

Amorphous silica biomineralizations in *Polytrichum strictum* Menzies ex Brid. (Bryophyta)

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In this study, the presence of amorphous silica biomineralizations (silicophytoliths) in individuals of *Polytrichum strictum* collected from Tierra del Fuego, Argentina was analysed. Gametophyte and sporophyte samples were subjected to calcination and staining techniques, through which the biomineralizations obtained were observed and described by optical and scanning electron microscopes. Lastly, their composition was analysed by energy dispersive spectroscopy. In both gametophyte and sporophyte silica skeletons comprising thin elongated silicophytoliths with blunt, round or acute ends were observed. These morphologies may correspond to the silicification of the costa or midrib region of the leaves (in the gametophyte) and the seta (in the sporophyte) and to the silicification of the awns of the leaves. The other morphologies found were silica skeletons comprising silicified walls of tabular polygonal cells, probably derived from the silicification of the cells of the unistratose lamina of leaves. Energy dispersive spectroscopy analyses and the use of a phenol-staining technique confirmed the silicon composition of these biomineralizations. The results obtained comprise the first description of amorphous silica biomineralization in mosses and the implications of the silicification process in this taxon are discussed. The data obtained will be relevant not only for anatomical, but also for palaeobotanical studies, since the preservation of these structures in the soil after the decomposition of organic matter, might allow the identification of mosses in the fossil record.

Keywords: Argentina, Mosses, Peatland, *Polytrichum strictum*, Silica, Silicophytoliths, Tierra del Fuego

Introduction

Biomineralizations are biogenic inorganic–organic composites, crystalline or amorphous, deposited in intracellular or extracellular spaces as the consequence of metabolic activity of organisms (Lowenstam, 1981; Osterrieth, 2004). The most common deposits in vascular plants are calcium carbonates and oxalates, and silicophytoliths (amorphous silica biomineralizations), while in lichens, the biomineralizations are mainly calcium carbonates and oxalates (Lowenstam, 1981; Metcalfe, 1985; Giordani *et al.*, 2003; Giordano *et al.*, 2005). In mosses, almost no studies have been carried out, except for the work of Estébanez Pérez *et al.* (2002) who identified biomineralizations both in gametophore stems and in sporophyte setae in

Homalothecium sericeum (Hedw.) Schimp. The analyses carried out in this species indicated the presence of amorphous silica, carbonates, and Fe and Al hydroxides in both the sporophyte and gametophore (Estébanez Pérez *et al.*, 2002).

Silicic acid is absorbed by root plants from soils, transported by the xylem and finally deposited as amorphous silica biomineralizations in extra or intracellular spaces (Ma & Takahashi, 2002). The presence of silica biomineralizations in plant species has numerous benefits such as improvement of biomass production, antiherbivore defense, and the amelioration of heavy metal toxicity, among others (e.g. Jones & Handreck, 1967; Epstein, 1994; Ma & Takahashi, 2002). The study of silica production in plants has not only a taxonomical and anatomical relevance but also a palaeobotanical, because amorphous silica biomineralizations are commonly used as indicators of past plant communities due to their

