Received: 30 March 2021 | Revised: 9 October 2021 | Accepted: 19 October 2021

DOI: 10.1111/1442-1984.12360

ORIGINAL ARTICLE

PLANT SPECIES BIOLOGY WILEY

Phenology of *Zamia boliviana* (Zamiaceae), a threatened species from a seasonally dry biodiversity hotspot in South America

Rosane Segalla[1,2] | **Fábio Pinheiro**[3] | **Gudryan J. Baronio**[4] | **Leonor Patrícia Cerdeira Morellato**[2]

[1]Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Cuiabá, Brazil

[2]Departamento de Biodiversidade, Laboratório de Fenologia, Instituto de Biociências, Universidade Estadual Paulista UNESP, Rio Claro, Brazil

[3]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

[4]Departamento de Ecologia, Instituto de Biociências (IB-USP), Universidade de São Paulo, São Paulo, Brazil

Correspondence

Rosane Segalla, Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Campus Cáceres – Prof. Olegário Baldo, Avenida Europa, n° 3000, Vila Real/Distrito Industrial, CEP: 78201-382, Cáceres, Mato Grosso, Brazil.
Email: rosane.segalla@ifmt.edu.br

Funding information

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES), Grant/Award Number: Finance Code 001; National Council for Scientific and Technological Development (CNPq); São Paulo Research Foundation (FAPESP)

Abstract

Dioecy, a character common to all cycads, requires obligatory outcrossing. The absence of potential reproductive mates of the opposite sex renders individuals effectively sterile. Therefore, reproductive synchrony is essential for the reproductive success of cycads. Here, we describe the reproductive phenology, morphology, and lifespan of strobili of *Zamia boliviana* (Cycadales, Zamiaceae), an endemic cycad in South America. We examined the variation in timing of maturation between polliniferous and ovuliferous individuals in two *Z. boliviana* populations. Lifespan of polliniferous and ovuliferous strobili was based on direct observations and systematic measurements of strobili development. Phenological study covered three reproductive cohorts in two distinct cycad populations. Lifespan of polliniferous strobili was comprised of four phases and lasted 50 days until the cycle's completion, while ovuliferous strobili underwent seven phases that extended over 330 days until seed dehiscence. Both sexes produced strobili during the dry season. We identified a seasonally synchronous pattern in the reproductive phenology of *Z. boliviana*, with a major overlap in the phases of emergence, pollen release, and strobili receptivity between sexes, populations, and subsequent years. Reproductive events of *Z. boliviana* followed the seasonality of the Cerrado vegetation and climate. Synchrony between the period of strobili production and reproductive activity peaks was found in both sexes, but seed dehiscence occurred in the dry season. Our study provides relevant and new biological data for *Z. boliviana* in its natural habitat, demonstrating a temporal distinction between the lifespan of polliniferous and ovuliferous strobili and the necessary overlap between the release and receptivity of pollen.

KEYWORDS

circular statistics, dioecy, phenograms, reproductive timing, *Zamia* strobili lifespan

1 | INTRODUCTION

Phenology is an integrative environmental science studying the timing of recurrent biological events related to climate and its effects on different species of plants and animals (Morellato et al., 2016). Studies examining the phenology of dioecious species, with different pollination modes and from diverse phylogenetic lineages, are relevant for understanding the influence of sexual selection on the evolution and maintenance of sexual dimorphism and sex allocation in plants (Escobedo-Sarti & Mondragón, 2016; Morellato, 2004; Munguia-Rosas et al., 2011) because overlapping between sexes is essential for reproductive success (Funamoto & Sugiura, 2021; Lazcano-Lara & Ackerman, 2018; Morellato, 2004; Obeso, 2002; Octavio-Aguilar, Iglesias-Andreu, et al., 2017; Segalla, Pinheiro, & Morellato, 2021).

