

# Culm anatomy: a contribution to the identification of vegetative Andean woody bamboos in southernmost America

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**Summary.** Six genera of woody bamboos (Poaceae, Bambusoideae, Bambuseae) occur along the Andes, but only two reach the southernmost portion of this mountain range, *Chusquea* Kunth and *Rhipidocladum* McClure. In woody bamboos, the identification of new anatomical characters bears a considerable taxonomic significance contributing to the determination of vegetative material. In the present work, culm anatomy of Andean woody bamboo species occurring in Argentina and neighbouring areas is described. Eight species are surveyed: *C. culeou* E. Desv., *C. deficiens* Parodi, *C. lorentziana* Griseb., *C. montana* Phil., *C. quila* Kunth, *C. valdiviensis* Phil., *R. neumannii* Sulekic, Rúgolo & L. G. Clark and *R. racemiflorum* (Steud.) McClure. Culm epidermal and cross sectional characters of each species are described and an identification key based on anatomical characters is provided. Culm anatomical characters of *C. montana*, *C. quila* and *C. valdiviensis* are presented for the first time. Also, anatomical evidence that support the idea of *C. quila* and *C. valdiviensis* as different species is presented and information on useful characters to distinguish between them is provided. The taxa studied are compared in tables based on culm anatomical characters of taxonomic value.

**Key Words.** Argentina, Bambuseae, Chile, cross section, epidermis.

## Introduction

Bamboos occur in almost every tropical, subtropical and temperate forest around the world. Neotropical woody bamboos range from northern Mexico to southern Argentina and Chile. In the fast disappearing Andean montane forest, bamboo is usually found along forest edges or in gaps. Bamboo can be the dominant plant in *páramo* regions. Also, in cool temperate southern America, woody bamboos are well known as understory dominants in beech forests (Judziewicz *et al.* 1999).

Six genera of woody bamboos occur in the Andes with an estimated 130 species, 90% of which are endemic (Clark 1995). Only two genera, *Chusquea* Kunth and *Rhipidocladum* McClure, reach the southernmost portion of the Andes. Approximately 18 species of woody bamboos occur in the austral Andes of Argentina, southern Bolivia and Chile (Renvoize 1998; Judziewicz *et al.* 2000; Morrone *et al.* 2008; Guerreiro & Rúgolo 2012; Rúgolo & Vega 2012).

*Chusquea* comprises an estimated 159 species; it is the largest bamboo genus in the world (Fisher *et al.* 2009). The genus is defined by papillate subsidiary cells in the foliar stomatal complexes in addition to its uniform spikelet structure comprising four glumes (two glumes and two sterile lemmas), one female-fertile floret and the absence of a rachilla extension (Clark 1997, Fisher *et al.* 2009). Primarily montane, it has the widest latitudinal

range of any bamboo genus, 24°N in Mexico to 47°S in Chile, and the widest altitudinal range, from 0 to over 4000 m. All major groups within this genus occur in the Andes, thus establishing this area as a primary centre of diversity (Clark 1995).

*Rhipidocladum* species range from Mexico to Argentina and from sea level to 2900 m, in humid forests or on forests margins (Judziewicz *et al.* 1999). There are 19 described species, which tend to have fairly restricted distributions, except for *Rhipidocladum racemiflorum* (Steud.) McClure which ranges from central Mexico down to northwestern Argentina (Clark 1995).

Parodi (1941) reviewed the species of *Chusquea* from Argentina, describing two new species, *C. deficiens* Parodi and *C. argentina* Parodi, which was later considered a synonym of *C. culeou* E. Desv. (Judziewicz *et al.* 1999). Sulekic *et al.* (1999) reviewed the genus *Rhipidocladum* for Argentina, describing a new species, *R. neumannii* Sulekic, Rúgolo & L. G. Clark, for northwestern Argentina and southern Bolivia.

In Argentina, Andean woody bamboo species occur in two distinct areas:

1. The high montane forest in northern Argentina known as *Yungas*. This ecosystem forms the southern end of a strip of montane forest that runs along the Andes of South America. Here, *Chusquea deficiens*,

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*C. lorentziana* Griseb., *Rhipidocladum neumannii* and *R. racemiflorum* occur (Guerreiro *et al.* 2011; Guerreiro & Rúgolo 2012; Rúgolo & Vega 2012).

2. The Andean-Patagonian beech forest of southern Argentina. The only genus present in this area is *Chusquea* with three species: *C. culeou*, *C. montana* Phil. and *C. valdiviensis* Phil. (Guerreiro & Rúgolo 2012).

The identification of woody bamboos is mainly based on vegetative characters because most of them have sporadic flowering, after long vegetative periods (Judziewicz *et al.* 1999). However, there are only a few studies dealing with the culm anatomy of woody bamboos from southern South America. The presence of woody bamboos at archaeological sites in Argentina motivated anatomical studies for their identification. Species of the genera *Chusquea* and *Rhipidocladum* dated among archaeological material from 8600 – 4700 years BP have been identified from culm cross sections. In two archaeological sites located in northwestern Argentina (Antofagasta de la Sierra, Province of Catamarca), several artifacts were recovered, including handles made with *C. lorentziana* and two culm fragments of *R. neumannii* with signs of use. The material was recovered in very good condition, without evidence of biodegradation, thus comparative anatomical studies with current material were possible (Rodríguez 1997; 1999a; 1999b). Rúgolo de Agrasar & Rodríguez (2002; 2003) described the characteristics of culm epidermis and cross section for fifteen species belonging to six genera of native woody bamboos of South America, including *C. culeou*, *C. lorentziana*, *R. neumanni* and *R. racemiflorum* in their study. Guerreiro *et al.* (2011) described culm characters of *C. deficiens*, a rare woody bamboo native to northwestern Argentina.

