

## Scanning electron microscopy of the leaf epidermis of *Merostachys* Spreng. (Poaceae: Bambusoideae)

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**RESUMO** – (Microscopia eletrônica de varredura da epiderme foliar de *Merostachys* Spreng. (Poaceae: Bambusoideae)). São apresentados dados relativos à micromorfologia foliar de 13 espécies de *Merostachys* Spreng. (Poaceae: Bambusoideae), um gênero neotropical, com numerosas espécies endêmicas, que tem sua maior diversidade nas regiões central e sul do Brasil, onde ocorrem 41 das 46 espécies descritas. O material coletado foi obtido nas seguintes localidades: Parque Nacional do Itatiaia e Parque Nacional da Tijuca (RJ); Parque Estadual das Fontes do Ipiranga (SP); Propriedade dos Irmãos Martinelli, Vargem Alta, Santa Teresa (ES) e Parque Estadual do Rio Doce (MG). As espécies herborizadas são provenientes do herbário do Instituto de Botânica de São Paulo (SP). Atenção especial é dada à presença de cerdas, corpúsculos silicosos e macropêlos, características que podem ser úteis na delimitação das espécies.

**Palavras-chave:** bambu, folha, *Merostachys*, microscopia eletrônica de varredura, Poaceae

**ABSTRACT** – (Scanning electron microscopy of the leaf epidermis of *Merostachys* Spreng. (Poaceae: Bambusoideae)). This study presents data on leaf micromorphology of 13 species of *Merostachys* Spreng. (Poaceae: Bambusoideae), a neotropical genus with numerous endemic species. Greatest species richness is found in Central and Southern Brazil, with 41 of the 46 species described. Species were collected in the field at the localities: Itatiaia National Park and Tijuca National Park, Rio de Janeiro State; Fontes do Ipiranga State Park, São Paulo state; Santa Teresa, Espírito Santo State and Rio Doce State Park, Minas Gerais State. Dried plant material came from the herbarium at the São Paulo Botany Institute. The presence of prickles, silica bodies and macro hairs may be especially useful in delimiting species.

**Key words:** bamboo, leaf, *Merostachys*, Poaceae, scanning electron microscopy

### Introduction

The Poaceae family is one of the largest among Angiosperm families, and is likely to be the most economically important for traditional people, since it is found within every phytogeographical region of the world. It comprises about 10,000 species, distributed in approximately 650 genera (Clayton & Renvoize 1986) in nine subfamilies (Judziewicz *et al.* 1999), as the Bambusoideae.

Bamboos usually grow associated with woody vegetation, typically occurring in tropical and subtropical forests (Soderstrom 1981). There are about 89 genera and 1,035 species of bamboos all over the world. In Brazil, 36 genera and 187 species are found, especially along the coast of Bahia state, the area with the greatest species diversity and endemism rates (Clark 1990).

Despite their wide distribution, little is known about bamboo morphology, ecology and evolutionary

relationships, particularly due to the long flowering intervals.

The understanding of taxonomic features in the Neotropical genus *Merostachys* Spreng., which comprises only woody species, has advanced slowly, particularly because of the unknown flowering periods of these plants, which may range from 30 to 33 years (Sendulsky 1992).

Like studies of leaf macro-morphology have been used to support Bambusoideae taxa identification, this micro-characteristics study will also be able to contribute to the taxonomy of this subfamily (Palmer & Tucker 1981; Renvoize 1985).

This study analyzed the leaf surface of *Merostachys fischeriana* Rupr. ex. Doell, *M. burmanii* Send., *M. kunthii* Rupr., *M. latifolia* R. Pohl, *M. neesii* Rupr., *M. skvortzovii* Send., *M. magellanica* Send., *M. scandens* Send., *M. caucaiana* Send., *M. fistulosa* Doell, *M. capixaba* Send., *M. gracilis* Send. and

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*M. ternata* Nees, both to contribute to a better understanding of the Bambusoideae and to support taxonomic studies.

## Material and methods

Plant material used in this study was either collected in the field or obtained in herbaria in Brazil. Species were collected in the field at the localities: *M. fischeriana* (Gomes *et al.* - R), Itatiaia National Park, Rio de Janeiro state; *M. burmannii* (Gomes *et al.* - R), Tijuca National Park, Rio de Janeiro state; *M. kunthii* (Gomes *et al.* - R), Tijuca National Park, Rio de Janeiro state; *M. latifolia* (Gomes *et al.* - R), Vista Chinesa, close to the Tijuca National Park, Rio de Janeiro state; *M. neesii* (Sarabyba, L. S. *et al.* - R, SP), Fontes do Ipiranga State Park, close to the Botany Institute, São Paulo state; *M. skvortzovii*, (Sarabyba, L.S. *et al.* - R, SP), Fontes do Ipiranga State Park, close to the Botany Institute, São Paulo state; *M. capixaba* (Gomes & Demoner 21 - R), Santa Teresa city, Espírito Santo state; *M. ternata* (Gomes *et al.* 19 - R), Rio Doce State Park, Minas Gerais state.

Dried plant material of 5 species came from the herbarium at the São Paulo Botany Institute as follows: *M. magellanica* Send. (SP 248339), *M. scandens* Send. (Sendulsky 1319), *M. caucaiana* Send. (SP 236273), *M. fistulosa* Doell (SP 246019) and *M. gracilis* Send. (G. Hatschbach 48646).

