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How are systematics and biological and ecological features related to silica content in plants?: a study in species from the Southern of South America

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Running title: Interrelation among factors affecting silica content

Keywords: angiosperms, growth form, life cycle, plant origin, plant tissues, silicophytoliths.

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ABSTRACT

Premise of the Research: Plant silica content depends on the phylogenetic position of a taxon; however, biological or ecological factors may also affect it. In this work, we analyzed data about silicophytolith content from 105 species of South America, examining, in a phylogenetic context, its relationship with the anatomy and ecological features such as life cycle, growth form, plant origin and environmental preferences.

Methodology: Data about silicophytolith content and bio-ecological features of the species were obtained from published and unpublished sources. The relation between systematics, silica content and bio-ecological variables were analyzed through measurements of phylogenetic signal and phylogenetic generalized least-squares regressions (PGLS).

Pivotal Results: 86% of the species produced between 0.38% and 19% dry weight of silicophytoliths in leaves. Silica content was variable between and within clades. λ and K values indicate a low phylogenetic signal for the variable silica content. Dicotyledons accumulated silica in typical epidermal cells, and a few families stored it also in cystoliths. Most of the monocot families showed high silicophytolith contents and high diversity of silicified cells. Plant origin affected silica contents: exotic species accumulated more than native ones. On the other hand, no statistical relationship was found between silica content and the other ecological variables.

Conclusions: Silicophytolith accumulation is a common feature in most of the species studied. The low phylogenetic signal of silica content is explained by the inter and intra clade variability, which in turn support the hypothesis that silicophytolith accumulation is a homoplasic character among plants. Based on the overall analysis of the silicophytolith content and their tissue distribution, high content could be related to specific accumulation mechanisms and roles of silica. The origin of the plants was the only bio-ecological variable that influenced in plant silica content. This finding may indicate some ecological role of silica in exotic plants, involving the success of them in novel environments.

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INTRODUCTION

Silicification is a widespread process in plant species (Hodson et al. 2005; Benvenuto et al. 2013a; Exley 2015; Katz 2015). The accumulation of amorphous silica (or silicophytoliths) has been attributed to multiple roles in plants, related to diverse plant features, and even proposed as a plant functional trait (Katz 2018). Numerous studies highlight the relevance of silica in the biology of plants and in terrestrial and aquatic ecosystems (e.g. Sommer et al. 2006; Cooke and Leishman 2011a; Schoelynck et al. 2014); however, less than 50% of the 412 angiosperms families have been analyzed to determine their silicophytolith content (Katz 2015).

Silica accumulation in plant tissues is usually associated with the phylogenetic position of a taxon (Hodson et al. 2005). For instance, commelinids generally accumulate more silica than dicotyledons and non-commelinid monocots (Prychid et al. 2004; Hodson et al. 2005). However, some studies also reported intra-clade variations, e.g. at family level (Katz 2014, 2015; Strömberg et al. 2016). This variability among clades supports the idea that the ability to accumulate silica evolved in different lineages at different times during plant evolution (Katz 2015; Trembath-Reichert et al. 2015; Strömberg et al. 2016).

The location of silicophytoliths in tissues and their morphologies vary among plant clades making silicophytoliths an important taxonomic tool, especially in Poaceae (Metcalfe 1960; Twiss 1992). The most common silicified tissue is the epidermis, but silica accumulation also occurs in parenchyma, xylem, endodermis (in roots) and sclerenchyma, both as inclusions or as complete lumen infillings (Piperno 2006). The relationship between silica content, systematics and anatomy has been studied much more frequently in grasses than in other monocots or dicots. This knowledge is essential in order to understand the roles of silica in plant biology and evolution (Strömberg et al. 2016).

Besides phylogenetic constraints, bio-ecological features such as life cycle, growth form, the origin of the plant, and soil water availability may affect the production of silicophytoliths in plants.

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Previous research showed that silica content is negatively correlated with leaf-life span or life cycle of plants (Cooke and Leishman 2011b). This was explained due to the differences in the leaf carbon balance strategies of annuals and perennials, and in the advantage of silicon as a cheaper alternative to lignin and cellulose (Raven 1983; O'Reagain and Mentis 1989; Cooke and Leishman 2011b). Studies in tussock grasses showed that these large herbs accumulate less silica per gram of leaf than smaller grasses (Fernández Honaine et al. 2017). Considering that the silicification process is irreversible and that it implies an addition of weight to the total biomass (Raven 1983), it was proposed that large grasses may accumulate low quantities of silica (measured as % dry weight) as a structural control (Fernández Honaine et al. 2017). This hypothesis may be extended to other larger growth forms, such as shrubs and trees.

The ability of exotic species to grow and invade novel regions depends in part on the defense mechanisms and the energy balance strategies of the invader. Silicophytoliths have been hypothesized to be both a physical anti-herbivore defense and a cheap structural material (Raven 1983; Hartley et al. 2015; Hartley and DeGabriel 2016). These two advantages of silicophytoliths may be favorable for exotic species growing in novel areas. Previous work in *Ligustrum lucidum*, an exotic and invasive species in South America, revealed that plants grown in novel areas (Argentina) had higher silica content than in native regions (China) (Fernández Honaine et al. 2019a). This result may suggest that under some special circumstances (e.g. the presence of soils with high Si availability), those species with the ability to accumulate silica may take advantage of it, either as a antiherbivore deterrence or as a structural reinforcement (Montti et al. 2016; De Rito et al. 2016; Fernández Honaine et al. 2019a). In this sense, we propose that silica accumulation could be a good strategy for exotics, and as a consequence silica content might be different between native and exotic species in a specific region.

Lastly, environmental factors such as soil water availability may also affect plant silica content; some authors relate a high water availability (either plants growing in wetter environments or subjected to irrigation managements) with a high silica accumulation (e.g. Jones and Handreck

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1967; Ma and Takahashi 2002; Jenkins et al. 2011; Quigley and Anderson 2014). In wet/ humid environments, monosilicic acid may be more readily available for plant uptake and evapotranspiration may not be as limiting as in dry environments. As a consequence silica uptake and accumulation may be higher in species associated to saturated soils or wetlands. In this sense, aquatic species have been described as highly silica accumulators (Schoelynck et al. 2010; Schoelynck and Struyf 2015).

In this work, we analyzed the relationship of plant silica content with systematic and anatomical aspects, as well as with specific bio-ecological features (life cycle, growth form, plant origin and soil water availability). We did this work in species from a scarcely studied region (southern of South America), using samples collected in a specific period of time (2003-2018). Data on silicophytolith content was obtained from published and unpublished sources, and was measured by the same technique to avoid differences in content due to methodology. We addressed the following questions: 1) how does the silicophytolith content vary between and within the different clades (monocots/ dicots, orders, families) in this group of species?; 2) what is the relationship between systematics, tissue silicification, and the potential role of silica?; 3) among silica accumulating species, is silicophytolith content variable according to the life cycle, growth form, plant origin (exotic vs. native) and/ or soil water conditions? We predict differences in silica content between taxa, and as a consequence, differences in the type of cells that are silicified. We also expect a great diversity of silicified cell types in highly accumulating families. Finally, we predict that annuals, herbs, exotics and species associated with wet environments have higher silica contents than perennials, trees/shrubs, natives and species not associated with wet environments, respectively.

MATERIALS AND METHODS

Plant material

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In order to include high phylogenetic biodiversity and different environments, we analyzed silicophytolith content data (compiled from both published and unpublished sources) from 105 species (covering 28 families and 79 genera) collected in Argentina and belonging to three main phytogeographic provinces from South America (Cabrera 1971) (Figure 1, Appendix Table A1). The Paranaense Province (Figure 1.1) represents a phytogeographic area covered by tropical and subtropical forests and savannas. It covers the northeast of Argentina, eastern Paraguay, and southern Brazil. The climate is warm and humid, with rainfall throughout the year. The Pampean Province (Figure 1.2.) is mainly characterized by a gramineous steppe extended in the east of Argentina and Uruguay, between 31º and 39º S latitude. The climate is temperate with a mean annual temperature between 13ºC and 17ºC and total annual precipitation of 600-1100 mm. Lastly, the Subantarctic Province (Figure 1.3) is an area characterized by deciduous and evergreen forests, grasslands and peatlands. It is extended along Austral Andes, from 37° S up to Cabo de Hornos, including the south of Chile, part of Tierra del Fuego and Los Estados Island. The climate is cold-temperate oceanic in the southern part and cold-temperate-subhumid in the northern one, with a mean annual temperature of ca. 5º C (Cabrera 1971).

