thanks to the enlargement of phototrophic elements. A unique feature of the leaf blade anatomy of this species is large cells that result from the combination of photosynthetic and water storage functions, as the species inhabits areas with saline soils (Burkovskaya, 2009).

Some authors (Selvi & Bigazzi, 2001; Barykina & Alyonkin, 2018) studied the leaf anatomy of different representatives of the Boraginaceae family. However the species from the *Mertensia* genus are not mentioned among the study subjects.

The goal of this paper is to study the parameters of the photosynthetic and stomata complex of *Mertensia sibirica* for the first time ever to specify the environmental features of this species and develop an optimal cultivation scheme to introduce this species into the southern taiga of Western Siberia.

Materials and Methods

Samples for the study were collected in 2010 from natural habitats in Chita Region (Chikoy Range) and then planted in the introduction area of the Siberian Botanic Garden (Tomsk) located in the southern taiga subzone of Western Siberia. Today, the species has been successfully induced in Tomsk where it regularly flowers and bears fruit.

The authors used generally accepted methods, publications by Pautov (2003, 2012), Barykina et al. (2004) as methodology to study leaf anatomy. We used rosette and cauline leaves with no visible damage collected from five generative plants at the fructification stage. Leaf sections were prepared with five-fold repeatability, so the authors analyzed at least 25 sections for each sample.

Temporary specimens of fresh leaves were cut via the freezing microtome MZ–2. Cross sections were taken from the middle part of the leaf. The section thickness was 75–100 µm. Epiderm was cut with a razor in the middle third of the blade between the leaf margin and the midrib.

The photos of leaf microsamples and microscopic measurements were made using the Carl Zeiss Axio Lab. A1 light microscope (Germany) with the AxioCam ERc 5s digital camera by means of the Axio Vision 4.8 software. Trichomes were imaged with a Leica MC170 HD microscope camera.

The measurement results were statistically processed with the Statistica 8.0 software. The authors determined the following parameters: M – arithmetic mean, m – arithmetic mean error, CV – coefficient of variation. Anatomic parameters are considered low-variance at CV < 20%, medium-variance – at CV = 20–40%, and high-variance – at CV > 40 % (Butnik & Timchenko, 1987). To assess the significance of variance in independent samples, the authors calculated a t-test statistic value assuming equal variance in samples; a t-test statistic value assuming unequal variance in samples; an F-test statistic value. Significant variance was determined at the confidence level of p < 0.05.

Results

The rosette leaves of *M. sibirica* have a long and flat petiole, are glabrous, glaucous-green, ovoid, ovate, wide-lanceolate, oblong-ovoid, with reticulate venation, are 18–25 cm long if taken with a petiole (the average blade length is 8.9±0.2 cm) and 4.2±0.2 cm wide, on average. The cauline leaves are scarce, sessile, oblong or ovoid, with a pointed tip. The study of the *Mertensia sibirica* leaf blade anatomy shows that the rosette and cauline leaves are dorsiventral, hypostomatous with anomocytic stomata.

The adaxial and abaxial leaf epidermis is single-layer, the cells are irregularly shaped, thin-walled, the anticlinal walls of the cells are sinuose, while the adaxial epidermal cells are more rounded (Figure 1).

On average, the adaxial epidermis has larger cells (by 1.2–1.3 times) vs. the abaxial epidermis. The adaxial epidermis of the rosette leaf consists of larger cells vs. the cauline leaf and, consequently, the number of cells in the adaxial surface of the rosette leaf (246.40±4.89 cells) is smaller vs. the cauline leaf (305.92±6.08 cells) (Table 1). The number of adaxial and abaxial epidermal cells is low-variance, CV < 10 %. The adaxial epidermis of the rosette and cauline leaves is 1.5–2.2 times thicker vs. the abaxial epidermis, while the cauline leaf cells (59.18±2.83 µm) are thicker vs. the rosette leaf (47.11±2.72 µm).