The present work continues our previous research on culm anatomy for the Andean woody bamboo species occurring in Argentina and neighbouring areas: *Chusquea culeou*, *C. deficiens*, *C. lorentziana*, *C. montana*, *C. valdiviensis*, *Rhipidocladum neumannii* and *R. racemiflorum*. In this study, we will also include *Chusquea quila* Kunth since its presence in Argentina is not clear (Parodi 1945; Zuloaga *et al.* 1994; Judziewicz *et al.* 2000; Morrone *et al.* 2008; Guerreiro & Rúgolo 2012). Epidermal and cross sectional characters are described and illustrated in order to enhance the current descriptions of the species and identify characters of taxonomic value, to be used in the determination at genus and/or species level. Culm characters of *C. montana*, *C. quila* and *C. valdiviensis* are presented for the first time.

## Material and methods

In order to analyse culm anatomical structure of woody bamboo species, herbarium material from SI

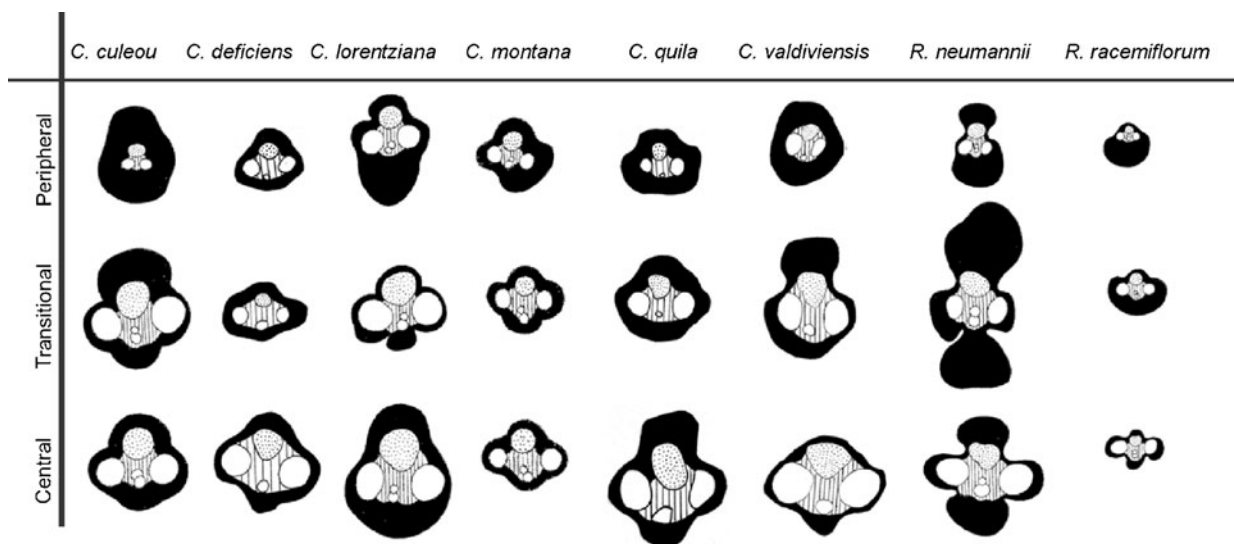
(Thiers 2012) and material preserved in 70% alcohol were used. The middle third of the internodes of developed culms was used. For epidermal studies, small epidermal fragments of culms were placed in glass tubes with xylol and exposed to ultrasound for approximately 2 h to eliminate superficial wax and impurities. The material was dehydrated and coated with gold-palladium. Photomicrographs of culm epidermis were obtained using a scanning electron microscope Philips XL30 TMP at the Museo de Ciencias Naturales 'Bernardino Rivadavia' in Buenos Aires, Argentina. The following superficial characters were observed: ribs and furrows, long cells, papillae, stomatal apparatus, silica bodies, prickle hairs, microhairs and macrohairs (Ellis 1979; Rúgolo de Agrasar & Rodríguez 2002).

In order to obtain culm cross sections, the material was placed in plastic tubes with 4% ethylenediamine for several days before making the cuts. Cross sections were obtained using a sliding microtome, stained with safranin and fast-green and mounted in Canada balsam (D'Ambrosio de Argüeso 1986). Culm cross sections were observed and photographed with a light microscope Nikon Microphot FXA. For descriptions, the following characters were considered: epidermis, hypodermis, sclerenchyma and vascular bundles (peripheral, transitional and central). The transitional vascular bundles are situated towards the middle of the culm walls. The number of vascular bundle cycles, their position and the size and shape of peripheral, transitional and central vascular bundles were also considered. To determine the position of vascular bundles, the phloem location in relation to the stem cortex was taken into consideration (Xishen *et al.* 2002; Rúgolo de Agrasar & Rodríguez 2003) and was drawn up diagrammatically (Fig. 1). The shape of the central vascular bundles was recorded based on Stearn's (1983) terminology, taking into consideration the most extreme points in their contour.

## Results

CULM ANATOMY: CROSS SECTION (Figs 1, 2 and 3; Table 1)

*Chusquea culeou*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 4 layers of thick walled cells. Vascular bundles in 4 – 5 cycles. Phloem facing different directions. Peripheral vascular bundles completely surrounded by sclerenchyma. Transitional vascular bundles surrounded by a continuous sclerenchymatic sheath with caps in connection with phloem, protoxylem and metaxylem more developed in relation to the phloem. Central vascular bundles 230 µm wide and 158 µm long, ovate, depressed,



**Fig. 1.** Variability in the shape of peripheral, transitional and central vascular bundles in *Chusquea* and *Rhipidocladum*. These are shown according to their orientation with the epidermis. Note: partially taken from Rógolo de Agrasar & Rodríguez (2003).

surrounded by a discontinuous sclerenchymatic sheath more developed in relation to the phloem and protoxylem (Fig. 2A).

