

The utility of Bambusoideae (Poaceae, Poales) leaf blade anatomy for identification and systematics

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Abstract

Bambusoideae is a diverse subfamily that includes herbaceous (Olyreae) and woody (Arundinarieae and Bambuseae) bamboos. Species within Bambusae are particularly difficult to identify due to their monocarpic lifecycle and the often long durations between mass flowering events; whereas the herbaceous bamboos are pluricarpic, but often are found with no reproductive structures. The leaf blade anatomy of 16 sympatric species of native Brazilian bamboos (Olyreae and Bambuseae) from the Atlantic Rainforest was studied in order to detect useful features for their identification. All the studied species share the following features: epidermis with a single stratum of cells; adaxial bulliform cells; mesophyll with arm cells, rosette cells, and fusoid cells; and collateral vascular bundles. Herbaceous bamboos share two features: papillae scattered on the abaxial surface and parallel-sided arrays of bulliform cells; whereas woody bamboos share: centrally organized papillae and fan-shaped arrays of bulliform cells. Also within the woody bamboos, intercostal fibers and a midrib with only one vascular bundle (simple midrib) characterize the subtribe Arthrotyliidiinae; whereas a midrib with more than one vascular bundle (complex midrib) and a stomatal apparatus with two papillae per subsidiary cell characterize the subtribe Chusqueinae. There are also diagnostic features for the sampled species, such as: papillae shape, and the outline and structure of the midrib. An identification key for all the studied species is provided based on the anatomical features.

Keywords: Arthrotyliidiinae, Bambuseae, Chusqueinae, leaf blade, Olyreae.

A utilidade da anatomia da lâmina foliar para a identificação e sistemática de espécies de Bambusoideae (Poaceae, Poales)

Resumo

Bambusoideae é uma subfamília que inclui diversas espécies de bambus herbáceos (Olyreae) e lignificados (Arundinarieae e Bambuseae). Bambus lignificados geralmente apresentam dificuldades de delimitação e identificação, devido principalmente ao ciclo monocárpico e longa amplitude temporal entre florações; enquanto que bambus herbáceos possuem ciclo pluricárpico, porém frequentemente são encontrados em estágio vegetativo. Foi estudada a anatomia da lâmina foliar de 16 espécies de Bambusoideae (Olyreae e Bambuseae), simpátricas e nativas do Brasil, visando levantar caracteres úteis para sua identificação. Todos os táxons estudados compartilham: epiderme uniestratificada; células buliformes na face adaxial; mesófilo com células invaginantes, células em roseta e células fusoides; e feixes vasculares colaterais. Bambus herbáceos compartilham: papilas dispersas na face abaxial e grupos de células buliformes organizadas paralelamente; enquanto que bambos lignificados compartilham: papilas organizadas em colunas centrais e grupos de células buliformes em forma de leque. Ainda dentre os bambus lignificados, fibras intercostais e nervura central com apenas um feixe vascular (nervura central simples) caracterizam a subtribo Arthrotyliidiinae; enquanto que nervura central com mais de um feixe vascular (nervura central complexa) e aparelho estomático com duas papilas por célula subsidiária caracterizam a subtribo Chusqueinae. Há ainda caracteres anatômicos diagnósticos, tais como: forma da papila, e forma e estrutura da nervura central. Uma chave de identificação é fornecida baseada nos caracteres anatômicos relevantes à identificação das espécies estudadas.

Palavras-chave: Arthrotyliidiinae, Bambuseae, Chusqueinae, lâmina foliar, Olyreae.

1. Introduction

The cosmopolitan family Poaceae comprise about 11,000 species found mainly in grasslands and forest formations (Watson and Dallwitz, 1992 onwards; GPWG II, 2012). Twelve subfamilies are recognized within Poaceae (GPWG II, 2012; Soreng et al., 2015), among them Bambusoideae, a monophyletic group that currently includes 1,482 described species (Clark et al., 2015). Three Bambusoideae tribes are recognized, two of which are found in the Neotropics: Bambuseae, which comprise the woody bamboos; and Olyreae, the herbaceous bamboos (Kelchner, 2013; Clark et al., 2015). The Atlantic Rainforest is considered an important center of bamboo diversity (Judziewicz et al., 1999), and Brazil occupies a leading position based on number of species (298) and high endemism (172) (Carvalho et al., 2016).