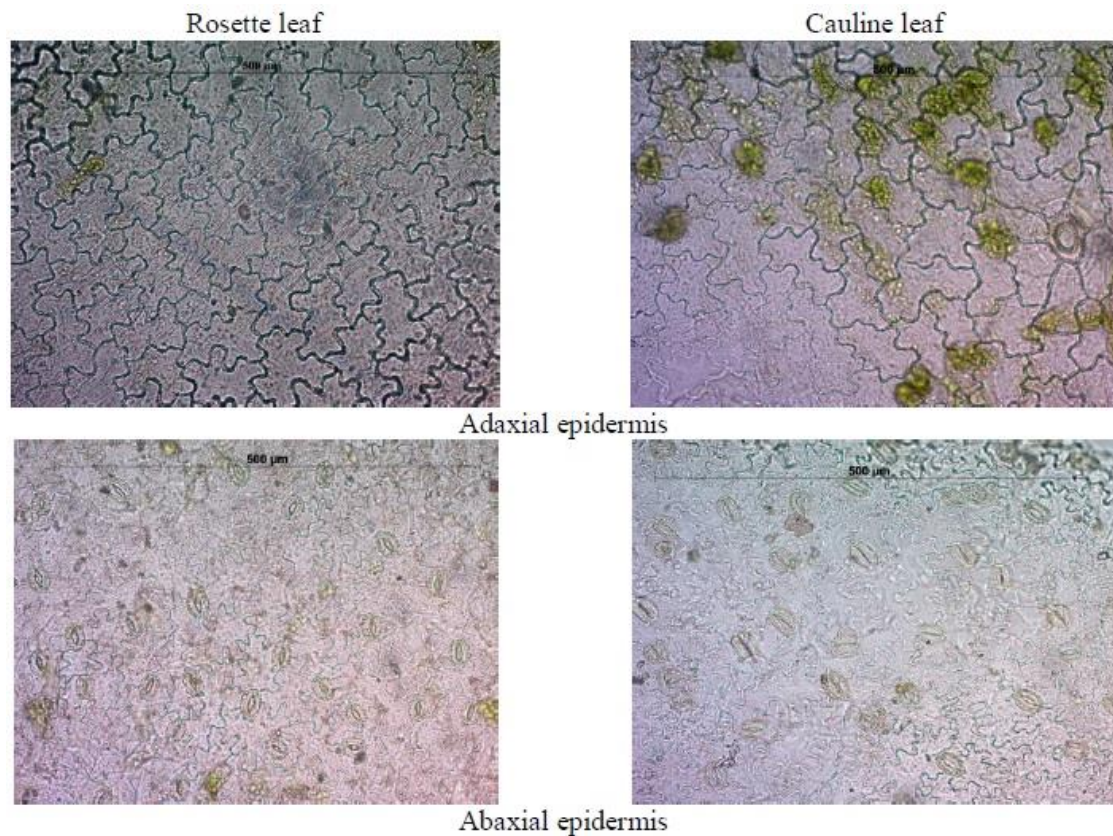


Fig. 1. Adaxial and abaxial epidermis of *Mertensia sibirica* cauline and rosette leaves

Table 1. *Mertensia sibirica* rosette and cauline leaf epidermis anatomy characteristics.

Characteristic	Rosette leaf	M ± m CV, %	Cauline leaf
	Number of adaxial epidermal cells per 1 mm ² , pcs		246.40 ^A ± 4.89 9.9
Adaxial epidermal cell size (area), µm ²	4394.07 ^A ± 177.15 20.2	3422.26 ^B ± 152.37 22.3	
Number of adaxial epidermal trichomes per 1 mm ² , pcs	5.12 ^A ± 1.78 174.0	8.96 ^A ± 2.08 116.2	
Adaxial epidermis thickness, µm	47.11 ^A ± 2.72 28.9	59.18 ^B ± 2.83 23.9	
Number of abaxial epidermal cells per 1 mm ² , pcs	343.04 ^A ± 5.85 8.5	367.36 ^B ± 6.69 9.1	
Number of abaxial epidermis stomata per 1 mm ² , pcs	134.40 ^A ± 5.77 21.5	158.72 ^B ± 6.19 19.5	
Abaxial epidermis stomatal index, %	27.97 ^A ± 0.82 14.6	30.02 ^A ± 0.79 13.1	
Abaxial epidermal cell size (area), µm ²	3411.38 ^A ± 262.41 38.5	2787.88 ^A ± 222.68 39.9	
Abaxial epidermis stomata length, µm	32.32 ^A ± 0.49 7.5	32.05 ^A ± 0.68 10.7	
Abaxial epidermis stomata width, µm	24.37 ^A ± 0.33 6.7	25.43 ^B ± 0.31 6.1	
Abaxial epidermis thickness, µm	30.63 ^A ± 1.85 30.2	27.15 ^A ± 1.10 20.2	

