Variation of micromorphological characters of lemma and palea in the genus *Bromus* (Poaceae)

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Micromorphological features of the lemma and palea were investigated for 77 species in the genus *Bromus* (Poaceae) using scanning electron microscopy. Several micromorphological characters of both long and short cells (cork cells, crown cells, prickles and macrohairs) were observed. All were treated as separate characters, although crown cells, prickles and macrohairs are treated as a single group (exodermic cells) owing to the protrusion of their periclinal wall. The main objectives of this work are to assess the value of the micromorphological typology in systematic studies, and to characterize the six subgenera of *Bromus*, using micromorphological features of the lemmas and paleas.

Key words: *Bromus*, lemma, micromorphology, palea, Poaceae, SEM

Introduction

Micromorphological characters are valuable for systematic studies in the family Poaceae. For example, the absence of microhairs is characteristic in Pooidae (Clayton & Renvoize 1986). Micromorphological features of the leaf epidermis are also of taxonomic value (Prat 1932, 1936, Metcalfe 1960, Ellis 1979, Baum *et al.* 1989, Amarasingue & Watson 1990), being especially useful for the characterization of the larger groups, particularly subfamilies and tribes.

Micromorphological characters of the floral bracts of grasses have been used to assess systematic relationships, as well as evolutionary trends. Furthermore, microcharacters can have taxonomic significance within genera, as in the case of microhairs in *Eragrostis* (Amarasingue...
& Watson 1990), or of the structure of the epicuticular waxes in *Avena* (Baum & Hadland 1975) and in * Hordeum* (Baum et al. 1989, Baum & Bailey 1990).

Nevertheless, the micromorphology of lemmas and paleas has not been widely studied in the Pooideae, except for some particular genera (see Thomasson 1986, Ortúñez Rubio & Fuente García 1995, Fuente García & Ortúñez Rubio 1996, among others). Accordingly, our objective was to carry out a micromorphological characterization of lemmas and paleas of *Bromus*, analyzing the value of their microcharacters for an infrageneric classification.

* Bromus * is the largest genus in the Bromeae. We estimate some 160 species worldwide for * Bromus*. They are either mesophytic or xerophytic, live in many different habitats (Watson & Dallwitz 1988), and are distributed nearly worldwide mainly in temperate zones of the Northern Hemisphere and on tropical mountains (Mabberly 1993). Bromae is included in the Pooideae, one of the six subfamilies of the Poaceae (Clayton & Renvoize 1986). Traditionally, the closest related species in *Bromus* have been grouped only on the basis of their morphological similarities, although recently, Acedo and Llamas (1999) have provided leaf anatomical data, allowing differentiation of some groups of species. The six subgenera of *Bromus* are: *Bromus, Stenobromus* Hackel, *Festucoides* Hackel, *Ceratochloa* ( Beauv.) Hackel, *Neobromus* Shear and *Nevskiella* Krecz. & Vvevd.

### Material and methods

The lemmas and paleas of 77 species (Table 1), representing the six subgenera of *Bromus*, were examined using herbarium specimens primarily from LEB. Initially, the 22 Iberian species were studied (Acedo & Llamas 1999), which belong to subgenera *Bromus, Stenobromus, Festucoides* and *Ceratochloa*. In most of these species, several specimens per population and several populations from different locations were