Silicophytolith data corresponded to samples collected from mature plants of natural environments and private fields as well as from herbarium specimens (Appendix Table A1). All species have between 2 and 10 replicates (individuals). All samples correspond to leaves, except in those species (*Schoenoplectus californicus* and *Eleocharis* spp.) where this organ was small, absent or rudimental; in this case, it was replaced by culm samples.

Silicophytolith content

Data about silicophytolith content was obtained from published and unpublished sources detailed in Table A1 (Appendix). In all the studies detailed in Table A1, silicophytoliths were extracted from leaves or culms following the same calcination technique (Labouriau 1983) and the content was

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calculated as the percentage of dry weight. The descriptions of the silicophytolith morphologies were obtained from the references mentioned for each species in Table A1 (Appendix). The assignment of each morphology to a specific tissue (Appendix Table A2) was obtained from the references cited in Table A1, from specific bibliography, or through the application of histological techniques. In this last case, free-hand transverse and longitudinal sections of samples were obtained, cleared, and mounted in immersion oil (Fernández Honaine et al. 2019b). This technique allows in a simple and fast way to visualize the cells that are silicified without the destruction of the tissue. The distribution of silicophytoliths in the tissues was observed under Zeiss Axiostar Plus microscope at 400X magnification.

Systematic, biological and ecological features

Taxonomic, life cycle (annual-perennial), growth form (tree/shrub-herbs) and plant origin (native-exotic) information of each species were obtained from Tropicos web site (www.tropicos.org) and the Darwinion Botanical Institute (http://www2.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm). Three life cycle types were considered for the statistical analyses: annuals (mostly herbs), deciduous perennials, and evergreen perennials. The classification of the species in wetland and not wetland (soil water condition) followed published literature on the regional flora (Cabrera and Zardini 1978; Moore 1983) and the Flora Argentina database (http://www.floraargentina.edu.ar/). Wetland species included taxa that commonly grow in wet/humid environments, saturated soils and/or near lagoons, ponds, rivers or any other wetland.

Data analyses

In order to analyze the relation between silicophytolith content and phylogenetic affinities among species (i.e. if closely related species are more likely to have similar silicophytolith content or if

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silicophytolith content varies randomly across phylogeny), we measured the phylogenetic signal of silica content. Two measures were applied: Pagel's lambda (Pagel 1999) and Blomberg's K (Blomberg et al. 2013). We used the 'phylosig' function from 'phytools' package in R version 3.6.1 (R Core Team 2019). This function calculates both values (λ and K) and also a *p* value for the tests where null hypothesis were λ=0 or K=0, respectively (Revell 2012). We only included 103 species, since two of the species could not be placed in the phylogenetic tree. The phylogenetic tree was obtained from Phylomatic Version 3 (https://phylodiversity.net/phylomatic/) (Webb and Donoghue 2005), and we used the megatree R20120829 for plants. Branch lengths were set according to Grafen's method (Grafen 1989). In order to obtain binary trees, we applied 'multi2di' and 'collapse.single' functions in R (Revell 2012).

The relationship between silica content and biological and ecological features (life cycle, growth form, plant origin, wetland/not-wetland species) was evaluated through phylogenetic generalized least squares regression models (PGLS). The PGLS method considers the phylogenetic non-independence of the data points (the species), incorporating into the model a matrix of phylogenetic covariance between species (Pagel 1999; Freckleton et al. 2002). One of the most widely known measurement of the phylogenetic signal for regression residuals is Pagel's λ, which was estimated by maximum likelihood. When λ=0 it indicates a complete independence between the regression residuals and phylogeny, and if λ=1, it indicates a Brownian phylogenetic dependence (Freckleton et al. 2002; Garamszegi 2014). As it was described above, the phylogenetic trees were obtained from Phylomatic Version 3 (https://phylodiversity.net/phylomatic/), and we used the megatree R20120829 for plants (Webb and Donoghue 2005). Branch lengths were set according to Grafen's method (Grafen 1989), and we applied 'multi2di' and 'collapse.single' functions in R (Revell 2012). In all the models, silica content was the dependent variable, and the biological or ecological features were the predictors or independent variables. Since these variables were qualitative (with two or three states), they were included into PGLS models using dummy coding. This coding consists of creating dichotomous variables in which each level of the categorical variable is

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contrasted with a specified reference level (Faraway 2005). R assigns levels to a factor in alphabetical order, and the reference category is the first. For each variable, the number of species included in the analyses was different, due to the lack of ecological or biological information (Table 1). In the case of life cycle, where deciduous perennial and evergreen perennial states were compared to the annual state, 86 species were included in the analyses. In the growth form variable, shrub/tree state was compared to the herb state, and 88 species were included; while in the plant origin variable, the native state was compared to the exotic state, and 87 species were included. Finally, in the soil water condition variable (wetland or not-wetland species), 85 species were considered. All the data (silica content values) were subjected to the arcsine square root transformation. Model residuals were checked for normality. A visual check of graphs, as usually suggested, was performed to test assumptions about the normality and homogeneity of the residuals in the PGLS model (Zuur et al. 2010). They were evaluated through qq-plots and scatter plots of the residuals of the models against their fitted values. The qq-plot graphs of the proposed models showed that the residuals are approximately normally distributed. In the scatter plots no pattern in the distribution of points was detected, i.e. there is homogeneity of the residuals. For PGLS we used the 'ape', 'nlme', 'phytools' and 'geiger' packages in R version 3.6.1 (R Core Team 2019).

In all the study, the values of silicophytolith/Si content are presented as mean $\pm$ standard deviation.

RESULTS

Silicophytolith content in relation to systematics and tissue origin

Ninety species, included in 19 families, accumulated silicophytoliths in their leaves/ culms. Among those species that produce silicophytoliths, the content (% of dry weight) ranged between 0.38% (*Ranunculus apiifolius*, Ranunculaceae) and 19% (*Chusquea ramossisima*, Poaceae), with a mean of 4.59 $\pm$ 3.46 % (Appendix Table A1).

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In order to analyze if silicophytolith contents of the species were related to their phylogenetic affinities, we measured the phylogenetic signal of the trait through two measures: Pagel's lambda and Blomberg's K. Both showed that there is some phylogenetic signal (we rejected null hypothesis K=0 and λ=0), but the values obtained were intermediate (λ=0.46, $p<0.0001$) or low (K=0.088, p=0.003).

In the analyses of silicophytolith content between clades we observed that the value was higher in monocotyledons (5.45 $\pm$ 3.31%) than in dicotyledons (1.92 $\pm$ 2.95%). Within each group (monocotyledons or dicotyledons), both non-silicophytolith producers and silicophytolith producers were found. If order level is analyzed, a high intra-clade variability in silica content was also observed (Figure 2). For instance, the order Rosales includes both high silicophytolith accumulator families (such as Urticaceae) and low accumulator families (Rhamnaceae). The same low/ high silicophytolith accumulation pattern was observed in Poales: this order comprises Poaceae and Cyperaceae families (high accumulator families) and, on the other hand, Typhaceae, a family that did not produce silicophytoliths.

