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# Anatomical traits of woody bamboos useful for taxonomic identification: A case study in neotropical species

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**Abstract:** Bamboos are perennial monocarps with long flowering intervals. To use floral morphology for species identification is difficult, because floral material is not always available. Anatomical characters may be used instead of floral traits for plant identification. However, in America there are few anatomical studies on woody bamboo species. Anatomical studies on two native neotropical bamboo species were made and unique traits of each species that can be used to help in their classification are presented. A complete description and comparative analysis of the anatomical characters of leaf, culm and root for *Chusquea ramosissima* and *C. tenella* are presented. Both species shared common characteristics to other members of Bambusoideae such as equal shape and position of silica cell in the leaf epidermis, presence of epidermal appendages, amphistomatic leaves and structure of culm vascular bundles. Both the species exhibited differences such as semi-massive culms, unique spatial structure of metaxylem vessels in the roots and abundant papillae in epidermal cells of *C. tenella,* or in *C. ramosissima,* second and third order leaf's vascular bundles and pericycle with more than five rows of cells.

*Key words:* Atlantic forest, bamboo, *Chusquea ramosissima, Chusquea tenella,* anatomical traits.

# Anatomical traits of woody bamboos useful for taxonomic identification: A case of study in neotropical species.

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**Abstract**: Bamboos are perennial monocarps with long flowering intervals. To use floral morphology for species identification is difficult, because floral material is not always available. Anatomical characters may be used instead of floral traits for plant identification. However, in America there are few anatomical studies on woody bamboos species. Anatomical studies on two native Neotropical bamboo species were made and unique traits of each species that can be used to help in their classification are presented. A complete description and comparative analysis of the anatomical characters of leaf, culm and root for *Chusquea ramosissima* and *Chusquea tenella* are presented. Both species shared common characteristics to other members of Bambusoideae such as equal shape and position of silica cell in the leaf epidermis, presence of epidermal appendages, amphistomatic leaves and structure of culm vascular bundles. Both species exhibited differences such as semi-massive culms, unique spatial structure of metaxylem vessels in the roots and abundant papillae in epidermal cells of C. tenella, or in *C. ramosissima* second and third order leaf's vascular bundles and pericycle with more than five rows of cells

*Key words:* Anatomy, Atlantic forest, bamboo, *Chusquea ramosissima*, *Chusquea tenella.*

## INTRODUCTION

The Poaceae are generally distinguished by characters associated to the inflorescences. Some members of this family, however, are monocarpic and flowering events occur after long periods of vegetative growth. When plants have infrequent flowering events, such as those observed in bamboos, anatomical characters can be useful for plant identification (Ellis, 1986; Londono *et al.*, 2003). The woody bamboos (tribe: Bambuseae), are abundant and diverse monocarpic grasses that exhibit flowering events recurring every 3 to 120 years (Janzen, 1976; Judziewicz *et al*., 1999). The morphology and anatomy of bamboos were described by several authors (Grosser and Liese 1971, 1973; Calderón and Soderstrom, 1973; Soderstrom, Hilu, Campbell and Barkworth, 1987; Ellis, 1986; Liese 1998; Liese and Grosser, 2000; Vieira *et al.*, 2002; Rúgolo and Rodriguez, 2002; Oliveria *et al.* 2008). Nevertheless, some species particularly in America remains poorly studied.

Bamboo differs from other grasses, in possessing a distinctive leaf anatomy that can be used for identification (Soderstrom *et al*., 1987; Londoño, 2003). The leaf epidermis surface has siliceous cells vertically oriented (Ellis, 1986; Soderstrom and Ellis, 1987;

Londoño, 2003), and in the transverse section has non- radiated mesophyll composed by arm and fusoid cells, and usually prominent midrib with complex vascular system (Soderstrom and Ellis, 1987; Judziewicz *et al*., 1999, Londoño, 2003). Other studies (*e.g*. Grosser and Liese 1971, 1973; Liese 1998; Soderstrom *et al.*, 1987; Ding and Zhao 1994; Liese and Grosser 2000), showed that the anatomy of the culm can also be useful to characterize and establish the taxonomic identity of tribes and sub-tribes of bamboo. However, the biological and anatomical information available, especially for an American woody bamboo is still incomplete and additional studies are needed.

In the Atlantic Forest of Argentina (Misiones province), *Chusquea* genus is represented by two widely distributed species, *C. ramosissima* Lindm. and *C. tenella* Nees. These species are important component of the forest understorey particularly in open natural areas and gaps created by human intervention becoming one of the most important determinants of the forest dynamics (Campanello *et al*., 2007). Both species, are very abundant and have similar distribution (southern Brazil, North-Eastern Argentina, Eastern Paraguay and Northern Uruguay) with the exception of *C. tenella* in Paraguay and *C. ramosissima* in Uruguay (Judziewicz *et al*., 1999; L. Clark, 'pers. comm.'). The utilities of these species are constrained to handicaps and tools, many medicinal and magical uses by Guaraní native people and for animal forage in the farms (Montti, unpubl. data).

Anatomical studies of these closely related species can help us to identify plant specimens without flowers. In addition, their anatomy could enhance our understanding of the adaptive significance of some biological and ecophysiological traits of the *Chusquea* spp. Here a comparative analysis of anatomical traits of *C. ramosissima* and *C. tenella* with samples collected from the Atlantic Forests in the Misiones province of Argentina is presented.

## MATERIALS AND METHODS

Samples of both species were collected in native forest near Iguazú National Park. Leaves, sheaths, culm and adventitious roots were fixed in ethanol 70° GL (Jensen, 1962) after harvest. Transverse sections of leaves, culms and roots were obtained with a rotary microtome. To characterize the internodes, transverse sections of culm internodes and sections of roots were autoclaved at 120°C for 4 h during three days in commercial glycerin and distilled water. The leaves and leaf sheaths were boiled in glycerin–alcohol 1:1 for a minute. The transverse sections were cleared in sodium hypochlorite (30%) and active clorox from one to two days. The sections were washed with distilled water and stained with Blue Alcian + Safranin 5:1 or 6:1, and were subjected to the series of alcohol solutions of increasing concentration (Johansen, 1940). Analysis of the frontal view of foliar epidermis was made to complement the anatomical observations. Small leaf samples were taken and dissociated in Franklin solution. The epidermis sections obtained were washed with distilled water and stained with aqueous methylene blue (1%) with borax (1%) which makes silica depositions easily observed. The sections were mounted in glycerin (50%). The histological descriptions were based on Metcalfe (1960) and Ellis

(1976, 1979). The sections were observed using a compound microscope (Olympus CX 31), and photographed with a digital camera (Olympus Camedia C7070).

## RESULTS

### *Chusquea ramosissima*

*Leaf transverse section: characteristics features*

*C. ramosissima* leaves were linear with little undulations on the adaxial surface and one principal vein (Fig. 1 A). Besides, it had lightly asymmetric middle leaf blades. The borders were rounded and asymmetric (Fig. 1 B, C). Both presented sclerenchymatic tissue in the margins, fibers and fundamental parenchyma associated with vascular bundles. This sclerenchymatic tissue was abundant on the abaxial surface and in the margins (Fig. 1 D, E).

The principal vein (keel) presented vascular bundles complex with vascular bundles of 1$^{st}$ order. The conductive tissue (xylem and phloem) was surrounded by two thin sclerenchymatic tissue sheaths which spread mainly along the abaxial surface and with arched shape. At both sides of the keel, fusoid cells were easily observed (Fig. 1 D).

Three types of vascular bundles were present (Fig. 1 C, D, E). The first order bundles were well developed and circular. Two incomplete bundles sheaths, with bi-seriated extensions of thin parenchymatic cells surrounded these vascular bundles (Fig. 1 D, E). The external sheath was formed by large parenchymatic cells, while the inner sheath presented small cells with thin walls. The vascular bundles of second order were very similar to those of first order; they were near the abaxial surface, with disposition 5-7 and intervenal space of more than 5 cells and were surrounded by bi-seriated and completed sheath with smaller and less lignified cells (Fig. 1 E). They were interrupted on the abaxial surface by girders of sclerenchyma. In the leaf margins, the vascular bundles of third order were little differentiated (Fig. 1 C).