| Table 1. Studied species/total number of species in each subgenus of *Bromus*. |
|---------------------------------|-----------------------------------------------------------------------------------------------|
| Bromus (29/40):                 | B. adoensis Hochst. ex Steud., B. alopecuros Poir., B. arenarius Labill., B. arvensis L.,  |
|                                | B. brachystachys Horn., B. briziformis Fisch. & Mey., B. bromoideus (Lej.) Crép., B.      |
|                                | cabrerensis Acedo & Llamas, B. caroli-henrici Greuter, B. chrysopogon Viv., B. danta-  |
|                                | thoniae Trin., B. hordeaceus L., B. intermedius Guss., B. lanceolatus Roth, B. lepidus    |
|                                | Holmberg, B. lusitanicus Sales & P.M. Sm., B. nervosus Acedo & Llamas, B. optimae        |
|                                | Scholz, B. oxyodon Schrenk, B. pectinatus Thunb., B. psammofilus P.M. Sm., B. pseu-  |
|                                | dothominni P.M. Sm., B. racemosus L., B. scoparius L., B. secalinus L., B.              |
|                                | severtzovi Regel, B. squarrosus L., B. tytthanthus Newsky,                                 |
| Stenobromus (6/6):             | B. diandrus Roth, B. fasciculatus Presl., B. matritensis L., B. rubens L., B. sterilis L.,  |
|                                | B. tectorum L.                                                                             |
|                                | Scholz, B. cappadocicus Boiss. & Bal., B. ciliatus L., B. cognatus Steud., B. condens- |
|                                | atus Hack., B. erectus Hudson, B. exaltatus Berh., B. firmior Stapt., B. frigidus  |
|                                | Boiss. & Hausskn. ex Boiss., B. inermis Leys., B. insignis Buse, B. kalni Gray, B.       |
|                                | kinabaluensis (Jansen) Veldk., B. kopetdagensis Drobov, B. macroglumis Wagnon, B.       |
|                                | natalensis Stapt., B. nottowayanus Fernald., B. pannonicus Kummer & Send., B.           |
|                                | paulsenii Hack. ex Paulsen., B. pseudolaevipes Wagnon, B. pseudowelkens Muhi. ex Wild., |
|                                | B. pumpelianus Scribn., B. ramosus Hudson, B. riparius Rhein., B. runssorensis        |
|                                | Schum., B. speciosus Ness., B. timorensis Veldk., B. tomentellus Boiss., B. variegatus  |
|                                | M. Bieb.                                                                                 |
| Ceratochloa (8/25):            | B. arizonicus (Shear) Stebbins, B. bonariensis Parodi & Camara, B. breviaristatus       |
|                                | Bukl., B. carinatus Hock. & Arn., B. catharticus Valh., B. lanatus Kunth, B. marginatus  |
|                                | Ness., B. purgans L.                                                                      |
| Neobromus (1/1):               | B. berterianus Dolla.                                                                     |
| Nevskiella (1/1):              | B. gracillimus Bunge.                                                                     |
analyzed in order to estimate intraspecific variation. An additional 55 species belonging to the previously mentioned subgenera, as well as those included in the subgenera Neobromus and Nevskiiella were also studied. As many specimens were studied as possible, within the limitations of the available material. The specimens were borrowed from the following herbaria: BCF, BM, BR, BRESA, C, COI, E, FCO, FI, G, GDA, GDAC, GH, GOET, JACA, K, L, LD, LINN, LISI, LISU, LY, MA, MAF, MICH, MO, MPU, MUB, NAP, NTM, OXF, P, PH, PRE, SALA, SALAF, SANT, SEV, UNEX, UPS, US, VAB, VALA, W, and Z. All of the identifications were confirmed using all relevant literature available.

Data were taken from the middle part of the side of the lemma, and from the middle part of the palea (Fig. 1). Observations were carried out first with a stereo microscope, a light microscope (LM), and finally a scanning electron microscope (SEM). Stains such as Sudan III were used to aid identification of cork cells using LM. Some samples were sonicated in xylene for at least thirty minutes to remove epicuticular waxes that can obscure surface features. For SEM observations, lemmas and paleas of the lowermost floret of mature spikelets were glued onto stubs and sputter-coated with 40 Å of gold-palladium under high vacuum. The samples were examined at 5–20 kV in a JEOL J.S.M. 6100 SEM.

We followed Ellis (1979) for the description of lemma micromorphology, since lemmas are homologous to leaves (Snow 1996), and we extended the terminology to paleas because they show similar epidermal characteristics. The only exception to Ellis’ terminology is that referring to the crown cells (see below for definition and references).

Results

Description of micromorphological characters

Long cells

Long cells are the dominant element of the epidermis of lemmas and paleas, which, despite their name, vary notably in length (Table 2 and Table 3). These are rectangular, long and narrow cells having convex periclinal walls. In contrast, the anticlinal walls are parallel and highly sinuous, with waves in shape of Ω. The ends are often angular, and their length ranges from 20 to 245 μm.

Short cells

Short cells are in general nearly equal-sided, although cells with a width exceeding the length are also relatively frequent (Tables 2 and 3). Short cells alternate with the long cells or occur solitary or in pairs. The frequency of short cells is resulting to some degree from the length of the long cells, and shows variations among several taxa. The anticlinal cell walls are straight or sinuous. Two types of short cells have been observed: cork cells and exodermic cells.

Cork cells

These are short cells containing solid deposits of organic substances and suberified cell walls
(Kaufmann et al. 1970). Their periclinal walls are usually slightly convex and collapse upon dehydration. The most frequent shape is polygonal or ± square. In some cases they adopt different morphologies (semicircular, reniform) that are consistent in the several groups.