Nine families did not produce silicophytoliths in any of their species analyzed: Adoxaceae (1 species), Amaranthaceae (2 species), Apiaceae (2 species), Brassicaceae (1 species), Convolvulaceae (1 species), Fabaceae (1 species), Onagraceae (1 species), Polygonaceae (2 species), Typhaceae (1 species). Among the silicophytolith accumulators, the families with the highest contents (> 5% dry weight) were Urticaceae, Poaceae and Cyperaceae, and the families with the lowest values (< 1% dry weight) were Rhamnaceae, Nothofagaceae, Ranunculaceae, Solanaceae and Araliaceae (Figure 2). As it was observed in other taxonomic levels, intra familial variability was found in the data. For instance, Asteraceae and Solanaceae include species that are accumulators and species that are not accumulators (Appendix Table A1). Finally, variability in silicophytolith content was observed within a genus (*Solanum*): *S. glaucophyllum* produced abundant silicophytoliths, while *S. chenopodioides* did not produce any (Appendix Table A1).

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Epidermis was the main silicified tissue, along with the xylem and the parenchyma (Appendix Table A2, Figure 3). Monocotyledons, which comprise families with high silicophytolith contents (Figure 2), accumulated silica in a high diversity of tissues (epidermis, xylem, aerenchyma and parenchyma), while dicotyledons mainly produced silica in epidermis and xylem (Figure 3). The highest producers of phytoliths studied in this work (Urticaceae, Poaceae, Cyperaceae, Moraceae, Cannabaceae and Asteraceae) mostly accumulated in epidermis, but in a high diversity of types of cells (short and long cells, hairs, hooks, cystoliths, typical epidermal cells) (Figures 2, 3, Appendix Table A2). In contrast, Arecaceae, the 5th highest producer, accumulated mainly in parenchyma. Those families whose silica content was lower than 1% (Rhamnaceae, Ranunculaceae, Nothofagaceae, Araliaceae and Solanaceae) accumulated silica in xylem, and in a lesser extent, in epidermis (Figure 3).

Silicophytolith content and biological and ecological features

The mean value of silicophytolith content obtained in annuals (5.85 $\pm$ 4.81%) was higher than in deciduous perennials (2.33 $\pm$ 1.78%) and evergreen perennials (4.73 $\pm$ 3.29%). However, PGLS regression showed no clear statistically significant differences between annual species and the two other states (Table 1). Moreover, although mean silicophytolith content was higher in herbs (5.14 $\pm$ 3.71%) than in shrubs and trees (2.99 $\pm$ 2.01%); and in species non strictly associated to wetlands or saturated soils (5.16 $\pm$ 3.84%) than in wetland species (3.95 $\pm$ 2.34%), no significant differences were obtained from PGLS analyses in these two variables (growth form and soil water condition) (Table 1). The only statistically significant regression was the one obtained in plant origin variable (Table 1), where exotic species have higher silicophytolith content (6.59 $\pm$ 4.52%) than natives (4.28 $\pm$ 3.09%). In all the analyses, the values of λ were intermediate.

DISCUSSION

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Silicophytolith content and systematics

A high percentage (86%) of the species analyzed –corresponding to 82% of the families and 68% of the genera studied- accumulate silicophytoliths in their leaves or culms, confirming the importance of the silicification process in plants (Katz 2018). Our results in relation to silica content and systematics agree with previous research, extend these relationships to southern species, and reflect some inter- and intra-clade variability (Prychid et al. 2004; Hodson et al. 2005; Katz 2015; Strömberg et al. 2016).

The methods here used for the measurement of phylogenetic signal did not clearly demonstrate that closer species have similar silica content. The very low ($K=0.088$) or intermediate ($\lambda=0.46$) values of phylogenetic signal could indicate that the silica content has evolved independently across the phylogeny here used, or that it has evolved under an evolutionary process other than Brownian motion, which is the one used in the models (Revell et al. 2008; Kamilar and Cooper 2013). However, these results are in concordance with the variability observed within clades, where species belonging to the same family have differences in silicophytolith content. As a consequence, these findings might support the idea previously proposed, that the ability of silica accumulation was gained or lost multiple times through plant history (Katz 2015; Strömberg et al. 2016). However, it is important to note that the methods used for phylogenetic signal depend on the available phylogenetic information for the species in our data set, and the treatments of branch lengths and polytomies (see material and methods section) (Blomberg et al. 2003; Revell et al. 2008; Kamilar and Cooper 2013).

Variability within clades was registered at all taxonomic levels, including families and one genus. Two groups could be differentiated at the family level. The first one includes families that share the ability to accumulate silica as a strong character, for example, Poaceae, Arecaceae, Cyperaceae and Urticaceae (Metcalfe 1960; Tomlinson 1990; Piperno 2006). The second group includes families,

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such as Asteraceae and Solanaceae, which contain some species that accumulate silica and others that do not. Considering these differences, it could be proposed that, in the first group, silica accumulation has become essential for its members' biology; the taxa has developed an adequate molecular framework (e.g. NIP transporters) and the accumulation of silicophytoliths in tissues occurs independently of the environmental or phenological factors. In the second case, silica accumulation is likely to be a consequence of other processes (e.g. senescence, transpiration, secretion) and it might not be associated to specific roles. The presence of silicophytoliths may be related to the ability to uptake monosilicic acid (transporters), to environmental conditions (such as Si soil availability, water availability, temperature), or to phenological stages (Jones and Handreck 1967; Motomura et al. 2004; Henriet et al. 2006). Further studies on the effect of diverse factors on low and intermediate accumulators may advance the knowledge on these differences among families.

The anatomical origin of silicophytoliths in different taxa and its relation to functionality

In dicotyledons, where the lowest contents were observed (except for Urticaceae, Moraceae and Cannabaceae, see below), most of the silicophytoliths produced are lumen infillings and are accumulated in typical epidermal cells, trichomes, and stomata complexes. Silica accumulation in epidermal cells has been associated with protection from fungi and small invertebrates (Ma 2004). It has also been explained as the result of the transpiration process through which silica is concentrated, polymerized, and deposited (Ma and Takahashi 2002). However, the antiherbivore or transpiration explanations do not seem to fit in the case of the silicification of the stomata complex, which implies the loss of its function. Instead, it might be explained as a consequence of a senescence process, as observed in bulliform cells in grasses (Fernández Honaine and Osterrieth 2012), or might be stomata-associated to areas of guttation, which have been suggested as the silicic acid exit in low silica accumulators (Exley 2015). Therefore, in this group of low accumulators, most of the silica accumulated seems to result from passive processes (the consequence of

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transpiration or senescence processes), and not strictly associated with a specific function. On the other hand, the highest accumulating dicots, families Urticaceae, Moraceae and Cannabacaeae, produce silica in cystoliths. Different roles have been associated to cystoliths, such as internal source of CO2 for photosynthetic assimilation and light scattering (Gal et al. 2012; Giannopoulos et al. 2019). Consequently, in this last group of dicot species, a higher silica content in leaves associated to cystoliths might be related to a specific role of silica in tissues.