The mesophyll was homogeneous, formed by arm cells, fusoid cells, and many layers of parenchymatic cells between the epidermal cells (Fig. 1 F). The arm cells contained chloroplasts and were distributed along two rows on the adaxial surface, but only one in the abaxial side. They were well developed, presenting thin and invaginated walls towards the inner area. Fusoid cells were translucent, long and narrow; they were situated at each side of the vascular bundles and in the region of the bulliforms cells. The adjacent fusoid cells were separated from one another by three or more elements of chlorenchyma.

The epidermis of the adaxial surface had thin cuticle, rectangular epidermal cells with uniform size and thickened walls in the upper periclinal walls (Fig. 1 F). The bulliform cells were translucent, inflated and forming groups of three or four elements in fan shape and can amount up to 50 per cent of the mesophyll. The silica cells were rectangular and singly distributed. They are frequently found near the fibers that surround the vascular bundles. The stomata on the adaxial surface were not very common but when present they were located at the same level as other epidermal cells with papillae that cover them and with sub-stomatic well developed cameras. The epidermis of the abaxial surface presented similar characteristics than those of the adaxial side but lacks bulliform cells and had high stomatal density (Fig. 1 F). In addition, long and aciculate macro-trichomes with bulbous base, opposite to the vascular bundles were observed.

*Leaf paradermical view: characteristic features on adaxial surface*

Leaf surface was divided in to two different areas: The costal zone formed by six rows of rectangular waved walls epidermal cells and two rows with siliceous cells. The intercostal zone consisted of nine or eleven rows of long cells with strongly waved walls, short cells, silica cells, bulliform cells, stomata and also micro and macro-hairs (Fig. 2 A).

The long intercostal cells were rectangular varying in size in different areas but they were shorter compared to the long cells of the abaxial surface. A pair of short cells was present between one or three long cells (Fig. 2 A).

The short intercostal cells were observed between the stomata and the long cells, forming pair of silica-suberous cells with crenate borders. Occasionally, they also appear isolated (Fig. 2 A). These cells were larger than the interstomatic short cells.

The bulliform cells were rectangular with sinuous and irregular walls; generally present in rows of three cells (Fig. 2 A). The stomata were situated at both sides of the costal zone and usually placed in one or two rows. One of the rows, between successive stomata had one interstomatic long cell. The other row had two, three or more interstomatic cells, occasionally separated by a pair of silica cells and short cells (Fig. 2 A). Stomata had sharply triangular with wide markedly angular subsidiary cells and the apex drawn towards the exterior. Refractive papillae were present in the guard cells. The prickle hairs were unicellular and were easily distinguished by a dilated medium sized base and a pointed apex, shorter than the base. They were spread in the costal zone as single row (Fig. 2 A). The micro-trichomes are also called micro-hair or short hair. They belong to the panicoid type; they were bi-cellular, constituted by a basal cell and an apical cell in rod form. Due to the fact that such elements have thin walls (especially in the apical cell) in many occasions they can not be seen. The macro-trichomes, also called macro-hair, were irregular, long and with very sharp extremes. They usually had thick walls and were located in the costal zone and apparently lacked specialized cells in the base.

In the costal zone, two types of silica cells in horizontal position were observed, which can be usually present in cross-shape with four apices not regularly rounded or occasionally in saddle shape (Fig. 2 A). Their vertical size was approximately the same as that of adjacent short or long cells. At the edges of intercostal zone, silica cells were observed near the stomata in vertical position. These cells were slightly rectangular or occasionally saddle shaped. Sometimes the borders were a bit crenate (Fig. 2 A). Suberous cells were present with the siliceous cells.

*Leaf paradermical view: characteristic features on abaxial surface*

The costal region was formed by six rows of rectangular epidermal cells, with crenate walls and two rows of siliceous cells. The intercostal region was constituted by twelve rows of long cells of strongly crenate walls, short cells, silica cells, stomata, micro and macro-trichomes, and prickle hairs (Fig. 2 A).

The intercostal long cells had rectangular shape of varied sizes, depending on the area. A pair of short cells was present between successive two or three long cells (Fig. 2 B). The intercostal short cells were present between the stomata and the long cells; they were like suberous-silica cell pairs of crenate borders. Occasionally they were isolated and usually located at the base of the micro-trichomes (Fig. 2 B). Bulliform cells were absent.

The stomata were very abundant in the adaxial face, and were present at both sides of the costal zone usually in three bands. There was only one interstomatic long cell between successive stomata. They had triangular shape because the subsidiary cells were broad and angular (Fig. 2 B). Similar to other species of this sub-tribe, they had refractive papillae.

Prickle hairs were distributed along the intercostal zone. The estimated size of the base was medium or large and the barb shorter than the base (Fig. 2 B). Micro-hairs were observed in the intercostal zone between the long cells; the distal cells were longer and more inflated than the basal cells, with the extremes generally rounded with dome shape (Fig. 2 B). Macro-hairs were similar to the macro-hairs from the adaxial side.

In the costal zone, there were two types of silica cells in horizontal position: cross shaped silica cells with irregular borders and similar vertical and horizontal dimensions and dumbbell shape elongated silica cells. These ones had the long axes parallel aligned to the long axis of the leaf (Fig. 2 B). At the edges of intercostal zone, silica cells were present near the stomata, in vertical position and associated with short cells. They could have a slightly rectangular shape. The vertical dimensions of these silica bodies were approximately the same than the adjacent short cells (Fig. 2 B). Suberous cells were present in pairs, together with the silica cells.

*Leaf sheath characteristics in transverse section*

The sheath had some similarities to the leaf blades already described, but differed in the involute shape with slight undulations on the abaxial surface, in the more developed central region and by the absence of the bulliform cells. The epidermis was formed by epidermal common cells, rectangular silica cells, micro, macro and prickle hairs. In the inner, epidermal cells were isodiametric and form a continuous layer. Stomata were present but were rare. The mesophyll was homogenous, with isodiametric parenchyma and without intercellular spaces. The fibers were distributed in a discontinuous form, generally associated to the vascular bundles or in sub-epidermal position in the abaxial surface. In the central region four or five vascular bundles of first and second orders were observed. They were structurally similar to those of the leaf blade and found intercalated near the abaxial surface and surrounded by two complete sheaths without extensions towards any of the surfaces (Fig. 3).

*Culm*

In transverse section, the culm of *C. ramosissima* was cylindrical and massive. It presented a thick cuticle and the epidermis was formed by square cells with lignified walls (specially the periclinal external walls), epidermal common cells, silica cells, stomata, micro-hairs and prickle hairs (Fig. 4 A) were present. In addition, the cortex was constituted by one layer of lignified sub-epidermal cells (the exodermis) and by three to four layers of parenchymatic cells of thin walls with intercellular spaces (Fig. 4 A).

The distribution of the vascular bundles was similar to other bamboos. In general, the size increases from the periphery towards the inner part of the culm, while the density decreases in the same direction due to the increase in parenchyma tissue (Fig. 4 B). Like some bamboos, the culm could be divided in three zones; the first one, also called peripheral region, presented numerous small and cylindrical vascular bundles of first order of type III (Grosser and Liese, 1971), surrounded by cortical sclerenchyma spread in the parenchymatic tissue (Fig. 4 C). The sclerenchyma was more abundant in this zone. The vascular bundles consisted of more or less developed phloem with protophloem and metaphloem, and with two metaxylem vessels (in some cases protoxylem lacunaes are visualized).

The second zone or transition region (Fig. 4 D), also had collateral vascular bundles of first order of type IV (Grosser and Liese, 1971), surrounded in small proportion by sclerenchyma sheaths developed over metaxylem, phloem and protoxylem. The third zone or central region (Fig. 4 E) was constituted by large parenchymatic cells with intercellular spaces and vascular bundles of first order of type IV. This parenchyma formed the ground tissue and contributes to the stability and flexibility of the culm. In this region, the bundles showed their highest stage of differentiation and the fibers that surround them were symmetrically located.