*Chusquea deficiens*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 3 – 4 layers of thick walled cells. Vascular bundles in 4 – 5 cycles. Phloem oriented towards the external face. Peripheral, transitional and central vascular bundles ovate, depressed surrounded by a continuous sclerenchymatic sheath of 2 – 3 cells thick. Central vascular bundles 216  $\mu\text{m}$  wide and 144  $\mu\text{m}$  long (Fig. 2B; Guerreiro *et al.* 2011).

*Chusquea lorentziana*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 1 – 3 layers of thin walled cells. Vascular bundles in 5 cycles. Phloem always oriented towards the external face of the culm. Peripheral vascular bundles completely surrounded by sclerenchyma, more developed in connection with the protoxylem and the metaxylem. Transitional vascular bundles surrounded by a continuous sclerenchymatic sheath, greater at protoxylem level, in a crescent-shaped cap. Elliptic central vascular bundles, 340  $\mu\text{m}$  wide and 374  $\mu\text{m}$  long, with a perivascular sheath of fibres and two remarkable crescent-shaped caps of similar development formed by 10 – 12 cells, thickest on phloem and protoxylem sides (Fig. 2C; Rógolo de Agrasar & Rodríguez 2003).

*Chusquea montana*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 7 – 8 layers of thin walled cells. Vascular bundles in 9 – 10 cycles. Phloem facing different directions. Peripheral and transitional vascular bundles surrounded by a continuous sclerenchymatic

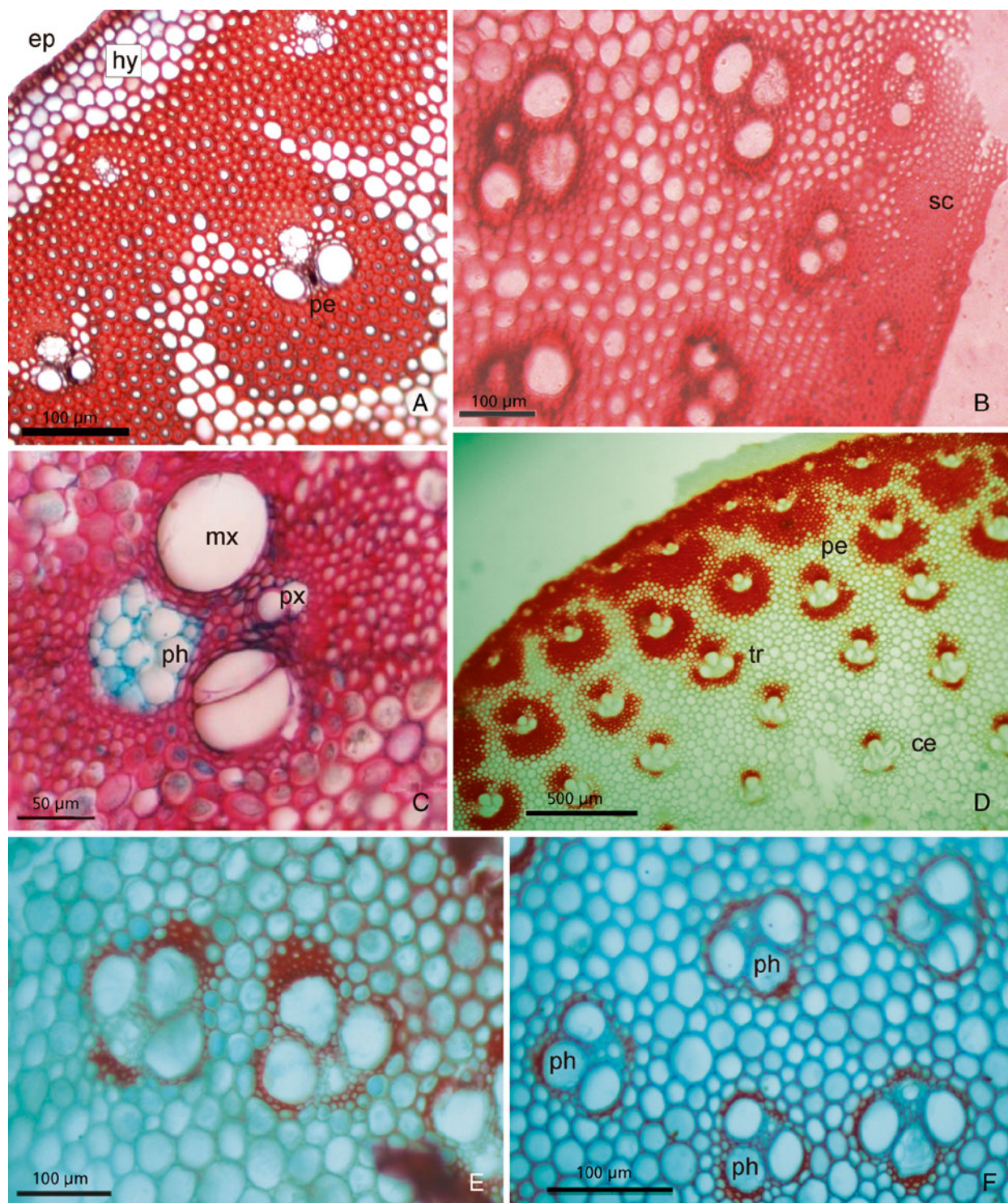
sheath with caps in relation to the phloem, protoxylem and metaxylem. Central vascular bundles 180  $\mu\text{m}$  wide and 209  $\mu\text{m}$  long, ovate, or depressed (293  $\mu\text{m}$  wide and 225  $\mu\text{m}$  long), with two crescent-shaped caps of sclerenchyma of similar development (2 – 3 cells thick) on phloem and protoxylem sides (Fig. 2D).