Within the monocot families that produce silicophytoliths, a high diversity of cells are silicified. Two types can be distinguished: a) those that are early, and almost always, silicified (short silica cells in Poaceae; epidermal cells with cone/conical silica in Cyperaceae; parenchyma cells with globular silicophytoliths in Arecaceae); and b) those that are silicified to a lesser extent. This classification of "typical" (group a) and "atypical" (group b) cells has also been proposed for grasses by Blackman and Parry (1968), and in the present study we extend it for the other high accumulating monocot families: Arecaceae and Cyperaceae. The two types of silicified cells may be associated with different mechanisms of silicification and functions. For instance, in the silicification process in short cells from grasses (group a cells), there is a biological control from the cell (Kumar et al. 2017a, 2017b), and their silicification is associated with herbivore deterrence (Keeping et al. 2009; Reynolds et al. 2009; Hartley et al. 2015), UV-radiation protection (Schaller et al. 2013), water loss prevention under drought stress (Ma 2004), or as a reinforcing structural element (Fernández Honaine et al. 2016). On the other hand, in bulliform cells from grasses (group b cells) the accumulation process is strongly associated with leaf senescence or transpiration (Takeoka et al. 1984; Fernández Honaine and Osterrieth, 2012), which might indicate some passive mechanism (Kumar et al. 2017b). In addition, no specific role has been associated to it thus far. Studies on Cyperaceae and Arecaceae are scarcer, especially in relation to potential roles of silica accumulation. Sedge conical silicophytoliths are located in epidermal cells associated with schlerenchyma (group a cells); they have some known metabolic regulation, but no function has been yet proposed (Mehra and Sharma 1965). On the other hand, aerenchyma silicification of culm

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sedges has been associated with senescence (Fernández Honaine et al. 2013). Lastly, the role of silica accumulation in palms is not clear; it has been proposed that its accumulation may indicate the failure to exclude silica from the absorbed water, thus the silica adaptive function may be secondary (Tomlinson 1990). However, its specific location around vascular bundles could indicate some special function, such as light scattering. In summary, it appears that in higher accumulating monocots, two groups of silicified cells are produced: one group with metabolic control of silicification that is associated with specific roles, and another group where the silicification process is passive (*sensu* Kumar et al. 2017b) as a consequence of other processes such as senescence or intense transpiration.

Silicophytolith content and ecological traits

Based on the PGLS results, no relationship between silica content and life cycle was found. This finding differs from the ones described by O'Reagain and Mentis (1989) and Cooke and Leishman (2011b) who observed a negative relationship between the two variables in grasses and other families. Moreover, no relationship between growth form and silicophytolith content was detected, contrary to what was hypothesized in this study.

The exotic species studied in this work accumulate more silicophytoliths than native species. If we analyze in detail the species included in the exotic group, most of them belong to high accumulating families (Moraceae, Urticaceae, and Cannabaceae). This may lead to the idea that there is a bias in the species included in the analysis, where the high accumulating families are only represented by exotic species. However, in those families with a high number of species analyzed, such as Poaceae and Asteraceae, the exotic ones have the highest silica contents (e.g. *Vulpia dertonensis* 12%, *Festuca arundinaceae* 12%, *Lolium multiflorum* 9% in Poaceae; *Achillea millefolium* 12% in Asteraceae) (Appendix Table A1). Therefore, high silica content in exotic species appears to be a robust pattern. As proposed in the present study, silica accumulation may facilitate the growth of

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exotic plants in novel sites by providing an advantage like a more economical reinforcing component that improves plant structure or as an antiherbivore strategy (Raven 1983; Reynolds et al. 2009; Hartley and DeGabriel 2016). For example, the ability to accumulate high levels of silica in some of the exotic and invasive species considered in this study, such as *Ligustrum lucidum* and *Rubus ulmifolius* (Grau and Aragón 2000; Mazzolari and Comparatore 2014), might explain why they are ecologically successful in novel environments. Future studies including additional native and exotic species growing under the same environmental conditions, will contribute to the understanding of this important issue in invasion ecology.

Many researchers have found higher silica accumulation in wetland species compared to dryland species, and this trend has been ascribed to higher water uptake (leading to higher silicic acid uptake) or to a role of silica as a reinforcing element in water-associated species (e.g. Schoelynck et al. 2010; Quigley and Anderson 2014). However, in our work, no relationship between preference for wet/ humid environments and the silicification process has been found. This result indicates that water availability does not directly affect amorphous silica content in plants, at least in this group of species from southern South America. Other environmental characteristics, such as soil Si availability, evapotranspiration rate, bio-ecological features, or the phylogenetic position, may have larger influence than water availability on the silicophytolith accumulation process in plants.

In summary, the results of the present study confirm that there is inter- and intra-taxa variation in the amorphous silica contents in plants. These findings are in concordance with the low phylogenetic signal of the variable silicophytolith content measured for the first time in a set of data with methodological uniformity. In turn, this supports the idea that silica accumulation is a homoplasic character, at least in angiosperms. Based on the overall analysis of the silicophytolith content and tissue distribution, it can be interpreted that in those taxa with high silica content and a high diversity of silicified cells, silicophytoliths are accumulated by both passive and controlled mechanisms, and the accumulation responds to specific functions in the plant. In low silica accumulators, this process may be associated with passive phenomena and interpreted as a

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secretion, not associated with specific functions in tissues. Finally, out of all the bio-ecological variables studied, plant origin (native vs. exotic) was the only one related to silica content. The fact that silicophytoliths represent an effective antiherbivore defense and an economical structural material may be advantageous for exotic species, and may explain their higher contents in comparison to native species. As abovementioned, and particularly for the exotic species, it is possible to relate a high silicophytolith content with a specific function, which in this case, would be an ecological function.

ACKNOWLEDGEMENTS

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Tables and Figures captions

Table 1. T-values and maximum likelihood estimate of λ from PGLS testing the relationship between silica content (dependent variable) and biological/ecological features (predictors). n= number of species included in the analyses.

Figure 1. Location of the main phytogeographic provinces (1. Paranaense Province, 2. Pampean Province, 3. Subantarctic Province) where most of the species were collected and panoramic view of some of the most representative communities (Map modified from Cabrera and Willink 1973).

Figure 2. Average + standard deviation of silicophytolith content (% Silica), measured as percentage of dry weight, of the different families studied. Families with no silicophytoliths were not included in the figure. Different colors or colors plus symbol indicate the order to which each family belongs.

Figure 3. Silica content and silicophytolith tissue origin in the families analyzed, in relation to phylogeny. The phylogenetic tree was obtained from Phylomatic Version 3 (https://phylodiversity.net/phylomatic/) (Webb and Donoghue 2005) (see materials and methods) and the branch lengths were arbitrary set to 1.

Appendix

Table A1. List of species studied, their ecological features, silicophytolith production (% Silica content) and references. N: number of replicates. P: perennial, A: annual. n: natives + endemics, e: exotics + introduced + adventitious. n.p.: non producer. The superscript in the species indicate the sampling location of the specimens.

Table A2. Main silicophytolith morphologies described in the families analyzed (based on published and unpublished resources) and their tissue assignment, according to this study.

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Table 1. T-values and maximum likelihood estimate of λ from PGLS testing the relation between silica content and biological/ecological features (predictors). N= number of species included for the analyses.

Predictor	T	P	λ	N
Life cycle (deciduous perennials vs. annuals)	1.68	0.09	0.46	86[1]
(evergreen perennials vs. annuals)	1.72	0.08	0.46	86[1]
Growth form (shrub/tree vs. herbs)	1.35	0.17	0.52	88[2]
Plant origin (native vs. exotics)	4.35	$0.01e^{-3}$	0.66	87[3]
Soil water condition (wetland vs. not wetland)	1.24	0.21	0.47	85[4]

1. *Calycera* sp., *Ranunculus* sp., *Bromus catharticus, Baccharis* sp were not included.
2. *Baccharis* sp. and *Ranunculus* sp. were not included.
3. *Calycera* sp., *Ranunculus* sp, *Baccharis* sp. were not included.
4. *Calycera* sp., *Ranunculus* sp, *Baccharis* sp., *Conyza* sp., *Zingiber* sp. were not included.