**Exodermic cells**

These cells protrude beyond the general level of the epidermis and have periclinal walls with differing degrees of convexity. Depending upon the size and shape of the process, three subtypes can be differentiated into:

— **Crown cells**: Short exodermic cells with small convex or conical protrusions, which occur frequently on the lemma and palea. This term was used by Prat (1932) and has been revived by Watson and Dallwitz (1988) in order to characterize the leaf epidermis of some genera of grasses. Many authors have neglected them, including all exodermic cells under the name of macrohairs. Ellis (1979) referred to these cells as hooks, using the term as a synonym of crochets and crown cells, although he only represented figures with hooked protrusions, thereby excluding those with rounded protrusions. The protrusions of the periclinal wall of the crown cells vary in shape and size. In general they have crenulate walls, with pronounced waves similar to those of the long cells.

— **Prickles**: Exodermic cells with elongated tips and swollen bases arising directly from the epidermis. They occur frequently especially on the veins, although in some groups they occur between long cells with a comparable frequency. Valdes-Reyna and Hatch (1991) found a high silica concentration in this structure.

— **Macrohairs**: Unicellular structures, soft or rigid, often with a bulbous base. They occur between other epidermal cells, and, in some cases, they have an associated small cork cell.

### Table 2. Micromorphological characters of lemma. Length in µm; cell density = number per mm²

<table>
<thead>
<tr>
<th>Lemma</th>
<th>Bromus</th>
<th>Stenobromus</th>
<th>Festucoides</th>
<th>Ceratochloa</th>
<th>Neobromus</th>
<th>Nevskiella</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long cells</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>length</td>
<td>50–150</td>
<td>35–100</td>
<td>30–90</td>
<td>20–150</td>
<td>50–100</td>
<td>30–130</td>
</tr>
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<tr>
<td>periclinal wall</td>
<td>convex</td>
<td>convex</td>
<td>convex</td>
<td>convex</td>
<td>convex</td>
<td>convex</td>
</tr>
<tr>
<td><strong>Short cells</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length</td>
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<td>5–27</td>
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<td>10–16</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>reniform</td>
<td>square</td>
<td>reniform</td>
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<td>10–13</td>
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<td>7–9</td>
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<td><strong>Exodermic cells</strong></td>
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<td>Crown cells</td>
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<td>cell shape</td>
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<td>square</td>
<td>square</td>
<td>square</td>
<td>square</td>
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<td>0–280</td>
<td>40–60(200)</td>
<td>0–40</td>
<td>200–240</td>
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<td>0–300</td>
<td>0–200</td>
<td>0–240</td>
<td>0–20</td>
</tr>
<tr>
<td>length macrohair</td>
<td>40–600</td>
<td>50–400</td>
<td>100–1000</td>
<td>30–300</td>
<td>70–350</td>
<td>60–70</td>
</tr>
</tbody>
</table>
Epicuticular waxes

Epicuticular waxes are deposits, formed mainly by a large mixture of different chemical compounds.

Micromorphological description of lemmas and paleas in Bromus

Bromus subgen. Bromus

Lemma. Long cells 50–150 µm. Cork cells square, 6–7 µm long, density 40–320 cork cells mm\(^{-2}\). Crown cells square, 15–20 µm long, with a spherical protrusion, density 240–920 crown cells mm\(^{-2}\). Prickles absent or scarce, up to 200 prickles mm\(^{-2}\), distributed mainly on the veins. Macrohairs highly variable in density, up to 400 macrohairs mm\(^{-2}\), 40–600 µm long. Waxes absent, except for outer zones of the lemma. Fig. 2A.

Palea. Long cells 35–245 µm. Cork cells square or polygonal, 15–21 µm long, density up to 360 cork cells mm\(^{-2}\). Crown cells square to ovate, 10–35 µm long, with a spherical protrusion, density up to 640 crown cells mm\(^{-2}\). Prickles absent. Macrohairs soft, with fine walls and bulbous base, highly variable in density, up to 120 macrohairs mm\(^{-2}\), 50–160 µm long. Waxes absent. Fig. 3A.

Bromus subgen. Stenobromus

Lemma. Long cells 35–100 µm. Cork cells semicircular, 5–9 µm long, density 40–500 cork cells mm\(^{-2}\). Crown cells square, 15–27 µm long, with a spherical protrusion, and density 280–1960 crown cells mm\(^{-2}\). Prickles 140–220 per mm\(^2\). Macrohairs absent or scarce, up to 80 macrohairs mm\(^{-2}\), 50–400 µm long. Waxes absent. Fig. 2B.

Palea. Long cells 30–75 µm. Cork cells semicircular, 6–8 µm long, density 40–560 cork cells mm\(^{-2}\). Crown cells rectangular, 13–18 µm

Table 3. Variation of micromorphological characters in palea. Length in µm; cell density = number per mm\(^2\).

