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Wood anatomy and dendroclimatological potential of *Ramorinoa girolae*: a species endemic to arid environments of Argentina

Anatomía del leño y potencial dendroclimático de *Ramorinoa girolae*: Una especie endémica de ambientes áridos de Argentina

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SUMMARY

Chica (*Ramorinoa girolae, Fabaceae)* is an endemic species with a valuable ecological role in arid environments of Argentina, protecting watersheds and wildlife and providing food for humans. The aim of this study was to describe its woody anatomy, growth dynamics, recruitment and relation to climate. Samples from three sites were collected, and anatomical woody analysis and standard dendrochronological techniques were applied. After dating the samples, residual tree-ring chronology was compared with two climate variables, temperature and southern oscillation index (SOI). Results show that chica has variable porosity (diffused, semicircular or circular) with single or grouped vessels. Vessel density varies among the annual growth rings. The storied rays are considered characteristics of a specialized wood. Wider vessels in the beginning of the growing season and terminal parenchyma allowed us to detect tree-ring boundaries. Missing rings and lenses were common, making the dating process difficult. Mean radial growth was 1.2 mm year^{-1} (SD ± 0.25; 1605 - 2009 period). Temperature and SOI have a positive impact on growth. Results showed the anatomical adaptations of chica wood to arid conditions and its potential to provide data from more than 400 years to environmental and temporal studies.

Keywords: monte region, xeric, storied rays, dendrochronology.

RESUMEN

Chica (*Ramorinoa girolae, Fabaceae)* es una especie esencial de los ambientes áridos de Argentina. Protege las cuencas y la fauna además de ser un recurso alimenticio para uso humano. A pesar de ser una especie de triple impacto, poco se conoce sobre su dinámica de crecimiento y sus adaptaciones anatómicas a estos ambientes. El objetivo de este trabajo es describir la anatomía de su leño, su dinámica de crecimiento, establecimiento y relación con el clima. Se obtuvieron muestras de tres sitios de estudio sobre las que se aplicaron estudios anatómicos y técnicas dendrocronológicas estándares. Después de la datación, la cronología residual se relacionó con las variables climáticas: temperatura e índice de oscilación del Sur (SOI). Los resultados muestran que chica posee porosidad variable (difusa, semicircular o circular) con vasos solitarios o múltiples. La densidad de los vasos cambia anualmente en cada anillo de crecimiento. Los radios estratificados son considerados características de leño altamente especializado, vasos de mayor diámetro en el comienzo de la estación de crecimiento y el parénquima terminal nos permitieron determinar el límite de los anillos de crecimiento. Los anillos ausentes y las lentes fueron frecuentes, dificultando el proceso de datación. El crecimiento radial anual promedio fue de 1,2 mm año^{-1} (DS ± 0,25; para el período 1605 - 2009). Tanto la temperatura como el SOI tuvieron un impacto positivo sobre el crecimiento anual. Estos resultados mostraron las adaptaciones anatómicas del leño de chica a los ambientes áridos y su potencial para el análisis ambiental temporal al proveer información por más de 400 años.

Palabras clave: monte, xérico, radios estratificados, dendrocronología.

INTRODUCTION

Arid and semiarid regions are geographical and ecological areas characterized by water deficiency, extreme temperatures, and poorly developed soils (Solh and van Ginkel 2014). In Argentina, they cover more than 630,000 km^2 (Cabrera 1976). The plant species which inhabit these regions are highly specialized to grow under dry conditions, however they are vulnerable to environmental changes linked to desertification processes (Vázquez *et al.* 2011). Thus, fragmentation and degradation of the environment can lead to increasing biodiversity loss affecting endemic species, the products of complex ecological and evolutionary processes (Godoy-Bürki *et al.* 2014). Some endemic species do not only play a key role as a source of biodiversity, but also as an essential food source for animal and human communities that inhabit these territories (Muñoz *et al.* 2015).

Chica (*Ramorinoa girolae* Speg.) (*Fabaceae*, *Dalbergiae* tribe) is a xerophilous endemic woody plant species in the Monte region of Argentina (Hadad *et al.* 2014). Chica grows in rocky soils in mountainous areas and valleys between 700 and 2,400 m a.s.l., covering about 1,220 km^2 (29º 30' - 32º 30' S) (figure 1) (Zapata 2017, Zapata *et al*. 2020), one of the few trees that grow in these environments. Ethnobotanical studies indicate that indigenous people have consumed their seeds since the pre-Hispanic period, as well as in the pre-Inca period before the 15th century. *Ramorinoa girolae* provides valuable ecosystem services such as watershed protection and food and habitat for wildlife (Zapata 2017). Today, *R. girolae* is considered a threatened species due to its restricted distribution area, low growth rate, limited natural regeneration and low fire resistance (Zapata 2017).