Cycads have prolonged sexual maturation periods and asynchronous reproductive activity within a single or multiple reproduction cohorts (Norstog & Nicholls, 1997). Furthermore, ovuliferous cycads invest more in reproduction than polliniferous, and may not have enough resources to generate strobili every year (Calonje et al., 2011; Clark & Clark, 1987; Tang, 1990). As a result, polliniferous individuals are expected to exhibit earlier and longer strobili emergence periods, as well as a greater number of strobili compared to ovuliferous individuals (Grobbelaar, 2002). This differential behavior might cause phenological differences between sexes (Martínez-Domínguez et al., 2018). In addition, dioecy, a common feature to all cycads, requires an obligatory outcrossing mechanism, and the absence of potential mates of the opposite sex renders individuals effectively sterile (Jones, 2002; Käfer et al., 2016). This characteristic makes reproductive synchronization essential for the reproductive success of the species in this group (Clugston et al., 2016; Heilbuth, 2000; Martínez-Domínguez et al., 2018; Morellato, 2004). Asynchronous phenological patterns can prevent successful reproductions in small and isolated cycad populations, negatively impacting their fitness and threatening their survival (Clugston et al., 2016; Laidlaw & Forster, 2012; Lopez-Gallego, 2007; Octavio-Aguilar, Iglesias-Andreu, et al., 2017; Octavio-Aguilar, Rivera-Fernández, et al., 2017; Okubamichael et al., 2016; Reed et al., 2012; Schneider et al., 2002; Segalla et al., 2019; Velasco García et al., 2016).

Despite the importance and applications of phenology, plant reproduction triggers in seasonal ecosystems remain poorly understood (Mendoza et al., 2017; Morellato et al., 2013). This knowledge is even more scarce for cycads in the Cerrado (Brazilian savanna), which are largely under-researched in their natural habitat. Many habitats in the Cerrado provide unique shelters for endemic dioecious species, such as those of the genus *Zamia* (Lopez-Gallego, 2015; Segalla et al., 2019). Limited knowledge of the biological patterns and ecological processes that sustain cycad populations (Krieg et al., 2017) makes studies on the phenology of cycads strategically and biologically significant and urgent in South American *Zamia* populations (Clugston et al., 2016; Martínez-Domínguez et al., 2018; Segalla et al., 2019). Phenological knowledge can elucidate patterns, propose new questions, and contribute to designing effective conservation and management strategies for *Zamia* in native ecosystems (Gorelick & Marler, 2012; Morellato et al., 2016). South America hosts 32 species of *Zamia* and 30 of these are considered endemic to the region (Calonje et al., 2013–2021; Segalla et al., 2019).

Here, we investigated the phenology of *Zamia boliviana* (Brongn.) A. DC. (Cycadales, Zamiaceae), a cycad species native to Cerrado habitats in Bolivia and Central Brazil (Segalla, Pinheiro, Barônio, & Morellato, 2021; Segalla, Pinheiro, & Morellato, 2021). Our objective was to describe the reproductive phenology of two populations *of Z. boliviana* and compare the lifespan of polliniferous and ovuliferous strobili. Specifically, we aimed to answer the following questions: (1) Does the reproductive phenology of *Z. boliviana* (time, duration, synchrony) vary between polliniferous and ovuliferous plants within and between populations? and (2) Is *Z. boliviana* phenology seasonal? As the reproductive success of dioecious species is intrinsically dependent on synchrony, we predicted that the phenological patterns between polliniferous and ovuliferous plants would be synchronous within their populations. Conversely, we expected polliniferous individuals to exhibit higher interannual frequency of strobili production and an earlier, more disjunctive emergence compared with ovuliferous individuals. In terms of seasonality, we expected phenological events to be seasonal, annual to interannual (as uninterrupted reproductive or discontinuous events) in polliniferous individuals and seasonal, interannual (discontinuous reproductive events) in ovuliferous individuals due to the differential costs between sexes, as predicted for dioecious and long-lived species (Delph, 1999; Labouche & Pannell, 2016; Zhang et al., 2014;) such as *Zamia* spp. (Clugston et al., 2016; Jones, 2002; Tang, 1990).