<table>
<thead>
<tr>
<th>Palaea</th>
<th>Bromus</th>
<th>Stenobromus</th>
<th>Festucoides</th>
<th>Ceratochloa</th>
<th>Neobromus</th>
<th>Nevskiella</th>
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<tr>
<td>periclinal wall</td>
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<td>convex</td>
<td>convex</td>
<td>convex</td>
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<tr>
<td>shape</td>
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<td>semicircular</td>
<td>rounded-elliptic</td>
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<td>oblong</td>
<td>not observed</td>
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<td>EXODERMIC CELLS</td>
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<td></td>
<td></td>
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<tr>
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<td>square-rectangular</td>
<td>square</td>
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<td>protrusion shape</td>
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</tr>
<tr>
<td>density</td>
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<td>20–900</td>
<td>140–860</td>
<td>400–500</td>
<td>–</td>
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<tr>
<td>length</td>
<td>10–35</td>
<td>13–18</td>
<td>12–30</td>
<td>14–25</td>
<td>18–22</td>
<td>–</td>
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<tr>
<td>Prickles</td>
<td>not observed</td>
<td>not observed</td>
<td>not observed</td>
<td>not observed</td>
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<td>not observed</td>
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<td>0–400</td>
<td>60–100</td>
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</tr>
<tr>
<td>length macrohair</td>
<td>50–160</td>
<td>20–130</td>
<td>50–75(175)</td>
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</tr>
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</table>
long, with a pointed protrusion, density 210–1600 crown cells mm$^{-2}$. Prickles and macrohairs absent. Waxes absent. Fig. 3B.

**Bromus subgen. Festucoides**

*Lemma*. Long cells 30–90 µm. Cork cells reniform, 7–13 µm long, density 40–460 cork cells mm$^{-2}$. Crown cells square, 15–30 µm long, with a spherical protrusion, density 40–1120 crown cells mm$^{-2}$, seldom as few as five cells mm$^{-2}$. Prickles with an ovate base, density up to 280 prickles mm$^{-2}$. Macrohairs very variable in density, up to 300 macrohairs mm$^{-2}$, 100–1000 µm long. Waxes dense, covering almost all the surface. Fig. 2C.

*Palea*. Long cells 30–200 µm. Cork cells rounded-elliptic, 10–18 µm long, density 20–600 cork cells mm$^{-2}$. Crown cells square-rectangular, 12–30 µm long, with a pointed protrusion, density 20–900 crown cells mm$^{-2}$. Prickles absent. Macrohairs rigid, highly variable in density up to 400 macrohairs mm$^{-2}$, 20–130 µm long. Waxes highly variable from absent to very abundant. Fig. 3C.

**Bromus subgen. Ceratochloa**

*Lemma*. Long cells 20–150 µm. Cork cells square, 10–13 µm long, density 100–320 cork cells mm$^{-2}$. Crown cells square, 12–16 µm long, with a pointed protrusion, density 140–1300 crown cells mm$^{-2}$. Prickles 40–60 per mm$^2$, seldom up to 200. Macrohairs soft or rigid, highly variable in density up to 200 macrohairs mm$^{-2}$, 30–300 µm long. Waxes generally abundant. Fig. 2D.

*Palea*. Long cells 30–125 µm. Cork cells rounded-elliptic, 10–15 µm long, density 120–480 cork cells mm$^{-2}$. Crown cells square, 14–25 µm long, with a pointed protrusion, density 140–860 crown cells mm$^{-2}$. Prickles absent. Macrohairs soft, density 60–100 macrohairs mm$^{-2}$, 50–75 µm long. Waxes absent. Fig. 3D.
**Bromus subgen. Neobromus**

*Lemma*. Long cells 50–100 µm. Cork cells reniform, 10–13 µm long, density 400–500 cork cells mm\(^{-2}\). Crown cells square, 17–22 µm long, with a pointed protrusion, density 1200–1500 crown cells mm\(^{-2}\). Prickles up to 40 per mm\(^2\). Macrohairs highly variable in density, up to 240 macrohairs mm\(^{-2}\), 70–350 µm long. Waxes dense. Fig. 2E.

*Palea*. Long cells 65–160 µm. Cork cells oblong, 7–13 µm long, density 20–60 cork cells mm\(^{-2}\). Crown cells rectangular, 18–22 µm long, with a pointed protrusion, density 400–500 crown cells mm\(^{-2}\). Prickles and macrohairs absent. Waxes absent. Fig. 3E.

**Bromus subgen. Nevskiella**

*Lemma*. Long cells 30–130 µm. Cork cells reniform, 7–9 µm long, density 340 cork cells mm\(^{-2}\). Crown cells square, 8–12 µm long, with a pointed protrusion, density 900–1200 crown cells mm\(^{-2}\). Prickles absent or scarce, 200–240 prickles mm\(^{-2}\). Macrohairs absent or scarce, up to 20 macrohairs mm\(^{-2}\), 60–70 µm long. Waxes absent or very scarce. Fig. 2F.