*Chusquea quila*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 3 layers of thin walled cells. Vascular bundles in 7 – 8 cycles. Phloem facing different directions. Peripheral and transitional vascular bundles completely surrounded by a continuous sclerenchymatic sheath of similar development on protoxylem, metaxylem and phloem sides. Elliptical central vascular bundles, 151  $\mu\text{m}$  wide and 118  $\mu\text{m}$  long, with a continuous sclerenchymatic sheath more developed (3 – 4 cells thick) in connection with the phloem (Fig. 2E).

*Chusquea valdiviensis*: Culm solid. Epidermis formed by a layer of sclerified cells with thick external wall. Hypodermis formed by 4 layers of thick walled cells. Vascular bundles in 8 cycles. Phloem oriented in different directions. Peripheral vascular bundles completely surrounded by a continuous sclerenchymatic sheath of similar development on protoxylem, metaxylem and phloem sides. Transitional vascular bundles with a continuous sclerenchymatic sheath more developed in connection with the phloem. Depressed central vascular bundles, 94  $\mu\text{m}$  wide and 72  $\mu\text{m}$  long, ovate, with a single continuous layer of sclerenchymatic cells (Fig. 2F).

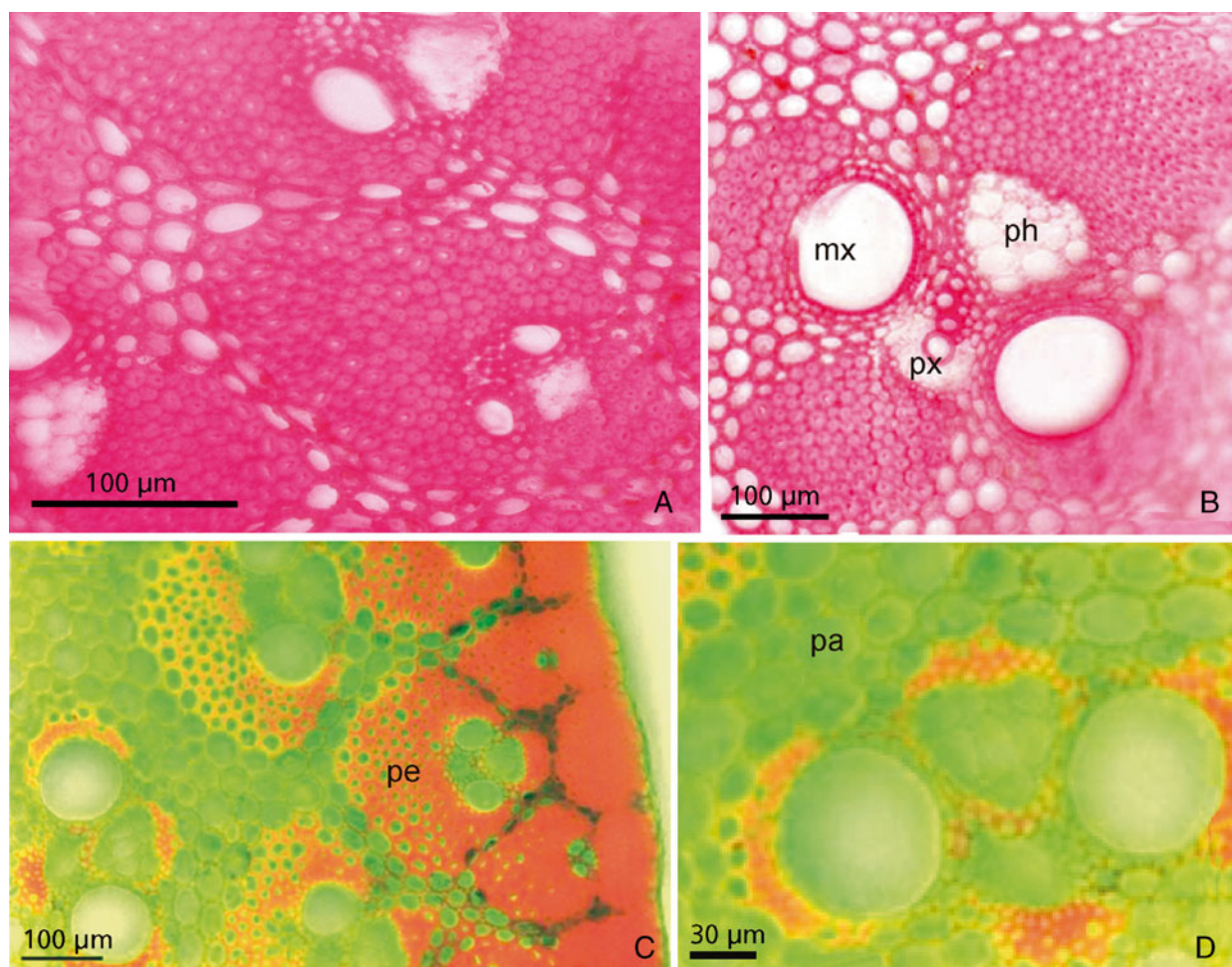
*Rhipidocladum neumannii*: Culm hollow. Epidermis formed by a layer of papillate epidermal cells with thick





**Fig. 2.** Culm cross section light microscope micrographs. **A** *Chusquea culeou*, epidermis, hypodermis and peripheral vascular bundles; **B** *C. deficiens*, general view; **C** *C. lorentziana*, central vascular bundle; **D** *C. montana*, general view; **E** *C. quila*, central vascular bundles; **F** *C. valdiviensis*, central vascular bundles with phloem facing different directions. Abbreviations **ce** central vascular bundle, **ep** epidermis, **hy** hypodermis, **mx** metaxylem, **pe** peripheral vascular bundle, **ph** phloem, **px** protoxylem, **sc** sclerenchyma, **tr** transitional vascular bundle. Note: **B** taken from Guerreiro *et al.* (2011); **C** taken from Rógolo de Agrasar & Rodríguez (2003).