*Root*

The root was cylindrical, and had a thin cuticle; the epidermis was formed by common epidermal cells and long root hairs (Fig. 5 A). The external cortex was formed by exodermis with one row of squared cells (Fig. 5 B) and three rows of small parenchymatic cells with asymmetrically thickened walls, without intercellular spaces. The inner cortex was formed by seventeen rows of asymmetric parenchymatic cells. The last row of cells did not have intercellular spaces but had considerable thickened walls (Fig. 5 C). The cells of the endodermis were tightly packed, lacking conspicuous intercellular spaces. The inner walls were thick and had a U-shape. Under the endodermis, more than five rows of cells developed the pericycle (Fig. 5 C). Inside it there were one phloem arc to follow one arc of xylem and more than twenty metaxylem vessels of different sizes which were distributed in two arches. In the inner part of the root, there were homogeneous thin walls of parenchyma cells with little intercellular spaces (Fig. 5 A).

### ***Chusquea tenella***

*Leaf transverse section: characteristics features*

The leaves appeared in linear form, with small undulations on the adaxial surface (Fig. 6 A). The edges were asymmetric, one was rounded and the other acicular (Fig. 6 B, C). They had sclerenchymatic tissue, fibers and silica cells in different proportion presenting terminal trichomas at the extremes.

The keel was well differentiated and formed by two vascular bundles of first order. On the adaxial surface there was girder of sclerenchyma fibers accompanied the keel bundle, but towards the abaxial surface, the sclerenchyma was wider and more arched (Fig. 6 D). Fusoid cells were not observed in the keel region.

The leaves presented vascular bundles of first and second order. They were rounded and well developed. They were located near the abaxial surface with disposition 5-9 and intervenal distance of more than 5 arm cells. Compared with *C. ramosissima*, the protophloem and the metaxylem were less developed. The vascular bundles of first order were surrounded by two complete sheaths of cells without extensions. The external sheath was constituted by large parenchyma cells, whereas the inner sheath was constituted by smaller cells of thickened walls that may or may not be interrupted (Fig. 6 E). The sheath that surround the bundles of second order were similar to those previously described, extending towards the adaxial zone, but with smaller and less lignified cells. The sclerenchyma was discontinuous and associated to the vascular bundles, as well as to the edges of the leaves. It was more developed on the abaxial surface, but less abundant than in *C. ramosissima*.

The mesophyll was homogenous and lacks associated airspaces. It was formed by little parenchymatic cells, arm cells and fusoid cells (Fig. 6 Aa). The arm cells were arranged in two layers on the adaxial and one layer on the abaxial side, the vertical invaginations in this species are less prominent than the ones observed in *C. ramosissima*. The fusoid cells were translucent, thin and sharp at both sides of the vascular bundles. Between the adjacent cells one or two sclerenchymatic cells were present.

The epidermis (adaxial surface) was covered by a very thin cuticle. It was formed by quite uniform rectangular epidermal cells, bulliform cells, silica cells, macro-and micro-hairs, stomata, prickle hairs and papillae (Fig. 6 A). The bulliform cells were present only on the adaxial surface; they were translucent, well developed with up to 50 per cent of mesophyll. They formed groups of three in the central zone and of six towards the borders of the leaves. The silica cells were rectangular in most of the cases and distributed near the vascular bundles.
Stomata were scarce on this side, but when present they were located at the same level as the other epidermal cells with well developed sub-stomatal cameras.

The abaxial surface presented similar characteristics in comparison to the adaxial side but lacks bulliform cells, and stomata were more common (Fig. 6 F). It also presented long and aciculate macro-trichomes with bulbous base commonly located opposite the vascular bundles.

*Leaf paradermical view: characteristic features on adaxial surface*

The costal zone consisted of seven rows of rectangular epidermal cells with winding walls and two rows of silica cells. The intercostal region (Fig. 7 A) was made of seventeen rows of long cells of strongly waved walls, short cells, silica cells, bulliform cells, stomata micro-and macro-trichomes. Both sides presented numerous cuticular papillae. Intercostal long cells were rectangular, with deeply undulated walls in U-shape, they also presented papillae. Two or three successive long cells were present between a pair of short and silica cells (Fig. 7 A). The intercostal short cells were present between long cells, forming pairs of silica cells like suberous-silica cell with crenate outline.

The bulliform cells were rectangular with sinuous and irregular walls, they were in rows of three or four cells and presented papillae (Fig. 7 A). The stomata were scarce, but when present, they were located at both sides of the costal zone arranged in one row. They had three or more interstomatic long cells and some pairs of silica and short cells (Fig. 7 A). The stomata were markedly triangular, with wide and notoriously angular subsidiary cells and the apex towards the exterior.

The papillae were present in long and short cells. The cells had more than one circular papillae of small diameter arranged in a single horizontal row. The papillae, presented in the bulliform cells were circular as well, but bigger and less abundant, sometimes arranged in an irregular way (Fig. 7 A). The hooks were present but not very common, from a lateral view the barb was more developed than the base of medium size. The estimated size of the prickle hair base was medium and the barb shorter than the base. They were very common in the costal zone but rarely present in the intercostal zone. Micro-hairs were rare and occur in the intercostal region. The macro-hairs were also infrequent, unicellular and irregular. They were soft and flexible so, they were often damaged during the preparation.

Costal silica cells were not very abundant, but when present have a horizontal position and a bilobate or dumbell shape (Fig. 7 A). The intercostal silica cells were present in vertical position and associated to short cells. They had a soft rectangular or saddle shape, the vertical dimensions were approximately the same or even bigger than the adjacent short or long cells (Fig. 7 A). Suberous cells were not observed.

*Leaf paradermical view: characteristic features on abaxial surface*

The costal zone was formed by seven rows of rectangular epidermal cells with winding walls and two rows of silica cells. The intercostal region had between seventeen to nineteen rows of long cells of strongly waved walls, short cells, silica cells, stomata, micro-and macro-trichomes. The intercostal long cells were rectangular with U-shaped deeply undulated walls. They presented small circular papillae of small diameter in horizontal position but they were less abundant than in the adaxial surface. Between two or three consecutive long cells, pair of short cells and silica cells were present (Fig. 7 B). Compared with *C. ramosissima* these cells were smaller and thinner. The intercostal short cells were present between long cells, with sinuous walls, forming pair with the silica cells, although we observed them isolated (Fig. 7 B). Bulliform cells were absent.

The stomata were present at both surface of costal zone and usually distributed in three bands. There was more than one interstomatic long cell between successive stomata. They were markedly triangular, with wide, clearly angular subsidiary cells and the apex orientated towards the exterior (Fig. 7 B). The hooks were commonly present in the costal region, and occasionally in the intercostal zone. From a lateral view the apex was more developed than the base, which was considered to be medium size. Prickle hairs were very common in the costal zone where they are arranged in a single row but in the intercostal zone they were rare. The base of prickles were medium size with the barb shorter than the base (Fig. 7 B). The micro-hairs were formed by cells of approximately the same size, having the extreme of the distal cell a rounded apex. By contrary, macro-hairs were unicellular, flexible, long and thin. They were very common in the intercostal zone but not so frequently in the costal one. They were shorter and more abundant than *C. ramosissima*'s macro-hair (Fig. 7 C).

The costal silica cells were scarce, but present horizontal position and irregular dumbbell and saddle shape (Fig. 7 C). The intercostals silica cells were present in vertical position along the intercostal zone associated to the short cells like in *C. ramosissima,* and had rectangular or saddle shape. Their vertical dimension was approximately the same or bigger than that of the adjacent short or long cells (Fig. 7 B). Suberous cells were found beside silica cells.

*Leaf sheath characteristics in transverse section*

Sheath shape was involute, more developed in the central region and with slight undulations on the abaxial side (Fig. 8 A). The cuticle was thin and the epidermis was formed by common epidermal cells with lignified walls, papillae, micro-and macro-trichomes, silica cells and single thorn on the abaxial surface (Fig. 8 B). On the adaxial side the epidermal cells lack intercellular spaces and form a continuous layer. Stomata were present but were scarce (Fig. 8 B). The mesophyll was homogeneous with isodiametric parenchyma without intercellular spaces. On the abaxial surface there was a continuous layer of fibers; while on the adaxial surface a discontinuous sub-epidermal row of fibers was present. In the central region, vascular bundles of first order with circular shape were observed; they are structurally similar to those of the leaf blade. The rest of leaf sheath, presented vascular bundles of first and second order located near the abaxial surface and surrounded by two incomplete sheaths of cells. These sheaths did not present extensions towards the adaxial and abaxial surfaces and were distributed within intervenal distances of more than 5 cells (Fig. 8 A, B).

