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# LIGULE ANATOMY AND MORPHOLOGY OF FIVE *POA* SPECIES

ZSUZSA K. SZABÓ[1*], MÁRIA PAPP[2], AND LAJOS DARÓCZI[3]

[1]*Department of Agricultural Botany and Plant Physiology,*
[2]*Department of Botany,* [3]*Department of Solid State Physics,*
*University of Debrecen, 4032 Debrecen, Hungary*

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The morphological and anatomical features of ligules of some *Poa* species (members of *Poa pratensis* group: *P. pratensis*, *P. angustifolia*, *P. humilis*; species outside the group: *P. compressa*, *P. alpina*) were studied by light and scanning electron microscopy. They are described in detail, emphasizing interspecific differences and habitat-dependent variation of shape, length and prickle hair density. All ligules studied are membranous, without veins and stomata-like structures, and may have only a few mesophyll-like cells. The *P. pratensis* ligule rarely contains short prickle hairs. Short leaflike prickle hairs occur densely on the ligule of *P. angustifolia*. The ligule of *P. humilis* is densely covered by long prickle hairs. The long prickle hairs of *P. compressa* end in elongated, curved apexes. There are no prickle hairs on the ligule of *P. alpina*. The density of hairs on the abaxial surface is habitat-dependent for all species studied. Ligule anatomy in the *P. pratensis* group is quite uniform, leading them to be grouped as closely related species, but the fine differences identified are useful for their identification.

**Key words:** *Poa pratensis* group, *Poa compressa*, *Poa alpina*, ligule, prickle hair.

## INTRODUCTION

Members of the *Poa pratensis* group (*P. pratensis* L., *P. angustifolia* L., *P. humilis* Ehrh. Ex Hoffm.) are very similar morphologically. There are only a few characteristics distinguishing them, especially in *P. pratensis* L. and *P. angustifolia* L. (Papp et al., 1999; Penksza and Böcker, 1999). Some morphological characteristics differ statistically, but there are overlaps between them. Apart from the two *Poa* species mentioned above, three others were examined in our study: *P. humilis* Ehrh. ex Hoffm. (also a member of the group), *P. compressa* L. and *P. alpina* L. (not members of this group). *P. compressa* individuals frequently coexist with members of the *P. pratensis* group, but *P. alpina* has a different distribution. It is not an element of the Hungarian flora but is included here for comparison as an example of a species from extremely different environmental conditions. In this study we use the results of our ligule investigations to show the taxonomic importance of the morphology and anatomy of this less studied leaf sheath appendage.

Grass ligules are small outgrowths at the junction of the leaf sheath and the blade. Their morphology and anatomy may be important for identification of some critical grass species, especially when the shoots are in the vegetative state (Zuloaga et al., 1998; Judziewicz and Clark, 1993; Fuente and Ortunez, 2001). They can provide information on the evolutionary relationships among certain species (Chaffey, 1984), contributing to a more natural taxonomic system. Because it is relatively constant, ligule anatomy is also important for identifying individuals living in non-optimal environments (Neumann, 1938). Variation of ligule anatomy has been shown in some species, however, for example in *Poa trivialis* (Chaffey, 1985), where the ligules of certain individuals are vascularized but those of others not. Stebbins and Zohary (1959) found differences between the ligules of ecotypes of *Dactylis glomerata*. Their results underscore the point that local ecotypes are manifestations of morphological plasticity and result from adaptation to different habitats.

Chaffey (1994) demonstrated the functional anatomy of ligules of 49 grass species from ten tribes. Most of the ligules consisted of three layers: the mesophyll and two epidermal cell layers. The mesophyll in those ligules contained chloroplasts. The main role of ligules is to protect the inner side of the leaf sheath from spores, water and dust, but in several species the ligule can also have a secretory function (Chaffey, 2000). Grasses with ligules have higher fitness (e.g., Moreno et al., 1997; Korzun et al., 1997).