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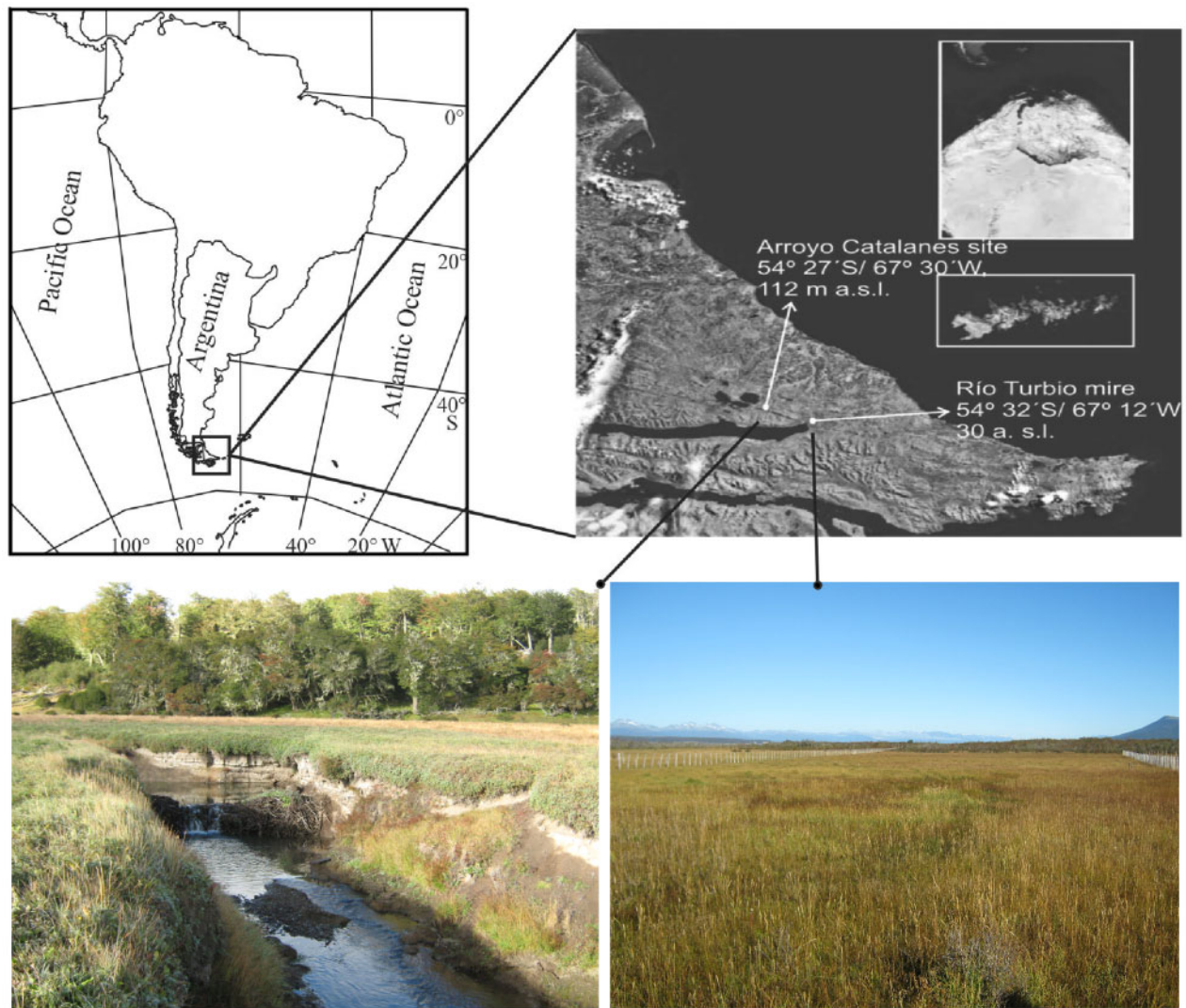


Figure 1 Location of the collection sites and photographs of the environment.

good preservation in soils and sediments (e.g. Twiss *et al.*, 1969; Piperno, 2006). Finally, there is a growing interest in the input of silica from plants to the environment, especially in studies related to the biogeochemical silica cycle (e.g. Derry *et al.*, 2005; Farmer *et al.*, 2005).

Silicophytolith studies have mainly involved families with a high and diverse production, such as monocotyledon taxa like Poaceae, Cyperaceae, and Arecaceae (e.g. Piperno, 2006; Fernández Honaine *et al.*, 2006, 2009). Bryophyte studies are very scarce, except for those from Ma & Takahashi (2002) who measured the silica content in *Sphagnum cymbifolium* Warnst. and *Marchantia polymorpha* L. and classified them as silica accumulators. However, no description or location of the silica morphologies was shown.