For the SEM (scanning electron microscopy) analysis, leaf pieces were fixed in an 8% paraformaldehyde + 2.5% glutaraldehyde solution in a 50mM phosphate buffer pH 7.2, over 24 hours, and put under vacuum until submerged. Afterwards, material was critical-point dried through a graded series of ethyl alcohols, in a Balzers CPD-020 apparatus (Silveira 1998). Leaf fragments were mounted on stubs coated with gold in a Balzers Union-FL-9496 and observed in a JEOL JSM-5310 scanning electronic microscope. Herbarium material was also mounted on proper stubs coated with gold before being observed in the SEM. Leaf fragments were extracted from the middle intercostal areas of both surfaces, as from the abaxial green marginal stripe.

## Results

The analysis of leaf epidermis in *Merostachys* species revealed that epidermal elements on both surfaces vary in shape and size. Leaf epidermis cells on the adaxial surface (Fig. 1-13) are both long and short, alternately arranged. The surface is smooth, with no ornamentation. Small silica bodies point away from the outer priclinal

wall of long cells, arranged in a row in *M. fischeriana* (Fig. 1), *M. burmannii* (Fig. 2), *M. latifolia* (Fig. 4), *M. skvortzovii* (Fig. 6), *M. magellanica* (Fig. 7), *M. scandens* (Fig. 8), *M. caucaiana* (Fig. 9), *M. fistulosa* (Fig. 10) and *M. gracilis* (Fig. 12); sparsely and minutely in *M. ternata* (Fig. 13); but absent in *M. kunthii* (Fig. 3), *M. neesii* (Fig. 5) and *M. capixaba* (Fig. 11). Long cells have sinuous walls, particularly in *M. fistulosa* (Fig. 10), *M. capixaba* (Fig. 11), *M. gracilis* (Fig. 12) and *M. ternata* (Fig. 13). Short cells could not be visualized easily, and are represented by suberose cells in *M. skvortzovii* (Fig. 6). The longest axis of these elements is arranged perpendicularly to the long cells, and is usually associated with prickles and micro-hairs located at their base (Fig. 29).

Bulliform cells are clearly present in the intercostal zones, especially in *M. latifolia* (Fig. 4), *M. capixaba* (Fig. 11), *M. gracilis* (Fig. 12) and *M. ternata* (Fig. 13), where three rows of cells were observed.

The abaxial surface has a wax layer (Fig. 16, 23), either grain-shaped (rough surfaced) or with no defined ornamentation. Like on the adaxial surface, both for the costal and intercostal zones, the epidermis on the abaxial surface (Fig. 14-27) consists of long cells, with markedly sinuous anticlinal walls, especially in *M. gracilis* (Fig. 25) and *M. ternata* (Fig. 27). There are numerous papillae in the long cells (Fig. 14-27). Papillae are either conical or globose structures, occurring in one row in *M. fischeriana* (Fig. 14), *M. burmannii* (Fig. 15), *M. kunthii* (Fig. 16), *M. latifolia* (Fig. 17), *M. neesii* (Fig. 18), *M. scandens* (Fig. 21) and *M. fistulosa* (Fig. 23), or arranged in two different ways, as in *M. skvortzovii* (Fig. 19), *M. caucaiana* (Fig. 22), *M. capixaba* (Fig. 24), *M. gracilis* (Fig. 25) and *M. ternata* (Fig. 26-27), where epidermal elements may present one or two rows of papillae.

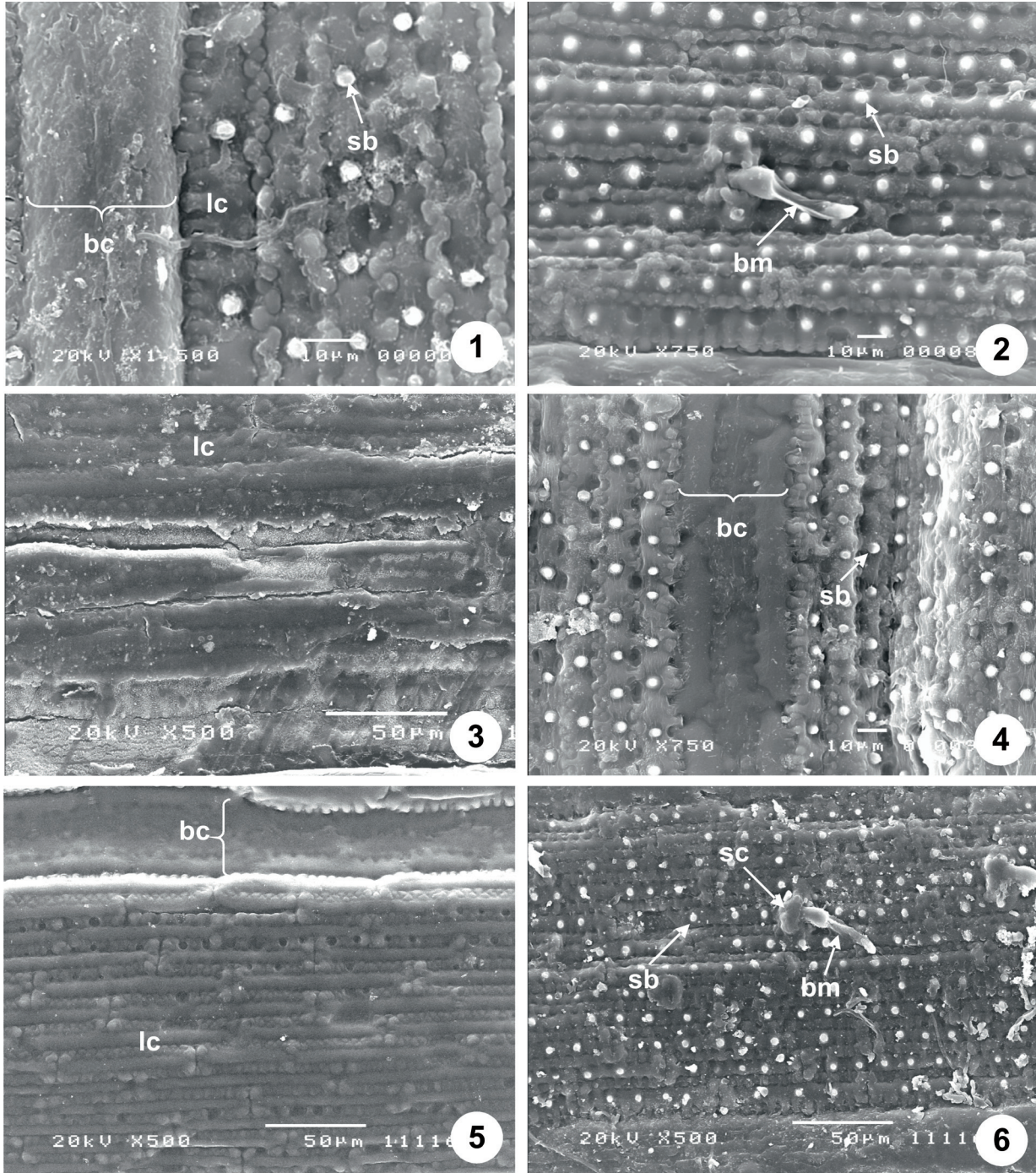
Three types of trichomes are observed in all of the 13 species: prickles, micro and macrohairs.