*e-mail: szabozs@agr.unideb.hu

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The ligules of the species studied here do not differ considerably in length (1–3 mm) or shape (truncate to blunt) Rothmaler, 1994; Tutin et al., 1980), so these features do not greatly assist classification. Simon (2000) and Soó and Kárpáti (1968) observed that the ligules of *P. pratensis* but not of *P. angustifolia* are decurrent on the sheath margins; our paper gives a fuller description of this.

## MATERIALS AND METHODS

*Poa* shoots were collected from various habitats in June 2003 at flowering. The sampling sites were wet meadow, dry sandy pasture, dry weedy vegetation and dry alkaline pasture in eastern Hungary near the city of Debrecen, except for *P. alpina*, the shoots of which were collected from a subalpine rocky grassland habitat on Mt. Hochtor in the Austrian Alps at 2505 m a.s.l. Five flowering shoots (culms) from each sampling site were studied.

Individuals of the same species from different habitats were treated separately for study of the variations. The ligules were separated from the leaves with tweezers, cleaned with commercial bleach and flushed with distilled water. Then the three uppermost mature ligules from each leaf were examined on both surfaces with a light microscope. Longitudinal cross sections were made in different zones to gain an overall picture of the structure of the interior. In *P. pratensis* and *P. angustifolia* the border zone of the ligule and leaf sheath was also cut. The surfaces were studied with an AMRAY 1830I scanning electron microscope. For histological measurements the Digit-Desk image-processing system was used (Demeter et al., 1995). Prickle hairs were counted on 1 mm$^2$ of the surface. Ligule hair density data were analyzed by ANOVA; species, habitats and the three levels of the shoots were compared. The normality of the data distributions was tested by the Kolmogorov-Smirnov test. The statistics employed SPSS for Windows (Rel. 11.0.1. 2001. SPSS Inc., Chicago). Ligule morphology nomenclature follows Chaffey (1984) (Fig. 1).

## RESULTS

The ligule of all studied species is a continuous layer of tightly packed cells without veins or stomata.

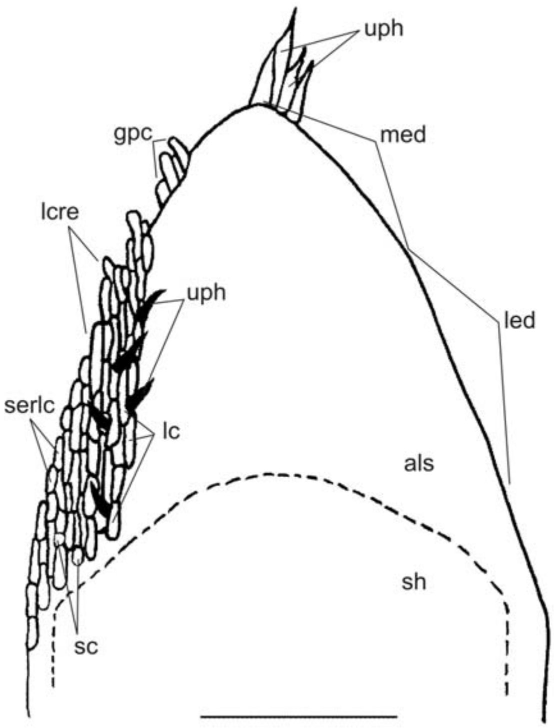


**Fig. 1**. Scheme of the abaxial surface of a ligule, showing the regions and types of cells for anatomical description after Chaffey (1984). uph – unicellular prickle hairs; gpc – group of papillate cells; lcre – long cells with rounded ends; serlc – shorter long cells; lc – long cells; sc – short cells; med – median edge; led – lateral edge; sh – sheath; als – abaxial ligule surface.

Mesophyll occurs only in *P. pratensis*, where some cells in the proximal part of the ligule form a discontinuous layer one-cell thick between the two epidermal surfaces (Fig. 2a,b). The ligule thickness of the other *Poa* species is built of a layer one-cell thick along almost their whole length (Fig. 2c). The cell shapes are similar to those of leaf epidermal cells. The long axes of the cells are parallel with the ligule axis.