As part of a study of the amorphous silica biomineralizations in plants from the peats and grasslands of Tierra del Fuego, Argentina, the moss *Polytrichum strictum* Menzies ex Brid. (Polytrichaceae) was analysed (Benvenuto *et al.*, 2011). This species is distributed in North America, N Europe (Scandinavia, Svalbard, UK, Turkey, the Caucasus and Iceland), NE Asia (Russian

and Japan), and Argentina (Tierra del Fuego, Islas Malvinas (Falkland Islands), Sandwich del Sur, Orcadas del Sur, Shetland del Sur, and Antarctic peninsula) (Schiavone, 1993; Smith Merrill, 2007). On Tierra del Fuego, *P. strictum* grows in peatlands on *Sphagnum magellanicum* Brid. mounds and on pond edges, where it forms continuous and pure carpets. It is also found in areas where grasslands have developed (Schiavone, 1993).

Materials and Methods

Fresh specimens of *Polytrichum strictum* were collected from two sites: the Río Turbio mire and the Arroyo Catalanes river banks; both located in Isla Grande de Tierra del Fuego, Argentina, at the southernmost end of South America (53–55°S; 66–74°W) (Figure 1). The Río Turbio site is a minerotrophic mire formed by Cyperaceae in an environment dominated by *Carex* L. sp., *Carex gayana*, Steud. *Empetrum rubrum* Vahl ex Willd., *Marsippospermum* Desv. sp., and *Bolax gummifera* Spreng., among other species. In this site, a few mounds of *Sphagnum magellanicum* in the central areas and *Nothofagus antarctica* (G.Forst.) Oerst. in

the marginal zone are developed (Roig, 2001). The Arroyo Catalanes locality is an alluvial sedimentary sequence in a stream bank which drains the northern slope of the Las Pinturas range, north of Lago Fagnano. This creek is a low discharge stream that runs into a vast fluvial valley, later modified by glacial and glaciofluvial processes. It presently comprises a wet grassland environment that is used for cattle raising (Collado, 2001).

Eleven samples from gametophytes and three samples from sporophytes were selected for phytolith extraction. Leaves from the gametophytes, and setae and capsules from the sporophytes were carefully isolated with tweezers. The samples were subjected to a calcination technique, in which ashes composed only of biogenic silica were obtained (Labouriau, 1983). The samples were first placed in an ultrasound bath for 15–20 minutes and washed with distilled water to remove mineral contaminants. The material was dried at 56°C for 24 hours, weighed, and charred at 200°C for 2 hours. Later, it was boiled in a 5 N HCl solution for 10 minutes, washed with distilled water, and filtered with ashless filter paper, until no more chloride ions were detected. Finally, the material was ignited at 760°C for 3 hours. Ashes obtained were weighted and silica content was calculated as a percentage dry weight.

The ashes obtained were mounted in immersion oil and the morphologies were observed and described with a Zeiss Axiostar Plus microscope at $\times 400$ magnification. Photographs were taken with a digital camera Canon Powershot G10. At least 100 phytoliths were counted in each sample and the morphologies were described. Some samples were gold-coated and observed using a scanning electron microscope (JEOL JSM-6460 LV; Japan) at Universidad Nacional de Mar del Plata, Argentina. The composition of the ashes was analysed by X-ray energy dispersive spectroscopy (EDS). The system used was an EDAX Genesis XM4-Sys 60, equipped with multi-channel analyser EDAX mod EDAM IV, Sapphire Si (Li) detector and super ultra-thin window of Be, and EDAX Genesis version 5.11 software.

To localize the phytoliths in the tissue, leaves were cleared with 50% (w/v) sodium hypochlorite, dehydrated in an ethanol series, and then stained with phenol crystals. Phenol crystals stained silica in the cells with a rose colour (Johansen, 1940).