Figure 1

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1. Paranaense Province

2. Pampean Province

3. Subantartic Province

Figure 2

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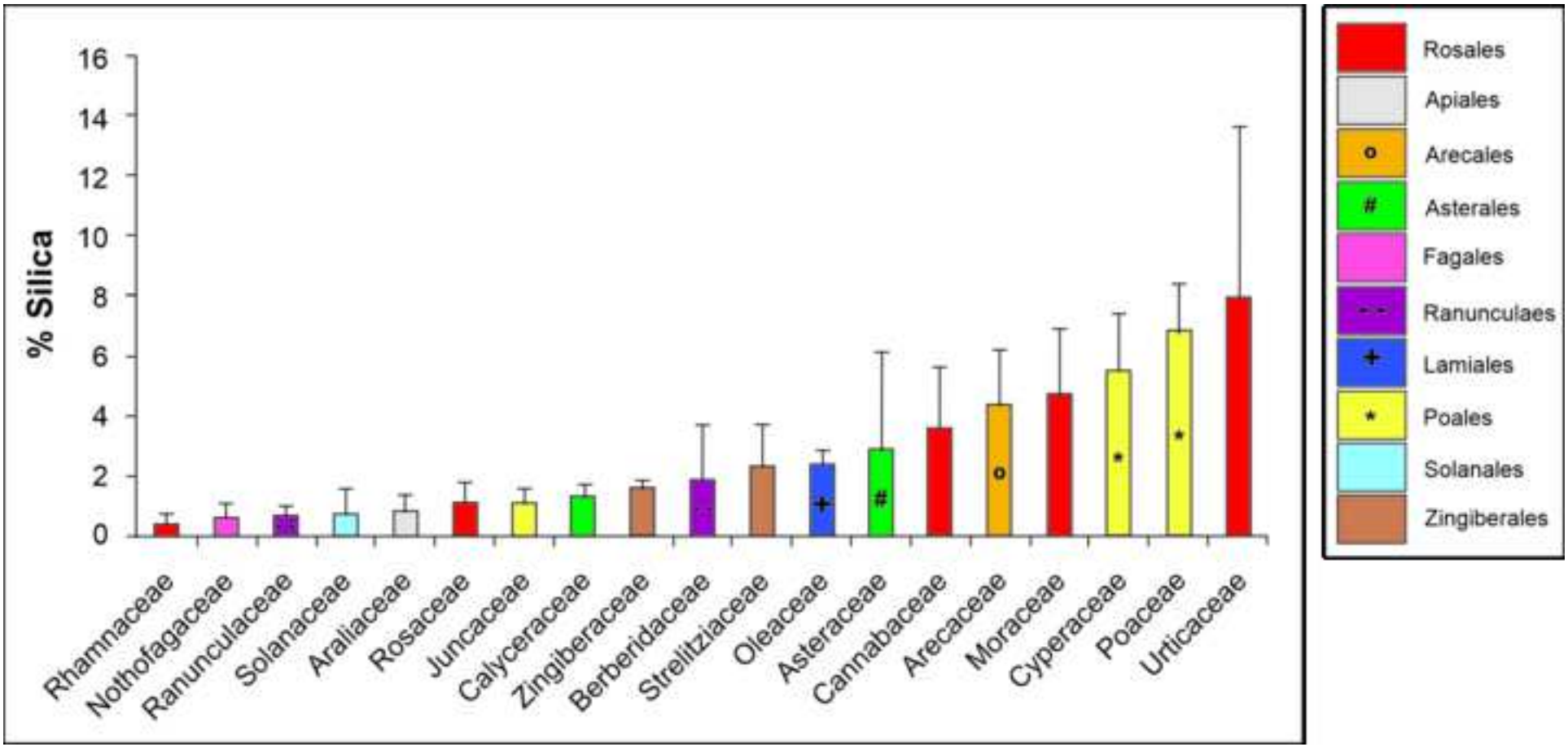

Figure 3

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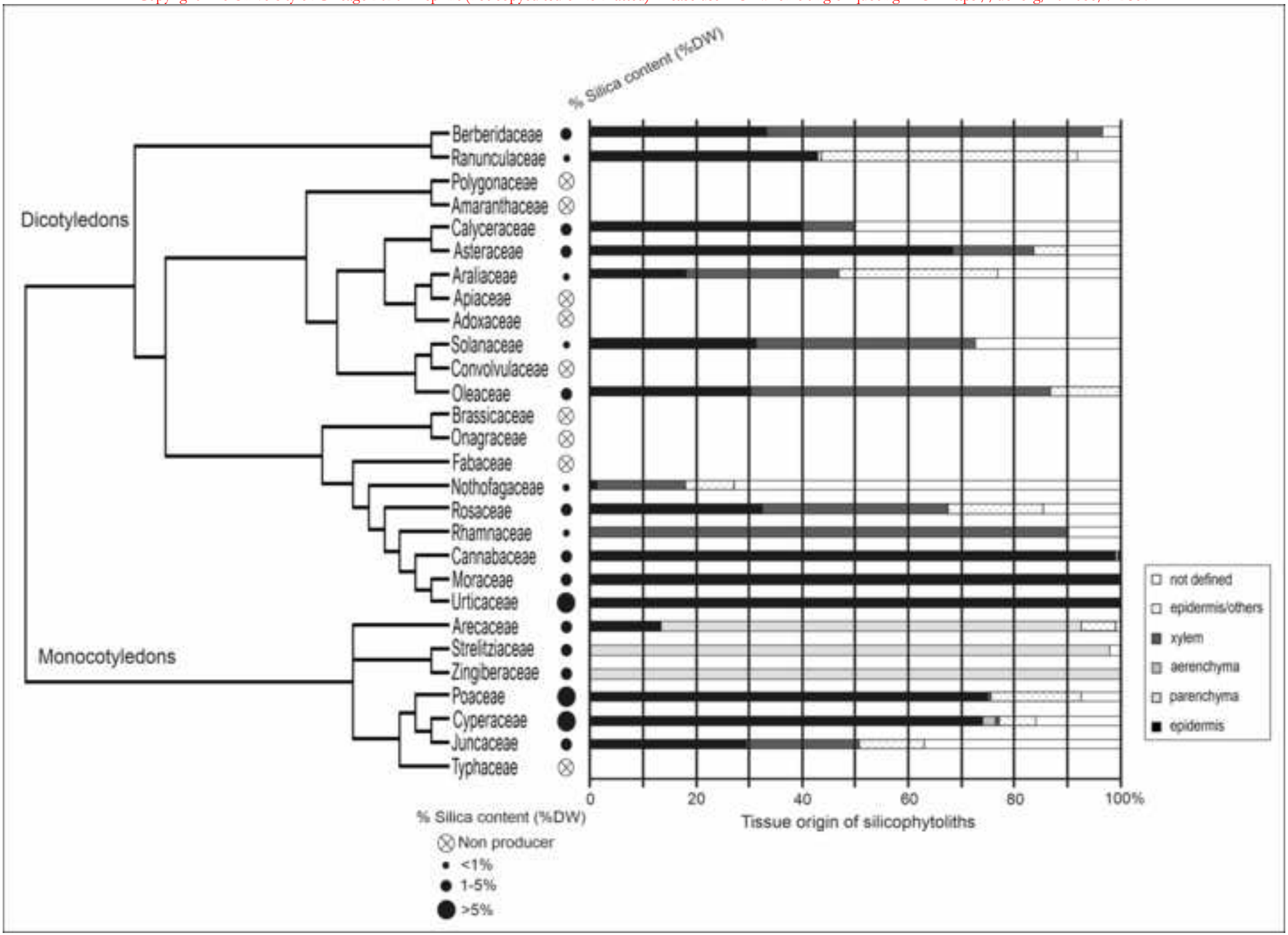