### ABAXIAL AND ADAXIAL SURFACES

The two surfaces differ morphologically in all species studied except *P. alpina*. The abaxial is always more variable than the adaxial surface. Short cells, long cells, shorter long cells and prickle hairs vary (Fig. 1). The anticlinal walls of long cells are

TABLE 1. One-way ANOVA table for upper ligule hair density of four *Poa* species

| Source | SS | df | MS | F | p-value | F-critical |
|---|---|---|---|---|---|---|
| Between groups | 3818.91 | 3 | 1272.97 | 10.00 | 0.00 | 4.06 |
| Within groups | 1018 | 8 | 127.25 | | | |
| Total | 4836.91 | 11 | | | | |

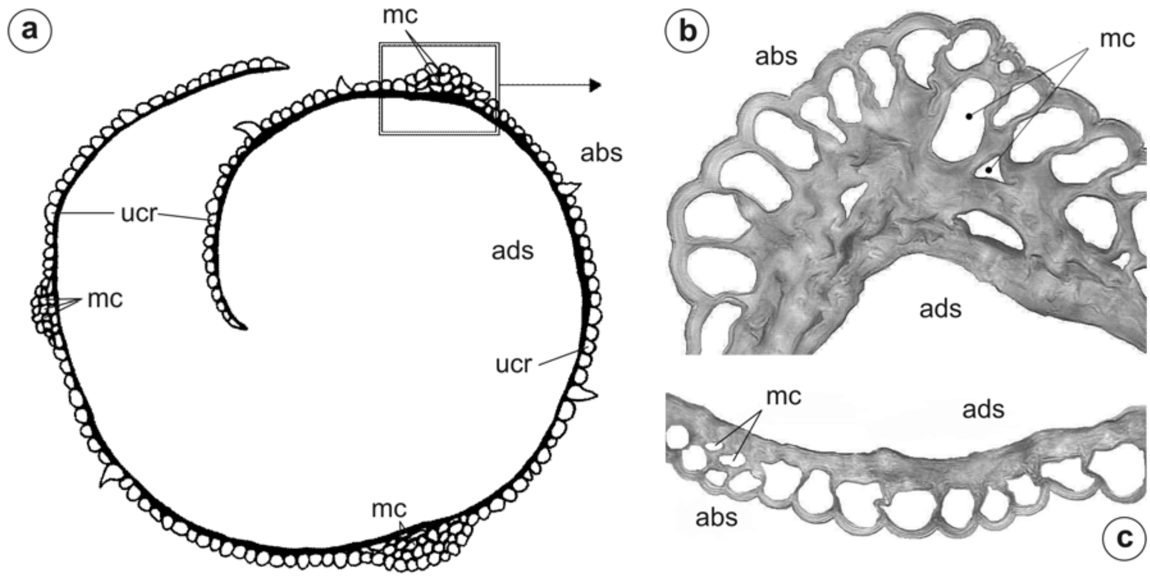


**Fig. 2.** Scheme (**a**) and part of microscopic cross sections of *P. pratensis* ligule (**b**) and *P. angustifolia* ligule (**c**). mc – mesophyll-like cells; ucr – unicellular region; abs – abaxial surface; ads – adaxial surface. (b) × 20, (c) × 40.

generally parallel. In a few places where the walls are thinner they are sometimes undulated. The short ends of the neighboring cells connect to each other at various angles. Shorter long cells can be found on the fringes, and their length only slightly exceeds their width. Their longer walls are slightly undulated and thin. Rounded short cells with thickened walls may occur among the long cells.