The study of functional patterns and species interaction in an ecosystem is a priority for research regarding sustainable management of native species in the world's natural landscapes (Fischer and Lindenmayer 2007). Forest management should be based on knowledge of forest structure and ecological dynamics such as growth and yield, key indicators that can be assessed through forestry and dendrochronological studies. In the case of *R. girolae*, studies that investigate its ecological role, forest structure and current status are limited. Available literature is related to its population distribution, wood characteristics and fruit properties (Hadad *et al.* 2014).

The study of wood anatomy provides useful information about the ecological adaptations of the species, mainly in relation to water stress tolerance, reflected in the structural characteristics of the xylem such as high pore density, short vessel elements and simple perforation plates. In arid environments, the xylem is adapted to avoid the embolism of vessels (Carlquist 2013). In conditions of significant aridity, the lower the vessel diameter, the higher and thicker the cell walls. In these environments, vessel frequency increases and they are grouped to favor conductivity. Currently, there is a lack of data about the water stress tolerance of *R. girolae,* information that could allow us to understand its evolution in such extreme areas and its future under changing global conditions. Additionally, the dendrochronological studies using tree rings may allow us to understand the growth dynamics of this species and the link between growth and climate (Speer 2010). Previous dendrochronological studies regarding species growing in arid regions of South America emphasized the potential of tree-ring analysis for the interpretation of ecological processes (Giantomasi *et al.* 2013). Furthermore, species growing in arid mountain sites under climatic conditions that favored wood preservation allow for study of chronologies longer than 600 years, making them a subject of encomiastic value for dendroclimatological studies (Morales *et al.* 2020).

Here, we propose the hypothesis that *R. girolae* has the anatomical structure of wood adapted to arid conditions, with detectable characteristics that allow the establishment of temporal growth dynamics and association with climatic variables. The objective of this study is to characterize the wood anatomy of *R. girolae*, to estimate its temporal growth dynamics and association with climate variables using dendrochronological studies.

METHODS

Distribution area and species characteristics. Ramorinoa girolae grows in the foothills of Sañogasta, Vilgo, Paganzo, Talampaya, Valle Fértil, de los Portezuelos, de la Huerta, Pié de Palo and Las Quijadas Mountains in the Monte biogeographical region in west-central Argentina (figure 1) (Zapata 2017).

The study area is characterized as a shrubby steppe dominated by *Zygophyllaceae* species, and *Prosopis* trees in sites with higher water availability (Cabrera 1976). The area shows minimal soil cover. Mean annual rainfall varies between 80 and 150 mm / year^{-1}, is concentrated mainly in the summer and often falls torrentially. The area is characterized by its high interannual rainfall variability. Mean temperatures of January and July are 25 °C and 11 °C, respectively (Abraham *et al.* 2009).

Ramorinoa girolae is an aphyla woody species with photosynthesizing stems that grows up to 10 m in height as monospecific woodlands or as isolated trees in the foothills (Zapata 2017) (figure 2AB). It can develop into a shrubby form under unfavorable environments and when branches reach the soil, adventitious roots are developed. The wood is hard and heavy (1,265 kg.dm^{-3}), primarily used as fuel wood due to its caloric potential (Hadad *et al.* 2014) (figure 2C).

Wood anatomy studies. Woody samples were taken from three sampling sites: Vilgo (29° 56´ S, 67° 23´ W); Talampaya (29° 46´ S, 67° 36´ W) and Ischigualasto (30° 12´ S, 67° 47´ W) (figure 1). Stem cross sections were cut with a chainsaw at 20 cm high from three specimens at each sampling site. In order to soften the wood, 1 cm^3 blocks of sapwood and hardwood were water boiled for 24 hours (figure 2D).

Light microscopy sections of 15 µm thickness were obtained from transverse and longitudinal sections (radial and tangential) using a Leica Hn 40 sliding microtome. Then, the samples were whitened with sodium hypochlorite and dehydrated with xylol. Finally, they were colored using Safranin (1 %), fast green and 80 % alcohol and sealed using Entellán (D'Ambrogio de Argüeso 1986). Wood was described according to terminology from the List for Sapwood and Hardwood Identification (Richter *et al.* 2004). Vessel diameter (n = 25) and the number of vessels per square millimeter was measured in 10 tree rings.