Bambusoideae may be distinguished from other grass subfamilies by morphological, anatomical, and ecological characters. Monocarpic perennial lifecycle, lignified culms, branching nodes, pseudopetiolate leaves, and an outer ligule are characters worth mentioning for the woody bamboos (GPWG, 2001; BPG, 2012); whereas herbaceous bamboos are pluricarpic, usually unbranched, with quite weak culms and an inner ligule (Judziewicz et al., 1999). Together with, the strongly and asymmetrically invaginated arm cells as seen in cross section are highly important for the recognition of Bambusoideae species (GPWG, 2001), and also represents one of the main synapomorphies for this group (Zhang and Clark, 2000; BPG, 2012).

In general, the Poaceae taxonomy is mainly based on reproductive characters, such as the shape and structure of spikelets and inflorescence types (Longhi-Wagner, 2012). This is true more particularly for the herbaceous species, which generally bloom many times in their life cycle. In contrast, the woody bamboos bloom only once during a life cycle (Janzen, 1976; Filgueiras, 1988), and sometimes even herbaceous species are found with no reproductive structures. For this reason, searching for vegetative characters in addition to the reproductive ones is highly important to aid in species identification, and anatomical features often have provided useful findings (e.g. Brandis, 1907; Prat, 1936; Brown, 1958; Metcalfe, 1960; Calderón and Soderstrom, 1973; Renvoize, 1987; Vieira et al., 2002; Guglieri et al., 2008; Oliveira et al., 2008; Pelegrin et al., 2009; Jesus Junior et al., 2012; Viana et al., 2013a, b; Leandro et al., 2016; Aliscioni et al., 2016).

Considering that mostly bamboo plants have unique life cycles, but also the importance of the leaf blade anatomy for the taxonomy of grasses in general, we studied 16 sympatric species of native bamboos from the Atlantic Rainforest. We examined the leaf blade anatomy of three species of herbaceous bamboos and 13 species of woody bamboos in order to provide useful features for their identification.

2. Material and Methods

2.1. Sampling area

The study was carried out with 16 native species sampled at *Parque Estadual das Fontes do Ipiranga - PEFI* (23° 38' 08" S and 23° 40' 18" S - 46° 36' 48" W and 46° 38' 00" W) [*Ipiranga State Park*], a fragment of Atlantic Rainforest located in the State of São Paulo, Brazil. We have analysed three specimens per species, but only one voucher per specimens was included in the herbarium of the Instituto de Botânica (SP) (Table 1). The choice of taxa was based on a floristic study of the area that indicated the necessity of providing additional data in order to aid in species identification and conservation (Shirasuna and Filgueiras, 2013). *Olyra lorentensis* Mez was not included in this study due to its uncertain occurrence in the PEFI (see Shirasuna and Filgueiras, 2013 for details about each species).

2.2. Anatomical analysis

For the woody bamboos, mature leaf blades were taken from the branches at the mid-culm, whereas for the herbaceous bamboos mature leaves were taken from the third node from the base. Fresh plant material was fixed in FAA₅₀ (Johansen, 1940) and later stored in 70% ethanol. Found on leaves of Arthrostylidiinae species, the green stripe was excluded from this work due to its anatomical peculiarities in relation to the remainder of the leaf blade (Judziewicz et al., 1999).

Samples from the middle portion of the leaf blade were embedded in polyethylene glycol 1500 solution (adapted from Richter, 1985) and cross-sectioned with a rotary microtome. Sections were cleared in sodium hypochlorite 50%, washed in distilled water, stained with Astra blue and Safranin (Bukatsh, 1972), and finally mounted on semi-permanent slides with glycerol. Also, a maceration technique was performed by the Jeffrey's method (Johansen, 1940) in order to describe the epidermal features.

Descriptions were primarily based on Ellis (1976, 1979), and optical images were obtained on a Leica DM4000B microscope using the software Leica Application Suite LASV4.0.

3. Results

3.1. Surface view

All the studied taxa share an epidermis with long-short cell alternation (Figures 1A-M). Short cells occur as silica bodies (Figures 1D, F, K - arrow) or suberized cells (cork cells - arrowhead) (Figure 1A) – sometimes as silico-suberose couples in the intercostal zone (e.g. Figure 1A). The wall sinuosity of long cells may be deep (Figures 1B, E, K), moderate (Figures 1A, F) or slight (Figures 1G, J). Papillae commonly occur on the abaxial surface: less pronounced in the herbaceous species (e.g. Figure 1D) and more pronounced in the woody species (e.g. Figures 1C, H). In *Merostachys argyronema* Lindm. papillae are very conspicuous (Figure 1H; Table 2), and in *Merostachys*

Table 1. Specimens used in this study, with classification and voucher information provided.