Prickles are unicellular, broad at base and with a sharply pointed apex, growing upward the leaf apex. Prickles are generally absent on the adaxial surface, and may eventually occur on the leaf boundaries. On the abaxial surface prickles are uniformly distributed, more frequently in the intercostal zones and more numerous on the green marginal stripe (Fig. 28-40). Prickles are rare in *M. fischeriana* (Fig. 14) and *M. ternata* (Fig. 26-27) which have prickles with a long apex, and absent in *M. fistulosa* (Fig. 23) and *M. capixaba* (Fig. 24).

Micro-hairs are formed by two thin-walled cells, especially the apex cell, which may be separated during plant material preparation. Occasionally apex cells appear to have collapsed walls. Micro-hairs are sparsely

arranged on the adaxial surface of all the species except *M. kunthii* and *M. neesii*, which have none. There are significantly more micro-hairs on the abaxial surface, where they are well distributed in all of the species (Fig. 14-27), preferentially in the intercostal zones, and more numerous on the green marginal stripe (Fig. 28-40).

Macro-hairs are unicellular, and they were only observed on the abaxial surface of *M. fischeriana* (Fig. 14) and *M. ternata* (Fig. 26-27). In the first species, they are long and uniformly distributed on the costal zones; in the latter species, macro-hairs are more sparsely arranged, and are shorter and either straight (Fig. 27) or sinuous (Fig. 26).