Prickle hairs occur only on the abaxial surface and on the edges. The statistics for their density are shown in Table 1. Except in *P. humilis*, we found differences in prickle hair density between the three upper ligules of the shoots. In all species the lower ligules are more densely hirsute than the upper ones (Tab. 2). *P. pratensis* has short prickle hairs with thick, rigid walls (Fig. 3a), and their density greatly depends on habitat and the level of the shoot (Tab. 2). Individuals collected from wet meadow had ligules more hirsute than those from dry habitats. The ligules of *P. angustifolia* are usually more hirsute than those of *P. pratensis*, and the prickle hairs are short, flat and leaflike (Fig. 4b). In *P. angustifolia* there is no significant difference in ligule hair density between individuals from different habitats (Tab. 2). The prickle hairs of *P. humilis* are 3–4 times longer than those of the species previously discussed, and are densest on the ligule surface (Fig. 3c). Hair density depends on

TABLE 2. Two-way ANOVA table for ligule hair density of the studied *Poa* species

| Species | Source | SS | df | MS | F | p-value | F-critical |
|---|---|---|---|---|---|---|---|
| *P. pratensis* | Shoot level | 298.66 | 2 | 149.33 | 34.46 | 0.003 | 6.94 |
| | Habitat | 206 | 2 | 403 | 23.76 | 0.006 | 6.94 |
| | Error | 17.33 | 4 | 4.33 | | | |
| | Total | 522 | 8 | | | | |
| *P. angustifolia* | Shoot level | 88.22 | 2 | 44.11 | 8.63 | 0.03 | 6.94 |
| | Habitat | 16.88 | 2 | 8.44 | 1.65 | 0.29 | 6.94 |
| | Error | 20.44 | 4 | 5.11 | | | |
| | Total | 125.55 | 8 | | | | |
| *P. humilis* | Shoot level | 233.33 | 2 | 116.66 | 8.33 | 0.10 | 19.00 |
| | Habitat | 1350 | 1 | 1350 | 96.42 | 0.01 | 18.51 |
| | Error | 28 | 2 | 14 | | | |
| | Total | 1611.33 | 5 | | | | |
| *P. compressa* | Shoot level | 272.66 | 2 | 136.33 | 8.70 | 0.03 | 6.94 |
| | Habitat | 268.66 | 2 | 134.33 | 8.57 | 0.03 | 6.94 |
| | Error | 62.66 | 4 | 15.66 | | | |
| | Total | 604 | 8 | | | | |

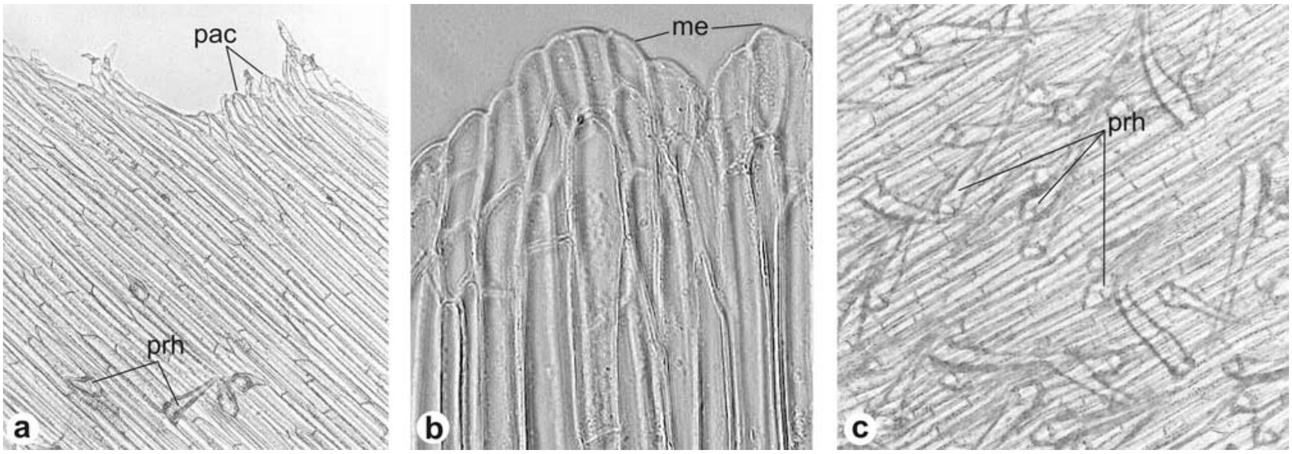


**Fig. 3**. Part of abaxial surface of *P. pratensis* ligule (**a**), *P. alpina* ligule (**b**), and *P. humilis* ligule (**c**). pac – papillate cells; prh – prickle hairs; me – median edge. (a) 20×; (b,c) 40×.