Taxa and classification	Voucher information
TRIBE OLYREAE	
Subtribe Olyrinae	
<i>Olyra glaberrima</i> Raddi	A. Custodio Filho 124 (SP 160961)
<i>O. humilis</i> Nees	R. T. Shirasuna 2617 (SP 415204)
<i>Parodiolyra micrantha</i> (Kunth) Davidse & Zuloaga	R. T. Shirasuna 2863 (SP 420323)
TRIBE BAMBUSEAE	
Subtribe Chusqueinae	
<i>Chusquea bambusoides</i> (Raddi) Hack.	R. T. Shirasuna & A. Costa 1809 (SP 409031)
<i>C. capituliflora</i> Trin var. <i>pubescens</i> McClure & L.B. Sm.	R. T. Shirasuna 2760 (SP 415735)
<i>C. meyeriana</i> Rupr. ex Doell	R. T. Shirasuna 2697 (SP 415247)
Subtribe Arthrostylidiinae	
<i>Aulonemia aristulata</i> (Doell) McClure	R. T. Shirasuna 2860 (SP 420320)
<i>Merostachys argyronema</i> Lindm.	R. T. Shirasuna 2868 (SP 420326)
<i>M. burmanii</i> Send.	J. F. Toledo s/n° (SP 238492)
<i>M. magellanica</i> Send.	M. T. Grombone s/n° (SP 412132)
<i>M. neesii</i> Rupr.	R. T. Shirasuna 2864 (SP 430324)
<i>M. pluriflora</i> Munro ex. E.G. Camus	R. T. Shirasuna 1811 (SP 409023)
<i>M. riedeliana</i> Rupr. ex Doell	R. T. Shirasuna 2872 (SP 426233)
<i>M. scandens</i> Send.	R. T. Shirasuna 2993 (SP 441817)
<i>M. skvortzovii</i> Send.	T. Sendulsky 1318 (SP 166796)
<i>M. speciosa</i> Spreng.	R. T. Shirasuna 2798 (SP 417980)

neesii Rupr. they have a concave apex (Figure 1C; Table 2). A scattered distribution of papillae is observed in the herbaceous bamboos (e.g. Figure 1D; Table 2), whereas the organization in the woody bamboos is often in a single central row (e.g. Figures 1G, I), but may be variable in some intercostal cells (1-2 rows) (e.g. Figure 1H).

Trichomes mainly occur on the abaxial surface and they may be of three types: (i) prickle hairs (short and silicified, microscopic unicellular) (Figures 1C, F); (ii) macrohairs (macroscopic unicellular) (Figures 1E); (iii) or microhairs (microscopic bicellular) (Figures 1E, L, M). The occurrence of these trichomes is variable among the studied species and only *Chusquea capituliflora* Trin var. *pubescens* McClure & L.B. Sm. has all the three types (Figures 1E, F). Prickle hairs of most of the species develop an enlarged base, usually as seen in *Chusquea capituliflora* var. *pubescens* (Figure 1F), but in *M. neesii* this base is more pronounced (Figure 1C). Macrohairs occur on the abaxial surface of *C. capituliflora* var. *pubescens* (Figure 1F) and *Chusquea meyeriana* Rupr. ex Doell (Figure 1I - scars). Bicellular microhairs often consist of cells of about the same size (e.g. Figure 1M), except for *C. capituliflora* var. *pubescens*, in which the apical cell is reduced (Figure 1L). Microhairs often occur on the abaxial surface in the woody species and *Parodiolyra micrantha* (Kunth) Davidse & Zuloaga.

Stomata are paracytic and occur on the abaxial surface of all the studied species, but also on the adaxial surface in *A. aristulata* and *Olyra humilis* Nees (Figure 1B; Table 2). Stomatal apparatus comprise triangular subsidiary cells (Figures 1B, D, H, K) or semi-circular (cupuliform) cells

(Figures 1A, E, G). In species of *Chusquea* the stomatal apparatus bears two papillae per subsidiary cell as seen in *Chusquea capituliflora* var. *pubescens* (Figure 1E detail inset; Table 2).

3.2. Cross section

The epidermis consists of a single stratum of cells with slightly thickened outer walls (Figures 2A-Q). Epidermal cells are visually about the same size (Figures 2E, G, J), but may be larger on the adaxial side (Figures 2F, H, M) – excluding the bulliform cells. Bulliform cells occur as part of the adaxial epidermis (Figures 2D-M) and form a fan-shaped array in the woody bamboos, (e.g. Figures 2G, J, K; Table 2); whereas the herbaceous bamboos share a parallel-sided array of bulliform cells (e.g. Figures 2E, I; Table 2).