Results

Silica content

The percentage dry weight of silica content ranged between 0.3 and 9.2% in the gametophytes, and 0.4 and 1.2% in the sporophytes.

Silica biomineralization description and tissue location

The ashes obtained after the calcination technique revealed the presence of diverse silicophytoliths

morphologies (amorphous silica biomineralizations) in both gametophyte and sporophyte. The most abundant morphology (76% of the total of the morphologies described on the slides) in both the gametophyte and sporophyte was silica skeletons 87.5–250 μm long and 25–35 μm wide, comprising thin elongated phytoliths with blunt, round, or acute ends (30–50 μm long and 2.5–5 μm wide). Also, in some cases, the edges had a triangular projection similar to a hook (Figure 2A). The EDS analyses carried on in these morphologies clearly showed the silicon composition of these biomineralizations (Figure 3).

The above morphologies mainly correspond to the silicification of the awns of the leaves, which constitute a prolongation of the costa region (Figure 4C). To a lesser degree, they may also correspond to the silicification of the costa or midrib region of the leaves (in the gametophyte) and seta (in the sporophyte). This was corroborated by the samples stained with phenol, where the main silicified areas (evidenced by a rose colour) corresponded to the awn and the central strand (Figure 4).

Other silica biomineralizations observed in ashes of the gametophyte (24%) were silica skeletons composed of the silicified walls of tabular polygonal cells (Figure 2B). These morphologies probably derived from the silicification of the cells of the unistratose lamina of leaves, areas which were also stained with phenol in our samples (Figure 4).

Discussion

Silica biomineralizations

The values of the silica content for the gametophytes analysed are slightly higher than those obtained for *Sphagnum cymbifolium* (1.37%) and *Marchantia polymorpha* (5.55%) by Ma & Takahashi (2002). Differences observed between the studies could be the consequence of genetic or environmental factors, because both affect the silica content of plants (Ma & Takahashi, 2002; Hodson et al., 2005).

Polytrichum strictum produces amorphous silica biomineralizations in both gametophyte and sporophyte. The calcination technique clearly showed the morphologies produced. The silicified areas were detected by a staining technique, where phenol coloured the silicified cells with rose. These areas mainly included the midrib or costa of leaves, stems and seta, and the awn of the leaves. To a lesser degree, the cells of the margin of the leaves were also silicified. The amorphous silica biomineralizations observed may be associated with areas with high evaporation rates, such as from the awns of the leaves, or areas implied in supporting, such as stereids in the costa. The distribution of the silicification observed in *P. strictum* resembles the pattern found in