**Fig. 3.** Culm cross section light microscope micrographs. *Rhipidocladum neumannii*: A peripheral vascular bundle; B transitional vascular bundle. *Rhipidocladum racemiflorum*: C general view; D central vascular bundle. Abbreviations **mx** metaxylem, **pa** parenchyma, **pe** peripheral vascular bundle, **ph** phloem, **px** protoxylem. Note: C taken from Rùgolo de Agrasar & Rodríguez (2003).

external walls. Subepidermal sclerenchyma with 3 – 4 layers of fibres with thick walls and pits. Vascular bundles in 9 – 10 cycles. Phloem always facing the exterior of the culm. Peripheral vascular bundles surrounded by a thick sclerenchymatic sheath. Transitional vascular bundles

with four caps of sclerenchymatic tissue, two crescent-shaped linked with the metaxylem and two subtriangular caps in connection with the protoxylem and the phloem. Central vascular bundles ovate, 476 µm wide and 476 µm long, with caps of fibre in connection with the protoxy-

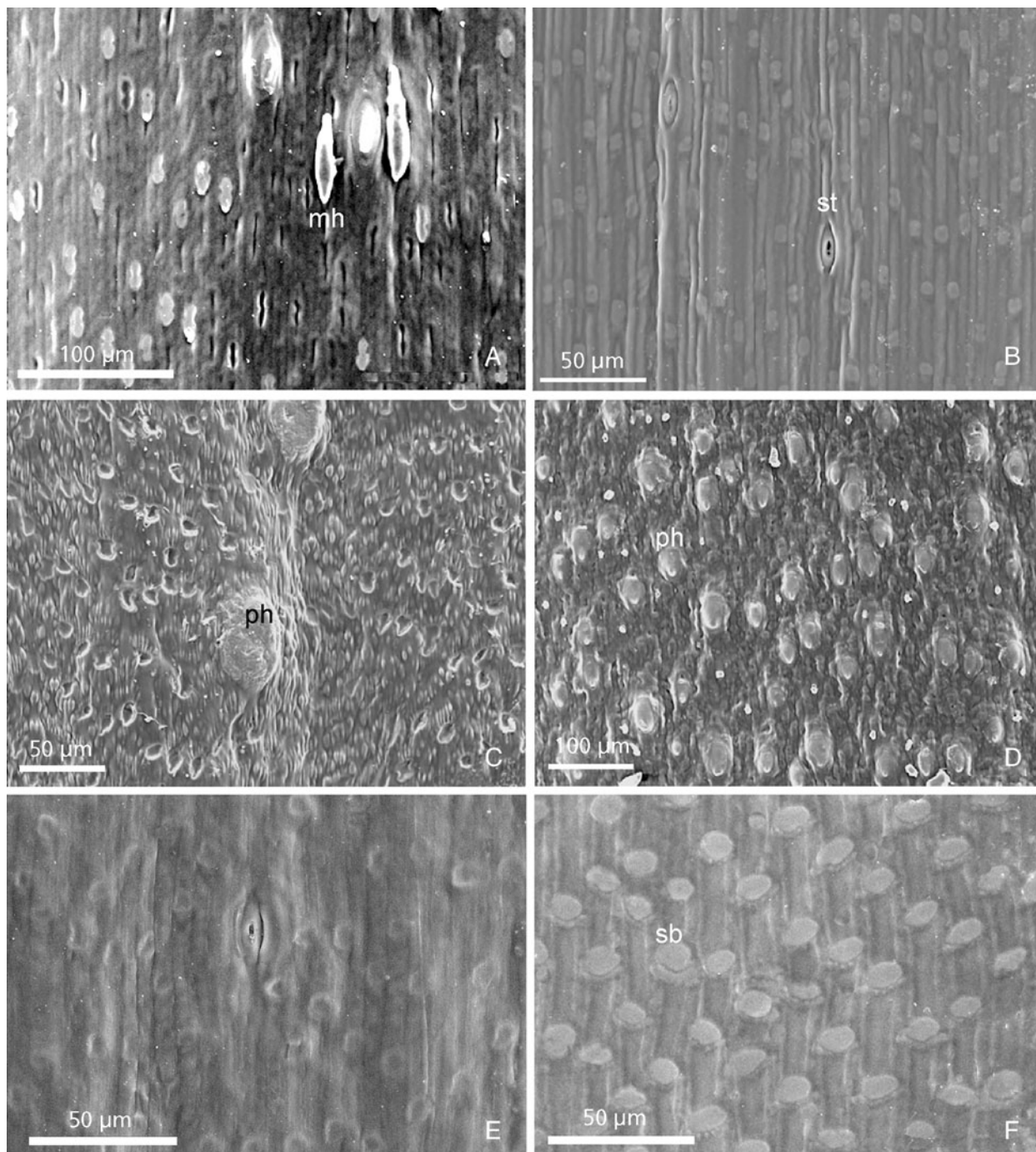
**Table 1.** Comparison of culm cross section characters among species of Andean woody bamboos, *Chusquea* and *Rhipidocladum*.

	Culm	Hypodermis	Vascular cycles	Phloem orientation	Central Vascular bundles	
					Shape	size (µm, width × length)
	<i>C. culeou</i>	solid	4 layers	4 – 5	different directions	ovate, depressed 230 × 158
	<i>C. deficiens</i>	solid	3 – 4 layers	4 – 5	exterior	ovate, depressed 216 × 144
	<i>C. lorentziana</i>	solid	1 – 3 layers	5	exterior	elliptical 340 × 374
	<i>C. montana</i>	solid	7 – 8 layers	9 – 10	different directions	ovate, depressed 180 (293) × 209 (225)
	<i>C. quila</i>	solid	3 layers	7 – 8	different directions	elliptical 151 × 118
	<i>C. valdiviensis</i>	solid	4 layers	8	different directions	ovate, depressed 94 × 72
	<i>R. neumannii</i>	hollow	3 – 4 layers	9 – 10	exterior	ovate 476 × 476
	<i>R. racemiflorum</i>	hollow	1 – 2 layers	4	exterior	depressed 138 × 160

lem and the phloem (Rúgolo de Agrasar & Rodríguez 2003; Fig. 3A, B).