Dendrochronological studies. For dendrochronological studies, 15 samples (cross-sections) at the base of both dead and alive individual trees from the three sampling sites were taken using a chainsaw in July 2010. They were then polished using sandpaper of different grit sizes (40-600 ANSI standards). Tree rings were dated using a magnifying glass (Olympus SZ61). Considering that this species grows in the Southern Hemisphere, the Schulman convention for assigning the precise calendar year was applied. Tree-ring growth was then measured using a VELMEX Inc. system (0.01 mm accuracy). The COFECHA program was applied in order to check the cross-dating to establish the correct calendar year of each tree ring and determine false or absent tree rings (Grissino-Mayer 2001).

After the dating process, the mean radial-growth chronology was standardized (double detrending: negative exponential and spline) using ARSTAN software (Holmes 2001). MS (Average mean sensitivity), EPS (Expressed population Signal) and SNR (Signal to noise ratio) were used to communicate the accuracy of the chronology. After the standardization, the residual chronology was correlated with the mean monthly temperature of San Juan meteorological Station (31° 31' 57.3" S, 68° 30' 51.2" W) (1969 - 2009 period) and the SOI (Southern Oscillation Index, 1867 - 2009 period), representing both local and global climate variables. Monthly SOI were obtained from the Climatic Research Unit of the University of East Anglia, England. SOI is a standardized index of sea level pressure oscillation between Tahiti and Darwin (Australia) that determines El Niño and La Niña events (Ropelewski and Jones 1987). Positive SOI indexes are linked to El Niño episodes. The association between growth and rainfall could not be assessed as rainfall events are localized and variable and there are no meteorological stations near the sampling sites. The association between growth of *R. girolae* and climate variables was assessed using Pearson's correlation coefficient (significant values at $P < 0.05$) from June before the growing season until July after the growing season.

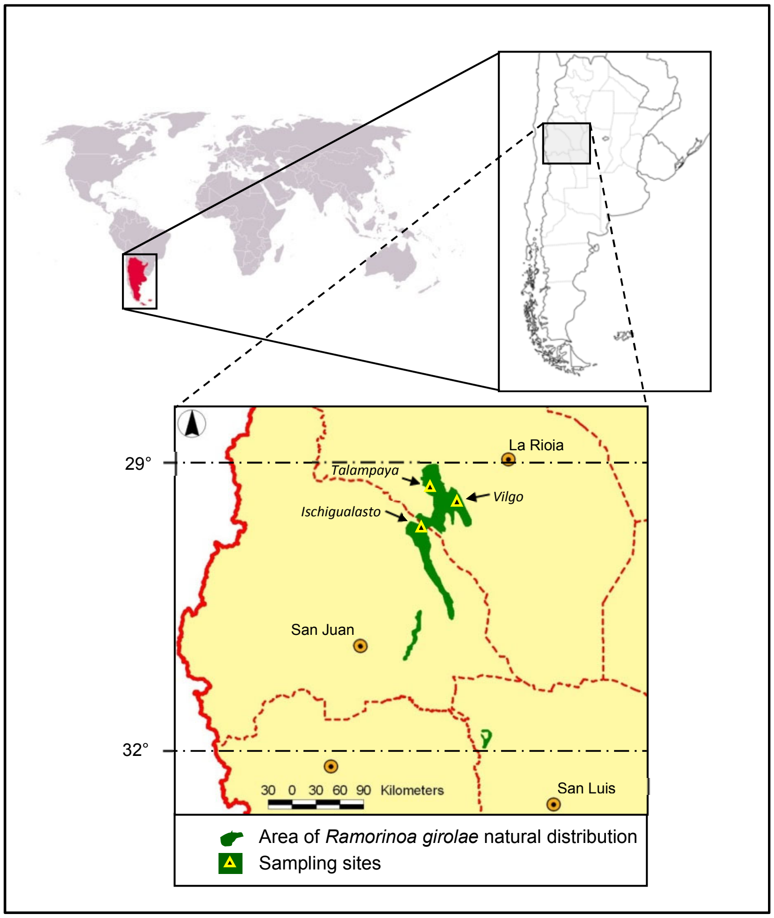

Figure 1. Natural distribution of *Ramorinoa girolae* in Argentina and locations of sampling sites.

Área de distribución natural de *Ramorinoa girolae* en Argentina y sitios de muestreo.

To assess the association between growth and climate variables, DENDROCLIM software was used (Biondi and Waikul 2004).