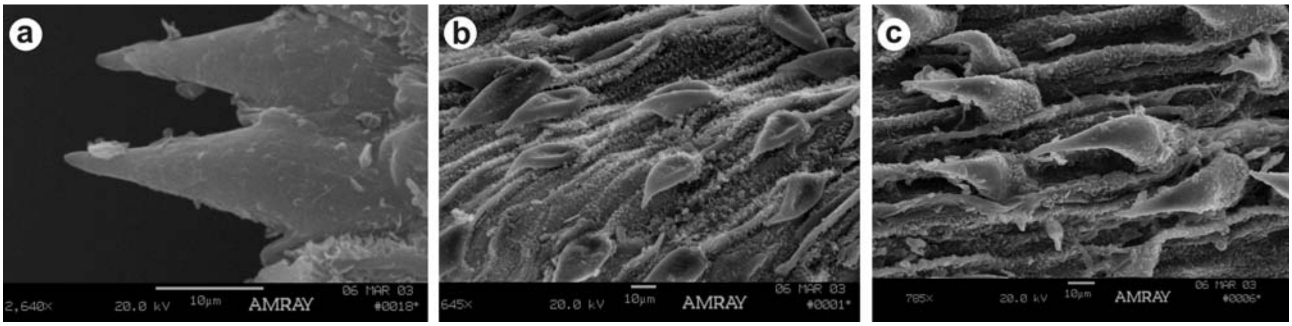


**Fig. 4**. SEM image of unicellular hairs from *P. angustifolia* ligule margin (**a**) and part of abaxial surface of *P. angustifolia* ligule (**b**) and *P. compressa* ligule (**c**).

habitat (Tab. 2). The ligules of *P. compressa* are rarely hirsute. Their prickle hairs, with elongated and curved apices, are longer than in *P. pratensis* (Fig. 4c). Ligules from dry, weedy vegetation are significantly more hirsute (Tab. 2). The ligule of *P. alpina* has no prickle hairs at all (Fig. 3b).

The bare adaxial ligule surfaces fit tightly to the cylindrical shape of the culm in all species. The walls of the cells are extremely thick on this surface (Fig. 2c); the surface looks very similar in all these species, and in the samples taken does not vary between habitats and shoot levels.

## MEDIAN EDGE AND LATERAL EDGES

The median edge and lateral edges of *P. pratensis* ligules are built of the rounded ends of long cells interrupted by a few groups of papillate cells with pointless apices (Fig. 3a). Papillate cell groups consist of 3–4 cells. The edges of *P. angustifolia* can be distinguished by the prickle hairs with thickened walls along almost the whole line (Fig. 4a). On the lateral edges the prickle hairs may alternate with the rounded ends of long cells. The prickle hairs can reach 180 $\mu$m in length but the average is ~45 μm. In *P. compressa* the prickle hairs constitute the median edge of the ligule; the lateral edges consist of long cells with rounded ends. The edges of *P. humilis* ligules consist of prickle hairs arranged as in *P. angustifolia*. *P. alpina* ligule edges are formed by only the rounded ends of long cells (Fig. 3b).