The mesophyll comprise arm cells, fusoid cells, rosette cells, fibers, and vascular bundles. Asymmetrically invaginated arm cells are parallel to the epidermis (Figures 2E-M), including the midrib portion (Figures 2A-D). Herbaceous species (Figures 2E, I) and *Chusquea bambusoides* (Figure 2H) develop arm cells with invaginations only from the abaxial side, whereas the other species develop invaginations from both sides (Figures 2F, G, J-M). The number of rosette cells between each fusoid cell is often variable (one to four) within the same sample/specimen (Figures 2E-M). Fusoid cells occur adjacent to the vascular bundles and arm cells (Figures 2E-M); and their outline may be short and wide, as seen in *A. aristulata* (Figure 2G), or long and narrow, as in *M. neesii* (Figure 2F). Intercostal fibers located adjacent to the bulliform cells (sometimes also opposite) occur just

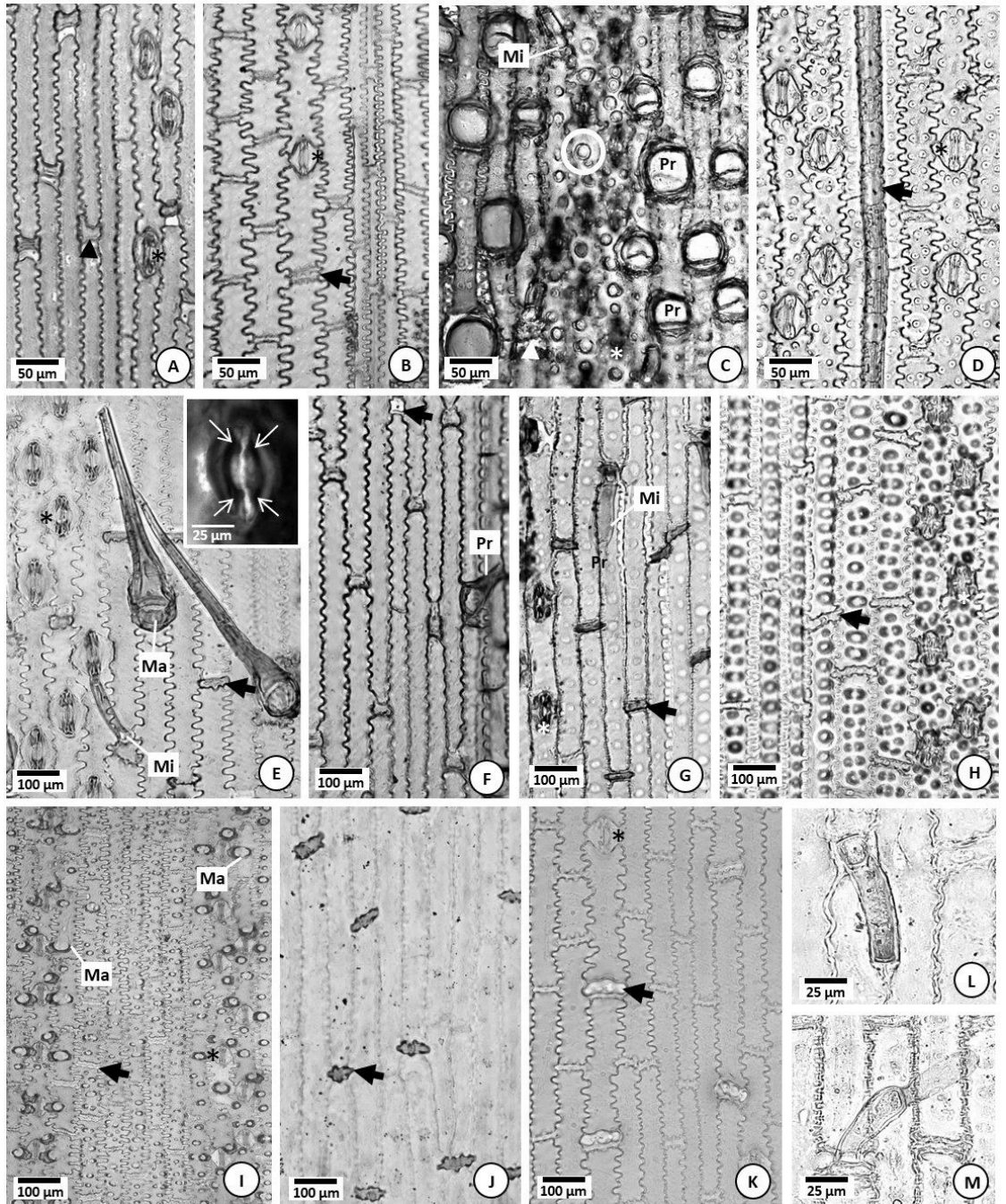


Figure 1. Surface view of leaf blades of Bambusoideae species. Adaxial surface (B, F, K) and abaxial surface (A, C-E, G-J). (A) *Chusquea bambusoides* with cupuliform subsidiary cells (*) and silico-suberose couples; (B) *Olyra humilis* with triangular subsidiary cells (*); (C) *Merostachys neesii* showing papillae with concave apex (white circle); (D) *Olyra glaberrima* with silica body (black arrow) and papillae scattered; (E) *Chusquea capituliflora* var. *pubescens* with macrohairs (Ma), microhairs (Mi), and a detail showing the stomata apparatus bearing two papillae per subsidiary cell (white arrows); (F) *Chusquea capituliflora* var. *pubescens* with prickle (Pr); (G) *Aulonemia aristulata* with microhair (Mi); (H) *Merostachys argyrocnema* with papillae organized in a central row (or double row in some long cells); (I) *Chusquea meyeriana* showing the epidermis with macrohair scars (Ma); (J) *Merostachys riedeliana* with silica body (black arrow); (K) *Parodiolyra micrantha* showing silica body (black arrow); (L) *Chusquea capituliflora* var. *pubescens* detail of a bicellular trichome (microhair) with reduced apical cell; (M) *Aulonemia aristulata* detail of a bicellular trichome (microhair) with cells about the same size. (Ma) macrohair; (Mi) microhair; (Pr) prickles. Black arrows: silica bodies; white arrows: papillae; arrowhead: suberized cells; asterisks: stomata; white circle: papillae with concave apex.

Table 2. Summary of leaf blade features useful for delimiting the tribes and subtribes, and also for recognizing the species.

FEATURES	SPECIES																	
	Tribe	Olyreae herbaceous bamboos						Bambuseae woody bamboos										
		Subtribe	Olyrinae			Chusqueinae			Arthrostylydiinae									