2 | METHODS

2.1 | Study region

The cycad *Z. boliviana* is found within the intertropical zone in the central portion of South America (Figure 1). *Z. boliviana* is restricted to an area along the border between Bolivia (Beni, Cochabamba, La Paz, and Santa Cruz) and the State of Mato Grosso (MT), in Brazil (Figure 1), where elevation ranges from 130 m to 450 m above sea level and landscape is covered by different types of Cerrado (Brazilian savanna) vegetation (Segalla, Pinheiro, Barônio, & Morellato, 2021; Stevenson, 2004). The study area is characterized by high solar radiation incidence throughout the year (SEPLAN, 2000) and an equatorial and tropical hot climate, with little seasonal or annual temperature variation (Köppen, 1918; Kottek et al., 2006). Annual temperature ranges from 22 to 26°C with low temperatures between 18 and 23°C and highs from 25 to 30°C (based on data from Geographic Information System analysis using bioclimatic variables from CHELSA (Climatologies at high resolution for the earth's land surface areas) (Karger et al., 2017). The region has two distinct seasons: a dry season (May to September) with monthly average rainfall of 42.3 mm, and a wet season (October to April) with monthly average rainfall of 186 mm (Karger et al., 2017).

Zamia boliviana is a small perennial plant with an average height of 0.8 m, subterranean stem or xylopodium (perennial thickened woody axis of the underground system), and one to three leaves per crown (Figure 1). The *Z. boliviana* populations studied were found mainly in Cerrado woodlands, also known as Cerrado sensu stricto (hereafter referred to as Cerrado), in sandy to rocky, well-drained oxisol, entisol, inceptisol, ultisol, and litolic soils, associated with rocky outcrops, generally characterized as alkaline, with low native fertility (SEPLAN, 2000; Skelley & Segalla, 2019). In the frontier border regions of Brazil–Bolivia, the cycad habitat of the cycad is relatively flat or exhibits minor topographical changes (Segalla, Pinheiro, Barônio, & Morellato, 2021; SEPLAN, 2000). Other habitats occur on slopes with the formation of deep valleys of the region of Chapada dos Guimarães, in the State of Mato Grosso (SEPLAN, 2000). *Z. boliviana* forms clumps of individuals of both "sexes," with different densities and reproductive ages (Segalla, Pinheiro, Barônio, & Morellato, 2021). Most of the populations of *Z. boliviana* populations have been destroyed or suffered drastic reductions in size across their distribution, mainly due to the intense Cerrado fragmentation caused by the expansion of agricultural lands and agribusiness activities in both countries (Segalla, Pinheiro, Barônio, & Morellato, 2021; Skelley & Segalla, 2019).

2.2 | Phenological sampling

To investigate the phenological pattern of *Z. boliviana*, we sampled populations from two distinct areas of Cerrado in the Brazil–Bolivia border region in the State