*Rhipidocladum racemiflorum*: Culm hollow. Epidermis formed by a layer of papillate epidermal cells with thick external walls. Subepidermal sclerenchyma with

packages of fibres surrounded by parenchyma and peripheral vascular bundles. Vascular bundles in 4 cycles. Phloem always facing the exterior of the culm. Peripheral vascular bundles surrounded by a thick sclerenchymatic sheath. Transitional vascular bundles surrounded by a continuous crescent-shaped



**Fig. 4.** Culm epidermis scanning electron micrographs. **A** *Chusquea culeou*, microhairs and silica bodies; **B** *C. deficiens*, elliptical stomata; **C** *C. lorentziana*, prickle hair; **D** *C. montana*, prickle hairs irregularly arranged; **E** *C. quila*, general view; **F** *C. valdiviensis*, abundant rounded silica bodies. Abbreviations mh microhair, ph prickle hair, sb silica body, st stomata. Note: **B** taken from Guerreiro *et al.* (2011).



sclerenchymatic cap, broadly developed in connection with protoxylem and metaxylem. Central vascular bundles 238  $\mu\text{m}$  wide and 160  $\mu\text{m}$  long, depressed, surrounded by a continuous sclerenchymatic sheath (1 – 3 cells thick) with a small cap linked with the protoxylem (Fig. 3C, D; Rúgolo de Agrasar & Rodríguez 2003).

CULM ANATOMY: EPIDERMIS (Figs 4 and 5; Table 2).

*Chusquea culeou*: Ribs and furrows not evident. Long cells with straight walls, without papillae. Very frequent stomatal complexes, 23  $\mu\text{m}$  long and 3  $\mu\text{m}$  wide, long and narrow, arranged in rows, slightly sunken. Abundant dumb-bell shaped silica bodies with rounded ends, alternating with long cells. Isolated big prickly hairs with brief apex. Infrequent bicellular microhairs with long distal cell and rounded apex. Macrohairs absent (Fig. 4A).

*Chusquea deficiens*: Ribs and furrows not evident. Long cells with straight walls, without papillae. Elliptical infrequent stomatal apparatus, 22  $\mu\text{m}$  long and 7  $\mu\text{m}$  wide. Abundant saddle and square shaped silica bodies, alternating with long cells. Prickle hairs, microhairs and macrohairs absent (Fig. 4B; Guerreiro *et al.* 2011).

*Chusquea lorentziana*: Ribs and furrows not evident. Long cells with slightly wavy walls and abundant simple papillae. Infrequent, elliptical stomatal complex 34  $\mu\text{m}$  long and 11  $\mu\text{m}$  wide, with low dome-shaped subsidiary cells, elevated. Abundant silica bodies alternating with long cells, dumb-bell shaped and squarish with irregular outlines. Infrequent prickly hairs and microhairs. Macrohairs absent (Fig. 4C).

*Chusquea montana*: Ribs and furrows not evident. Long cells with wavy walls and frequent simple papillae irregularly arranged. No stomata visible on surface examined. No silica bodies visible on surface examined. Abundant prickly hairs, irregularly arranged. Isolated microhairs. Macrohairs absent (Fig. 4D).

*Chusquea quila*: Ribs and furrows not evident. Long cells with straight walls and without papillae. Isolated low dome-shaped stomatal complex, 23  $\mu\text{m}$  long and 9  $\mu\text{m}$  wide, subsidiary cells rounded. Abundant square and rectangular silica bodies, with rounded ends. Prickle hairs, microhairs and macrohairs absent (Fig. 4E).

*Chusquea valdiviensis*: Ribs and furrows not evident. Long cells with straight walls, but wavy on the proximal side next to the adjacent silica cell. Papillae absent. Infrequent, ovoid stomatal complex, 9  $\mu\text{m}$  long and 3  $\mu\text{m}$  wide. Abundant round and dumb-bell shaped

silica bodies. Prickle hairs, microhairs and macrohairs absent (Fig. 4F).

*Rhipidocladum neumannii*: Prominent ribs and furrows. Long cells with straight walls and compound papillae with two or three smaller papillae. Elliptical stomatal apparatus 30  $\mu\text{m}$  long and 10  $\mu\text{m}$  wide, aligned in furrows. No silica bodies visible on surface examined. Prickle hairs, microhairs and macrohairs absent (Fig. 5A; Rúgolo de Agrasar & Rodríguez 2002).

*Rhipidocladum racemiflorum*: Prominent ribs and furrows. Long cells with straight walls and compound papillae with five to nine smaller papillae. Elliptical stomatal apparatus 27  $\mu\text{m}$  long and 9  $\mu\text{m}$  wide, aligned