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Epidermis																		
Papillae scattered on the abaxial surface	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Papillae centrally organized in a single or double row	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Papillae with concave apex	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
Papillae (two) per subsidiary cell	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
Microhairs with reduced apical cell	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	
Amphistomatic leaves	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
Mesophyll																		
Parallel-sided arrays of bulliform cells	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fan-shaped arrays of bulliform cells	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intercostal fibers	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	
Midrib																		
Adaxially projected	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Complex midrib	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
Simple midrib	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	
With one vascular bundle opposite to the central one	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	

1. *Olyra humilis*; 2. *O. glaberrima*; 3. *Parodiolyra micrantha*; 4. *Chusquea bambusoides*; 5. *C. capituliflora* var. *pubescens*; 6. *C. meyeriana*; 7. *Aulonemia aristulata*; 8. *Merostachys argyronema*; 9. *M. burmanii*; 10. *M. magellanica*; 11. *M. neesii*; 12. *M. pluriflora*; 13. *M. riedeliana*; 14. *M. scandens*; 15. *M. skvortzovii*; 16. *M. speciosa*. (+) presence, (-) absence.

among species of Arthrostylydiinae (e.g. Figures 2D, F, G; Table 2). Collateral vascular bundles are surrounded by a double sheath (Figures 2A-M): the outer one is parenchymatic and may be interrupted by fibers from both sides, as seen in *Merostachys speciosa* Spreng. (Figure 2M) or only from the abaxial side, as in *Merostachys skvortzovii* Send. (Figure 2L); and the inner one is pericyclic (mestome) with thick-walled cells (Figures 2A-M). First and third order vascular bundles are observed in all the studied species (Figures 2F-M).

In most of the studied species the midrib is flat (e.g. Figure 2D), but it is abaxially projected in species of *Chusquea* (Figures 2A, B) and adaxially projected in *Parodiolyra micrantha* (Figure 2C; Table 2). The midrib comprise one first order vascular bundle (Figures 2C, D), except among species of *Chusquea*, in which the midrib includes minor vascular bundles adjacent to the central one (Figures 2A, B; Table 2).