RESULTS

Wood anatomy. The *Ramorinoa girolae* wood shows a clear color difference between sapwood and heartwood (figure 2C). Sapwood was yellow or pink-ochre in color, while heartwood was dark brown, reddish-brown or violet brown with dark irregularities (figure 2C). Rays and parenchyma could be observed with a hand glass (10 X). Macroscopic observation of wood indicated the presence of growth rings defined by a light band of tissue at the end of growth band (figure 3A), and that the wood corresponds to a cell layer of terminal parenchyma (figure 3B).

Vessels were predominantly single with circular or oval sections. Multiple vessels (2-3) arranged parallel to the rays were also detected, with few larger groups (4-5). Vessel distribution in the early wood was variable, with diffuse, semicircular or circular porosity. In the late wood, vessels are grouped in clusters and/or in ulmoide to semi-ulmoide arrangement. Numerous vessels were occluded by tyloses with xylochrome substances (figure 3CD).

Vessel elements are short, show slightly oblique terminal borders, were arranged in stories, and their height was similar to the length of other woody elements. They had simple perforation plates and ornate and alternate intervascular pits (figure 4).

The mean diameter of lumen varied between 49 and 102 µm (Mean = 71.88 µm; SD ± 16.15; n = 25) and 5 to 20 vessels could be counted per square millimeter (Mean = 11.24; SD ± 4.61; n = 21).

The axial parenchyma is vasicentric paratracheal, aliform and confluent by narrow bands (figure 3ACD). The terminal parenchyma band of each tree ring comprises 4-5 cell strata (figure 3). Rays are uniseriate with, on average, seven cells of height on average. Biseriate rays were scarce or absent. Most of the rays were heterocellular, constituted by square cells at the boundaries and by procumbent

Figure 2. Tree of *Ramorinoa girolae* from Vilgo sampling site (A), Detail of photosynthesizing stems (B), Cross section showing sapwood and hardwood (samples were taken using a chainsaw and polished using sandpaper) (C); 1 cm^3 blocks of sapwood and hardwood used for anatomical studies (D).

Ejemplar de *Ramorinoa girolae* que pertenece al sitio de muestreo Vilgo (A), detalle de sus tallos fotosintéticos (B), sección transversal que muestra la albura y el duramen (las muestras se obtuvieron usando una motosierra y con posterior lijado) (C); 1 cm^3 de albura y duramen para estudios anatómicos (D).

cells at the center (figure 4). Homocellular rays predominated in exceptional cases with procumbent cells. The wood fibers have irregular shape, thick walls and reduced lumen. They form dense groups in rectangular packages. Vasicentric tracheids (short cells linked to the radios) and fiber tracheids (long cells with lignified walls) were also present (figure 4).

A complete storied woody structure was observed including axial and radial parenchyma as well as elements and segments of vessels (figure 4). Numerous calcium oxalate crystals arranged in axial series of 5, 7, 8 and up to 12 were present in the axial parenchyma cells and, eventually, in fibers and ray parenchyma.

Dendrochronological results. Dendrochronological results showed that it is possible to cross-date 21 series from 15 sampled trees taken from the three sampling sites. The dating process was difficult due to many missing rings and lenses. Master series covered 405 years (1605-2009) and after cross-dating the mean intercorrelation between samples was 0.33 and MS 0.38. Mean annual radial growth was 1.2 mm year^{-1} (SD ± 0.25), with extreme minimum and maximum values measured at 0.18 and 2.09 mm year^{-1}, respectively. After standardization EPS was 0.69 and STR: 1.32 (from 19 series onwards) (figure 5).

Ramorinoa girolae showed a significant positive correlation with mean monthly temperature in June before the growing season and in September and March during the growing season. SOI positively affected growth during the growing season (December, January and March) (figure 6).

DISCUSSION

This study recognizes for the first time the wood anatomy and dendrochronological potential of *Ramorinoa girolae,* covering a period of more than 400 years. The wood of this species is characterized by highly specialized woody structures such as completely storied rays and short vessels that reduce risk of embolism. Terminal parenchyma at the end of the growing season and higher vessels at