*Palea*. Long cells 130–180 µm. Cork cells absent. Crown cells generally absent in most of the palea, although sometimes a few crown cells can be found near the base or beside the keel. Prickles and macrohairs absent. Waxes absent. Fig. 3F.

**Discussion and conclusions**

One of the main problems with micromorphological studies is to determine the optimal site of observation. We have noticed variation in lemma and palea depending on the zone on observation. The lemma shows variations in the expression of micromorphological characters among the apex, base and margins, whereas the palea varies mostly near the apex and in some
cases in the wings. Because of this, we have selected the middle part of the lemma side, and the central part of the palea body (Fig. 1). These regions appear to be quite constant in their micromorphological characteristics among the taxa studied.

The analysis of the epidermal micromorphology of lemma and palea of Bromus reveals considerable variation in some subgenera, particularly regarding the presence of waxes and density of short cells. Several differences also exist as regards the presence or absence of macrohairs, but in some species this character can show a high variation even among specimens from the same population.

On the basis of the epidermal micromorphology of lemma and palea, it can be inferred that all species of the genus Bromus have the same epidermal cell types, as it can be suspected in species of the same genus. It means that it is possible to identify a fragment of a floral bract as Bromus, or one of its subgenera, using the micromorphological characteristics presented here. Nevertheless, the similarities among species of the same subgenus, or lower taxonomic groups, are higher than those among species of different subgenera. The most different one is the subgenus Nevskiella. If these differences were as high as those on the other genera of Bromee (Boissiera and Littledalea) this could support its segregation from Bromus as proposed by Kreczetowicz and Vvedensky (1934).

There are two characters that, in general, have a close positive correlation. These are the length of the long cells (in lemma and palea) and the number of short cells. For example, subgenus Stenobromus has the shortest short cells (5 µm) and the highest density of short cells (ca. 2000, up to 560 cork cells mm⁻² and up to 1600 crown cells mm⁻²). However, as the long cells increase in length, the density of short cells decreases. An extreme case is found in the palea of the subgenus Nevskiella, where long cells are relatively long, and short cells are lacking entirely. The lowest values for lemmatal long cells are found in subgenus Ceratochloa, where longer long cells also occur, thereby making long cells size not useful for characterizing the epidermal type.

More differences occur on short cells than long cells. Thus, the crown cells show considerable variation in density and in their type of protrusions, whereas cork cells show variation in their size and density (Table 2).

The exodermic cells, like the cork cells, are distributed in longitudinal arrays, alternating with the long cells and other epidermal cells.

The density of cork cells, in relation to the other epidermal elements is also variable, ranging from 0–600 cells mm⁻². In a given subgenus the number of cork cells is higher in the palea than in lemma, except in the subgenus Nevskiella, where this type of cell has not been observed on the palea. The highest values for the length of cork cells are found on the palea of subgenera Festucoides and Stenobromus. The size of the cork cells varies from 5–13 µm in the lemma and 6–21 µm in the palea. The highest values are found in the palea of subgenus Bromus, where cork cells are between 15–21 µm. (Table 3).

In Bromus all the exodermic cells are short cells. Snow (1996) reported the first evidence of macrohairs originated in short cells in Eleusininae. We have observed the same thing in Bromus where all the exodermic cells are short cells.

Crown cells vary from square to rectangular, although sometimes they have an irregular shape, as in the palea of subgenus Stenobromus. The protrusion or protuberance can be spherical or pointed and vary in its relative size to the cell supporting it. It can be either similar or slightly shorter than the cell, or of 1/3 or less of the diameter of the cell. In the first case the protrusion is formed by most of the external periclinal wall of the cell, or at least more than 1/3. In the second case the protrusion is formed only by the central portion of the outer periclinal cell wall (palea of Bromus mucroglumis, var. Festucoides).

The density of the crown cells varies notably among and within different subgenera. In the palea they vary from complete absence (subgenus Nevskiella) to 1600 cells mm⁻² (subgenus Stenobromus), where these differences are more significant. In the lemma their density always exceeds that of the palea, in species as well as in subgenera. It ranges from 40 cells mm⁻² in subgenus Festucoides to 1960 cells mm⁻² in Stenobromus.

The size of the crown cells ranges from 8 to
30 µm. Subgenus *Nevskiella* has the smallest (8–12 µm), whilst in the remaining subgenera they are 12–30 µm.

Intercostal prickles appear in variable densities, but differences are not significant, since in most cases there is high intraspecific variation. Sometimes prickles are longer than usual, and then, it is difficult to differentiate between prickles and macrohairs. This intergradation has been formerly reported by Metcalfe (1960), Ellis (1979) and Snow (1996).

