HOW ARE SYSTEMATICS AND BIOLOGICAL AND ECOLOGICAL FEATURES RELATED TO SILICA CONTENT IN PLANTS? A STUDY OF SPECIES FROM SOUTHERN SOUTH AMERICA

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Premise of 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 on silicophytolith content from 105 species of South America, examining, in a phylogenetic context, its relationship with anatomy and ecological features such as life cycle, growth form, plant origin, and environmental preferences.

Methodology. Data on the silicophytolith content and bioecological features of the species were obtained from published and unpublished sources. The relations between systematics, silica content, and bioecological variables were analyzed through measurements of phylogenetic signal and phylogenetic generalized least squares regressions.

Pivotal results. Eighty-six percent of the species produced between 0.38% and 19% of silicophytolith dry weight in leaves. Silica content was variable between and within clades. Values of λ and K indicate a low phylogenetic signal for the variable silica content. Dicotyledons accumulated silica in typical epidermal cells, and a few families also stored it in cystoliths. Most of the monocot families showed high silicophytolith contents and a high diversity of silicified cells. Plant origin affected the 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 inter- and intraclade variability, which in turn supports the hypothesis that silicophytolith accumulation is a homoplastic character among plants. On the basis of the overall analysis of silicophytolith content and tissue distribution, high content could be related to the specific accumulation mechanisms and roles of silica. The origin of the plant was the only bioecological variable that influenced plant silica content. This finding may indicate some ecological role for silica in exotic plants involving their success in novel environments.

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

Online enhancements: appendix tables.
dicotyledons and noncommelinid monocots (Pychid et al. 2004; Hodson et al. 2005). However, some studies have also reported intralclade variations, for example, at the 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 silified tissue is the epidermis, but silica accumulation also occurs in the parenchyma, xylem, endodermis (in roots), and sclerenchyma, both as inclusions and as complete lumen infillings (Piperno 2006). The relationships between silica content, systematics, and anatomy have been studied much more frequently in grasses than in other monocots or dicots. This knowledge is essential for understanding the roles of silica in plant biology and evolution (Strömberg et al. 2016).

Besides phylogenetic constraints, bioecological features such as life cycle, growth form, the origin of the plant, and soil water availability may affect the production of silicophytoliths in plants. Previous research has shown that silica content is negatively correlated with leaf life span or the life cycle of plants (Cook and Leishman 2011b). This was explained by the differences in the leaf carbon balance strategies of annuals and perennials and the advantage of silicon as a cheaper alternative to lignin and cellulose (Raven 1983; O’Reagain and Mentis 1989; Cooke and Leishman 2011b). Studies of 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 silification 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 a percentage of 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. It has been hypothesized that sili
cophytoliths are both a physical antiherbivore 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 on *Ligustrum lucidum*, an exotic and invasive species in South America, revealed that plants grown in native areas (Argentina) had higher silica content than those in native regions (China; Fernández Honaine et al. 2019b). This result may suggest that under some special circumstances (e.g., in the presence of soils with high Si availability), those species with the ability to accumulate silica may take advantage of it, either as an antiherbivore deterrent or as a structural reinforcement (De Rito et al. 2016; Montti et al. 2016; Fernández Honaine et al. 2019b). 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.

Finally, environmental factors such as soil water availability may also affect plant silica content; some authors relate high water availability (plants either growing in wetter environments or subjected to irrigation management) with high silica accumulation (e.g., Jones and Handreck 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 with saturated soils or wetlands. In this sense, aquatic species have been described as high 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 bioecological features (life cycle, growth form, plant origin, and soil water availability). We did this work with species from a scarcely studied region (eastern South America) using samples collected in a specific period of time (2003–2018). Data on silicophytolith content were obtained from published and unpublished sources and were measured with the same technique to avoid differences in content due to methodology. We addressed the following questions: (1) How does 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 silification, and the potential role of silica? (3) Among silica-accumulating species, is silicophytolith content variable according to life cycle, growth form, plant origin (exotic vs. native), and/or soil water conditions? We predicted differences in silica content among taxa and, as a consequence, differences in the types of cells that are silificated. We also expected a great diversity of silified cell types in highly accumulating families. Finally, we predicted 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.