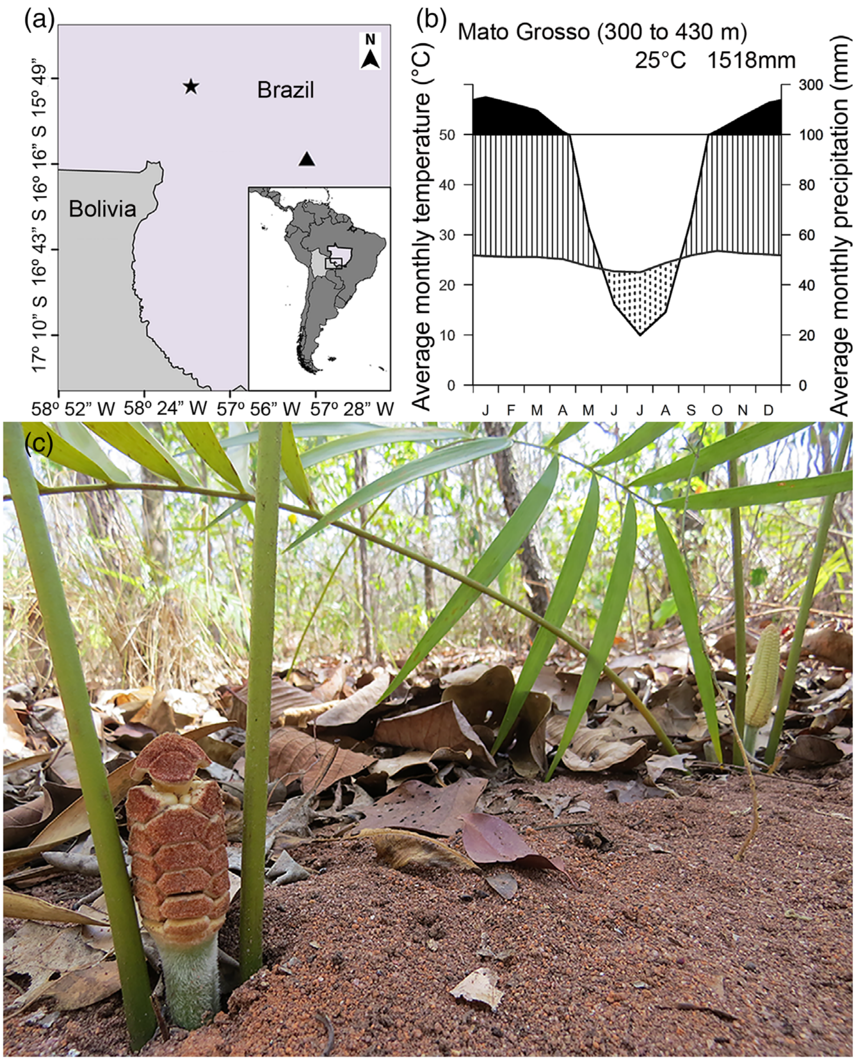


FIGURE 1 *Zamia boliviana* distribution. (a) Central Portion of South America, State of Mato Grosso, Brazil. The star indicates the population studied in Glória do Oeste, and the triangle marks the population in the municipality of Cáceres. (b) Climatic diagram for the distribution region of the studied populations from 1979 to 2013. The diagram distinguishes dry (stippled area), humid (black), and wet periods (hatching). "Temperature" refers to average monthly mean temperature, and "precipitation" refers to average monthly precipitation. (*Source*: climatic variables from CHELSA [Climatologies at high resolution for the earth's land surface areas]; Karger et al., 2017). (c). Ovulliferous (left) and polliniferous (right) plants in habitat. Specific geographical coordinates of the areas of populations are omitted to minimize the risk of illegal extraction of this species from the wild

TABLE 1 Phenological phases across two populations of *Zamia boliviana* in Mato Grosso, Brazil

Strobili phases	Description	Duration in days
Ovuliferous strobili		
1. Immature emerging (IE)	A1. Strobili emerge from the ground surrounded by cataphylls	30–35
2. Immature fully emerged (IFE)	B1. Immature strobili emerge from the ground B2. Exposure and growth of peduncle B3. Development and maturation of ovules B4. Prereceptive phase	10–15
3. Open and receptive (R)	C1. Slight fissures open between the megasporophylls (base/apex) C2. Prominent ovules, translucent white coloring C3. Pollination phase	7–15
4. Closed postreceptive (CPR)	D1. Slit closure D2. Late ovule – pollinated ovules develop	15–20
5. Seed in development (MSD)	E1. Increased rigidity of the megasporophylls E2. Gradual change in color (white to yellow to orange) E3. Growth stops E4. Increased rigidity of the sclerotesta E5. Maturation of sarcotesta	270–300
6. Seed dehiscent (MD)	F1. Dehydration of central axis and disruption of sterile apex F2. Release of seeds starts (can last for up to 30 days)	300–330
7. Senescent/dry (MSD)	G1. Disintegration of the central axis, persistent peduncle	Indefinite
Polliniferous strobili		
1. Immature emerging (IE)	A1. Strobili emerge from the ground surrounded by cataphylls	25–30
2. Immature fully emerged (IFE)	B1. Peduncle growth B2. Growth of microsporophylls and microsporangia	20–25
3. Pollen release (PR)	C1. Microsporangia in maturation (base to the apex) C2. Central axis, condensed, microsporangia visible but closed C3. Central axis elongates/distension C4. Microsporophylls separate (base to the apex), prerelease pollen C5. Beginning of the opening of the sporangia	2–4
4. Senescent/dry (SD)	D1. Dehydration of central axis and organ curvature D2. Strobilus structure disintegrating	Indefinite