*Culm*

Distinguishing from most bamboos of the *Chusquea* genera, the culm of *C. tenella*, was semi massive (Fig. 9 A). It presented a thick cuticle with an epidermis by epidermal common cells, silica cells, stomata and prickle hairs (Fig. 9 B).The cortex was formed by one or two layers of lignified sub-epidermal cells, the exodermis and one layer of parenchymatic cells, that constitute the amiliferous sheath (Fig. 9 A). The pericycle was formed by approximately eight layers of cells and small groups of fibers between the parenchymatic tissues. The collateral vascular bundles of the peripheral region were cylindrical of first order and were embedded in cortical sclerenchyma inside the parenchymatic tissue (Fig. 9 A, B). The sclerenchyma however was more abundant in this zone but less than *C. ramosissima*. The vascular bundles had more or less developed phloem (with protophloem and metaphloem), two-metaxylem vessel at both sides of protoxylem and in some cases some protoxylem lacunae. The transition region (Fig. 9 A) also presented collateral vascular bundles of first order of type IV (Grosser and Liese, 1971) of a bigger size, surrounded by fibers, which were more developed near the metaxylem. The central region (Fig. 9 A, C) consisted mainly of a hollow marrow and some large parenchymatic cells with small intercellular spaces. The vascular bundles had little fibers generally surrounded by parenchymatic cells. The phloem was well defined and the protoxylem lacunaes were well developed.

*Root*

It was cylindrical, with a thin cuticle and an epidermis formed by common cells and with long and short root hairs at the nodal region (Fig. 10 A). The external cortex was formed by one row of squared cell, the exodermis and three rows of parenchymatic small cells with asymmetrical thickened walls but without intercellular spaces. The inner cortex was formed by eleven rows of asymmetric parenchymatic cells with intercellular spaces and aeriferous cavities. The last row of cells did not have these spaces and the cells presented a thickened, periclinal wall (Fig. 10 B). The cells of the endodermis were tightly packed together and lacked intercellular spaces. The inner walls of these cells were thickened in U-shape more prominently than in *C. ramosissima*. The pericycle was represented by three to five layers of cells, some time interrupted by phloem cells. Inside the pericycle, there was one arc of phloem surround by parenchymatic cells, followed by more than fifteen metaxylem vessels distributed in an arch and surrounded by parenchymatic tissues. Inside the arc cells of metaxylem vessels some cells of the phloem were observed. The marrow had thin-walled parenchyma cells with triangular intercellular spaces (Fig. 10 A).

## DISCUSSION

The two species were similar to other members of Bambusoideae subfamily having fusoid cells, arms cells, vascular bundles surrounded by two sheaths, long cell with thin and sinuous walls, and abundant silica accumulation, especially on the leaf epidermal tissue with silica cell. On the other hand both species had anatomical traits that were different from other bamboo species, and had traits unique to each species which can be utilized for their identification.

Silica cells are vertically oriented in bamboos, a feature used for the diagnosis of Bambusoideae (Ellis, 1987; Soderstrom and Ellis, 1999; Clark and Londoño, 1991). However, in some species of *Chusquea,* silica cells are arranged horizontally (Soderstrom and Ellis 1999, in Judziewicz *et al.*, 1999). In both species, silica cell were arranged vertically in the intercostal zone, but horizontally in the costal zone. Both, the position and shape of silica cells in the costal and intercostal zone are important anatomical traits that can be used to identify the genus *Chusquea.*

There are three types of epidermal appendages in the bamboo leaves (Calderon and Soderstrom, 1973, Soderstrom and Ellis, 1988): the micro-hairs, the prickles, and the papillae. Both species presented bi-cellular micro-hairs with thin walls and caduceus extremes. The prickles were unicellular, with dilated base and pointed apex that were easily identified in the costal zone. The presence of papillae seems to be an exclusive character of certain tribes among Bambusoideae (Calderon and Soderstrom, 1973; Soderstrom and Ellis, 1988; Judziewicz *et al.*, 1999; Rúgolo *et al.*, 2002) that can be used as taxonomic information at the species, genera and sub-tribe levels (Clark, 1986; Soderstrom and Ellis, 1999). For example, members of sub-tribe Guaduinae have distinctive combination of papillae associated with stomata on the abaxial surface, while the adaxial surface has subsidiary cells that lack papillae (Judziewicz *et al*., 1999). The members of sub-tribe Chusqueinae apparently lack refractive papillae but can have papillae associated with the subsidiary cells, a trait unique to Chusquinae (Judziewicz *et al*., 1999). Both species had refractive papillae associated with the subsidiary cells, but *C. tenella* also had numerous papillae in epidermal cells; a trait absolutely unique to *C. tenella*.

Bamboos are different from other grasses in having well developed and easily distinguishable leaf vascular tissue (Brandis, 1999; Calderon and Soderstrom, 1973; Soderstrom and Ellis, 1999; Judziewicz *et al*., 1999). The vascular tissue of leaves was conspicuous in both species, with one or two vascular bundles surrounded by sclerenchyma along the veins. Although, the species differ in the leaf vascular anatomy, *C. tenella* leaves had first and second order bundles with well defined protophloem and poorly developed metaxylem, while *C. ramosissima* in addition had third order bundles along the leaf margins.

The stomata are very important as character for taxonomic studies, but also have ecological relevance. The abundance and distribution of stomata are strongly influenced by the ecological conditions in which the plant lives (Ellis, 1979). Most bamboos, with the exception of the Guaduinae tribe that contains a large number of stomata on both surfaces, have the greatest concentration of stomata usually on the abaxial surface of the leaves (Renvoize, 1985; Judziewicz *et al*., 1999). This could give bamboos certain adaptive advantage for survival in the understory of forests (Renvoize, 1985). The leaves of the species studied were amphistomatic, although, the stomata were more abundant on the abaxial surface and were arranged in longitudinal rows.

The culm tissue consisted of parenchymatic cells, sclerenchyma and vascular bundles. Like the majority of bamboo species, the vascular bundles of the culm of both *Chusquea* species had two big metaxylem vessels with one or two protoxylem elements, and phloem surrounded by sclerenchyma fibers. In this study, we did not compare different internodes in the same culm, but it is known that the bundles differ considerably in size, according to their position along the culm (Liese, 1980). In both species of *Chusquea* the size of vascular bundles increased from the periphery towards the inner part of the culm and the vascular bundles were distributed irregularly. According to the classification proposed by Liese and Grosser (2000), considering the form of vascular bundles, *C. ramosissima* and *C. tenella* correspond to the types II, IV and subtype IVa. The difference between these species was that the culm of *C. ramosissima* is cylindrical and massive, while *C. tenella* had slender culms with less abundance of sclerenchyma tissue in the peripheral region.

Bamboo root anatomy has received scant attention (Raechal and Curtis, 1990) but their anatomy could help to differentiate some genera (Makino and Shibata, 1901; Raechal and Curtis, 1990). Some works seem to indicate that little variation occurred in bamboo root (Raechal and Curtis, 1990). However, we found that we can use some traits of root anatomy to distinct *C. ramosissima* from *C. tenella*. In particular *C. tenella´s* roots had aerenchymatous cells, short and long hairs and fewer metaxylem vessels than *C. ramosissima*. Metaxylem vessels in *C. tenella* were organized along a single arch unlike other bamboo species where the metaxylem vessels were organized along two arches. The phloem tissue in *C. tenella,* is less abundant, and has smaller cells.

**CONCLUSION**

In this study, we pointed out some anatomical variations of *C. ramosissima* and *C. tenella* that can help for identification and to recognize other aspects that are common in the Bambusoideae taxonomy. Similar to other members of the Bambusoideae, these two species had conspicuous fusoid cells, arms cells, vascular bundles surrounded by two sheaths, long cell with thin-sinuous walls, and abundant silica accumulation, especially on the leaf epidermal tissue. Common characteristic shared by both species were: shape and position of silica cell in the epidemic leaves, the same type of epidermal appendices (bi-cellular micro-hairs, unicellular prickles and refractive papillae in subsidiary cells), amphistomatic leaves (with more density of stomata on the abaxial surface) and culm´s vascular bundles of types II, IV and subtype IVa (Table 1).