## THE LIGULE-LEAF SHEATH CONNECTION IN *P. PRATENSIS* AND *P. ANGUSTIFOLIA*

In contrast to the observations of Simon (2000) and Soó and Kárpáti (1968), we found that the fringes (lateral edges) of the ligule are decurrent on the margin of the leaf sheath not only in *P. pratensis* but also in *P. angustifolia* (Fig. 5). In *P. angustifolia* the connection between ligule blade and leaf sheath is at right angles to the longitudinal axis of the leaf sheath (Fig. 6), while the ligule of *P. pratensis* describes a curve in its connection to the leaf sheath (Fig. 6). This is seen in cross sections made at the point of connection (Figs. 6, 7). At the cut only the median part of the ligule connects to the leaf sheath tissue. The lateral parts are free and converge at the cut (Fig. 7).

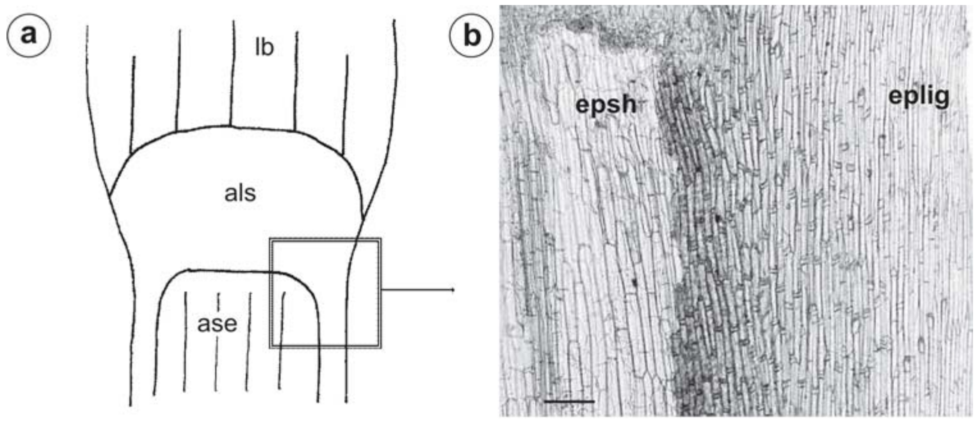


**Fig. 5**. Drawing (**a**) and photograph (**b**) of ligule-leaf sheath connection in *P. angustifolia*. lb – leaf blade; als – adaxial ligule surface; ase – adaxial sheath epidermis; epsh – epidermis of sheath; eplig – epidermis of ligule. Bar = 100 μm.

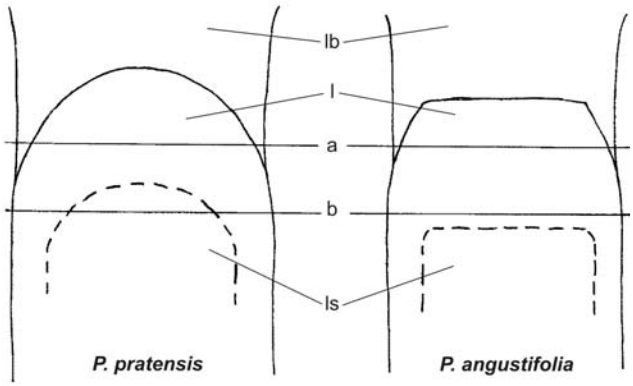


**Fig. 6**. Scheme of the ligule of *P. pratensis* and *P. angustifolia*. Lines show the positions of cross sections (a, b). lb – leaf blade; l – ligule; ls – leaf sheath.

## DISCUSSION

Chaffey (2000) reported that ligules do not differ greatly within the grass genera he studied. The ligules shown in the present paper are broadly similar, but with certain significant differences between species on the abaxial surfaces with respect to hair density and type as a consequence of habitat. The studied species can be distinguished by the structure of the abaxial surfaces of ligules (Tab. 3). The ligules of *P. pratensis* and *P. compressa* are rarely hirsute, while that of *P. angustifolia* is densely covered by hairs. The dense prickle hairs of *P. humilis* are 3–4 times longer than those of the other species studied. The hair cells of the ligule of *P. angustifolia* are very similar in shape to those of *P. nemoralis* studied by Chaffey (1984). The ligule of *P. alpina* has no hairs at all.