of Mato Grosso (MT), in the municipalities of Glória do Oeste (Vale Bonito [VB]: 39.605 ha) and Cáceres (Vale do Chapadão [VC]: 15.376 ha), which are separated by approximately 80 km (Figure 1). Phenological monitoring started in January 2017 after completion of a pilot project that was carried out from August to December 2016 to better understand the biological system and define *Z. boliviana* phenophases. The study covered three reproductive cohorts in 2017, 2018, and 2019. Sampling was carried out in fixed plots (VB $=$ 1300 m^2 and VC $=$ 500 m^2), following haphazard criteria, in predominantly uniform patches of individuals, and by actively searching for and marking adult plants within the plots. The inclusion criterion of individuals was according to plant size ($\geq$0.80 m tall) and the number of leaflets per leaf ($\geq$12). Sampling included 197 individuals in VB (0.14 ind.$\cdot$m^{-2}), 60 of which were polliniferous individuals and 45 ovuliferous, and 216 sampled individuals in VC (0.43 ind.$\cdot$m^2), including 79 polliniferous and 48 ovuliferous plants. Phenological observations were performed monthly to monitor strobili (reproductive organs) phenophases, from their emergence from the soil to the senescence of the polliniferous strobili (in polliniferous plants) and seed dehiscence of the ovuliferous strobili (in ovuliferous plants). In each plot, we recorded the phenological categories for Zamiaceae species by adapting the methodology of Hall et al. (2004) and Clugston et al. (2016). The complete morphological description of the categories is presented in Table 1. Due to technical problems with the equipment collecting climate variables at the sampling sites, as well as incomplete data in the bank provided by the Instituto Nacional de Meteorologia (INMET – www.inmet.gov.br: "Dados Meteorológicos: Estações Automáticas"), we were unable to include climate variables in our phenology analyses from January 2017 to June 2019.

To analyze our data, we applied two methods of analysis, according to criteria defined by Bencke and Morellato (2002): (a) The Fournier index, a method proposed by Fournier (1974) based on the phenological sampling principles used for flowering plants. Here we adapt the method according to the magnitude of observable progress (subjective observation) of the appearance of the polliniferous and ovuliferous strobili in each phenophase (see phenological phases in Table 1). Each phenophase was visually estimated in the field by observing each sampled individual using a semiquantitative interval scale of five categories (0 to 4) (see Fournier, 1974). Our categorical scale was defined as follows: 0, individual without strobili; 1, individual with phenophase occurring between 1 and 25% of strobili; 2, individual with phenophase occurring between 26 and 50% of strobili; 3, individual with phenophase occurring between 51 and 75% of strobili; and 4, individual with phenophase occurring between 76% and 100% of strobili. Subsequently, these categories were expressed as a percentage (i.e., 0%, 25%, 50%, 75%, and 100%) and statistically analyzed. (b) The activity index (or percentage of individuals in each population displaying a particular phenophase) was used to estimate synchrony, indicating the proportion of sampled individuals manifesting a particular phenological event.