*C. tenella* is distinguished from most bamboos of the *Chusquea* genera because their culm was semi-massive and metaxylem vessels in root were distributed in one arch. Additional anatomical traits that are helpful for classification include: vascular bundles of leaf of I, II and III order in *C. ramosissima* and pericycle constituted by more than five rows of cells. In *C. tenella*, abundant, papillae in epidermal cells, vascular bundles of leaf of I and II order; root with aerenchyma tissue, pericycle formed by three to five rows of cells and less metaxylem vessel than in *C. ramosissima*.

Lastly, this study represents the first complete description of anatomical traits of *Chusquea* bamboos from the Upper Paraná Atlantic Forest region. The descriptions confirm the utility of anatomical studies as important tools for species identification.

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## TABLE

Table 1. Anatomical traits of the woody bamboos *Chusquea ramosissima* and *C. tenella*.

| | | ***C. ramosissima*** | ***C. tenella*** |
|---|---|---|---|
| **Leaf** | *Leaf shape* | linear, little undulation | linear, little undulation |
| | *Borders* | rounded | rounded and acicular |
| | *Epidermic cells* | EC, BC, ST, Mih, SC | EC, BC, ST, Mih, SC, Pih, Pap |
| | *Silica cell* | vertically and horizontally | vertically and horizontally |
| | *Stomata* | amphistomatic | amphistomatic |
| | *Bulliform cell* | inflated, forming groups of 3-4 in fan shape | translucent, well developed, forming groups of 3 |
| | *Arm cell* | 2 rows on the adaxial side, 1 in the abaxial side | 2 rows on the adaxial side, 1 in the abaxial side |
| | *Fusoid cell* | translucent, long and narrow. | translucent, thin and sharp |
| | *Vascular bundles* | 1°, 2° and 3° order | 1° and 2° order |
| | *Keel* | 2 vascular bundles of $1^{st}$ order | 2 vascular bundles of $1^{st}$ order |
| | *Sheaths of VB* | 2 with bi-seriated extensions | 2 can extending to the adaxial zone |
| | *Disposition* | 5 a 7 | 5 a 9 |
| | *Localization* | near the abaxial surface | near the abaxial surface |
| | *Intervenal space* | + of 5 cells | + of 5 cells |
| **Leaf sheath** | *Shape* | involute, slight undulations | involute |
| | *Epidermic cells* | EC,SC, Mih, Mah Pih. | EC,SC, Mih, Mah, Pih, Pap |
| | *Mesophyll* | homogenous | homogenous |
| | *Parenchyma* | isodiametric, without intercellular spaces. | isodiametric, without intercellular spaces. |
| | *Fibers in the BV* | discontinuous, in sub-epidermal position in the abaxial surface | abaxial surface: continuous layer of fibers. adaxial surface: discontinuous layer of fibers |
| | *Vascular bundles* | 1° and 2° order | 1° and 2° order |
| | *Sheaths of VB* | two complete sheaths | two incomplete sheaths |
| **Culm** | *Shape* | cylindrical and massive | cylindrical and semi massive |
| | *Cuticle* | thick | thick |
| | *Epidermic cells* | lignified, Ph; Mah, Mih EC, SC, ST. | lignified, Pih; Mah, Mih EC, SC |
| | *Cortex* | exodermis and 3-4 layers of parenchymatic cells, without fibers | exodermis and 1 layer of parenchymatic cells with fibers |
| | *Amiliferous sheath* | no present | present |
| | *Pericycle* | no | eight layers of cells and small groups of fibers |
| | *Peripheral region* | VB of first order of type III | VB of first order |
| | *Transitional region* | VB of first order of type IV | VB of first order of type IV |
| | *Central region* | VB of first order of type IV | hollow. VB had little fibers. lacunae |
| **Root** | *Epidermic cells* | EC, long root hairs | EC, long and short root hairs |
| | *External cortex* | exodermis 1 row of squared cells and 3 rows of parenchymatic cells | exodermis 1 row of squared cells and 3 rows of parenchymatic cells |
| | *Internal cortex* | 17 rows of parenchymatic cells. The last row of cells did not have intercellular spaces | 11 rows of parenchymatic cells. aeriferous cavities. The last row of cells did not have intercellular spaces |
| | *Endodermis* | The inner walls thicked in U-shape | The inner walls thicked in U-shape |
| | *Pericycle* | + of 5 rows of cells | 3-5 rows of cells |
| | *Metaxylem arches* | + of 20 metaxylem vessels in two arches | + of 15 metaxylem vessels in one arc. |
| | *Marrow* | homogeneous with little intercellular spaces | homogeneous with little intercellular spaces |

EC: epidermal cell, BC: bulliform cell, SC: silica cell, AC: arm cell, FC: fusoid cell, Mih: micro-hair, Mah: macro-hair, Pih: prickle hair, Pap: papillae, ST: stomata, VB: vascular bundles.

**FIGURE LEGENDS**

**Figure 1. Foliar leaf blade transverse section of *C. ramosissima***. A- General view. 20X. B and C- Leaf blade borders and epidermal fibers 40X. D- The keel: vascular bundle of 1$^{st}$ order, (BC) bulliform cell, (SC) silica cell, (AC) arm cell, (FC) fusoid cell, (Sch) sclerenchyma tissue, (Ph) phloem, (MX) metaxylem. 40X. E- Discontinued sclerenchyma in sub-epidermal position and adaxial girder (GrSch).Vascular bundle surrounded by inner and outer bundle sheaths (IBS, OBS). 40X. F- Homogeneous mesophyll, fusoid cell (FC) and stomata in adaxial surface (ST). 40X scale bar = 1mm.

**Figure 2. Foliar leaf blade paradermical section of *C. ramosissima*.** A- General view of adaxial surface, showing the costal (CZ) and intercostal zone (IZ), silica cell (SC) in vertical and horizontal position, stomata (ST), long cell (LC) and bulliform cell (BC). 10X scale bar = 1mm. B- General view of adaxial surface, showing the costal (CZ) and intercostal zone (IZ), silica cell (SC) in vertical and horizontal position, stomata (ST), long cell (LC) with sinuous walls, short cell, micro-hair (Mh) and prickle hair (Pih). 10X Scale bar = 1mm.

**Figure 3. *C. ramosissima* sheath in transverse section**. General view. Detail in zoom show the epidermis and their component, the mesophyll with the vascular bundles and borders. 10X scale bar = 1mm.

**Figure 4. *C. ramosissima* Culm cross section.** A- Epidermis constituted by square cell with lignified walls and epidermal common cells (EC). Stomata (ST). Cortex constituted by parenchymatic cells of fine walls. 40X. B- General view. 10X. C- Peripheral region and vascular bundles surrounded by fibers (Fr). 40X. D- Transition region and collateral vascular bundles. 40X. E- Central region: constituted by parenchymatic tissue with intercellular spaces and vascular bundles of I order. 10X. scale bar = 1mm.

**Figure 5. *C. ramosissima* root cross sections.** A- General view: metaxylem vessels distributed in two arches, marrow and cortex with root hairs showed in zoom. 4X. B- Exodermis composed of epidermal cell and long root hair. Hypodermis formed by one row of squared cell. 40X. C- Inner cortex: see the last row of cell without intercellular spaces and the periclinal wall with an important thick. 40X. scale bar = 1mm.

**Figure 6. *C. tenella* Foliar leaf blade transverse section.** A- General view. 40X. B- Leaf blade border. 40X. C- Leaf blade border. 40X. D- The keel with two vascular bundles of 1$^{st}$ order, a very broad and arched sclerenchyma on the abaxial surface, (Ph) phloem, (MX) metaxylem and (EC) epidermal cell. 40X. E- Vascular bundle surrounded by inner and outer sheaths (IBS and OBS), fusoid cell (FC) and bulliform cell (BC). 40X. F- General view of stomata, fusoid and bulliform cells distribution. 40X. scale bar = 1mm.