*Different letters show significant variance at the confidence level of $p < 0.05$.

The abaxial epidermal cells of the cauline leaf are smaller vs. the rosette leaf, but this variance is not significant. The number of abaxial epidermal cells and stomata per 1 mm² is larger in the cauline leaf (367.36±6.69 cells and 158.72±6.19 stomata) vs. the rosette leaf (343.04±5.85 cells and 134.40±5.77 stomata) while the stomatal index of the rosette (27.97%) and cauline (30.02%) leaves is practically the same and has no significant variance. The stomata are oblong-rounded, 25.03–38.05 µm long and 21.20–

28.66 μm wide, chaotic and surrounded with epidermal cells (Table 1, Figure 1). The abaxial epidermis thickness of the rosette and cauline leaves has no significant variance.

The adaxial side of the leaf comprises glandular trichomes surrounded with pronounced rosettes of cells in the base part (Figure 2). The number of trichomes in the rosette and cauline leaves has no significant variance and amounts to 0–32 pcs per 1 mm^2 .



Fig. 2. *Mertensia sibirica* glandular trichomes in juvenile (A) and old leaves (B)

The rosette leaf is thicker (1360.7–1629.8 μm) within the midrib vs. the cauline leaf (839.49–1006.29 μm), which is due to its more developed vascular system: the vascular bundle and its parts are 2–2.5 times larger vs. the rosette leaf. However, the vascular tissue area ratio (xylem/phloem) does not have significant variance (Table 2).

Table 2. *Mertensia sibirica* rosette and cauline leaf anatomy characteristics.

Characteristic	Rosette leaf	M \pm m CV, %	Cauline leaf
	Leaf thickness (midrib), μm	1526.65 ^A \pm 14.51	
	4.8		5.9
Leaf thickness (blade), μm	373.10 ^A \pm 8.31		369.67 ^A \pm 7.43
	11.1		10.1
Mesophyll thickness, μm	290.60 ^A \pm 6.55		289.56 ^A \pm 3.84
	11.3		6.6
Palisade mesophyll thickness, μm	74.88 ^A \pm 2.06		89.59 ^B \pm 2.60
	13.8		14.5
Spongy mesophyll thickness, μm	214.86 ^A \pm 6.69		204.21 ^A \pm 5.47
	15.6		13.4
Palisade mesophyll / spongy mesophyll ratio	0.36 ^A \pm 0.01		0.45 ^B \pm 0.02
	19.4		23.7
Cell length of the upper mesophyll layer, μm	80.80 ^A \pm 3.06		90.66 ^B \pm 3.03
	18.9		16.7
Cell width of the upper mesophyll layer, μm	29.53 ^A \pm 2.46		28.51 ^A \pm 1.78
	41.6		31.2
Bundle cross section area, μm^2	207411.68 ^A \pm 3651.90		82777.69 ^B \pm 1746.60
	8.8		10.6
Xylem cross section area, μm^2	61177.74 ^A \pm 1555.00		31229.01 ^B \pm 684.42
	12.7		11.0
Phloem cross section area, μm^2	49110.47 ^A \pm 1508.62		24744.63 ^B \pm 796.88
	15.4		16.1
Xylem/phloem area ratio	1.26 ^A \pm 0.04		1.29 ^A \pm 0.05
	15.0		19.8

* Different letters show significant variance at the confidence level of $p < 0.05$.