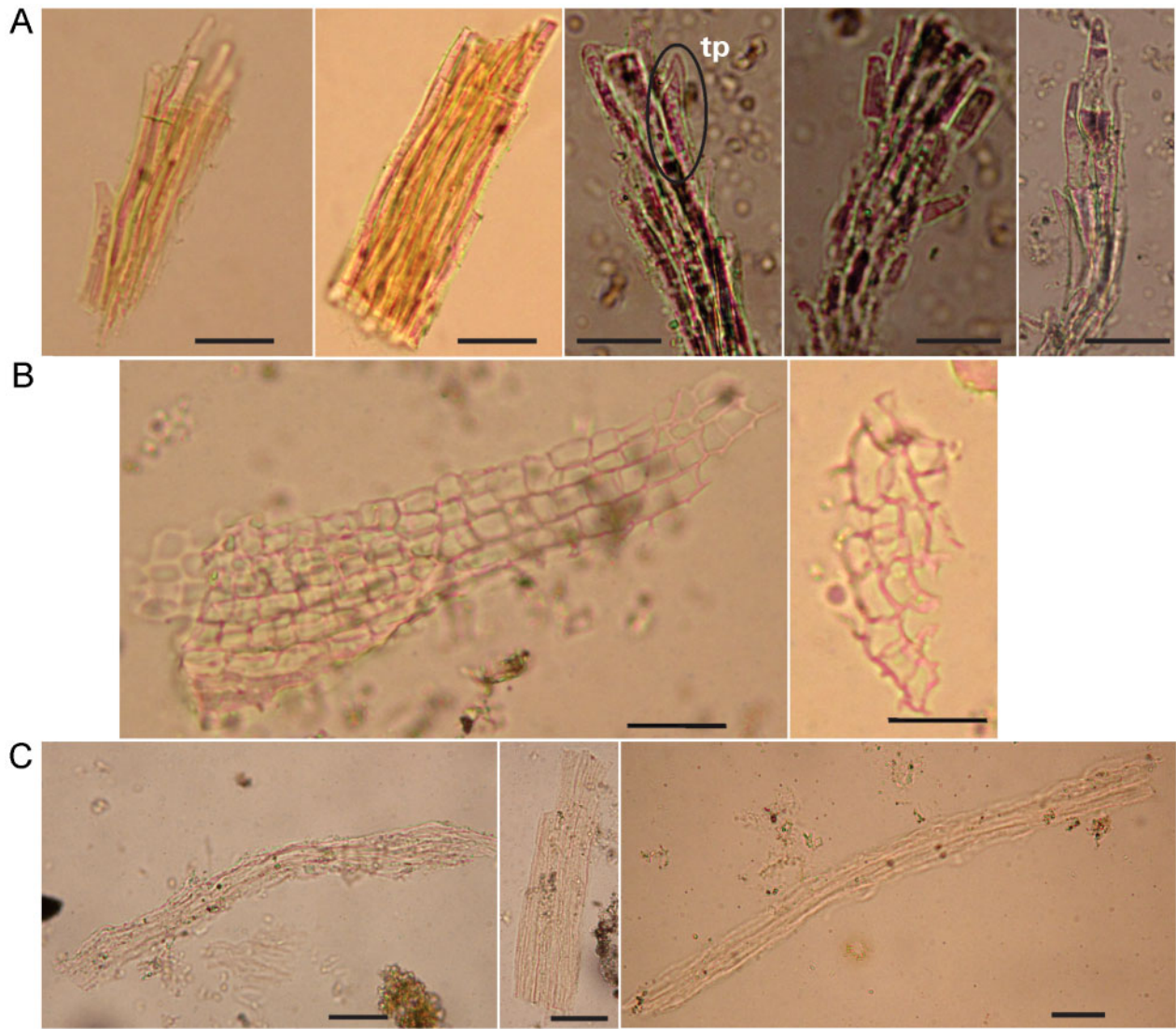


Figure 2 Morphologies observed by optical microscope. (A, B) Phytolith morphology observed in the gametophyte: (A) silica skeletons composed by thin elongated phytoliths with blunt, round, or acute ends; (B) silica skeletons composed by the silicified walls of tabular polygonal cells; (C) silica skeletons composed by thin elongated phytoliths from sporophyte. tp=triangular projection similar to a hook. Bar=25 μ m.

vascular plants, where the epidermis, and to a lesser extent the fibres and xylem, are silicified (e.g. Metcalfe, 1960; Jones *et al.*, 1963; Ma & Takahashi, 2002; Fernández Honaine *et al.*, 2006).

Implications of the silicification process in Polytrichum strictum

It is known that roots of vascular plants take up monosilicic acid (H_4SiO_4) in water from the soil solution. H_4SiO_4 is transported via the xylem to the different organs and tissues, where, in general, as water is lost by evaporation, this molecule is precipitated, thus forming the amorphous silica biomineralizations (Sangster & Parry, 1971; Ma & Takahashi, 2002). Although diverse factors, such as genetics and age of the organ, affect the silica content in plants, the silica content of soils is important in the silicification process. In the case of bryophytes, which lack an absorptive root system and thus cannot take up water from soil solution where monosilicic acid is