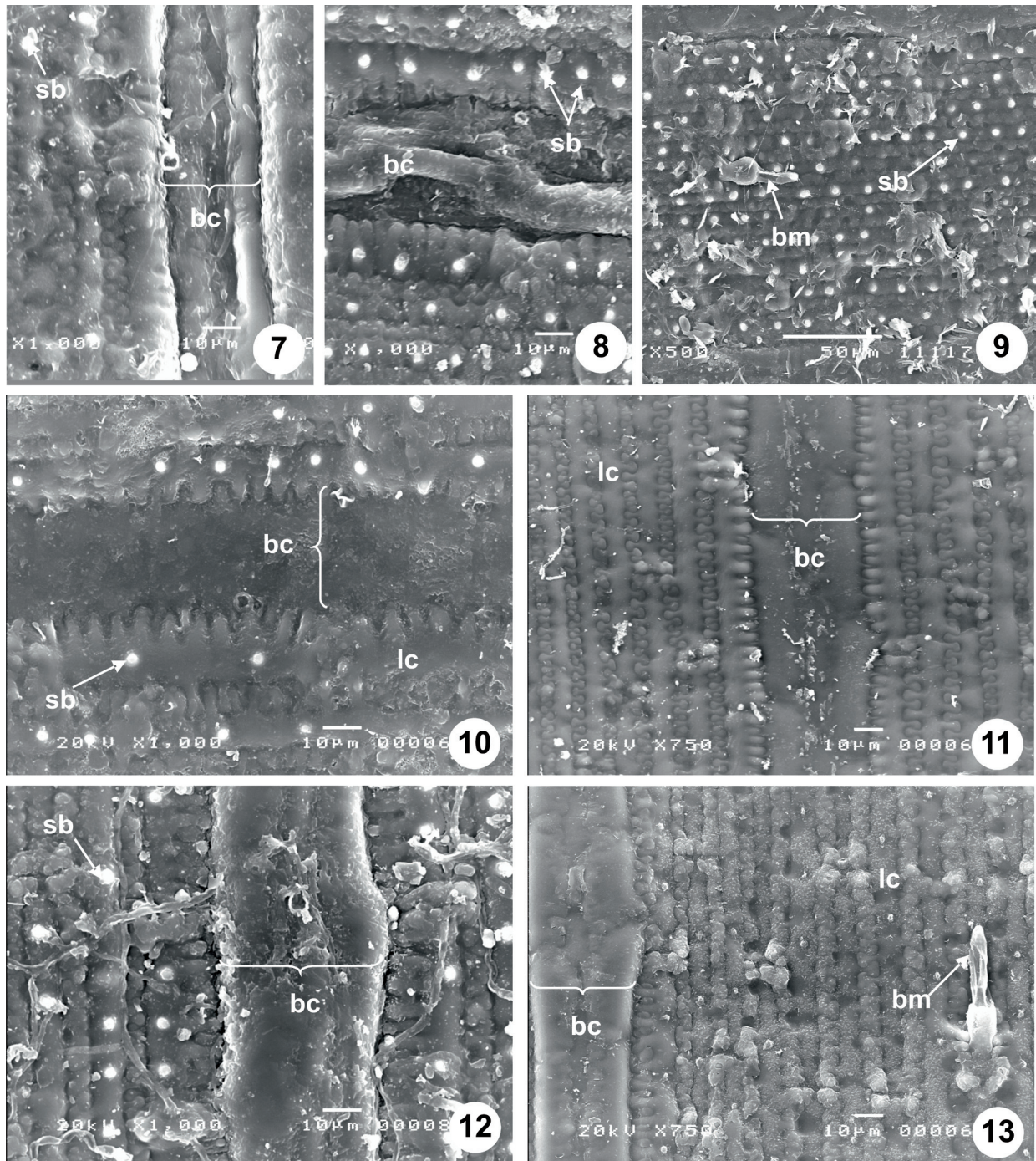


Figures 1-6. Scanning electron microscopy of leaf blade of *Merostachys fischeriana* Rupr. ex. Doell (1), *M. burmanii* Send. (2), *M. kunthii* Rupr. (3), *M. latifolia* R. Pohl (4), *M. neesii* Rupr. (5) and *M. skvortzovii* Send. (6). Adaxial surface showing small silica bodies (sb), bulliform cells (bc), bicellular microhairs (bm), suberose cells (sc) and long cells (lc) with sinous walls.

The number of rows of stomata found on the abaxial surface varies greatly (Fig. 14-15, 19-20, 27). Stomata were not easily observed, since papillae grow over stomatal cells.

There is a green marginal stripe on the narrowest portion of the abaxial surface (Fig. 28-40), characterized by the apparent lack of a wax layer, lack of papillae and

macro-hairs, and presence of silica bodies, as was observed on the adaxial surface of all studied species, except *M. capixaba* (Fig. 28). Stomata are more evident on that stripe, and are situated at a lower level when compared to other epidermal elements, as seen in Fig. 40, where projections from the cutinized wall cover the stomata.



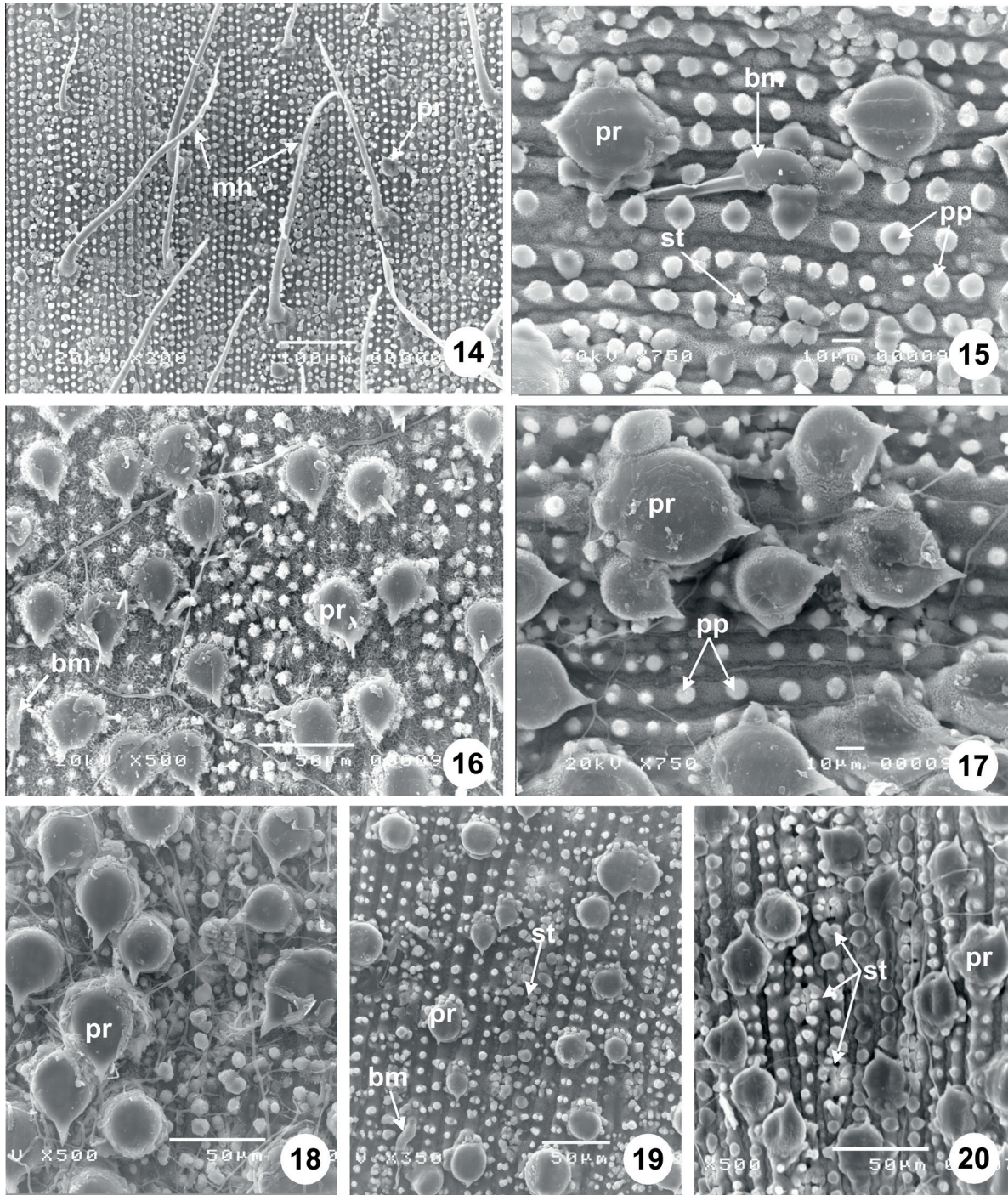
Figures 7-13. Scanning electron microscopy of leaf blade of *Merostachys magellanica* Send. (7), *M. scandens* Send. (8), *M. cauciana* Send. (9), *M. fistulosa* Doell (10), *M. capixaba* Send. (11), *M. gracilis* Send. (12) and *M. ternata* Nees (13). Adaxial surface showing small silica bodies (sb), bulliform cells (bc), bicellular microhairs (bm), and long cells (lc) with sinuous walls.