**Figure 7. *C. tenella* Foliar leaf blade paradermical section.** A- General view of adaxial surface showing the costal (CZ) and intercostal zone (IZ), interstomatal cell, stomata (ST), bulliform cell (BC), silica cell (SC) in vertical and horizontal positions and

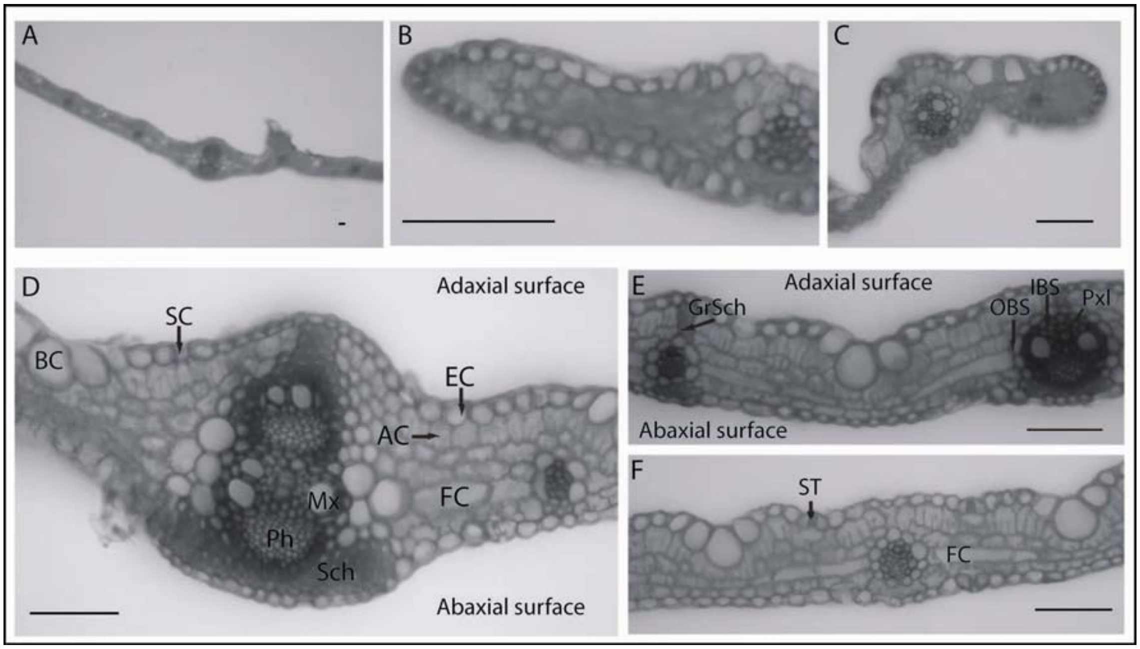
papillae.10X. B- General view of abaxial surface, showing the costal and intercostals zone, silica cell and prickle hair that are very common in the costal zone. 10X. Detail show: stomata, two types of silica cell, long cell with papillae and short cell. 40X. C- Details in phase contrast show unicellular soft flexibility macro-hair. 40X. scale bar = 1mm.

**Figure 8. *C .tenella´*s sheath in transverse section.** A- General view. 4X. B- Detail shows: the thin cuticle, fiber and vascular bundle surrounded by two sheaths of cells. 10X. Scale bar = 1mm.

**Figure 9. Culm cross sections of *C. tenella*.** A- General view of peripheral, transition and central region and the distribution of vascular bundles, fiber and parenchymatic tissue. 4X. Detail shows the vascular bundle. B- Cortex with stomata (ST) in detail and amiliferous sheath. 40X. C- Central region and vascular bundles. 10X. Scale bar = 1mm.

**Figure 10. Root cross sections of *C. tenella*.** A- General view: metaxylem vessels distributed in two arches. 10X. B- Epidermis constituted by common epidermal cell and long root hair and hypodermis. The inner cortex presents the last row of cell without intercellular spaces and the periclinal wall with and important thick. The inner walls of endodermis cells are thickened in U-shape. 40X. Scale bar = 1mm.

**FIGURES**

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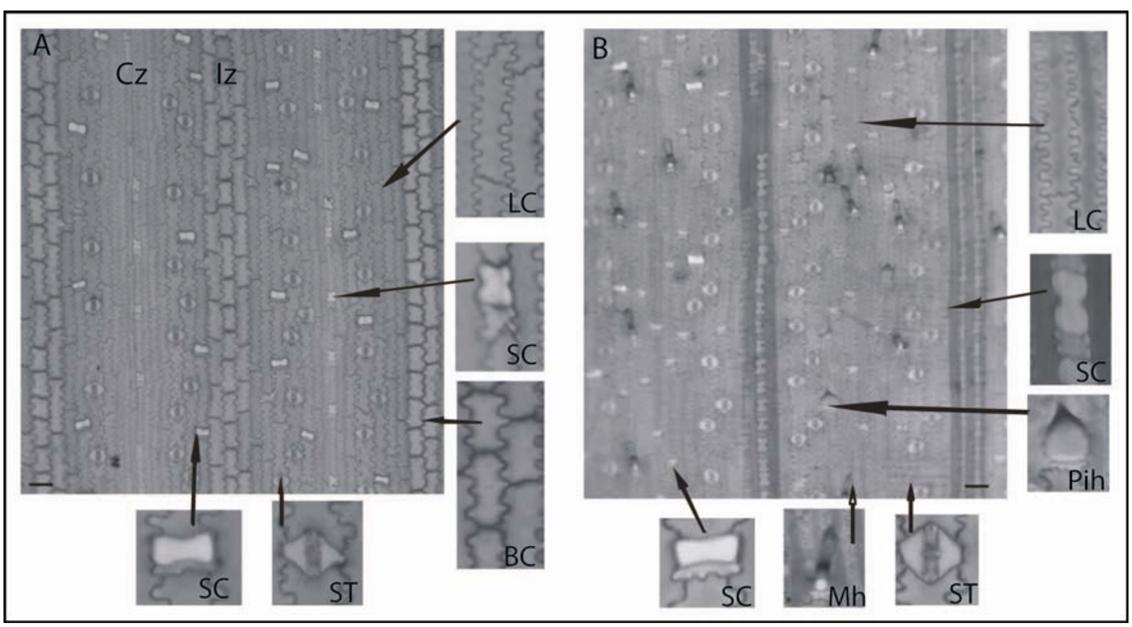


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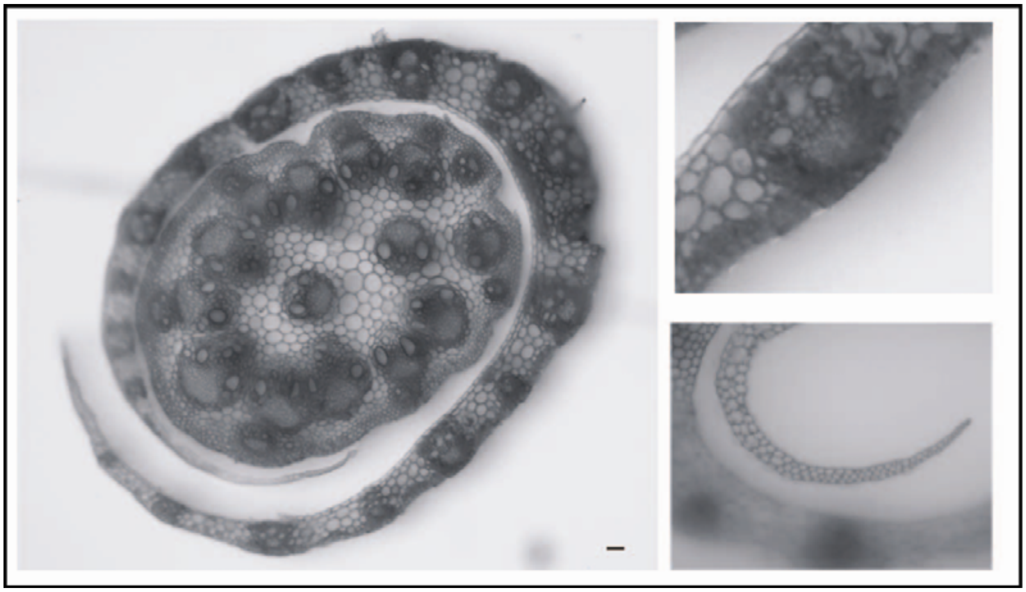