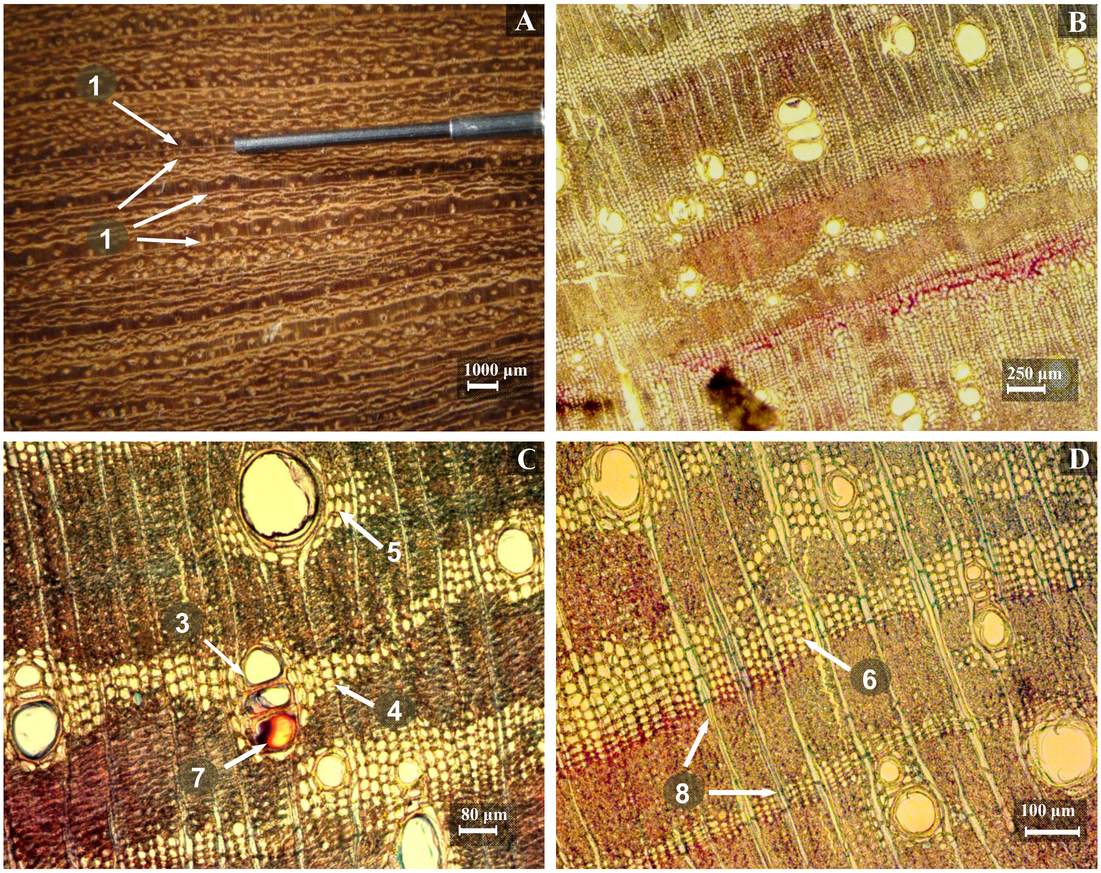

Figure 3. Transversal section of *Ramorinoa girolae* wood. Optical magnifying glass view (A) microscope views (B,C and D). Tree ring boundary (1); single vessels (2); grouped vessels (3); aliform parenchyma (4); vasicentric paratracheal parenchyma (5); banded parenchyma and apotracheal termina parenchyma (6); vessels with contents (7); uniserial rays (8).

Sección transversal del xilema de *Ramorinoa girolae*. Vista en lupa óptica (A) y en microscopio (B,C y D). Límite del anillo de crecimiento (1); vasos solitarios (2); vasos agrupados (3); parénquima aliforme (4); parénquima paratraqueal vasicentrico (5); parénquima en bandas y terminal apotraqueal (6); vasos con contenidos (7); radios uniseriados (8).