widely available, it remains unclear how these plants take up this molecule. It is known that they typically take up water and nutrients throughout the shoot, intercepting and absorbing solutes in rainwater, cloud and mist droplets, and airborne dust (Proctor, 2000). Rainwater has a low silica content and its absorption would not be sufficient for silica biomineralizations production (Borrelli *et al.*, 2010). However, in *Polytrichum strictum*, it is important to consider two significant aspects in order to understand why this species can produce silica biomineralizations: the rhizoids and the environment studied. Firstly, this moss has a dense layer of rhizoids (Groeneveld, 2002), which would allow the absorption, at least to some extent, of water and minerals from soil solutions, where silicic acid is available. In addition, it is known that the stems of the species of *Polytrichum* have a well-developed central strand of hydroids that allows internal water conduction

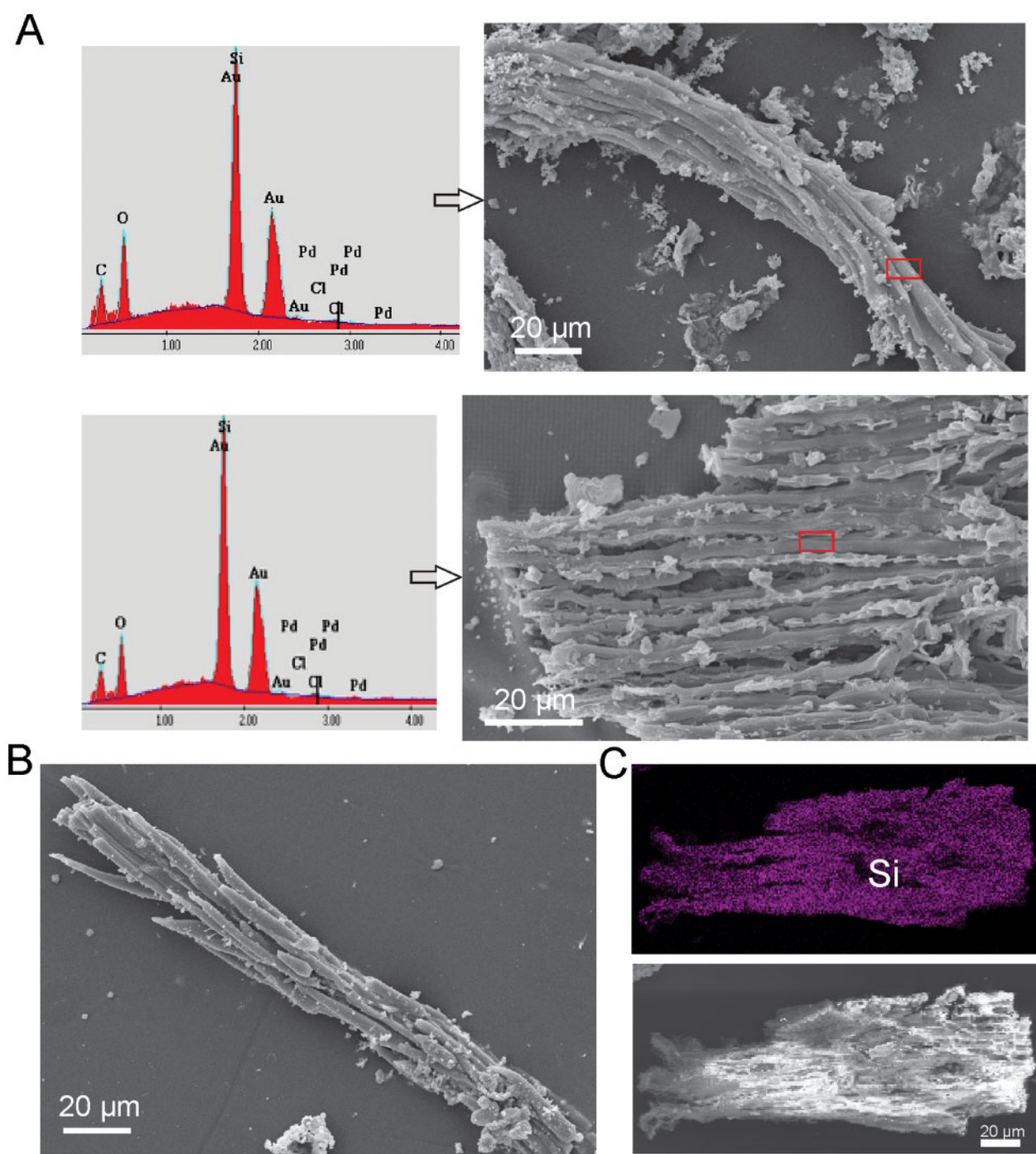


Figure 3 Morphologies observed by scanning electron microscopy. (A) EDAX analyses carried on silica skeletons comprising thin elongated phytoliths; (B) silica skeletons comprising thin elongated phytoliths with several acute ends from awns of the leaves; (C) mapping of silicon on silica skeletons observed in the ashes of gametophyte.

(Delgadillo & Cárdenas, 1990; Proctor, 2000; Estébanez Pérez *et al.*, 2011). Secondly, the plants were sampled from flooded peatlands and/or grasslands areas, where variations in waterlogging are usual. When waterlogging events occur, water from soil solution (containing silicic acid) makes contact with the plant. Finally, the Si concentration of the soil solutions of the sites where the specimens were collected is considerable (e.g. higher than rainwater content). Preliminary data showed that the values of Si content ranged between 93–109 mg per litre of

SiO₂ in Río Turbio mire and 55–105 mg per litre of SiO₂ in Arroyo Catalanes river banks (Borrelli *et al.*, data not published; Borrelli *et al.*, 2010). Therefore, regardless whether water is absorbed by rhizoids or the whole plant, *P. strictum* will be able to uptake the necessary monosilicic acid for silica biomineralization production, since Si is available in these soils. It also can be suggested that plants that grow in other soils or substrates, and where the only water source is rain, would have a lower silica biomineralization production. Once silicic acid is absorbed, it may be