With regard to the margin, the leaf blade may be acute (Figure 2N-P) or obtuse (Figures 2Q); always with thick-walled epidermal cells and fibers immediately subjacent to the epidermis (e.g. Figures 2N-Q).

3.3. Taxonomic treatment

The main anatomical features are summarized in Table 2. These data in tabular form are available upon request from the first author.

Identification key to the native Bambusoideae species from PEFI, SP, based on the leaf blade anatomical data (surface view and cross section):

1. Papillae scattered on the abaxial surface; parallel-sided arrays of bulliform cells (Tribe Olyreae)
2. Prickle hairs on the abaxial surface developed; midrib adaxially projected*Parodiolyra micrantha*
- 2'. Prickle hairs on the abaxial surface lacking; midrib slightly convex on both surfaces
3. Leaves amphistomatic; adaxial epidermal cells larger than abaxial epidermal cells (excluding the bulliform cells) *Olyra humilis*
- 3'. Leaves hypostomatic; adaxial epidermal cells equal to sub-equal to the abaxial epidermal cells (excluding the bulliform cells) *Olyra glaberrima*

- 1' Papillae centrally organized in a single or double row on the abaxial surface; fan-shaped arrays of bulliform cells (Tribe Bambuseae)
4. Intercostal fibers developed; midrib with only one vascular bundle (simple midrib); stomata apparatus without papillae (Subtribe Arthrotyliidiinae)
5. Stomata on both surfaces; papillae on the adaxial surface developed *Aulonemia aristulata*
- 5'. Stomata only on the abaxial surface; papillae on the adaxial surface lacking
6. Central vascular bundle in the midrib (major one) with outer sheath interrupted by fibers from both sides
7. First order vascular bundle with outer sheath interrupted by fibers from the abaxial side *Merostachys scandens*
- 7'. First order vascular bundle with outer sheath interrupted by fibers from both sides
8. Fusoid cells long and narrow... *Merostachys magellanica*
- 8'. Fusoid cells short and wide
9. Adaxial epidermal cells larger than abaxial epidermal cells (excluding the bulliform cells) *Merostachys riedeliana*
- 9'. Adaxial epidermal cells equal to sub-equal to the abaxial epidermal cells (excluding the bulliform cells)
10. Prickle hairs on the abaxial surface developed *Merostachys burmanii*
- 10'. Prickle hairs on the abaxial surface lacking *Merostachys pluriflora*
- 6'. Central vascular bundle in the midrib (major one) with outer sheath interrupted by fibers only from the abaxial side
11. First order vascular bundle with outer sheath interrupted by fibers from both sides
12. Bicellular microhairs on the adaxial surface developed; adaxial epidermal cells larger than abaxial epidermal cells (excluding the bulliform cells) *Merostachys neesii*
- 12'. Bicellular microhairs on the adaxial surface lacking; adaxial epidermal cells equal to sub-equal to the abaxial epidermal cells (excluding the bulliform cells) *Merostachys skvortzovii*
- 11'. First order vascular bundle with outer sheath interrupted by fibers only from the abaxial side
13. Fusoid cells short and wide; bicellular microhairs on the adaxial surface developed *Merostachys argyronema*
- 13'. Fusoid cells long and narrow; bicellular microhairs on the adaxial surface lacking *Merostachys speciosa*
- 4'. Intercostal fibers lacking; midrib with more than one vascular bundle (complex midrib); stomatal apparatus bearing two papillae per subsidiary cell (Subtribe Chusqueinae)

14. Midrib with two vascular bundles subjacent to the adaxial epidermis and opposite to the central one *Chusquea meyeriana*
- 14'. Midrib with one vascular bundle subjacent to the adaxial epidermis and opposite to the central one
15. Prickle hairs on the adaxial surface developed; macrohairs on the abaxial surface developed; bicellular microhairs developed *Chusquea capituliflora* var. *pubescens*
- 15'. Prickle hairs on the adaxial surface lacking, macrohairs on the abaxial surface lacking; bicellular microhairs lacking *Chusquea bambusoides*

4. Discussion

Our anatomical study demonstrates that papillae scattered on the abaxial surface and parallel-sided arrays of bulliform cells are exclusive features among the herbaceous bamboos sampled; whereas centrally organized papillae and fan-shaped arrays of bulliform cells are exclusive features among the woody bamboos sampled.