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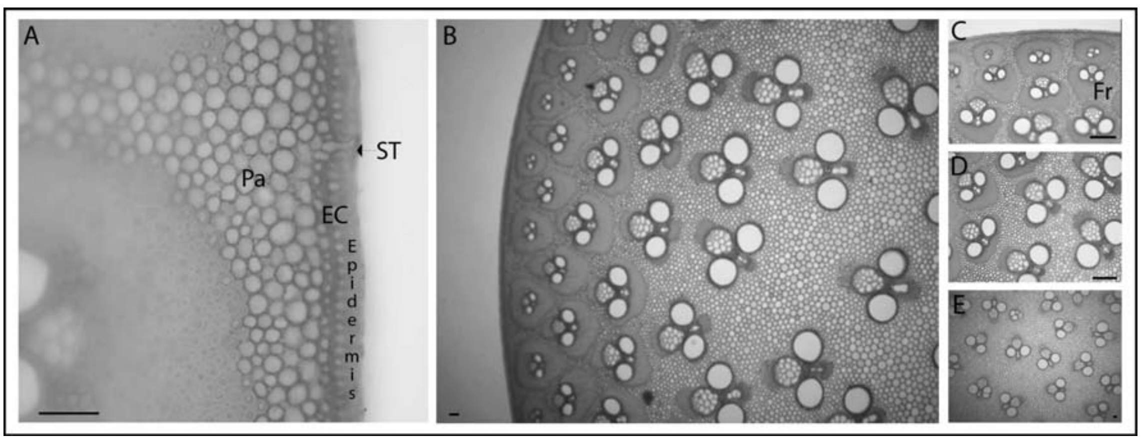


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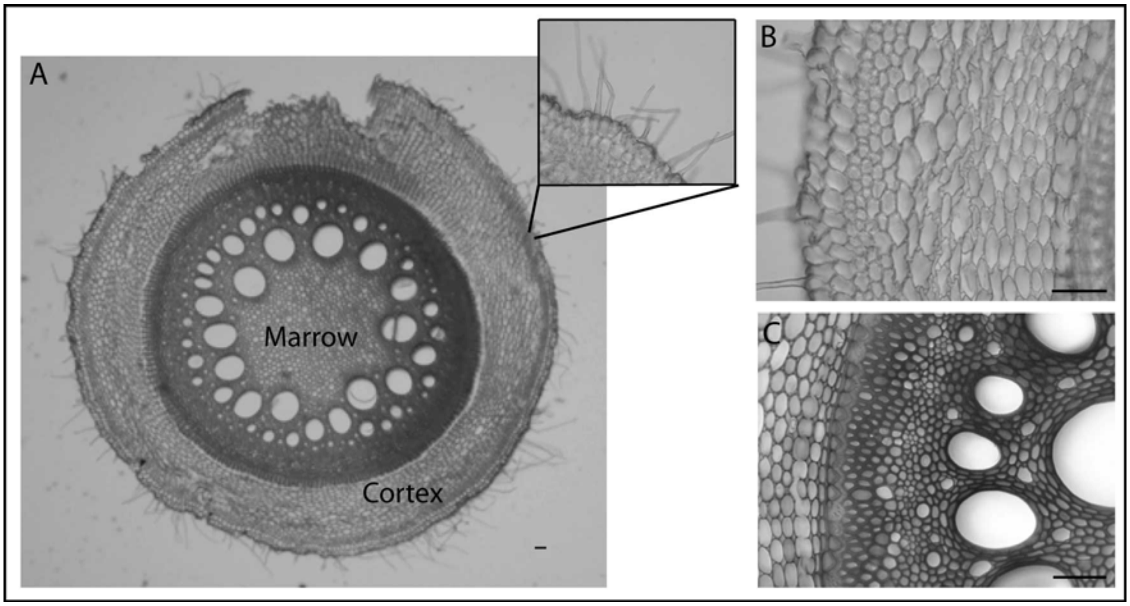


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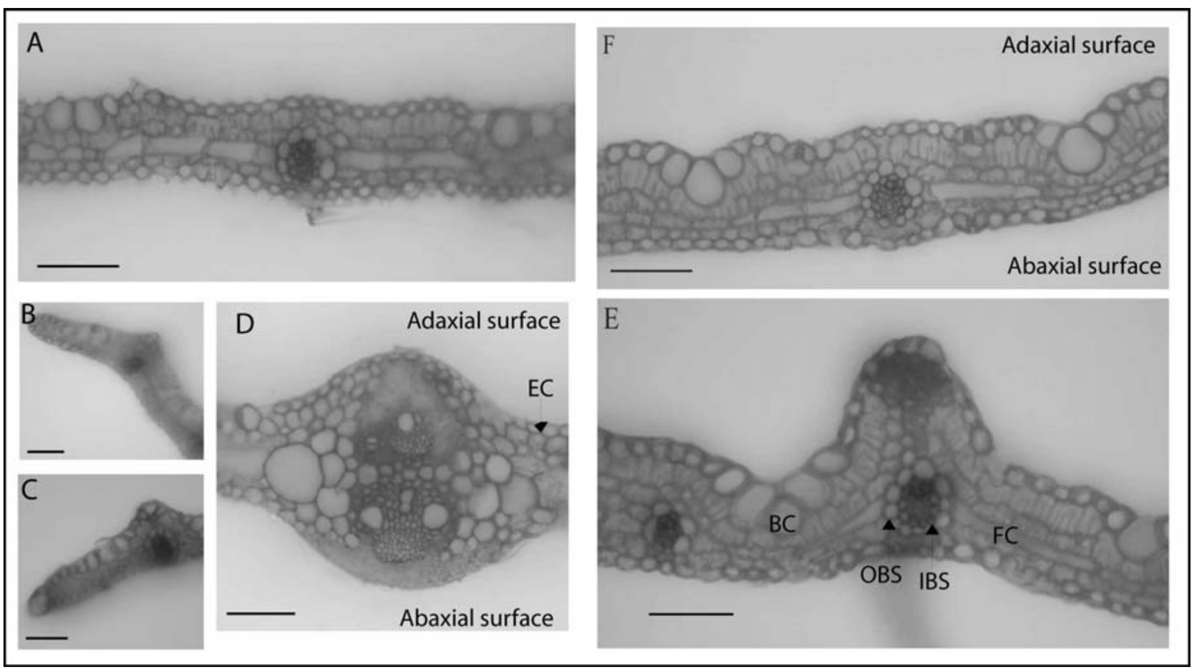


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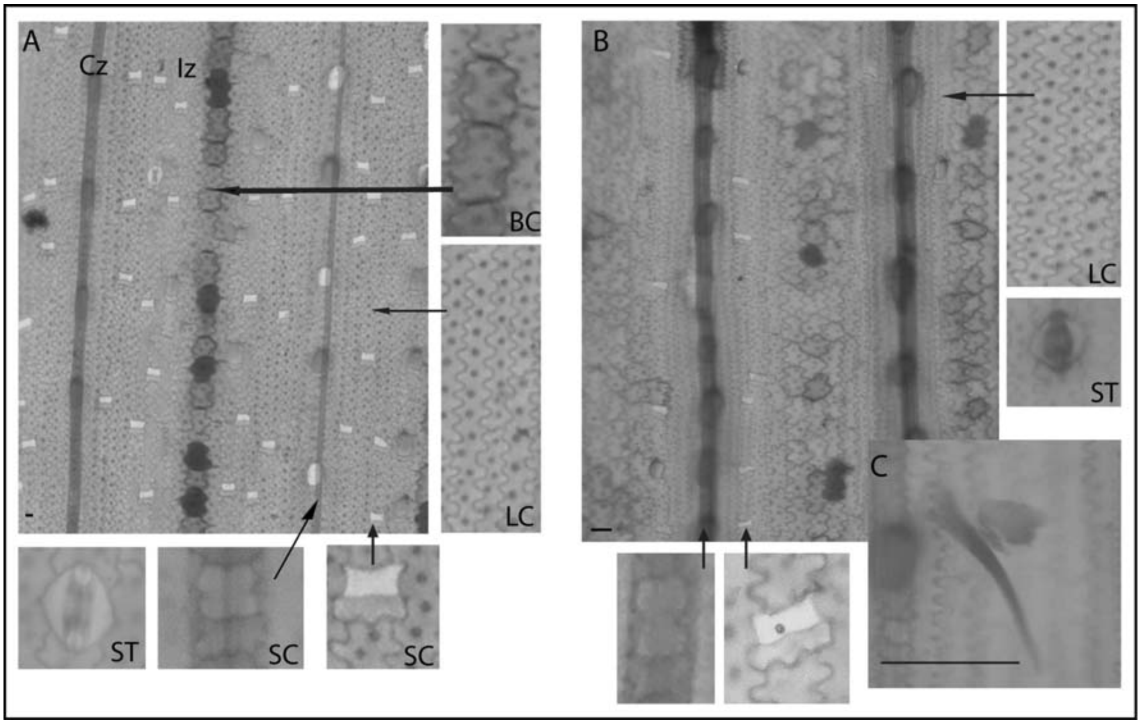