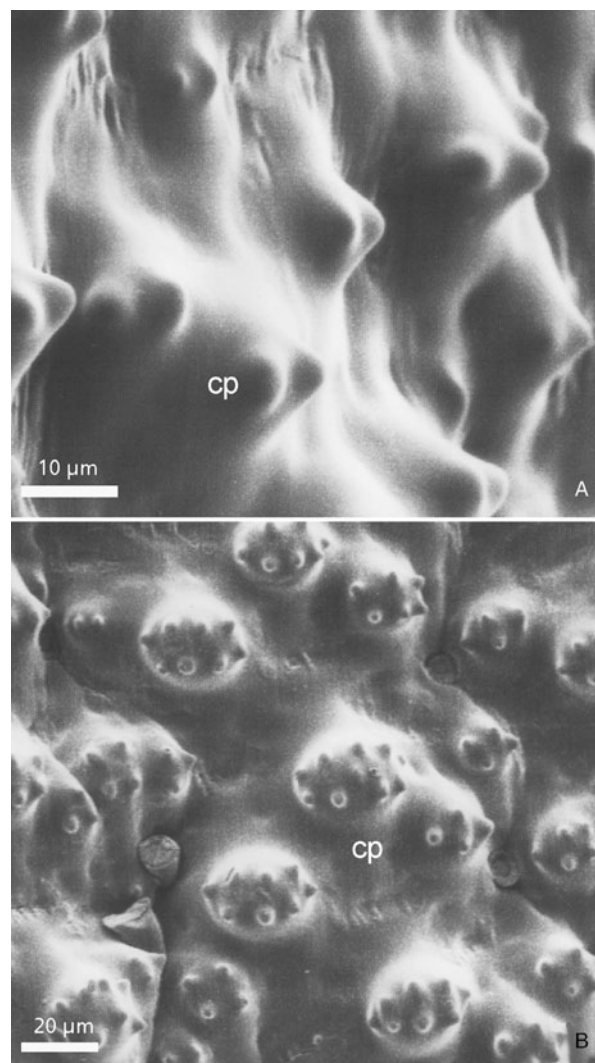


Fig. 5. Culm epidermis scanning electron micrographs. A *Rhipidocladum neumannii*: compound papillae. B *R. racemiflorum*: compound papillae. Abbreviation cp compound papillae. Note: A taken from Rúgolo de Agrasar & Rodríguez (2002).

**Table 2.** Comparison of culm epidermis characters among species of Andean woody bamboos, *Chusquea* and *Rhipidocladum*.

	Long cell walls	Papillae	Shape of silica bodies	Prickle hairs	Microhairs	Macrohairs
<i>C. culeou</i>	straight	absent	dumb-bell	scarce	scarce	absent
<i>C. deficiens</i>	straight	absent	saddle and square	absent	absent	absent
<i>C. lorentziana</i>	slightly wavy	abundant, simple	dumb-bell and square	scarce	scarce	absent
<i>C. montana</i>	wavy	abundant, simple	absent	abundant	scarce	absent
<i>C. quila</i>	straight	absent	square and rectangular	absent	absent	absent
<i>C. valdiviensis</i>	straight	absent	round and dumb-bell	absent	absent	absent
<i>R. neumannii</i>	straight	abundant, compound	absent	absent	absent	absent
<i>R. racemiflorum</i>	straight	abundant, compound	absent	absent	scarce	scarce

in furrows, exposed. No silica bodies visible on surface examined. Prickle hairs absent. Microhairs and

macrohairs in furrows (Rúgolo de Agrasar & Rodríguez 2002; Fig. 5B).

### Key to Andean woody bamboo species based on culm characters (epidermis and cross section)

- 1a. Culm hollow; prominent ribs and furrows; epidermal long cells with compound papillae . . . . . 2
- 1b. Culm solid; ribs and furrows not evident; epidermal long cells with or without papillae; if present, simple papillae . . . . . 3
- 2a. Compound papillae with 5 – 9 smaller papillae; microhairs and macrohairs present; central vascular bundles depressed . . . . . **Rhipidocladum racemiflorum**
- 2b. Compound papillae with 2 – 3 smaller papillae; microhairs and macrohairs absent; central vascular bundles ovate . . . . . **Rhipidocladum neumannii**
- 3a. Vascular bundles with phloem facing different directions; hypodermis formed by 3 – 8 layers of cells. . . . . 4
- 3b. Vascular bundles with phloem facing the exterior of the culm or different directions; hypodermis formed by 1 – 4 layers of cells. . . . . 6
- 4a. Vascular bundles in 9 – 10 cycles; hypodermis formed by 7 – 8 layers of thin walled cells; epidermal long cells with wavy walls; stomata absent; papillae, microhairs and prickle hairs present . . . . . **Chusquea montana**
- 4b. Vascular bundles in 4 – 8 cycles; hypodermis formed by 3 – 4 layers of cells; epidermal long cells with straight walls; papillae, microhairs and prickle hairs absent . . . . . 5
- 5a. Depressed central vascular bundles, ovate, with a single continuous layer of sclerenchymatic cells; epidermal long cells with straight walls, but wavy on the proximal side next to the adjacent silica cell; round and dumb-bell shaped silica bodies . . . . . **Chusquea valdiviensis**
- 5b. Elliptical central vascular bundles with a continuous sclerenchymatic sheath more developed in connection with the phloem; epidermal long cells with straight walls; square and rectangular silica bodies . . . . . **Chusquea quila**
- 6a. Frequent stomata, arranged in rows; epidermal long cells without papillae; vascular bundles with phloem facing different directions; central vascular bundles ovate, depressed, surrounded by a discontinuous sclerenchymatic sheath more developed in relation to the phloem and protoxylem. . . . . **Chusquea culeou**
- 6b. Infrequent stomata; long cells with or without papillae; vascular bundles elliptical or ovate, depressed with phloem facing the exterior of the culm . . . . . 7
- 7a. Long cells without papillae; prickle hairs and microhairs absent; central vascular bundles ovate, depressed. . . . . **Chusquea deficiens**
- 7b. Papillate long cells; prickle hairs and microhairs present; central vascular bundles elliptical . . . . **Chusquea lorentziana**

### Discussion

In this paper, culm characters of taxonomic value found in Andean woody bamboo species from Argentina and neighbouring areas were described. Previous descriptions of some of the species included in this study were improved and extended by adding new characters. Culm characters of *Chusquea montana*, *C. quila* and *C. valdiviensis* were presented for the first time. Also, an identification key based on culm anatomical characters

was provided. Since most of the species studied here are monocarpic, with long vegetative periods, the identification of new anatomical characters bears a considerable taxonomic significance contributing to the determination of vegetative material.