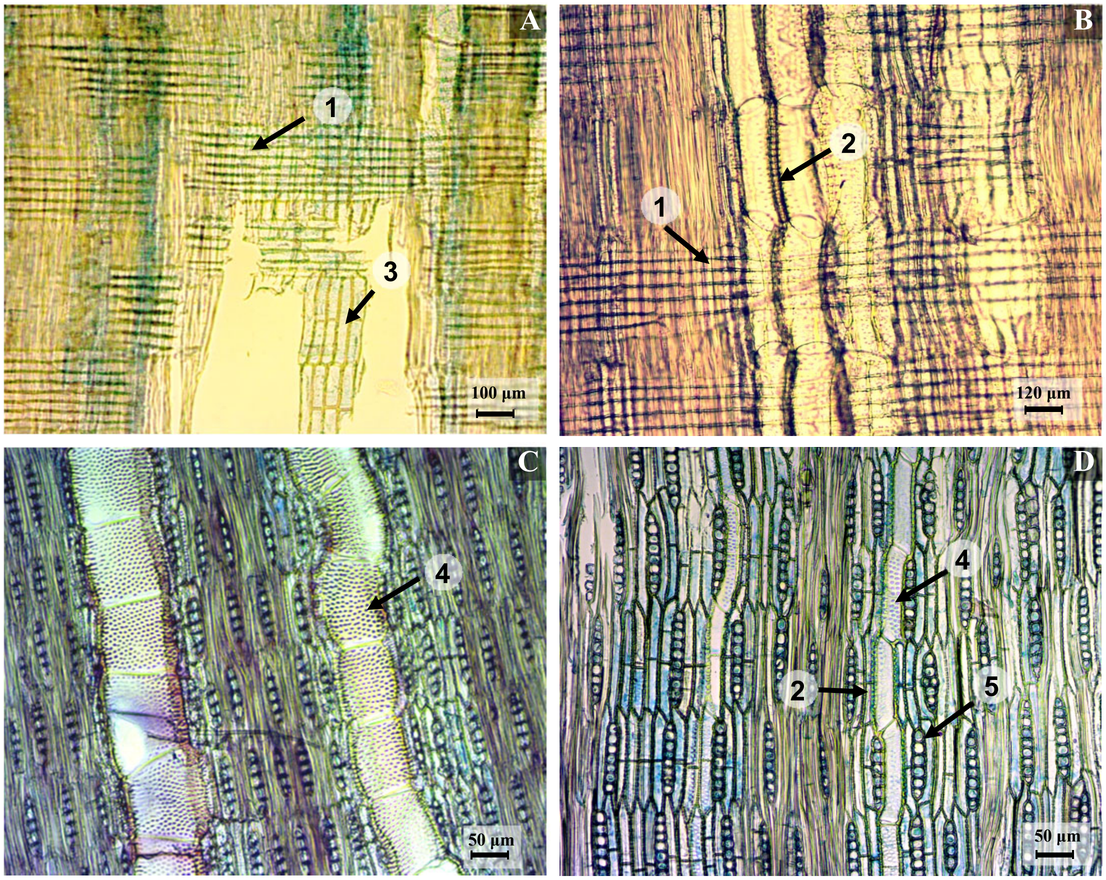

Figure 4. Radial (A, B) and tangential longitudinal (C, D) sections of *Ramorinoa girolae* wood. Rays (1); vessel elements (2); cells of the axial parenchyma (3); vessels with ornate and alternate intervascular pits (4); homocellular rays (5).

Secciones radial (A, B) y tangencial longitudinal (C, D) del leño de *Ramorinoa girolae*. Radios (1); elementos de vaso (2); células del parénquima axial (3); segmentos de vaso con puntuaciones intervasculares ornadas alternadas (4); radios homocelulares (5).

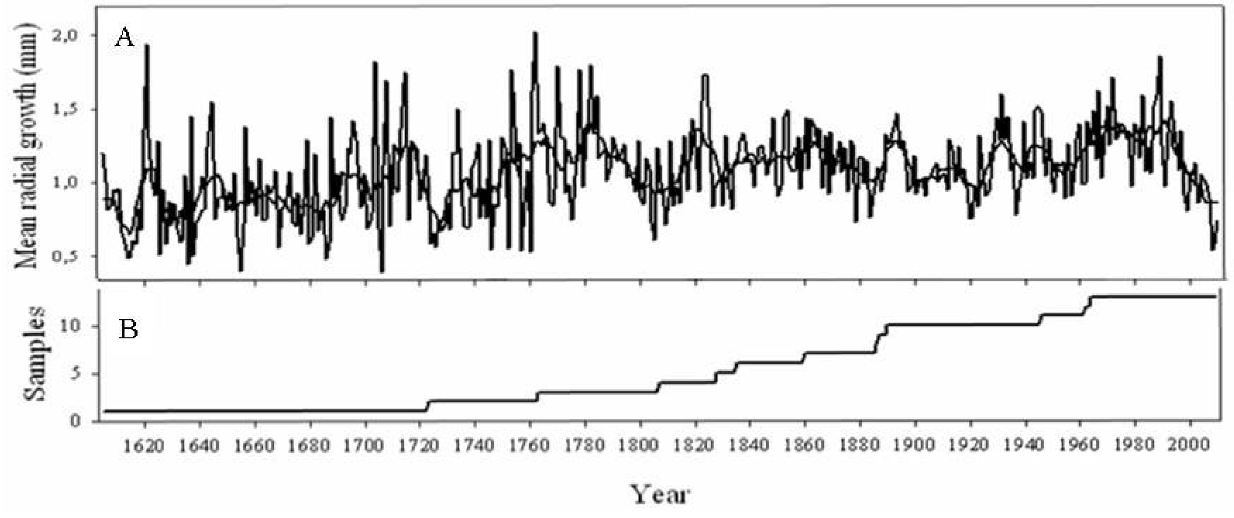

Figure 5. Tree-ring width chronology of *Ramorinoa girolae* (A) and number of samples (B).