Figure 2 black and white

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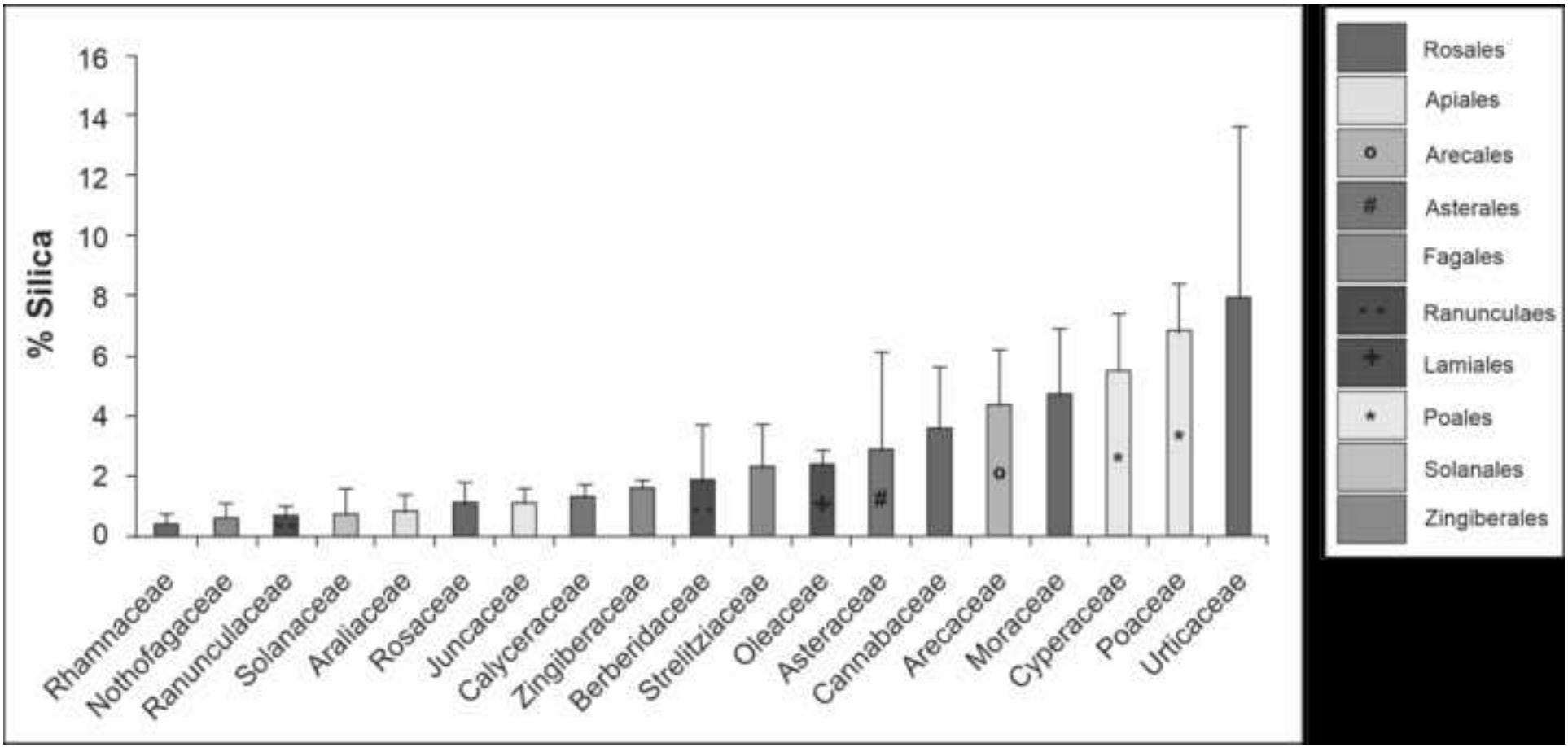

Supplemental Material Table A1 Click here to access/download;Supplemental Material;Appendix Table A1.docx

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Appendix

Table A1. List of species studied, their ecological features, silicophytolith production (% silica content) and references. N: number of replicates. P: perennial, A: annual. n: native/endemics, e: exotic/introduced/adventitious. n.p.: non producer. The superscript in the species indicate the sampling location of the specimens.

Family	Species	N	Life-cycle	Plant origin	Growth form	Wetland species?	% Silica content (mean ± s.d.)	References
Adoxaceae	*Sambucus australis* Cham. & Schltdl. [2]	2	P	n	tree/shrub	no	n.p.	De Rito et al. 2018
Amaranthaceae	*Alternanthera philoxeroides* (Mart.) Griseb.[2]	2	P	n	herbs	yes	n.p.	Borrelli et al. 2011
Amaranthaceae	*Sarcocornia perennis (Mill.) A.J. Scott* [2]	3	P	e	tree/shrub	yes	n.p.	Altamirano et al. 2018
Apiaceae	*Azorella trifurcata* (Gaertn.) Pers.[2]	2	P	n	herbs	yes	n.p.	Benvenuto et al. not published
Apiaceae	*Hydrocotyle bonariensis* Lam.[2]	3	P	n	herbs	yes	n.p.	Altamirano et al. not published
Araliaceae	*Hedera helix* L.[2]	3	P	n	herbs	no	0.79 ±0.45	De Rito et al. 2018
Arecaceae	*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.[1]	3	P	n	tree/shrub	no	4.86 ±1.29	Benvenuto et al. 2015
Arecaceae	*Allagoptera campestris* (Mart.) Kuntze [1]	3	P	n	tree/shrub	no	1.67 ±0.78	Benvenuto et al. 2015
Arecaceae	*Butia capitata* (Mart.) Becc.[1,2]	3	P	n	tree/shrub	no	4.61±0.83	Benvenuto et al. 2015

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Arecaceae	*Butia paraguayensis* (Barb. Rodr.) L.H. Bailey [1,2]	3	P	n	tree/shrub	no	2.18 ±0.96	Benvenuto et al. 2015
Arecaceae	*Copernicia alba* Morong[1]	3	P	n	tree/shrub	no	5.11 ±1.07	Benvenuto et al. 2015
Arecaceae	*Euterpe edulis* Mart.[1]	3	P	n	tree/shrub	no	5.52 ±3.15	Benvenuto et al. 2015
Arecaceae	*Phoenix canariensis* Chabaud [2]	2	P	e	tree/shrub	no	5.00 ±0.17	Benvenuto et al. 2015
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman[1]	3	P	n	tree/shrub	no	2.85 ±0.30	Benvenuto et al. 2015
Arecaceae	*Trachycarpus fortunei* (Hook.) H. Wendl.[2]	2	P	e	tree/shrub	no	7.97 ±0.04	Benvenuto et al. 2015
Arecaceae	*Trithrinax campestris* (Burmeist.) Drude & Griseb.[2]	3	P	n	tree/shrub	no	3.80 ±0.97	Benvenuto et al. 2015
Asteraceae	*Achillea millefolium L.*[2]	2	P	e	herbs	no	12.51 ±3.51	Fernández Honaine et al. 2006
Asteraceae	*Baccharis articulata* (Lam.) Pers. [2]	3	P	n	tree/shrub	no	1.56 ±0.16	Fernández Honaine not published
Asteraceae	*Baccharis* sp. Speg. [2]	3	P	n	tree/shrub	no	0.71 ±0.11	De Rito et al. 2018
Asteraceae	*Bidens laevis* (L.) Britton, Stern & Poggenb.[2]	3	P	n	herbs	yes	1.02 ±0.90	Borrelli et al., 2011
Asteraceae	*Carduus acanthoides* L. [2]	7	A	n	herbs	no	2.38 ±0.96	Fernández Honaine et al. 2006; De Rito et al., 2018
Asteraceae	*Cirsium vulgare* (Savi) Ten.[2]	5	A	n	herbs	no	2.99 ±0.49	Fernández Honaine et