**Material and Methods**

**Plants**

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 in South America (Cabrera 1971; fig. 1; table A1; tables A1, A2 are available online). The Paranaense Province (fig. 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 (fig. 1.2) is mainly characterized by a gramineous steppe extending into the east of Argentina and Uruguay, between lat. 31° and 39°S. The climate is temperate, with a mean annual temperature between 13° and 17°C and total annual precipitation of 600–1100 mm. Finally, the Subantarctic Province (fig. 1.3) is an area characterized by deciduous and evergreen forests, grasslands, and peatlands. It extends along the Austral Andes from 37°S up to Cape de Hornos, and it also includes 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 part, with a mean annual temperature of ca. 5°C (Cabrera 1971).

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

**Silicophytolith Content**

Data on silicophytolith content were obtained from published and unpublished sources detailed in table A1. 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 calculated as a percentage of dry weight. The descriptions of the silicophytolith morphologies were obtained from the references mentioned for each species in table A1. The assignment of each morphology to a specific tissue (table A2) was determined based on the references cited in table A1, from a specific bibliography, or through the application of histological techniques. In this last case, freehand transverse and longitudinal sections of samples were obtained, cleared, and mounted in immersion oil (Fernández Honaine et al. 2019a). This technique is a simple and fast way of visualizing the cells that are silicified without destroying the tissue. The distribution of silicophytoliths in the tissues was observed under a Zeiss Axiosstar Plus microscope at × 400 magnification.

**Systematic, Biological, and Ecological Features**

Taxonomic, life cycle (annual/perennial), growth form (tree/shrub/herb), and plant origin (native/exotic) information for each species was obtained from the Tropicos website (http://www.tropicos.org) and the Instituto de Botánica Darwinion (http://www2.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm). Three life cycle types were considered for the statistical analyses, as follows: annuals (mostly herbs), deciduous perennials, and evergreen perennials. The classification of the species in wetlands...
and nonwetlands (soil water condition) followed published literature on the regional flora (Cabrera and Zardini 1978; Moore 1983) and the Flora Argentina database (http://www.florargentina.edu.ar/). Wetland species include taxa that commonly grow in wet/humid environments, in saturated soils, and/or near lagoons, ponds, rivers, or any other wetlands.

**Data Analyses**

To analyze the relation between silicophytolith content and phylogenetic affinities among species (i.e., whether closely related species are more likely to have similar silicophytolith content or whether silicophytolith content varies randomly across a phylogeny), we measured the phylogenetic signal of silica content. Two measures were applied, as follows: Pagel’s \( \lambda \) (Pagel 1999) and Blomberg’s \( K \) (Blomberg et al. 2003). We used the phylosig function from the phytools package in R version 3.6.1 (R Core Team 2019). This function calculates both values (\( \lambda \) and \( K \)) and also a \( P \) value for the tests where the null hypotheses were \( \lambda = 0 \) or \( K = 0 \), respectively (Revell 2012). We included only 103 species since two of the species could not be placed in the phylogenetic tree. The phylogenetic tree was obtained from Phylomatic version 3 (http://www.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). To obtain binary trees, we applied the 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/nonwetland species) was evaluated through phylogenetic generalized least squares (PGLS) regression models. The PGLS method considers the phylogenetic nonindependence 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 measurements of the phylogenetic signal for regression residuals is Pagel’s \( \lambda \), which was estimated with maximum likelihood. When \( \lambda = 0 \), it indicates a complete independence between the regression residuals and the phylogeny, and when \( \lambda = 1 \), it indicates a Brownian phylogenetic dependence (Freckleton et al. 2002; Garmszegi 2014). As described above, the phylogenetic trees were obtained from Phylomatic version 3 (http://www.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 the 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 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 because of the lack of ecological or biological information (table 1). In the case of life cycle, when deciduous perennial and evergreen perennial states were compared with the annual state, 86 species were included in the analyses. For the growth form variable, the shrub/tree state was compared with the herb state, and 88 species were included; for the plant origin variable, the native state was compared with the exotic state, and 87 species were included. Finally, for the soil water condition variable (wetland or nonwetland species), 85 species were considered. All the data (silica content values) were subjected to 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 with quantile-quantile (Q-Q) plots and scatterplots of the residuals of the models against their fitted values. The Q-Q plot graphs of the proposed models showed that the residuals are approximately normally distributed. In the scatterplots, no pattern for the distribution of points was detected; that is, there is homogeneity among the residuals. For the PGLS, we used the ape, nlme, phytools, and geiger packages in R version 3.6.1 (R Core Team 2019). Throughout the study, the values of silicophytolith/Si content are presented as means ± standard deviation.