Within the herbaceous bamboos sampled, the midrib outline and amphistomatic leaves may distinguish *Parodiolyra* Soderstr. & Zuloaga from *Olyra* L. Although this may be true, it is not clear if these features are consistent among all Brazilian species of *Olyra* (20) and *Parodiolyra* (four) (Oliveira and Filgueiras, 2016a, b). Comparatively, within the woody bamboos sampled, intercostal fibers and a midrib with only one vascular bundle (simple midrib) characterize the subtribe Arthrotyliidiinae; whereas a stomata apparatus bearing two papillae per subsidiary cell and a midrib with more than one vascular bundle (complex midrib) characterize the subtribe Chusqueinae. The presence of two papillae per subsidiary cell herein supports the assumption of this feature as a synapomorphy for *Chusquea* (Fisher et al., 2009, 2014), although there are not enough studies on micromorphology and anatomy to clarify its value. Currently, the set of features herein observed for Arthrotyliidiinae and Chusqueinae is common among all species known within each subtribe and extremely applicable for recognizing these groups (BPG, 2012; Clark et al., 2015).

The comparative anatomical analysis herein performed demonstrates that the variation in the distribution of papillae is useful for delimiting tribes. There are some reports showing the importance of this feature in bamboo systematics (e.g. Soderstrom and Ellis, 1987; Paisooksantivatana and Pohl, 1992; Yang et al., 2008; Gomes and Neves, 2009; Mota, 2013), but also for other closely related groups (e.g. Pelegrin et al., 2009). Our study is not able to define the value of this feature to the systematics of Olyreae and Bambuseae, therefore a detailed work to evaluate both distribution and type of papillae within different groups would be informative.

Our study also indicates that some features may be considered diagnostic at the species level. Among them, the stomata on the adaxial surface in *Aulonemia aristulata*