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								al. 2006
Asteraceae	*Conyza* sp. [2]	2	A	n	herbs	no	1.37 ±0.14	De Rito et al. 2018
Asteraceae	*Mikania parodii* Cabrera [2]	2	P	n	herbs	yes	2.72 ±0.50	Borrelli et al. 2011
Asteraceae	*Pluchea sagittalis* (Lam.) Cabrera [2]	3	P	n	herbs	yes	2.24 ±0.26	Fernández Honaine not published
Asteraceae	*Senecio madagascariensis* Poir. [2]	2	P	e	herbs	no	1.42 ±0.23	De Rito et al. 2018
Asteraceae	*Senecio magellanicus* Hook. & Arn. [3]	3	P	n	herbs	no	5.08 ±3.88	Benvenuto et al. 2013b
Asteraceae	*Taraxacum officinale* F.H. Wigg [2]	5	P	e	herbs	no	n.p.	Fernández Honaine et al. 2006
Berberidaceae	*Berberis microphylla* G. Forst. [3]	3	P	n	tree/shrub	no	1.86 ±1.52	Benvenuto et al. 2013b
Brassicaceae	*Cakile maritima* Scop. [2]	3	A	e	herbs	yes	n.p.	Fernández Honaine et al. not published
Calyceraceae	*Calycera* sp. [2]	3	P		herbs	not defined	1.32 ±0.30	Fernández Honaine et al. not published
Cannabaceae	*Celtis ehrenbergiana* (Klotzsch) Liebm. [2]	9	P	n	tree/shrub	no	2.13 ±1.23	De Rito et al. 2018
Cannabaceae	*Celtis occidentalis* L. [2]	3	P	e	tree/shrub	no	5.03 ±0.82	De Rito et al. 2018
Convolvulaceae	*Dichondra microcalyx* (Hallier f.) Fabris [2]	3	P	n	herbs	no	n.p.	De Rito et al. 2018
Cyperaceae	*Carex chilensis* Brongn. ex Duperrey [2]	4	P	n	herbs	yes	5.57 ±2.26	Fernández Honaine et al. 2009
Cyperaceae	*Carex magellanica* Lam. [3]	3	P	n	herbs	yes	3.56 ±1.03	Benvenuto et al. 2013b
Cyperaceae	*Carex phalaroides* Kunth [2]	3	P	n	herbs	yes	4.85 ±2.05	Fernández Honaine et al. 2009

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Cyperaceae	*Carex tweediana* Nees [2]	3	P	n	herbs	yes	5.68 ±1.80	Fernández Honaine et al. 2009
Cyperaceae	*Cyperus corymbosus* var. *subnodosus* Rottböll [2]	3	P	n	herbs	yes	2.91 ±1.56	Fernández Honaine et al. 2009
Cyperaceae	*Cyperus digitatus* Roxburgh [2]	2	P	n	herbs	yes	7.18 ±0.12	Fernández Honaine et al. 2009
Cyperaceae	*Cyperus eragrostis* Lam. [2]	5	P	n	herbs	yes	5.67 ±2.55	Fernández Honaine et al. 2009
Cyperaceae	*Cyperus odoratus* L. [2]	3	P	n	herbs	yes	5.40 ±0.85	Fernández Honaine et al. 2009
Cyperaceae	*Cyperus reflexus* Vahl [2]	3	P	n	herbs	yes	7.80 ±4.82	Fernández Honaine et al. 2009
Cyperaceae	*Eleocharis bonariensis* Ness. [2]	4	P	n	herbs	yes	9.55 ±1.83	Fernández Honaine et al. 2009
Cyperaceae	*Eleocharis macrostachya* Britton [2]	3	P	n	herbs	yes	3.33 ±0.75	Fernández Honaine et al. 2009
Cyperaceae	*Rhynchospora corymbosa* var. *bonariensis* Barros ex Cabrera & G. Dawson [2]	3	P	n	herbs	yes	6.60 ±1.95	Fernández Honaine et al. 2009
Cyperaceae	*Schoenoplectus californicus* (C.A. Mey.) Soják [2]	8	P	n	herbs	yes	3.75 ±1.90	Fernández Honaine et al. 2009
Fabaceae	*Melilotus albus* Desr. [2]	3	A	e	herbs	no	n.p.	Fernández Honaine et

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								al. not published
Juncaceae	*Juncus acutus* L. [2]	3	P	n	herbs	yes	0.70 ±0.02	Altamirano et al. 2018
Juncaceae	*Juncus imbricatus* Laharpe [2]	2	P	n	herbs	yes	1.25 ±0.28	Borrelli et al. 2011
Juncaceae	*Juncus microcephalus* Kunth. [2]	2	P	n	herbs	yes	1.75 ±0.02	Borrelli et al. 2011
Juncaceae	*Luzula alopecurus* Desv. [3]	2	P	n	herbs	no	0.57 ±0.11	Benvenuto et al. 2013b
Moraceae	*Morus alba* L.[2]	3	P	e	tree/ shrub	no	4.71 ±1.80	Fernández Honaine et al. 2018
Nothofagaceae	*Nothofagus pumilio* (Poepp. & Endl.) Krasser [3]	2	P	n	tree/ shrub	no	0.61 ±0.35	Benvenuto et al. 2013b
Oleaceae	*Ligustrum lucidum* W.T. Aiton [2]	6	P	e	tree/ shrub	no	2.00 ±0.86	De Rito et al. 2018
Oleaceae	*Ligustrum sinense* Lour. [2]	6	P	e	tree/shrub	no	2.69 ±0.56	De Rito et al. 2018
Onagraceae	*Ludwigia peploides* (Kunth) P.H. Raven [2]	3	P	n	herbs	yes	n.p.	Borrelli et al. 2011
Poaceae	*Alopecurus magellanicus* Lam. [3]	3	P	n	herbs	yes	4.89 ±2.82	Benvenuto et al. 2013b
Poaceae	*Bothriochloa laguroides* (DC) Herter [2]	8	P	n	herbs	no	8.33 ±2.90	Fernández Honaine et al. 2006
Poaceae	*Briza subaristata* Lam. [2]	8	P	n	herbs	no	9.38 ±5.32	Fernández Honaine et al. 2006
Poaceae	*Bromus auleticus* Trin. Ex Ness [2]	8	P	n	herbs	no	8.76 ±2.34	Fernández Honaine et al. 2006
Poaceae	*Bromus catharticus* Vahl. [2]	8	undefined	n	herbs	no	6.53 ±3.62	Fernández Honaine et al. 2006

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Poaceae	*Chusquea ramosissima* Lindm. [2]	4	P	n	herbs	no	19.36 ±5.41	Montti et al. 2009
Poaceae	*Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn.[2]	4	P	n	herbs	no	4.04 ±1.45	Fernández Honaine et al. 2017
Poaceae	*Dactylis glomerata* L. [2]	8	P	e	herbs	no	6.52 ±1.68	Fernández Honaine et al. 2006
Poaceae	*Danthonia montevidensis* Hack. & Arechav. [2]	3	P	n	herbs	no	6.15 ±1.44	Fernández Honaine et al. 2006
Poaceae	*Deschampsia antarctica* E. Desv. [3]	3	P	n	herbs	yes	2.91 ±0.75	Benvenuto et al. 2013b
Poaceae	*Deyeuxia poaeoides* (Steud.) Rúgolo [3]	3	P	n	herbs	yes	4.87 ±2.08	Benvenuto et al. 2013b
Poaceae	*Elymus angulatus* J. Presl. [3]	3	P	n	herbs	yes	4.52 ±3.18	Benvenuto et al. 2013b
Poaceae	*Festuca arundinacea* Schreb. [2,3]	10	P	e	herbs	no	12.65 ±5.46	Benvenuto et al. 2013b
Poaceae	*Festuca gracillima* Hook. [3]	2	P	n	herbs	no	5.06 ±0.13	Benvenuto not published
Poaceae	*Festuca magellanica* Lam. [3]	2	P	n	herbs	no	3.18 ±0.56	Benvenuto et al. 2013b
Poaceae	*Jarava plumosa* (Spreng.) S.W.L. Jacobs & J. Everett [2]	7	P	n	herbs	no	6.95 ±2.68	Fernández Honaine et al. 2006
Poaceae	*Lolium multiflorum* Lam. [2]	4	A	e	herbs	no	9.04 ±1.98	Fernández Honaine et al. not published
Poaceae	*Melica brasiliana* Ard. [2]	9	P	n	herbs	no	6.56 ±3.13	Fernández Honaine et al. 2006
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth [2]	9	P	n	herbs	no	6.40 ±2.55	Fernández Honaine et al. 2006