Snow (1998) pointed out that these characters showing intergradation cannot be considered homologous and hence lack phylogenetic value although they may still be of local diagnostic value. The *Bromus ramosus* group (rank not defined yet, perhaps section) has macrohairs only on a marginal strip of the lemma. In this case the character is diagnostic for this group against the remaining species of the subgenus.

Since the density of macrohairs on the lemma and palea is highly variable, their presence or absence has little taxonomic significance, which confirms our experience in the genus. One exception is the disposition of the macrohairs in subgenus *Festucoides*, where many species have macrohairs restricted to the margin of the lemma. Even if they appear over much of the surface, their density in subgenus *Festucoides* increases notably in the marginal zone.

It is known that the trichomes are common in plants growing in xeric habitats (Esau 1977). We agree with Dávila and Clark (1990) that papillae, prickles and waxes could play a similar role, and, as pointed out by Stace (1965), may reduce the transpiration rate of the plant organ having them. However, we did not find a positive correlation between the density of these structures and the habitat of origin of the specimens.

The interest of the epicuticular waxes resides, according to some authors, in the fact that the configuration of the deposits of wax seems to have a genetic basis (Baum & Hadland 1975). The study of the waxes in several species could therefore be justified for taxonomic purposes. Taking this premise as valid, the epicuticular waxes have been studied in numerous taxa and for several purposes. We tried to analyze only the intrageneric variation, with the aim of knowing if this character has differential value in the systematic of the genus *Bromus*.

The epicuticular waxes are common on the lemma, where they often appear in the zone not covered by the glume or the lemma of the lower floret. Waxes were not observed on the lemmas of the two annual subgenera *Stenobromus* and *Nevskiella*. In the palea, waxes are only frequent in some species of subgenus *Festucoides*.

Considering the studied samples, we can infer that variation in epicuticular waxes in *Bromus* (when present) is not significant. In the lemma, the absence or presence of waxes depends on the zone of observation, i.e. if it is covered or not by another bract (perhaps, there is some relationship between the presence of epicuticular waxes and the environmental conditions). The morphology of the crystals is similar in all the studied species (and therefore homogeneous in the six subgenera) being rosettes of platelets in all cases (nomenclature following Barthlott et al. 1998). In the palea we have only observed epicuticular waxes in subgenus *Festucoides*, although they are also lacking in some species (*B. erectus, B. paulsenii, B. pseudolaevipes, B. ramosus, B. speciosus, B. timorenensis*).

Thus we can remark the following conclusions: For the micromorphological studies it is necessary to make a careful selection of the site for its observation. The palea reveals itself as an important place of observation in this type of survey. The genus *Bromus*, or any of the six subgenera, can be identified using the micromorphological characteristics of palea and/or lemma.

It should be interesting to study the closely related genera in the Bromaea and to compare them with the differences among the subgenera. In such a way the segregation of *Nevskiella* could be justified as an independent genus, because it is the most different one in *Bromus*. This would justify the proposal made by Kreczetoich and Vvedensky (1934).

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References


Appendix: Specimens examined

_Bromus adoensis._ **France.** Sementes vindas do H13 Bordeaux; Cult. na EAN, sob o n° 15749, 1947 _B. Rainha_ (MA 284889). **Germany.** In Karlsruhe i. Baden kultiviert, 1909–1912, 117 m, _A. Kneucker_ (MA 246377).

_Bromus albidus._ **Russian Federation.** An steinigen Abhangen in den Landschaften Digoria und Ossetia im Kaukasus, 1500 m, 1899 _B. Markowicz_ (MA 246378).

_Bromus alopecuros._ **Greece.** Rhodos, 1 km NE Salakos; Cultivated area, 1982 _A. Carlström_ 5633 (LD). Samos, N of the town of Samos, roadside and former terraced fields, 50–100 m, 1992 _S. & B. Snogerup_ 8832 (LD).


_Bromus angustifolius._ No data (C).

_Bromus arenarius._ **Australia.** Albury, 1916 _T.H. Patterson_ NSW 10596 (K). New South Wales, hill E of the road from Cobar to Bourke, c. 6 km bifurcation Byrock., low-growing wood of _Eucalyptus_, _Acacia_, and grassy clearings, 200–250 m, 1988 _W. Greater_, _Plants from NSW_, AT, 20774 (K).

_Bromus arizonicus._ **United States.** Arizona, St. Johns Mission, next to the house Maricopa County, 1200 ft, 1961 _R. J. Kramer_ 40 (K).