The leaf mesophyll is 242.90–369.90 μm thick, dorsiventral, consists of a single layer of palisade and multiple layers of spongy parenchyma (Figure 3). The thickness of the rosette and cauline leaf mesophyll, spongy mesophyll, blades has no significant variance, while the palisade tissue is thicker in the cauline leaf (Table 2) and the palisade/spongy mesophyll ratio is also higher for the cauline leaf (0.45 vs. 0.36). The palisade coefficient is low, amounts to 25.8% and 30.9% for the rosette and cauline leaves, respectively.

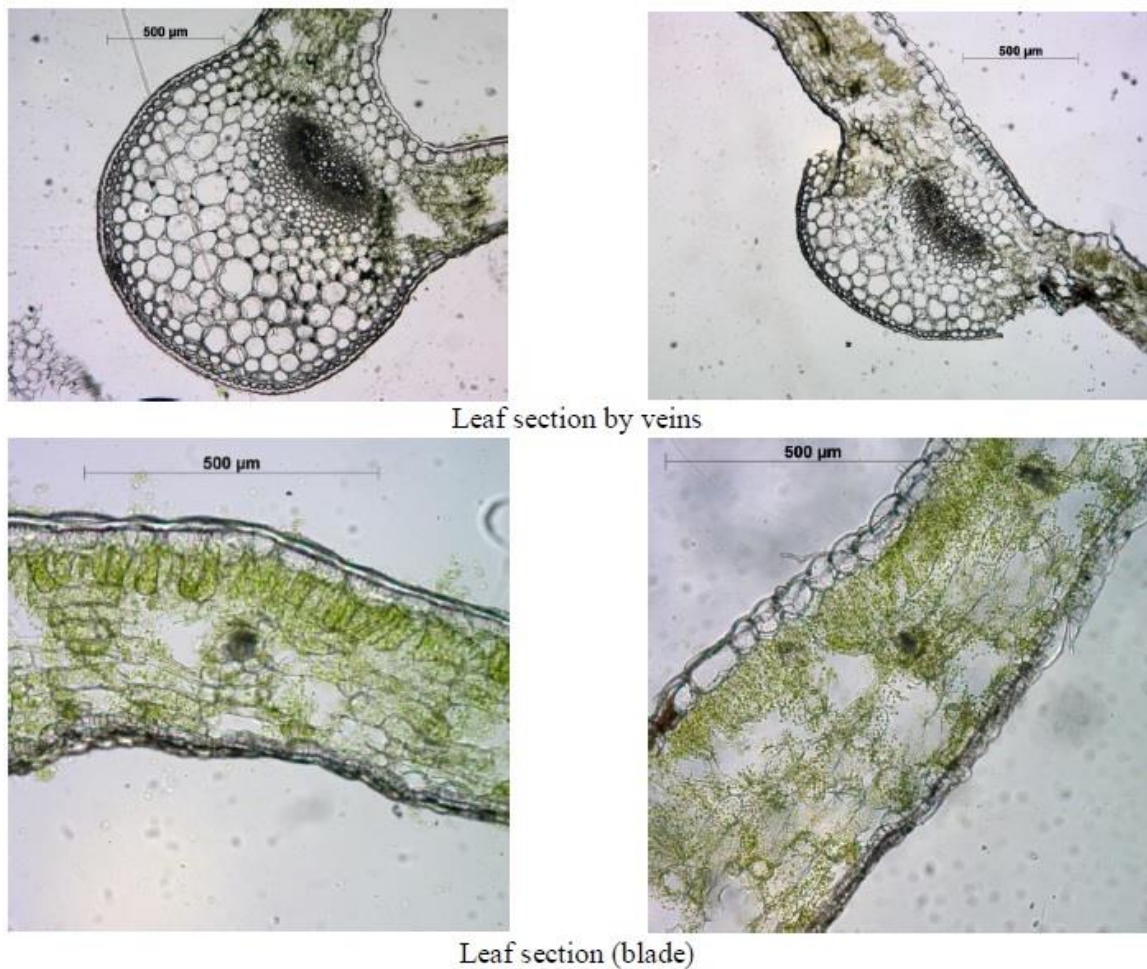


Fig. 3. *Mertensia sibirica* rosette and cauline leaf blade anatomy respectively.