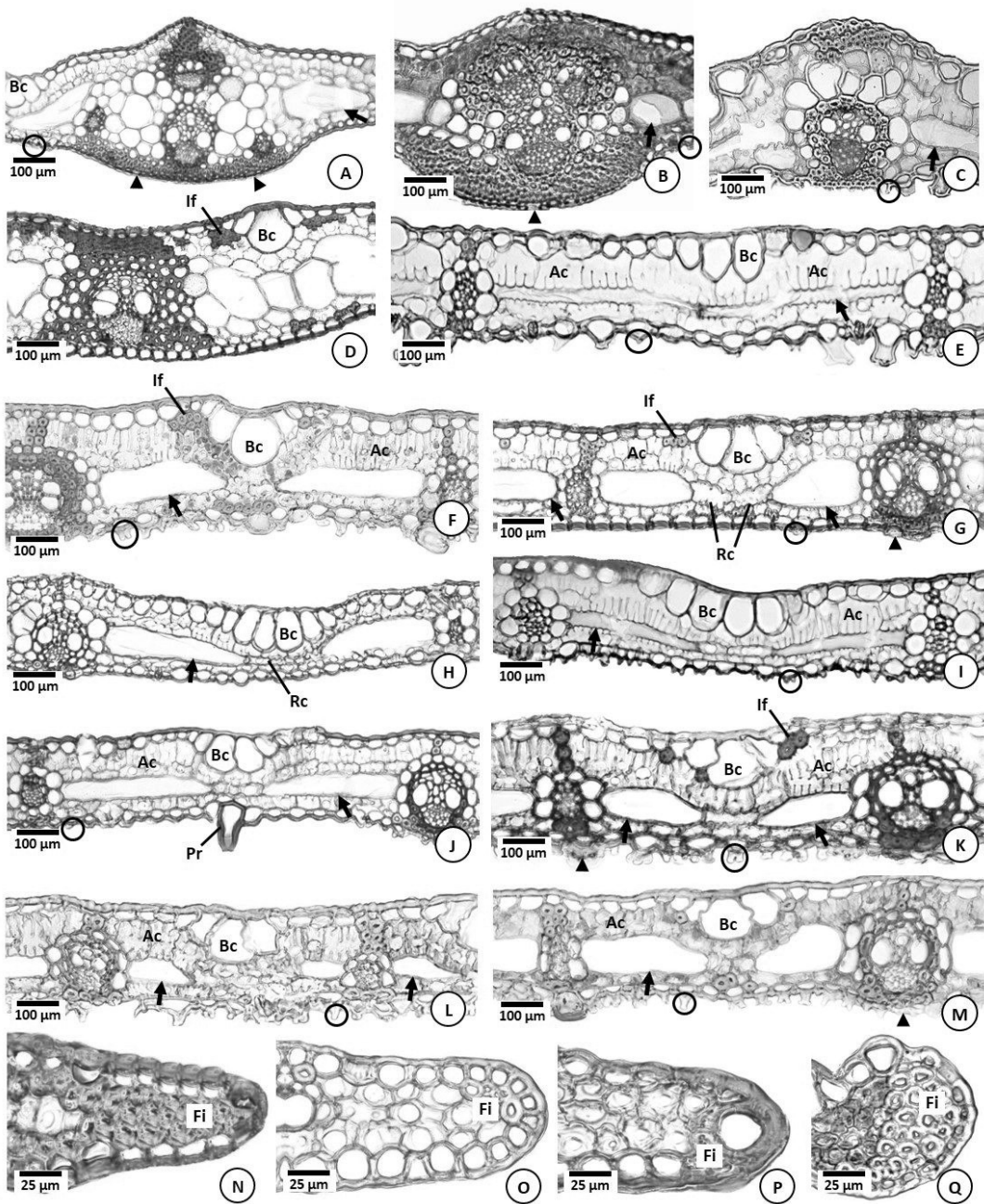


Figure 2. Cross section of leaf blades of Bambusoideae species. Midrib (A-D), mesophyll (E-M) and margin (N-Q). (A) *Chusquea capituliflora* var. *pubescens* showing complex midrib with one vascular bundle opposite to the central one; (B) *Chusquea meyeriana* showing complex midrib with two vascular bundles opposite to the central one; (C) *Parodiolyra micrantha* showing midrib with only one vascular bundle (simple midrib); (D) *Aulonemia aristulata* with intercostal fibers (If); (E) *Olyra humilis* showing arm cells with invaginations only from the abaxial side; (F) *Merostachys neesii* with intercostal fibers (If), bulliform cells (Bc) and arm cells (Ac); (G) *Aulonemia aristulata* showing arm cells with invaginations from both sides; (H) *Chusquea bambusoides* showing rosette cells (Rc); (I) *Parodiolyra micrantha* with papillae (black circle); (J) *Chusquea capituliflora* var. *pubescens* showing prickly hair (Pr); (K) *Merostachys burmanii* with silica body (arrowhead); (L) *Merostachys skvortzovii* showing vascular bundles with outer sheath interrupted by fibers from the abaxial side; (M) *Merostachys speciosa* showing vascular bundles surrounded by a double sheath; (N) *Aulonemia aristulata* with acute margin and thick-walled epidermal cells; (O) *Chusquea capituliflora* var. *pubescens* with acute margin and a few fibers; (P) *Chusquea bambusoides* with thick-walled epidermal cells; (Q) *Chusquea meyeriana* with obtuse margin and many fibers. (Ac) arm cells; (Bc) bulliform cells; (Fi) fibers; (If) intercostal fibers; (Pr) prickly hair; (Rc) rosette cells. Arrows: fusoid cells; arrowheads: silica bodies; black circles: papillae.

must be mentioned, since their occurrence is considered as rare for *Aulonemia* (Arthrotyliidiinae) (Viana et al., 2013a), but usually typical for species within the subtribe Guaduineae (Soderstrom and Ellis, 1987). Adaxial stomata were also recently observed in other species within *Aulonemia* (Viana, 2010; Viana et al., 2001), and thus it reinforces the anatomical affinity between the subtribes Arthrotyliidiinae and Guaduineae (Bambuseae) (Soderstrom and Ellis, 1987; Zhang and Clark, 2000; Ruiz-Sánchez et al., 2008), as well as the necessity of a broad anatomical study in order to elucidate the systematic value of this feature for Bambuseae.

It is important to highlight that the size and shape of bulliform cells may be influenced by environmental factors (Shields, 1951), but the structural variation herein observed deserves more attention in order to verify its constancy among bamboo groups. Also, the fusoid cell is another feature that requires additional attention since its environmentally influenced morpho-anatomical variations (March and Clark, 2011; T. D. Leandro, unpubl. data). In the present study, we consider the structure of bulliform cells and the outline of fusoid cells as relevant features for delimiting species given that all specimens were sampled under the same environmental conditions.

5. Conclusion

Although most of the information herein provided is not a novelty for Bambusoideae, our results reinforce the importance of leaf blade anatomy studies for grass systematics, specially when we consider the great number of questions that are still unclear. The inclusion of anatomical data as a routine on bamboo studies may be really useful for identifying diagnostic features and additional synapomorphies, in which certainly will aid in species circumscription.

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