_Bromus armenus._ **Turkey.** Prov. Tunceli, Munzur dag above Ovacik, rocky limestone slopes, 2800 m, 1957 _Davis & Hedge_ 31264 (K).


_Bromus biebersteinii._ **Afghanistan.** Kabul, in declivibus australibus jugi Salang, substr.gran., 3200 m, 1967, _H. K. Rechinger_ 37092 (MA 416864).

_Bromus bonariensis._ **Argentina.** Prov. de Buenos Aires-Pdo. de Tornquist-Sa. de la Ventana, Abra de la Ventana, -78, Proyecto Ventania, _M. A. Torras_ (K).


_Bromus cabrerensis._ **Spain.** Campus de Vegazana (León), Zona nitrificada, 30TTN81, 1994 _C. Acedo_ (LEB 51652). Truchas (La Cabrera, León), borde de reguero con _Holcus lanatus_, _Poa_ vari. _,..._ 29TQG07, 1992 _F. Llamas_ & _C. Acedo_ (LEB 52595).


_Bromus chrysopogon._ **Cyprus.** Entre Xylophagou et Aya Thékla (Larnaca), Cham (blé) abandonné et pseudosteppe
à Sarcopote, 0–5 m, 1991 *Iter Mediterraneum IV* (Chipre) 104 (MA 496422). Larnaca, lac salé, 1991 *Iter Mediterraneum IV* (Chipre) 197 (MA 495585).


*Bromus condensatus*. **Austria.** Tirolia australis, Bozen, ad saxa porphyrita monti Kalvarienberg, -98 Sauter (C).

*Bromus danthoniae*. **Iran.** Gotvend (Rio Karvum), 400 m, C. Pau & C. Vicioso (MA 14042). No data (Willkom).


*Bromus insignis*. **Malaysia.** Java, Marakkan, Junghuhn s.n. (L det. J. F. Veldkamp).

*Bromus intermedium*. **Spain.** Santa Cruz de Mudela (Ciudad Real), borde de camino en la Encomienda, 30SVH57, 1951 J. Borja (MA 199370). Sierra de Cázulas (Granada), 30SVF37, 1980 A. T. Romero (GDAC 24303). **Germany.** In Karlsruhe i. Baden, kultiviert, 117 m, 1903 A. Kneucker (MA 246436).

*Bromus kaiini.** **United States.** Indiana, nr. Clark Road in NW Gary Lake County, in sandy swale between low dune beach ridges, 1955 H. R. Benett (MA 167409-D).

*Bromus kinabalensis*. **Malaysia.** Borneo, Mount Kinabalu, 6000–13 500 ft, 1931–32 J. & M. S. Clemens 29174 (L).

*Bromus kopetdagensis*. **Turkmenistan.** Turkmeniya, centralnii Kopet-dag, 1953 V. N. Nikitin (MA 364670).

*Bromus lanatus*. **Colombia.** Andes Cordillera Central, Dep. Tolima, Nevado del Tolima, arenales, 1932 J. Cuatreccasas (MA 175644).


*Bromus frigidus*. **Iran.** Zardeh Kuh Mts., Bakhtiar, dry limestone rock, 12 000 ft, 1965 R. Timmis 119 (K).


*Bromus insignis*. **Malaysia.** Java, Marakkan, Junghuhn s.n. (L det. J. F. Veldkamp).

*Bromus intermedium*. **Spain.** Santa Cruz de Mudela (Ciudad Real), borde de camino en la Encomienda, 30SVH57, 1951 J. Borja (MA 199370). Sierra de Cázulas (Granada), 30SVF37, 1980 A. T. Romero (GDAC 24303). **Germany.** In Karlsruhe i. Baden, kultiviert, 117 m, 1903 A. Kneucker (MA 246436).

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*Bromus lanatus*. **Colombia.** Andes Cordillera Central, Dep. Tolima, Nevado del Tolima, arenales, 1932 J. Cuatreccasas (MA 175644).

*Bromus lanceolatus*. **Italy.** Naples, Genijola Porrentina,


**Bromus lasianicus.** **Portugal.** Souzelas, near Coimbra (Beira Litoral), growing in dramp soil between a road and a stream, 70 m, 29TNE45, 1983 F. Sales (E 146/93 2).

**Bromus marginatus.** **United States.** Moist meadow in open valley above Crazy Head Spring 8 miles E of Lame Deer, Rosebud County, Montana, 4300 ft, 1955 H. R. Benett (MA 167407).

**Bromus matrientis.** **Spain.** Puzol (Valencia), en terreno labrado, 30TYK37, 1982 J. L. Carretero (VALA 4109). Sorribera (León), 30TUN23, 1988 F. Llamas & C. Acedo (LEB 52604).