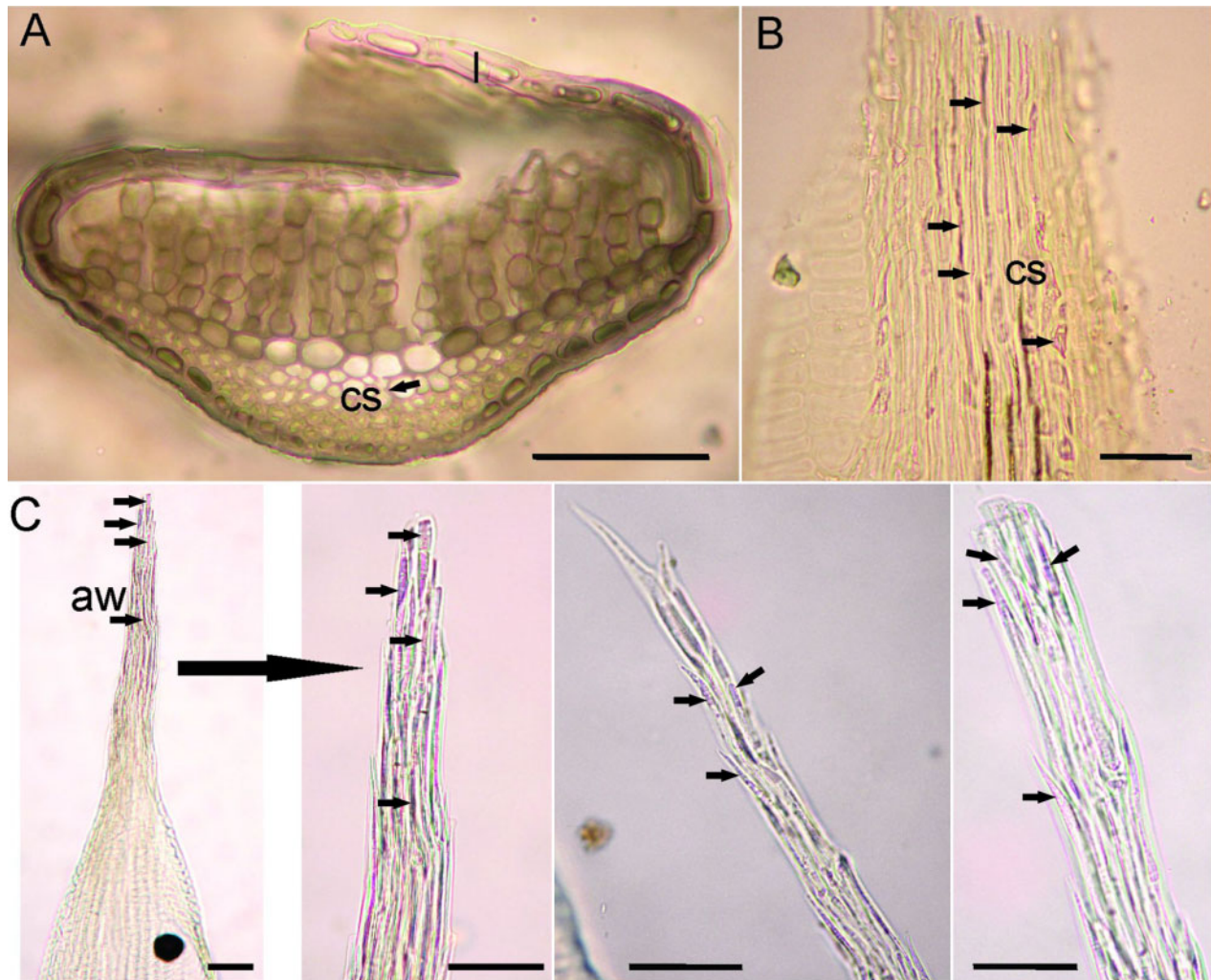


Figure 4 Silicified areas from gametophyte, detected by the staining technique. (A) Transverse view of the leaf; (B) silica deposits observed in the central strand; (C) silica deposits observed in awn region (aw). l=lamina of the leaf, cs=central strand. The arrows indicate the areas of silica deposits (stained rose colour). Scale bar=25 µm.

transported by hydroids or by the ectohydric pathway to the areas where water loss is higher, e.g. the apical sections of leaves like the awns (Proctor, 2000; Estébanez Pérez *et al.*, 2011).

Lastly, considering that bryophytes lack a structural resistance substance such as lignin, and taking into account the role of silica as a reinforcing material (as an alternative to lignin) in vascular plants, it is possible to suggest that these biomineralizations could improve the supporting tissues as for example, in the stereids (Raven, 1983; Ma & Takahashi, 2002; Estébanez Pérez *et al.*, 2011).

Conclusion

This study is the first report of amorphous silica biomineralizations in mosses. Both the gametophyte (leaves and stem) and the sporophyte (seta) were silicified, especially those areas of the midrib or costa and the awn of the leaves. Although only one species was analysed, it is probable that these structures are present in more species of this genus, and also in the

family, and so we encourage further studies in this interesting area of bryophyte biology.

In addition to the taxonomic and anatomical relevance of the study of silica biomineralizations in bryophytes, it is necessary to emphasize their palaeobotanical importance. As was detailed previously, silicophytoliths are widely used as indicators of past floras, so knowledge of the presence of these biomineralizations in bryophytes may be of interest for paleontological studies.

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Taxonomic Additions and Changes: Nil.

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