## Discussion

Leaves of Poaceae have traditionally been analyzed with optical microscopy, but Palmer & Tucker (1981; 1983) and Palmer *et al.* (1985) have demonstrated that the use of SEM in the study of leaf epidermal surface may strongly support taxonomic studies for this family.

SEM exposes some structures more clearly, showing distinct aspects of some features.

The use of SEM allowed the observation of aspects such as papillae, prickles, bicellular hairs, silica bodies, bulliform cells, macro-hairs and stomata, confirming that the type, frequency, arrangement, as well as the presence or absence of some structures may be important for



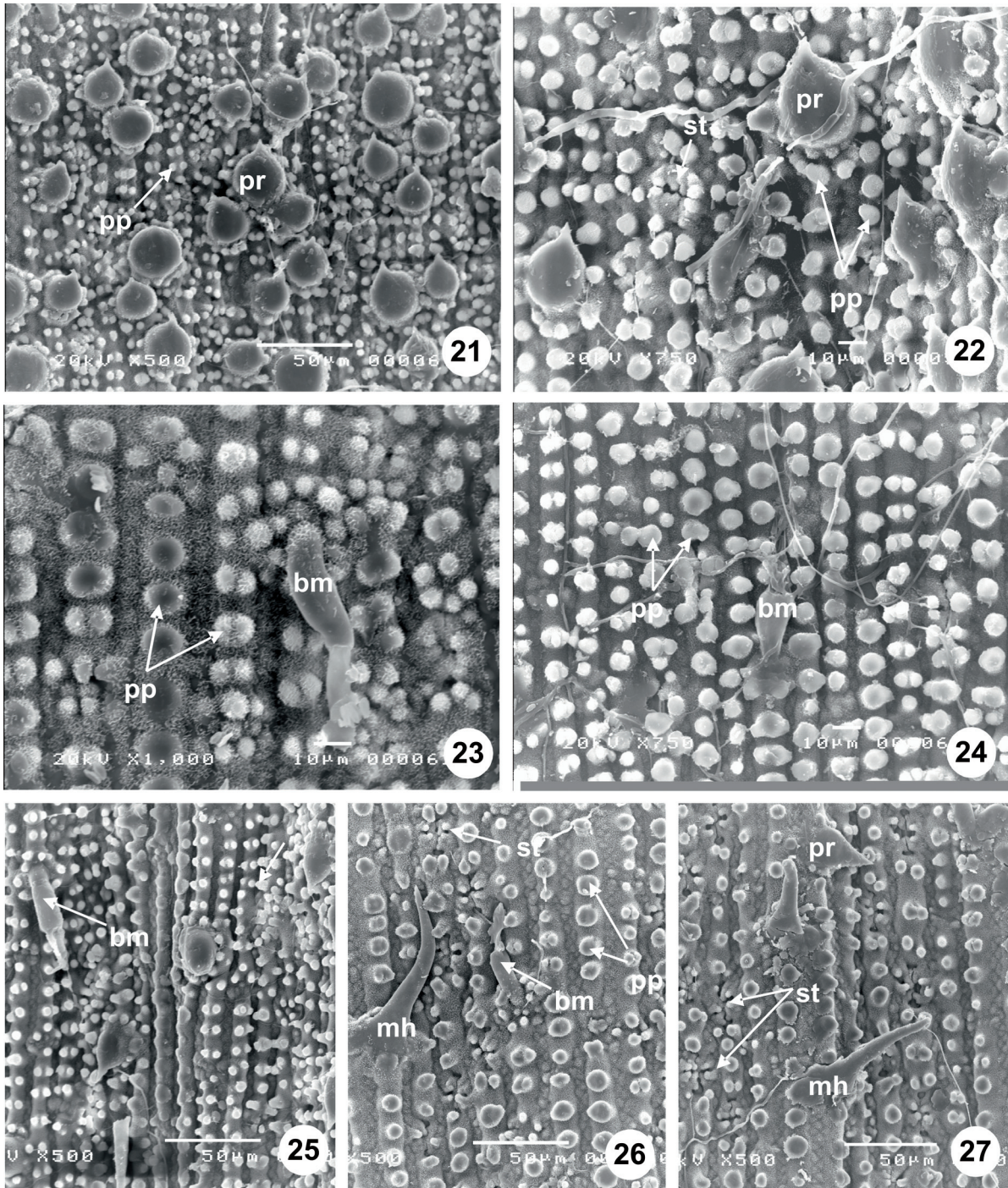
Figures 14-20. Scanning electron microscopy of leaf blade of *Merostachys fischeriana* Rupr. ex. Doell (14), *M. burmanii* Send. (15), *M. kunthii* Rupr (16), *M. latifolia* R. Pohl (17), *M. neesii* Rupr. (18), *M. skvortzovii* Send. (19) and *M. magellanica* Send. (20). Abaxial surface showing abundant papillae (pp), prickles (pr), macrohairs (mh), bicellular microhairs (bm) and stomata (st).