The size and shape of culm vascular bundles vary from the periphery toward the centre of the culms. Thus, vascular bundles may be classified as peripheral, transitional and central (Fig. 1). The peripheral



vascular bundles generally are embedded in the cortical sclerenchyma, the transitional and the central ones in the parenchyma. All vascular bundles have a more or less developed sclerenchyma sheath associated with the phloem and the xylem, surrounding the vascular bundle and defining their contour. This sheath varies greatly in size and distribution of fibres around vascular bundles, showing noticeable differences at generic and specific levels. In some cases, it is continuous and more or less uniform as in *Chusquea deficiens*, *C. montana* or the peripheral and central vascular bundles of *C. valdiviensis*. While in other cases, greater development of the sclerenchyma is observed in relation to the protoxylem and the metaxylem, as in *R. racemiflorum* and the peripheral vascular bundles of *C. lorentziana*, or the phloem, as in the transitional vascular bundles of *C. culeou* and *C. valdiviensis* and the central vascular bundles of *C. quila*. It is worth noticing that, while in most species peripheral and transitional vascular bundles are similar, in *Rhipidocladum neumannii* the transitional vascular bundles differ from all others because of their abundance of sclerenchymatic tissue.

Culm epidermal characters observed with electronic microscopy contributed valuable information for taxa recognition and identification. All *Chusquea* species have epidermal long cells without papillae or with simple papillae. Compound papillae were observed in both *Rhipidocladum* species. The morphology of the compound papillae provided information to distinguish between the species studied, *R. neumannii* and *R. racemiflorum*. In most species, silica bodies were readily observed, with the exception of *C. montana* and both *Rhipidocladum* species where they were not evident. The form and disposition of the silica bodies provided characters of taxonomic value at species level within *Chusquea*; dumb-bell shaped cells were observed in *C. culeou*, *C. lorentziana* and *C. valdiviensis*. Square silica cells were observed in *C. deficiens*, *C. lorentziana* and *C. quila*. Also, rectangular, round and saddle-shaped silica bodies were found. Prickle hairs were observed in *C. culeou*, *C. lorentziana* and *C. montana*. Also, in these species and in *R. racemiflorum*, bicellular microhairs were observed. Microhairs were absent, as well as prickle hairs and macrohairs, in *C. deficiens*, *C. quila*, *C. valdiviensis* and *R. neumannii*. Apparently, macrohairs on culm epidermis are not common, they were found only in *R. racemiflorum*.

Some authors have considered *Chusquea quila* and *C. valdiviensis* as synonyms (Parodi 1945; Nicora 1978) while others, as separate species (Matthei 1997; Judziewicz *et al.* 2000; Morrone *et al.* 2008). They occur sympatrically in the Andean-Patagonian beech forests of southern Argentina and Chile. However, a list of characters separating the two species was never presented. Only Matthei (1997) made a small comment on this matter using

height and distribution as the main characters to distinguish between them. In the present work, anatomical evidence was presented that supports the idea of *C. quila* and *C. valdiviensis* as different species and helps to distinguish between them. Regarding cross sectional characters, vascular bundles are noticeably different in size and shape between the two species (Fig. 1, Table 1) and as for epidermal characters, although they are very similar, they can be distinguished by the shape of their silica bodies (Table 2).

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### Appendix

#### SPECIMENS EXAMINED

*Chusquea culeou* E. Desv.: ARGENTINA. Neuquén: Los Lagos, Villa La Angostura, 23 April 2009, Rúgolo 2332 (SI); 12 Sept. 2009, Rúgolo 2334 (SI).

*Chusquea deficiens* Parodi: ARGENTINA. Jujuy: Valle Grande, 15 Feb. 1995, Deginani *et al.* 835 (SI). Salta: Anta, Maíz Gordo mountain range, 10 Jan. 1939, Devoto *et al.* 1010 (SI).

*Chusquea lorentziana* Griseb.: ARGENTINA. Salta: Guachipas, Pampa Grande, 2 May 1942, Hunziker 1548 (SI). Tucumán: Monteros, Quebrada de los Sosa, 26 Dec. 1971, Krapovickas & Cristóbal 20452 (SI).

*Chusquea montana* Phil.: ARGENTINA. Neuquén: Los Lagos, Villa La Angostura, Bayo hill, 8 Jan. 2010, Rúgolo 2343 (SI). Río Negro: Bariloche, Lake Frías, 18 April 1977, Rúgolo *et al.* 752 (SI).

*Chusquea quila* Kunth: CHILE. X Región: Llanquihue, Puerto Varas, no date, Junge s.n. (SI); Osorno, 24 Jan. 1992, Rúgolo 1370 (SI). XI Región: Aisén, Palena river valley, 28 Jan. 1994, Rúgolo 1980 (SI).

*Chusquea valdiviensis* E. Desv.: ARGENTINA. Neuquén: Los Lagos, Nahuel Huapí National Park, Victoria Island, Feb. 1946, Pérez Moreau 58 (SI). CHILE. XIV Región: Valdivia, Puyehue cross, 23 Feb. 1978, Rúgolo 749 (SI).

*Rhipidocladum neumannii* Sulekic, Rúgolo & L. G. Clark: ARGENTINA. Salta: José de San Martín, Río Pescado deposit, 2 May 2003, Morrone *et al.* 4555 (SI); Orán, Arrazayal ranch, 23 April 1998, Sulekic & Cano 2053 (SI). BOLIVIA. Santa Cruz: Andrés Ibañez, 27 March 1998, Nee *et al.* 48768 (SI).

*Rhipidocladum racemiflorum* (Steud.) McClure: ARGENTINA. Salta: Santa Victoria, Baritú National Park, 22 June 1999, Hilgert & Hill 2367 (SI); El Lipeo, 11 Feb. 2001, Sulekic *et al.* 3199 (SI). BOLIVIA. La Paz: Sur Yungas, Marimonos mountain range, 25 July 1987, Killeen 2628 (SI).

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