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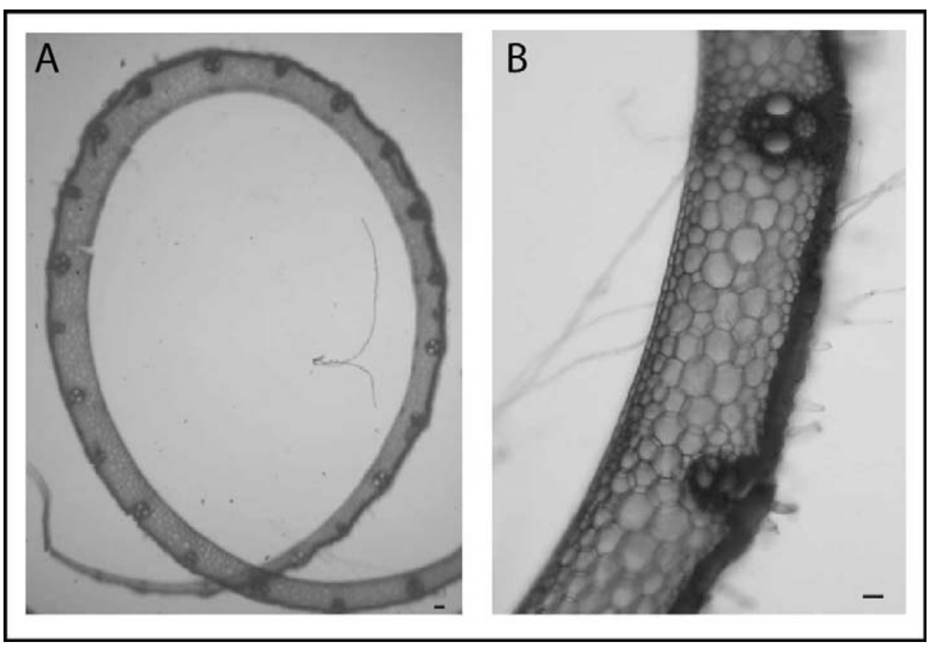


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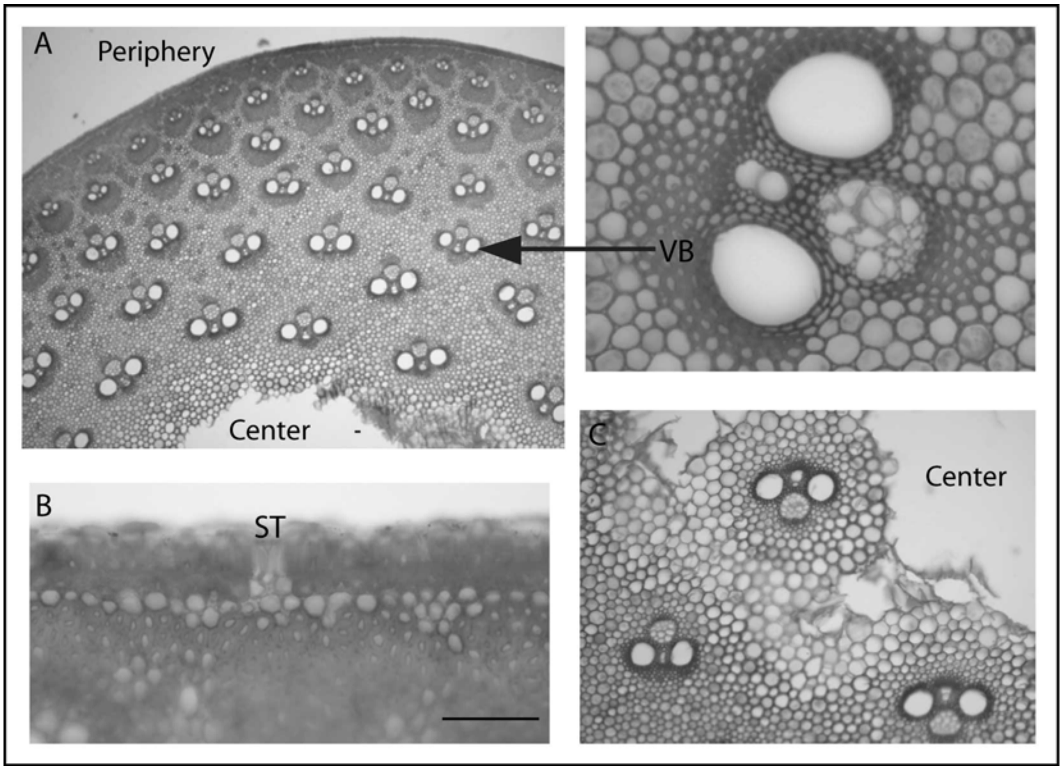


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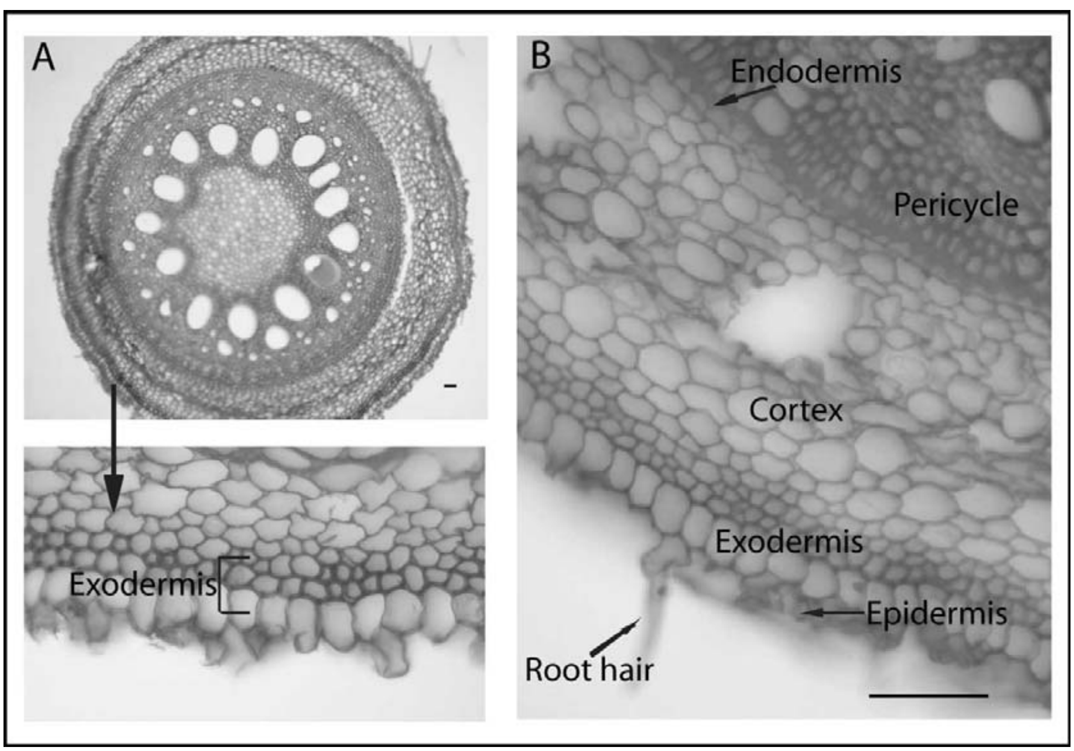


Figure 10.