taxonomic studies (Palmer & Tucker 1981).

Both costal and intercostal zones of Poaceae epidermal surfaces are formed by either short or long cells. Long cells do not vary significantly, generally showing sinuous walls, as in *M. fistulosa*, *M. capixaba*, *M. gracilis* and *M. ternata*, and short cells are predominantly represented by suberose cells. The

presence of short suberose cells is also mentioned by Palmer & Tucker (1981) for *Arundinaria alpina* K. Schumann.

Short silicated cells and silica bodies were not observed in the studied species, in opposition to Soderstrom *et al.* (1987), who state that the presence of silicated cells is an important feature in Bambusoideae.

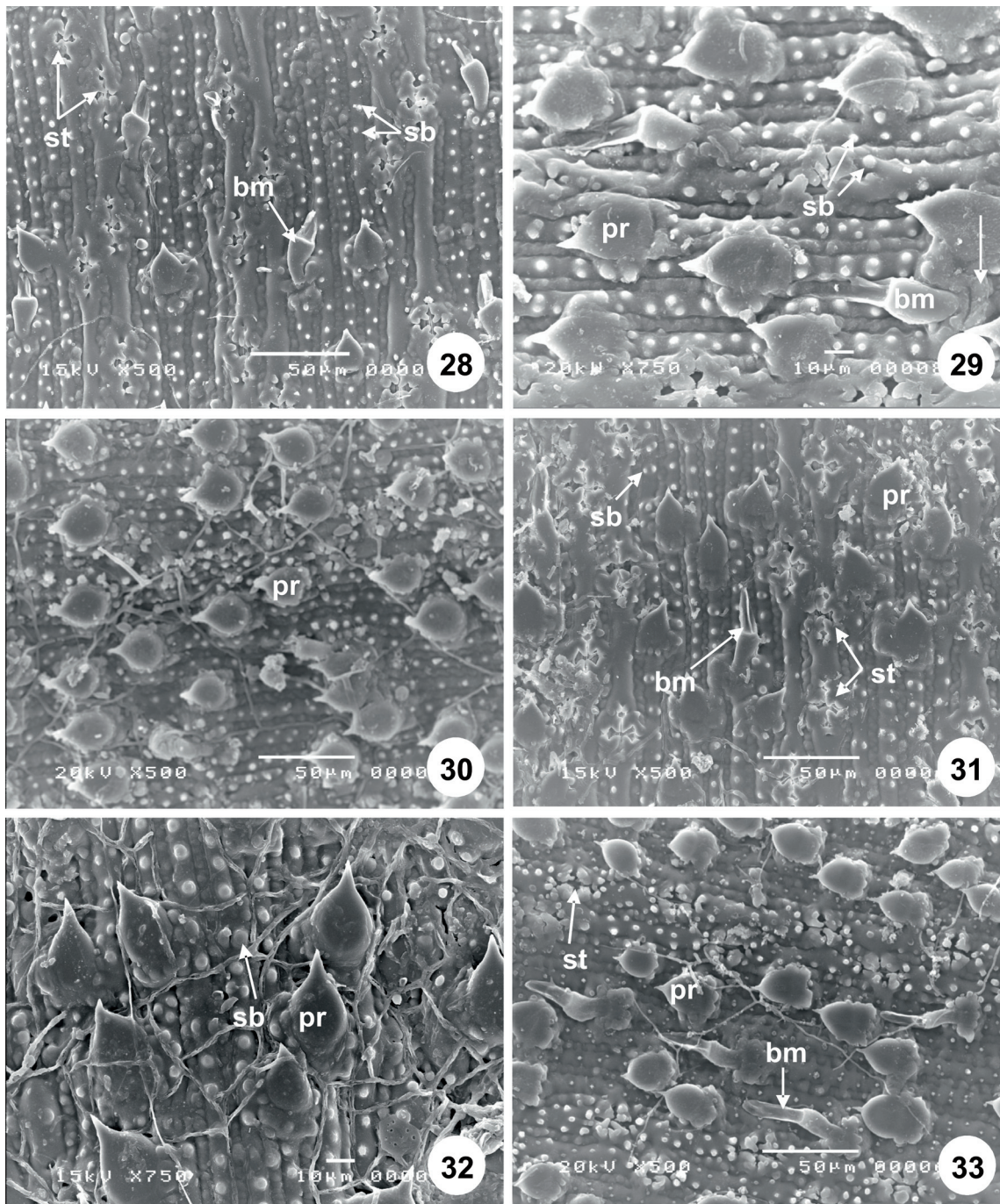


Figures 21-27. Scanning electron microscopy of leaf blade of *Merostachys scandens* Send. (21), *M. cauciana* Send. (22), *M. fistulosa* Doell (23), *M. capixaba* Send. (24), *M. gracilis* Send. (25) and *M. ternata* Nees (26-27). Abaxial surface showing abundant papillae (pp), prickles (pr), macrohairs (mh), bicellular microhairs (bm) and stomata (st).