Discussion

As for dermal tissue parameters, medium variance is typical of the thickness of the abaxial (CV=20.2–30.2%) and adaxial (CV=28.9–23.9%) epidermis and the cell size of the adaxial (CV=20.2–22.3%) and especially abaxial (CV=38.5–39.9%) epidermis. The stomatal complex and mesophyll parameters under study are primarily characterized by low variance. Coefficients of variation for the cells of the upper mesophyll layer (CV=31.2–41.6%) and the number of stomata on the lower epidermis of the rosette leaf (CV=21.5%) demonstrate medium and high variance. A very high coefficient of variation (116.2–174.0%) is registered for the adaxial epidermis parameter characterizing the density of trichomes per 1 mm². Glandular trichomes having a similar structure are also relevant to some genera of the Boraginaceae family, according to F. Selvi and M. Bigazzi (Selvi & Bigazzi, 2001). Significant variance was established for 13 (56.5%) of the 23 studied parameters of rosette and cauline leaf blades belonging to this species.

Thus, the cauline leaf significantly differs from the rosette leaf in finer cells of its adaxial and abaxial epidermis (and, consequently, their larger number per 1 mm²), while the adaxial epidermal cells are thicker, and in a larger number of stomata in the abaxial epidermis. The palisade mesophyll in the cauline leaf is more developed vs. the rosette leaf, while the cells are longer and the palisade/spongy mesophyll ratio is higher. This variance is due to the fact that cauline leaves develop in better light environments vs. the rosette leaves and therefore have more pronounced signs of being heliophytic. The rosette leaves have a more developed system of vascular tissues vs. cauline ones, as they play the main role in providing plants with water and nutrients. However, the cauline and rosette leaf blades have no variance in terms of the xylem/phloem ratio and mesophyll thickness.

The contribution of the cauline leaf palisade mesophyll to the photosynthetic potential of *M. sibirica* is higher vs. that of the rosette leaf (the ratio between palisade and spongy mesophyll is 0.45 vs. 0.36, respectively), which characterizes the cauline leaf as more heliophytic.

M. sibirica differs from *M. maritima* in thinner hypostomatous rather than amphistomatous leaves being 369.67–373.10 µm thick vs. 471.34–550.46 µm for the second species, a smaller number of stomata per unit of area (134.4–158.72 pcs per 1 mm² vs. 1.5–3.25 pcs/cm² or 150–325 pcs/mm²), which is probably reasoned by *M. maritima* adaptation to open, well-lighted areas with saline soils (sand beaches and pebbles of sea littorals) and winds.

The established thickness of the *M. sibirica* leaf blade (373.10±8.31 µm) is comparable with data provided by M.R. Slaton et al (2001) for the North American species *Mertensia viridis* (A. Nels.) A. Nels. (366±13 µm).

Thus, the *M. sibirica* leaf blade structure has hygromesophytic and scioheliophytic signs: quite fleshy hypostomatous leaves with a thin cuticle, large-cell stomata and epidermis, dorsiventral mesophyll, increased contribution of spongy parenchyma to the photosynthetic potential of this species, large amounts of water-storage tissues, predominant rising current development.

Apparently, the large thin-walled mesophyll cells have a water storage function. The poor development of trichomes found only on the adaxial epidermis, the large-cell mesophyll also reflect species preference for high air and substrate humidity.

Conclusion

The studies of the *M. sibirica* leaf blade anatomy allow classifying this species as hygromesophytes. We have determined 13 signs, according to which the rosette leaves significantly differ from the cauline leaves. We have also established the presence of glandular trichomes on the adaxial leaf epidermis.

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