Cronología de ancho de anillos de *Ramorinoa girolae* (A) y número de muestras (B).

the beginning of the growing season allow for determination of annual growth-ring boundaries. Storied rays in trees are an important evolutionary characteristic (Carlquist 1984), which suggests that *R. girolae* is a highly evolved species. Variability of porosity among tree rings is related to changes in water availability that characterize the arid environments where this species grows (Bissing 1982). On the other hand, a high number of small vessels allows for more efficient growth in plants vegetating in dry, stressed environments (Carlquist 2013). Low vessel number of *R. girolae* (5 - 20 vessel mm^{-2}) is similar to *Aspidosperma quebracho-blanco* Schltr. (9.77 vessel mm^{-2}) (Moglia and López 2001) and lower compared to other species growing under arid conditions, such as *Monttea aphylla* (Miers) Benth. & Hook (240 vessel mm^{-2}) (Giménez *et al.* 1998) and *Ziziphus mistol* Griseb. (28.77 vessel mm^{-2}) (Giménez *et al*. 2014). Small vessels, short vessel members, vasicentric tracheids and well-defined and narrow growth rings

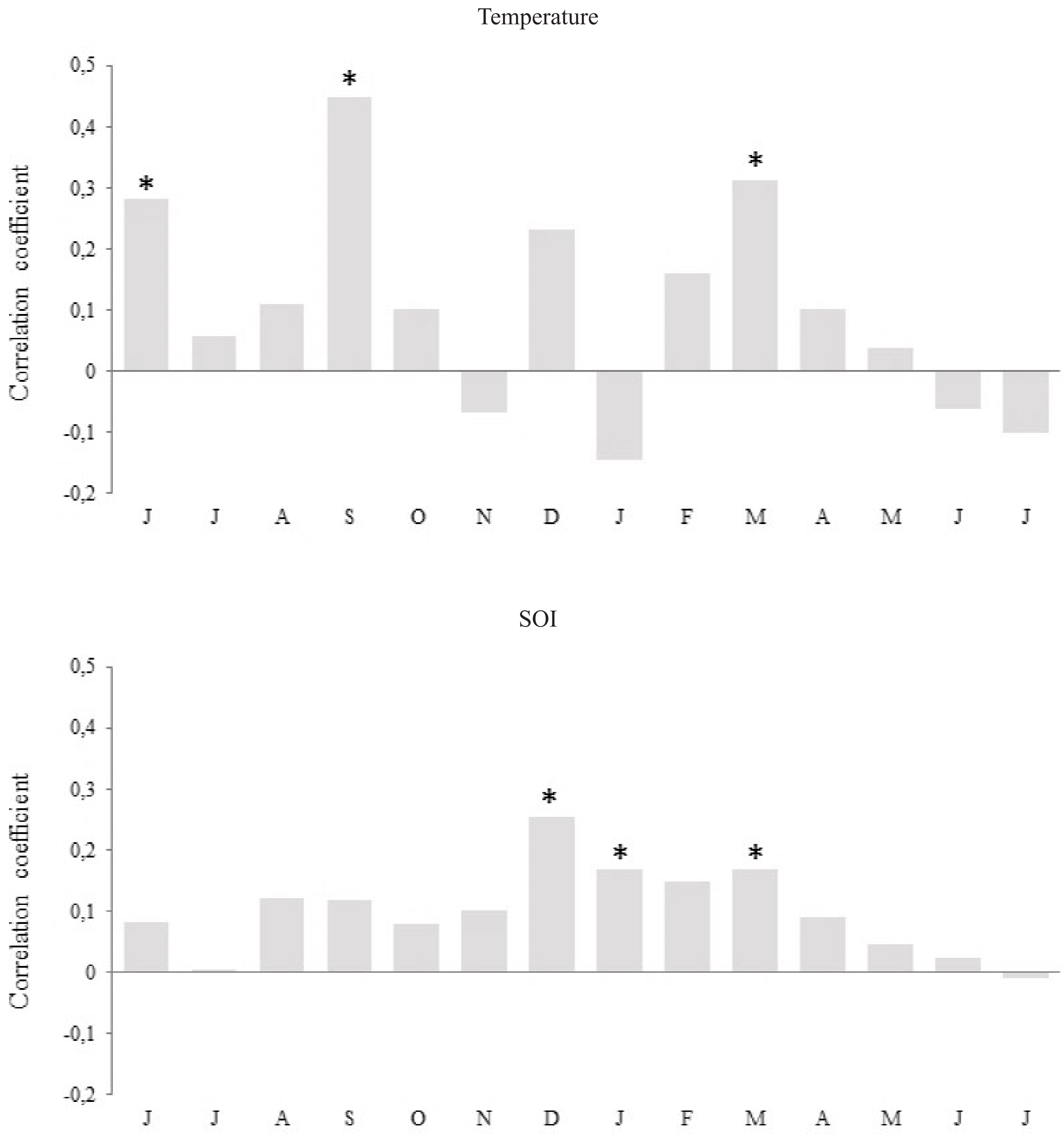

Figure 6. Pearson's correlation coefficients comparing residual tree-ring chronology of *Ramorinoa girolae* and climate variables: mean monthly temperature and monthly southern oscillation index (SOI). Asterisks denote months with significant correlation ($P < 0.05$).

Coeficiente de correlación de Pearson entre la cronología residual de *Ramorinoa girolae* y las variables climáticas: temperatura media mensual e índice de oscilación del Sur (SOI). Los asteriscos indican los meses con correlación significativa ($P < 0,05$).

are characteristics of xerophyte environments (Carlquist 2013), and *R. girolae* wood shows similar features. Variable vessel distribution (diffuse, circular and semicircular) is also an anatomical woody characteristic of the species used as an adaptation to arid conditions (Lugones and López 2001).

The mean intercorrelation between samples of 0.33 was significant and the mean sensitivity among series of 0.38 showed a high variability in growth between successive tree rings (Speer 2010). The average annual wood growth of *R. girolae* (0.8 mm.year-1) is below that which was determined for *Prosopis flexuosa* DC (2 - 4 mm year^{-1}) (32 - 34° S), but similar to *Prosopis ferox* Grisebach, both species that grow in arid environments of Argentina (Villagra *et al*. 2005). The samples analyzed in this work represent a period of more than 405 years that has not been covered for other species using dendrochronological methodology outside the Andes in South America (Boninsegna *et al.* 2009, Morales *et al*. 2020).