Silica is more frequent in the outer periclinal wall, like bodies pointing away from the wall. The presence of similar structures is also mentioned by Arruda (2005) for Cyperaceae. The absence of silica bodies on the adaxial surface in *M. kunthii* and *M. neesii*, and on both surfaces of *M. capixaba*, may allow the distinction of those species.

There are generally three types of epidermal elements in Bambusoideae: papillae, micro-hairs and prickles (Calderón & Soderstrom 1973).

Papillae have been found in the Poaceae family both in short and long cells, particularly in the intercostal zones, ranging from one to many papillae on each cell (Ellis 1979). The outer walls of long cells in bamboos

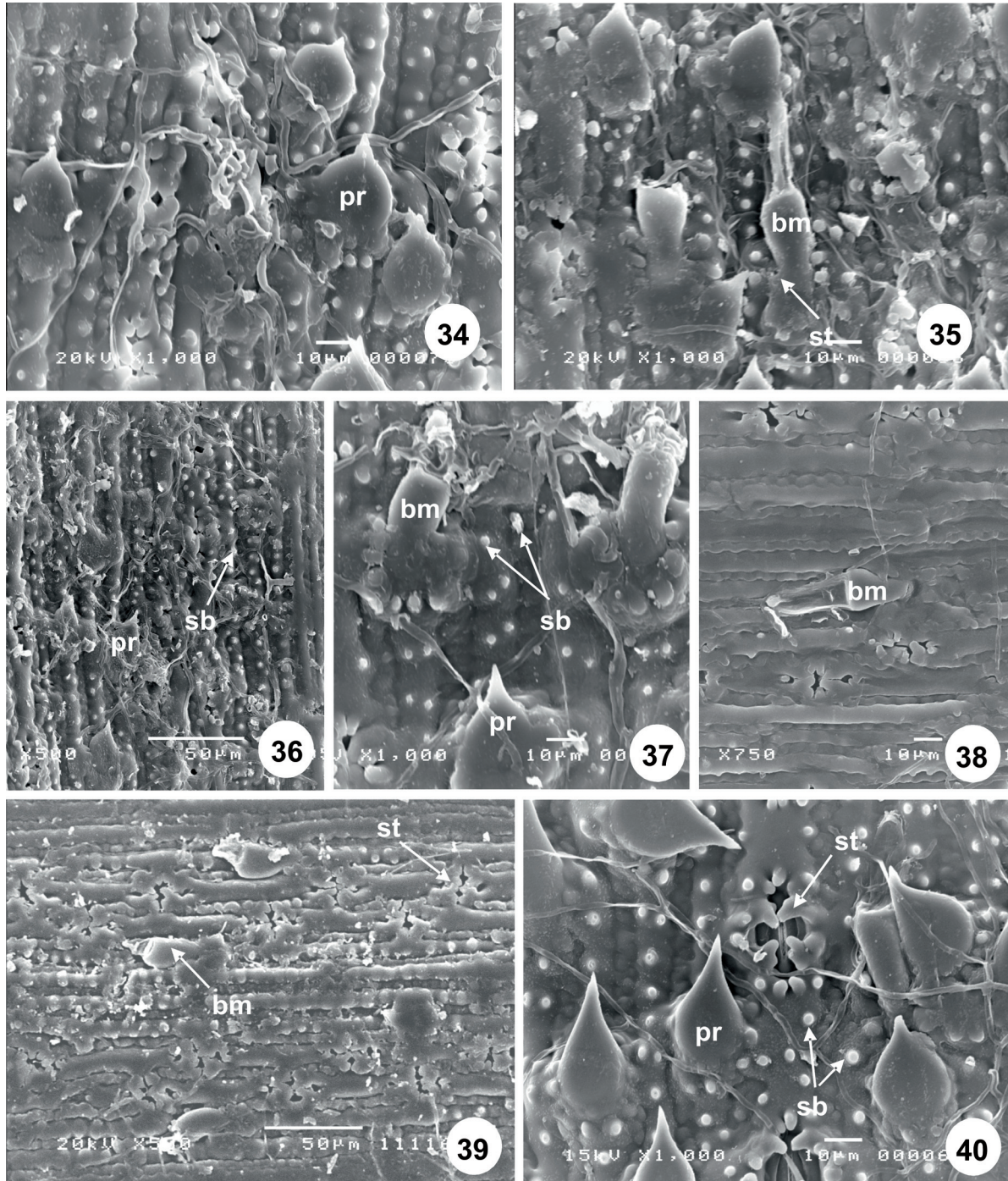


Figures 28-33. Scanning electron microscopy of leaf blade of *Merostachys fischeriana* Rupr. ex. Doell (28), *M. burmanii* Send. (29), *M. kunthii* Rupr. (30), *M. latifolia* R. Pohl (31), *M. neesii* Rupr. (32) and *M. skvortzovii* Send. (33). Abaxial surface in the marginal stripe showing small silica bodies (sb), prickles (pr), bicellular microhairs (bm), suberose cells (sc) and stomata (st).

are especially rich in cutinized papillae, and the differences in papillae frequency and distribution may be used for specific diagnosis (Metcalf 1956).