**Results**

**Silicophytolith Content in Relation to Systematics and Tissue Origin**

Ninety species from 19 families accumulated silicophytoliths in their leaves/culms. Among those species that produce silicophytoliths, the content (percentage of dry weight) ranged from

| Predictor                              | \( t \)   | \( P \)   | \( \lambda \) | \( N \) |
|----------------------------------------|----------|----------|---------------|-------|
| Life cycle (deciduous perennials vs. annuals) | 1.68     | .09      | .46           | 86*   |
| Life cycle (evergreen perennials vs. annuals) | 1.72     | .08      | .46           | 86*   |
| Growth form (shrubs/trees vs. herbs)   | 1.35     | .17      | .52           | 88b   |
| Plant origin (natives vs. exotics)     | 4.35     | .01 e^{-3} | .66          | 87c   |
| Soil water condition (wetland vs. nonwetland) | 1.24     | .21      | .47           | 85d   |

Note. \( N \) = number of species included for the analyses.

\( ^* \) Calycera sp., Ranunculus sp., Bromus catharticus, and Baccharis sp. were not included.

\( ^b \) Baccharis sp. and Ranunculus sp. were not included.

\( ^c \) Calycera sp., Ranunculus sp., and Baccharis sp. were not included.

\( ^d \) Calycera sp., Ranunculus sp., Baccharis sp., Conyza sp., and Zingiber sp. were not included.
phylogenetic signal of the trait with two measures: Pagel’s $\lambda$ and Blomberg’s $K$. Both showed that there is some phylogenetic signal (we rejected the null hypotheses of $K = 0$ and $\lambda = 0$), but the values obtained were intermediate ($\lambda = 0.46, P < 0.0001$) or low ($K = 0.088, P = 0.003$).

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

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

The epidermis was the main silicified tissue, along with the xylem and the parenchyma (table A2; fig. 3). The monocotyledons, which comprise families with high silicophytolith contents (fig. 2), accumulated silica in a high diversity of tissues (epidermis, xylem, aerenchyma, and parenchyma), while dicotyledons mainly produced silica in the epidermis and xylem (fig. 3). The producers of the highest levels of phytoliths studied in this work (Urticaceae, Poaceae, Cyperaceae, Moraceae, Cannabaceae, and Asteraceae) mostly accumulated them in the epidermis but in a high diversity of types of cells (short and long cells, hairs, hooks, cystoliths, and typical epidermal cells; figs. 2, 3; table A2). In contrast, Arecaceae, the fifth-highest producer, accumulated it mainly in the parenchyma. Those families with silica content lower than 1% (Rhamnaceae, Ranunculaceae, Nothofagaceae, Araliaceae, and Solanaceae) accumulated silica in the xylem and, to a lesser extent, in the epidermis (fig. 3).

**Silicophytolith Content and Biological and Ecological Features**

The mean value of silicophytolith content obtained in annuals (5.85% ± 4.81%) was higher than that in deciduous perennials (2.33% ± 1.78%) and evergreen perennials (4.73% ± 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% ± 3.71%) than in shrubs and trees.
and in species not strictly associated with wetlands or saturated soils (516% ± 3.84%) than in wetland species (3.95% ± 2.34%), no significant differences were obtained from PGLS analyses of these two variables (growth form and soil water condition; table 1). The only statistically significant regression was the one obtained with the plant origin variable (table 1); exotic species had higher silicophytolith content (6.59% ± 4.52%) than natives (4.28% ± 3.09%). In all the analyses, the values of $\lambda$ were intermediate.

**Discussion**

**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 intraclade variability (Prychid et al. 2004; Hodson et al. 2005; Katz 2015; Strömberg et al. 2016).

The methods used here for the measurement of phylogenetic signal did not clearly demonstrate that closer species have similar silica contents. The very low ($K = 0.088$) or intermediate ($\lambda = 0.46$) values for phylogenetic signal could indicate that silica content has evolved independently across the phylogeny used here 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; 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 to accumulate silica 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 the data set and the treatments of branch lengths and polytomies (see “Material and Methods”; Blomberg et al. 2003; Revell et al. 2008; Kamilar and Cooper 2013).