Establishment of *R. girolae* during the first decade of the 20th century coincides with that of species from other semi-arid environments such as *Prosopis caldenia* Burkart (Bogino *et al.* 2015). However, anthropogenic factors such as the construction of railways and the changes in land tenure that impacted the growth dynamics of these species were not identified as a factor in the case of *R. girolae.* Further studies are needed in order to determine the driving factors impacting its growth dynamics.

The significant correlation between *R. girolae*'s growth and mean monthly temperature is consistent with previous studies regarding species in semiarid environments such as Prosopis *caldenia,* for which temperature showed a positive impact on growth. However, the positive impact of temperature on growth is not a common driver of all species in arid environments, as the case of *Geoffroea decorticans* (Gillies *ex* Hook. & Arn.) Burkart and *Parkinsonia praecox* (Ruiz & Pav. *ex* Hook.) Hawkins shows an inverse relationship with temperature (Cendoya *et al.* 2021). These results bring up new questions regarding the link between species' growth in arid environments and the influence of temperature. Due to the lack of a consistent correlation (positive or negative) it is possible to assume differing behavior of species under changing climate conditions. Considering that data regarding historical precipitation in the region are not available, the value of species such as *R. girolae* for research is highlighted, given the possibility of establishing links with global climatic variables such as that of SOI and woody growth (Ropelewski and Jones 1987). The positive association of annual wood growth of *R. girolae* with SOI indices (linked to El Niño and La Niña events) during the growing season may be a useful tool for the reconstruction of climatic events throughout the last 400 years. The relation between growth of *R. girolae* and climate variables shows the value of this species for use in dendroclimatological reconstruction, considering the lack of dendrochronological studies in the area.

We conclude that *R. girolae* possesses encomiastic anatomical characteristics important for adaptation to extreme arid environments, such as variable porosity and storied rays. Additionally, it shows great value for the temporal study of these environments covering a period superior to 400 years, providing an unprecedented resource for the study of arid regions in Argentina.

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