All studied species presented numerous papillae on the long cells of the abaxial surface, which is an important feature of the Arthrotyliidiinae subtribe (Soderstrom *et al.* 1987). On the other hand, the occurrence of two rows

of papillae on each cell in *M. magellanica*, *M. scandens* and *M. fistulosa*, one or eventually two rows on each cell in *M. skvortzovii*, *M. caucaiana*, *M. capixaba*, *M. gracilis* and *M. ternata*, and one row on each cell in the other species, cannot be considered a safe taxonomic feature (Metcalf 1956), since either one or two rows can be found in 5 of the species mentioned above.



Figures 34-40. Scanning electron microscopy of leaf blade of *Merostachys magellanica* Send. (34), *M. scandens* Send. (35), *M. caucaiana* Send. (36), *M. fistulosa* Doell (37), *M. capixaba* Send. (38), *M. gracilis* Send. (39) and *M. ternata* Nees (40). Abaxial surface in the marginal stripe showing small silica bodies (sb), prickles (pr), bicellular microhairs (bm) and stomata (st).



Either presence or absence of micro-hairs on the leaf epidermis, as well as shape, may be used to distinguish certain groups of Poaceae (Soderstrom *et al.* 1987; Amarasinghe & Watson 1988). Micro-hairs are usually formed by two cells. The apical cell has a very thin wall, which may even be considered caducous (Tateoka *et al.* 1959), and seems to be the most frequent type of trichome in Bambusoideae (Prat 1936; Calderón & Soderstrom 1973; 1980; Renvoize 1985; Soderstrom & Ellis 1988; Paisooksantivatana & Pohl 1992).

All studied species have bicellular micro-hairs, whose cells vary both in arrangement and shape. The micro-hair apical cell also has a very thin wall, not always visualized, which makes determination of its size difficult. Therefore micro-hairs are not a good feature for delimiting species, as well as the low density at which they are found.

Prickles are sharply pointed epidermal appendages with a broad base (Ellis 1979). They generally do not have thick walls, and are found both in intercostal and costal zones, where they are more commonly observed. Prickles had been previously mentioned for several Bambusoideae species (Renvoize 1985; Soderstrom & Ellis 1988; Judziewicz *et al.* 1999; Vieira *et al.* 2002).

Prickles are considered to be a useful anatomical feature in this study, since they were found to be rare in *M. fischeriana* and *M. ternata*, absent in both *M. capixaba* and *M. fistulosa*, and numerous in the other species. It must be pointed out that prickles were more numerous on the abaxial green marginal stripe.

Besides the difference in prickle density in the green marginal stripe when compared to the rest of the leaf blade, there are neither papillae nor silica bodies on this stripe. Like prickles, silica bodies are supposed to be

another mechanism that protects plants from insect attack, which can be explained by the outer position of the green stripe before leafing. The green marginal stripe was been considered an important feature of the subtribe Arthrotyliidiinae, and was characterized by no wax deposition (Judziewicz *et al.* 1999).

Wax has been considered a universal epidermal cell component, and may either impregnate cuticular layers or be exuded over the plant surface (Martin & Juniper 1970; Mauseth 1988). In the case of the abaxial and adaxial green marginal stripes, wax deposition on the leaf surface seems to be continuous, which is mostly frequent in vascular plants (Barthlott & Wollenweber 1981). The ornamentation pattern of the wax in the studied species was very uniform, and not considered to be useful for taxonomic purposes.

Macro-hairs are not commonly found in Bambusoideae, and were only mentioned for this subfamily by Judziewicz & Soderstrom (1989) and Judziewicz *et al.* (1999). In this study, only *M. fischeriana* and *M. ternata* presented macro-hairs.

Stomata in Poaceae are generally found in well defined stripes in the intercostal zones, and most bamboos (except the guaduinae) have their stomata on the abaxial surface (Judziewicz *et al.* 1999), as was observed in this study.

The subtribe Arthrotyliidiinae may be characterized by a series of features, such as the presence of refractive papillae and the presence of a bright green marginal stripe on the abaxial leaf surface (Soderstrom *et al.* 1987), that were observed in all studied species.

In this study, presence or absence of prickles and silica bodies, as well as presence of macro-hairs, could support the definition of some species. The most important differences are presented in Table 1.

Table 1. Different leaf epidermis characteristics of *Merostachys* species (Poaceae: Bambusoideae) observed by SEM.

	Silica bodies on the adaxial surface	Prickles on the abaxial surface	Macrohairs	Number of rows of papillae	Number of rows of stomata
<i>M. fischeriana</i>	present	rare	present	1	2
<i>M. burmanii</i>	present	numerous	absent	1	2
<i>M. kunthii</i>	absent	numerous	absent	1	2-3
<i>M. latifolia</i>	present	numerous	absent	1	2
<i>M. neesii</i>	absent	numerous	absent	1	2-3
<i>M. skvortzovii</i>	present	sparse	absent	1-2	1-2
<i>M. magellanica</i>	present	numerous	absent	2	2
<i>M. scandens</i>	present	numerous	absent	2	1-2
<i>M. caucaiana</i>	present	numerous	absent	1-2	1-3
<i>M. fistulosa</i>	present	absents	absent	2	2-4
<i>M. capixaba</i>	absent	absents	absent	1-2	1-2
<i>M. gracilis</i>	present	numerous	absent	1-2	2
<i>M. ternata</i>	sparse	rare	present	1-2	2

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