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Poaceae	*Nassella trichotoma* (Nees) Hack. ex Arechav.[2]	5	P	n	herbs	no	8.64 ±4.73	Fernández Honaine et al. 2006
Poaceae	*Paspalum quadrifarium* Lam.[2]	5	P	n	herbs	no	3.98 ±1.15	Fernández Honaine et al. 2006
Poaceae	*Phleum alpinum* L. [3]	3	P	n	herbs	no	0.99 ±0.41	Benvenuto et al. 2013b
Poaceae	*Piptochaetium bicolor* (Vahl) E. Desv. [2]	9	P	n	herbs	no	9.67 ±3.85	Fernández Honaine et al. 2006
Poaceae	*Piptochaetium hackelii* (Arechav.) Parodi [2]	5	P	n	herbs	no	6.22 ±2.98	Fernández Honaine et al. 2006
Poaceae	*Piptochaetium lasianthum* Griseb. [2]	6	P	n	herbs	no	5.04 ±1.95	Fernández Honaine et al. 2006
Poaceae	*Piptochaetium medium* (Speg.) Torres [2]	8	P	n	herbs	no	6.95 ±2.71	Fernández Honaine et al. 2006
Poaceae	*Puccinellia magellanica* (Hook. f.) Parodi [3]	3	P	n	herbs	no	5.77 ±3.22	Benvenuto et al. 2013b
Poaceae	*Sorghastrum pellitum* (Hack.) Parodi [2]	8	P	n	herbs	no	3.91 ±1.28	Fernández Honaine et al. 2006
Poaceae	*Trisetum spicatum* (L.) K. Richt. [3]	2	P	n	herbs	no	3.38 ±0.93	Benvenuto et al. 2013b
Poaceae	*Vulpia bromoides* (L.) Gray [2]	7	A	e	herbs	no	12.83 ±8.90	Fernández Honaine et al. 2006
Polygonaceae	*Polygonum hydropiperoides* Michx [2]	2	P	n	herbs	yes	n.p.	Borrelli et al. 2011
Polygonaceae	*Rumex crispus* L. [2]	3	P	e	herbs	yes	n.p.	Borrelli et al. 2011

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Ranunculaceae	*Ranunculus apiifolius* Pers. [2]	2	A	n	herbs	yes	0.38 ±0.08	Borrelli et al. 2011
Ranunculaceae	*Ranunculus* sp. [3]	2			herbs	not defined	0.92 ±0.60	Benvenuto et al. 2013b
Rhamnaceae	*Colletia paradoxa* (Spreng.) Escal. [2]	7	P	n	tree/ shrub	no	0.39 ±0.30	De Rito et al. 2018
Rosaceae	*Acaena magellanica* (Lam.) Vahl [3]	4	P	n	herbs	yes	1.95 ±1.15	Benvenuto et al. 2013b
Rosaceae	*Rubus geoides* Sm. [3]	3	P	n	herbs	no	0.57 ±0.23	Benvenuto et al. 2013b
Rosaceae	*Rubus ulmifolius* Schott [2]	3	P	e	tree/ shrub	no	0.60 ±0.20	De Rito et al. 2018
Solanaceae	*Cestrum parqui* L`Her [2]	3	P	n	tree/ shrub	no	1.17 ±0.27	De Rito et al. 2018
Solanaceae	*Salpichroa origanifolia* (Lam.) Baill. [2]	2	P	n	herbs	no	n.p.	De Rito et al. 2018
Solanaceae	*Solanum chenopodioides* Lam. [2]	4	A	n	herbs	no	n.p.	De Rito et al. 2018
Solanaceae	*Solanum glaucophyllum* Desf. [2]	3	P	n	tree/ shrub	yes	1.73 ±2.11	Borrelli et al. 2011
Strelitziaceae	*Strelitzia reginae* Aiton [2]	2	P	e	herbs	no	2.30 ±0.98	Benvenuto et al. 2015
Typhaceae	*Typha latifolia* L. [2]	3	P	e	herbs	yes	n.p.	Borrelli et al. 2011
Urticaceae	*Parietaria debilis* G. Forst. [2]	3	A	e	herbs	no	14.44±1.66	Fernández Honaine et al. 2018
Urticaceae	*Parietaria judaica* L. [2]	3	A	e	herbs	no	5.82 ±3.16	Fernández Honaine et al. 2018
Urticaceae	*Urtica urens* L. [2]	2	A	n	herbs	no	3.44 ±1.69	Fernández Honaine et

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						al. 2018
Zingiberaceae	*Zingiber* sp. [2]	2	herbs	not defined	1.60 ±0.21	Benvenuto et al. 2015

[1] Paranaense Province, [2] Pampean Province, [3] Subantartic Province.

Supplemental Material Table A2
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Appendix

Table A2. Main silicophytolith morphologies described in the families analyzed (based on published and unpublished resources) and their tissue assignment, according to this study.

Plant Family	Silicophytolith morphologies described	Tissue assignment	References
Araliaceae	Elongate psilate	Epidermis/others	1
	Epidermal tabular polygonal	Epidermis	
	Cylindrical sulcate	Xylem	
Arecaceae	Elongate psilate	Epidermis/others	2
	Stomatal complexes	Epidermis	
	Globular echinate	Parenchyma	
Asteraceae	Elongate psilate, crenate or echinate	Epidermis/others	1; 3; 4; 5
	Trichomes, stomatal complexes, epidermal tabular lobate, epidermal tabular polygonal	Epidermis	
	Cylindrical sulcate	Xylem	
Berberidaceae	Stomatal complexes	Epidermis	5
	Cylindrical sulcate	Xylem	
Calyceraceae	Stomatal complexes, epidermal tabular polygonal	Epidermis	6

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	Cylindrical sulcate	Xylem	
Cannabaceae	Trichomes, epidermal tabular polygonal, cystoliths	Epidermis	1
	Cylindrical sulcate	Xylem	
Cyperaceae	Elongate psilate and crenate	Epidermis/others	5; 7
	Cones, trichomes, epidermal tabular polygonal, stomatal complexes	Epidermis	
	Cylindrical sulcate	Xylem	
	Stellate	Aerenchyma	
Juncaceae	Elongate psilate	Epidermis/others	4; 5; 8
	Epidermal tabular polygonal, stomatal complexes	Epidermis	
	Cylindrical sulcate xylem	Xylem	
Moraceae	Trichomes, cystoliths, stomatal complexes	Epidermis	9
Oleaceae	Epidermal tabular polygonal, trichomes	Epidermis	1
	Cylindrical sulcate xylem	Xylem	
	Polyhderical/Blocky	Epidermis/others	

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Poaceae	Short silica cells (bilobates, rondels, trapeziforms, crosses), stomatal complexes, bulliforms, elongate with concave ends, trichomes	Epidermis	3; 5; 10; 11
	Elongate psilate, crenate and echinate	Epidermis/others	
	Cylindrical sulcate	Xylem	
Ranunculaceae	Elongate psilate	Epidermis/others	4; 5
	Epidermal tabular polygonal, stomatal complexes, trichomes	Epidermis	
Rhamnaceae	Cylindrical sulcate	Xylem	1
Rosaceae	Elongate psilate, crenate	Epidermis/others	1; 5
	Epidermal tabular lobate, epidermal tabular polygonal, trichomes, stomatal complexes	Epidermis	
	Cylindrical sulcate	Xylem	
Solanaceae	Epidermal tabular polygonal, stomatal complexes	Epidermis	1; 4
	Cylindrical sulcate xylem	Xylem	
Strelitziaceae	Irregular multifaceted	Parenchyma	2

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Urticaceae	Cystoliths, trichomes, epidermal tabular polygonal	Epidermis	9
Zingiberaceae	Globular psilate	Parenchyma	2

[1]De Rito et al. 2018, [2]Benvenuto et al. 2015, [3]Fernández Honaine et al. 2006, [4]Borrelli et al. 2011, [5]Benvenuto et al. 2013, [6]Fernández Honaine et al. not published; [7]Fernández Honaine et al. 2009; [8]Altamirano et al. 2018; [9]Fernández Honaine et al. 2018; [10]Montti et al. 2009; [11]Fernández Honaine et al. 2017.

Epidermis/others: this category includes those silicophytolith morphologies described in the references which cannot be assigned to a unique tissue, because the morphology can derived from diverse type of cells.