Variability within clades was registered at all taxonomic levels, including in 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, Arecaaceae, Cyperaceae, and Urticaceae (Metcalf 1960; Tomlinson 1990; Piperno 2006). The second group includes families, such as Asteraceae and Solanaceae, that 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 have developed an adequate molecular framework (e.g., Nod26-like major intrinsic protein transporters), and the accumulation of silicophytoliths in tissues occurs independently of the environmental or phenological factors. In the second case, silica accumulation is likely a consequence of other processes (e.g., senescence, transpiration, secretion), and it might not be associated with 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, or 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 of these differences among families.

**Anatomical Origin of Silicophytoliths in Different Taxa and Their Relation to Functionality**

In dicotyledons, in which the lowest contents were observed (except for in 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 Osterrich 2012), or it might be stomata associated with 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 (as a consequence of transpiration or senescence processes) and is not strictly associated with a specific function. On the other hand, the highest-accumulating dicot families, Urticaceae, Moraceae, and Cannabaceae, produce silica in cystoliths. Different roles, such as acting as an internal source of CO₂ for photosynthetic assimilation and light scattering, have been associated with cystoliths (Gal et al. 2012; Giannopoulos et al. 2019). Consequently, in this last group of dicot species, higher silica content in leaves associated with cystoliths might be related to a specific role for silica in tissues.

Within the monocot families that produce silicophytoliths, a high diversity of cells are silicified. Two types can be distinguished, as follows: group a are those that are early and almost always silicified (short silica cells in Poaceae, epidermal cells with cone/conical silica in Cyperaceae, and parenchyma cells with globular silicophytoliths in Arecaaceae), and group b are 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 to the other high-accumulating monocot families, Arecaaceae and Cyperaceae. The two types of silicified cells may be associated with different mechanisms of silicification and functions. For instance, in the silicification process of the short cells of 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 a reinforcing structural element (Fernández Honaine et al. 2016). On the other hand, in the bulliform cells of grasses (group b cells), the accumulation process is strongly associated with leaf senescence or transpiration (Takeoka et al. 1984; Fernández Honaine and Osterrich 2012), which might indicate some passive mechanism (Kumar et al. 2017b). In addition, no specific role has been associated with it thus far. Studies on Cyperaceae and Arecaaceae are scarcer, especially in relation to the potential roles of silica accumulation. Sedge conical silicophytoliths are located in epidermal cells associated with sclerenchyma (group a cells); they have some known metabolic regulation, but no function has been proposed yet (Mehra and Sharma 1963). On the other hand, aerenchyma silicification of culm sedge has been associated with senescence (Fernández Honaine et al. 2013). Finally, 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, and thus the silica adaptive function may be secondary (Tomlinson 1990). However, its specific location around the 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, as follows: one group with metabolic control of silicification that is associated with specific roles and another group with a passive silicification process (sensu Kumar et al. 2017b) that is a consequence of other processes such as senescence or intense transpiration.

**Silicophytolith Content and Ecological Traits**

On the basis of 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, with the high-accumulating families represented only 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., in Poaceae: Vulpia dertonensis, 12%; Festuca arundinacea, 12%; Lolium multilorum, 9%; in Asteraceae: Achillea millefolium, 12%; 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 exotic plants in novel sites by providing advantages like a more economical reinforcing component that improves plant structure or an antitherbivore strategy (Raven 1983; Reynolds et al. 2009; Hartley and De-Gabriel 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 that include 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 with dryland species, and this trend has been ascribed to higher water uptake (leading to higher silicic acid uptake) or to a role for 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 a preference for wet/humid environments and the silicification process was 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, bioecological features, or phylogenetic position, may have a 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 intrataxa variation in the amorphous silica contents of 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 homoplastic character, at least in angiosperms. On the basis of the overall analysis of 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 secretion, not associated with specific functions of the tissues. Finally, of all the bioecological variables studied, plant origin (native vs. exotic) was the only one related to silica content. The fact that silicophytoliths represent an effective antitherbivore defense and an economical structural material may be advantageous for exotic species and may explain the higher contents in exotic in comparison with native species. As mentioned above, and particularly for the exotic species, it is possible to relate high silicophytolith content with a specific function, which in this case would be an ecological function.

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