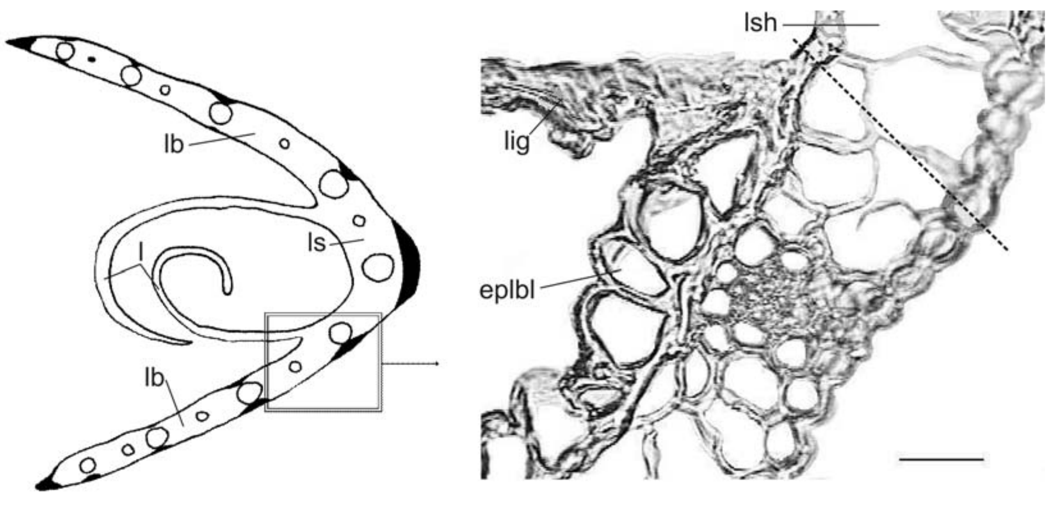


**Fig. 7**. Connecting region of sheath, blade and ligule of *P. pratensis* (20×) cut at position b in Fig. 6. ls – leaf sheath; l – ligule; lb – leaf blade; lig – ligule; lsh – leaf sheath; eplbl – epidermis of leaf blade; dashed line: connection of blade and sheath. Scale bar = 50 μm.

TABLE 3. Summary of abaxial surface differences of ligules of the five studied species

| Hair characters | *P. pratensis* | *P. angustifolia* | *P. humilis* | *P. compressa* | *P. alpina* |
|---|---|---|---|---|---|
| Density | rare | dense | dense | dense | |
| Length | short | short | long | long | without hairs |
| Shape | prickle | leaf-like | hair-like | hair-like; elongated, curved apices | |

The adaxial surfaces are uniform in every respect and do not have taxonomic significance. This surface consists of cells with thick walls, and is devoid of hairs. The concave ligules make a tight connection on this surface to the culm, supporting the suggestion that they secure the free development of the shoots as sliding/clinging surfaces, in addition to their protective functions (Chaffey, 2000).

The adaxial surfaces consist of long and short cells, but they may contain only long cells, as in other *Poa* species studied by Chaffey (1984); the ligules he investigated consisted of more cell layers in cross section, so that an upper and a lower epidermis formed the two surfaces. In all ligules studied the same cell layer forms the two surfaces along almost their whole length; only in *P. pratensis* do mesophyll-like cells come proximally to the surface. Chloroplasts are not found in the mature cells. Whether the mesophyll-like cells of the membranous ligule of *P. pratensis* can photosynthesize when they are young remains an open question.

We showed that the ligules of *P. angustifolia* are decurrent on the leaf sheath margin, as in *P. pratensis*. In *P. angustifolia*, however, the connection of the ligule blade and the adaxial epidermis of the leaf sheath is a straight line forming a right angle to the longitudinal axis of the leaf sheath, while the ligule of *P. pratensis* describes a curve in its connection to the adaxial epidermis of the leaf sheath. This provides a good discriminating character between the two closely related taxa, irrespective of modifications caused by habitat conditions.

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