**Bromus macroglumis.** **United States.** Grown at the Botanical Gardens, University of Michigan, 1948 H. K. Wagon 1520 (MICH).


**Bromus nervosus.** **Portugal.** Reguengos de Monsaraz (Estremadura), 29SPC25, 1949 J. Tapum (LISI s.n.).

**Bromus nottowayanus.** **United States.** Virginia, Sussex County, Nottoway River, east of Huske, bottomland woods, 1940 M. L. Fernald & Bayard 12239 (GH 23249), 12239 (GH 23248).

**Bromus optimae.** **Cyprus.** Chipre, Cape Greco (Larnaca), calcaires coralliens du Miocène in serieux et saib, 10–20 m, 1991 (MA 495913, Iter Mediterranean IV, Chipre 157). Larnaca, lac salé, ± 5 m, 1991 H. Scholz (MA 495684, Iter Mediterranean IV).

**Bromus oxyodon.** **Tadzhikistan.** Montes meridionales, Pamir-alaj exterior, ad declibia herbosa montium Turkestancorum, 1927 Drobov (MA 14038).

**Bromus pannonicus.** **Belgium.** Montagne d’Auronse, bas de la combe d’Auronse, a l’Est du Roc des Hirondelles, 1650 m, 1991 M. Kerguen (MA 526523).

**Bromus paulsenii.** **Tadzhikistan.** Monte Pamir, prope lacus Jashilkul, 3900 m, 1998 O. Paulsen 1108 (C).

**Bromus pectinatus.** **India.** Jambhari, 4000 ft, Surq. Lil-Harrin, 1895 T.A. Cope 16825 (K). Lahul, Manali, 1941 N. L. Bor (K).

**Bromus psmammoitus.** **Turkey.** Içel, Tarsus, Dunes, 1973 T. Zul (E).

**Bromus pseudolaevipes.** **United States.** Grown at the Botanical Gardens, University of Michigan, 2850 ft, 1948 H. K. Wagon 1507 (MICH).

**Bromus pseudohominii.** **Spain.** Muñorodero (Santander), Borde de carretera, 5 m, 30TUP80, 1992 F. Llamas, R. Muñoz Valencia & C. Acedo (LEB 52562). **United Kingdom.** Haddington, Aberlady, sandy grass, VC82, 1984 O. M. Stewart 037684 & P. M. Smith (E).

**Bromus pubescens.** **United States.** Muhlenberg Herbarium, “M. 154”(PH).


**Bromus purgans.** **United States.** Felsiger Waldran, Cabin John, Maryland, 1907 A. Chase (MA 247117).


**Bromus ramosus.** **Spain.** Hervás (Cáceres), 30TTK56, 1975 Bote, Ladero & Pérez Chisano (MAF 93281). Posada de Valdeón (León), borde de cursos de agua, 30TUN47, 1968 J. Borja (MAF 76329). **United Kingdom.** Dorsetshire, Corfe Castle, 1944 A. Dunston (K).


**Bromus rubens.** **Spain.** Castropuepe (Zamora), Comunidades nitrofilas de plantas anuales, 30TTM75, 1988 J. M. Fernández (LEB 50103). León, Campus Universitario de Vegazana (León), comunidades nitrofilas de plantas anuales, 1994 C. Acedo (LEB 52457). Sierra de Mahimon (Almeria), borde de camino, 1250 m, 30SWG77, 1986 F. Gómiz (LEB 31787).

Bromus sclerophyllus. (G-BOISS).


Bromus tectorum. **France.** Nantes, 1879 without collector (MAF 26764). **Spain.** Cazorla, c. Ermita de la Virgen de la Cabeza (Jaen), en rellanos terrosos, 30SWG0096, 1983 A. M. Hernández (GDA 15411). León, Campus Universitario de Vegazana (León), comunidades nitrófilas de plantas anuales, 30TNN81, 1994 C. Acedo (LEB 52455).

Bromus timorensis. **Indonesia.** Timor, Mt. Tatamailau, in eucalypt forest, 2800 m, 1954 Van Steenis 18467 (L).


Bromus turkestanicus. **Uzbekistan.** Montes Meridionales, Tadshikorum, ad declivia saxosa (inter rupes) Tschulbair, ca. 3600, 1930, Botschantzev & Vvedensky (MA 13358).

Bromus tytthanthus. **Uzbekistan.** Montes Meridionales, Tian-schan occidentalis, inter rupes graniticas in montibus Mongol-tau prope, 1924 Popov & Vvedensky (MA 14044).

Bromus variegatus. **Lebanon.** Auf den Gipfeln und obersten Abhängen des Dschebel Sannin im, Libenom in Syrien, Kreidekal 2300–2600 m, 1903 E. Hartmann (MA 247178).