2.3 | Strobili: morphology, development, and lifespan

To describe the morphological development along the lifespan of the polliniferous ($n = 6$) and ovuliferous strobili ($n = 6$), we observed and measured these reproductive organs, or strobili. Polliniferous strobili were monitored weekly after soil emergence until the phase preceding pollen release (PR), and daily from the PR phase until complete pollen dehiscence or senescence. The ovuliferous strobili were followed weekly after soil emergence until the end of receptivity or closing of cracks, and then, monthly until seed dehiscence. We considered senescence followed by dehydration and disintegration, and dehydration followed by apical detachment of the seeds, as the final phases of reproduction in polliniferous and ovuliferous strobili, respectively. The photographic records of the strobili were organized on plates according to their respective phenophases and the strobili' growth dynamics.

2.4 | Statistical analyses

To estimate and compare seasonality of each phenophase (activity and intensity) for both polliniferous and ovuliferous individuals and among years, we used circular statistics, as proposed by Morellato et al. (2000, 2010). Intensity refers to the Fournier index and measures semiquantitatively the phenophases exhibited in each individual, which can be extended to the entire population. Activity refers to the percentage of individuals presenting a given phenophase, regardless of its intensity. For each of these components, months of occurrence were converted into angles, with 15° = January, successively

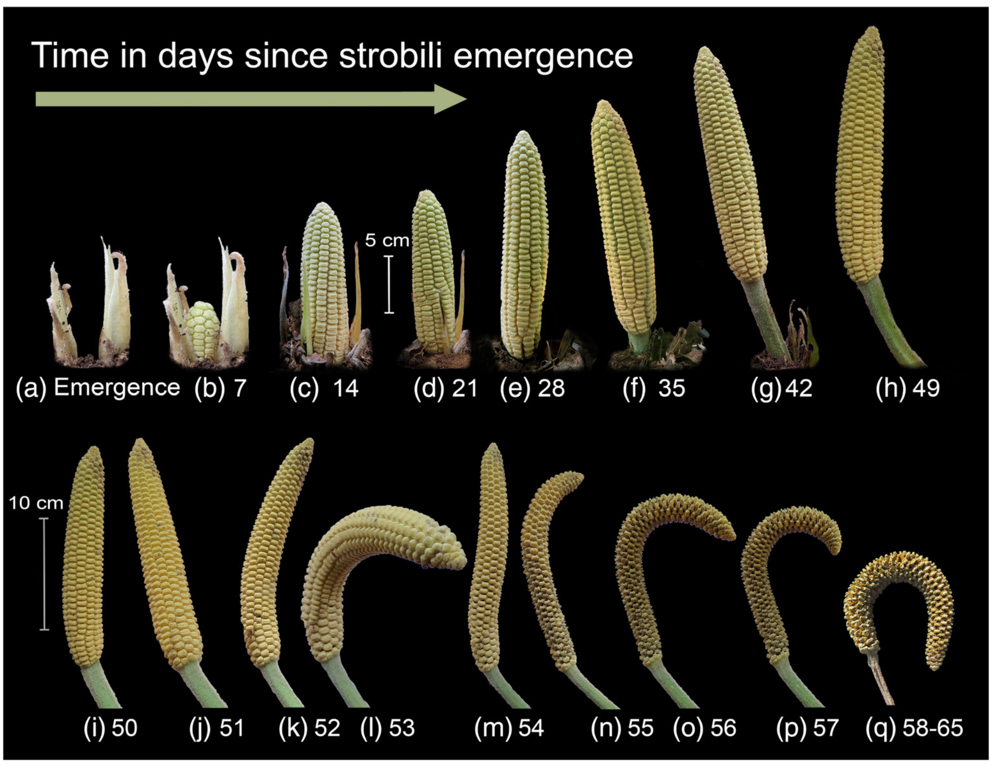


FIGURE 2 Biology of polliniferous strobili of *Zamia boliviana*. Morphology and lifespan of microstrobili. Phases: (a,b) emergence (7 days); (c–f) emergence from the ground (14–35 days); (g) fully emerged/ developed (35–40 days); (h) prerelease (40–50 days); (j) distension of sporophylls (50–51 days); (k–n) pollen release (52–55 days); (o–p) postrelease (56–57 days); and (q) dehydration/senescence and disintegration (58–65 days)

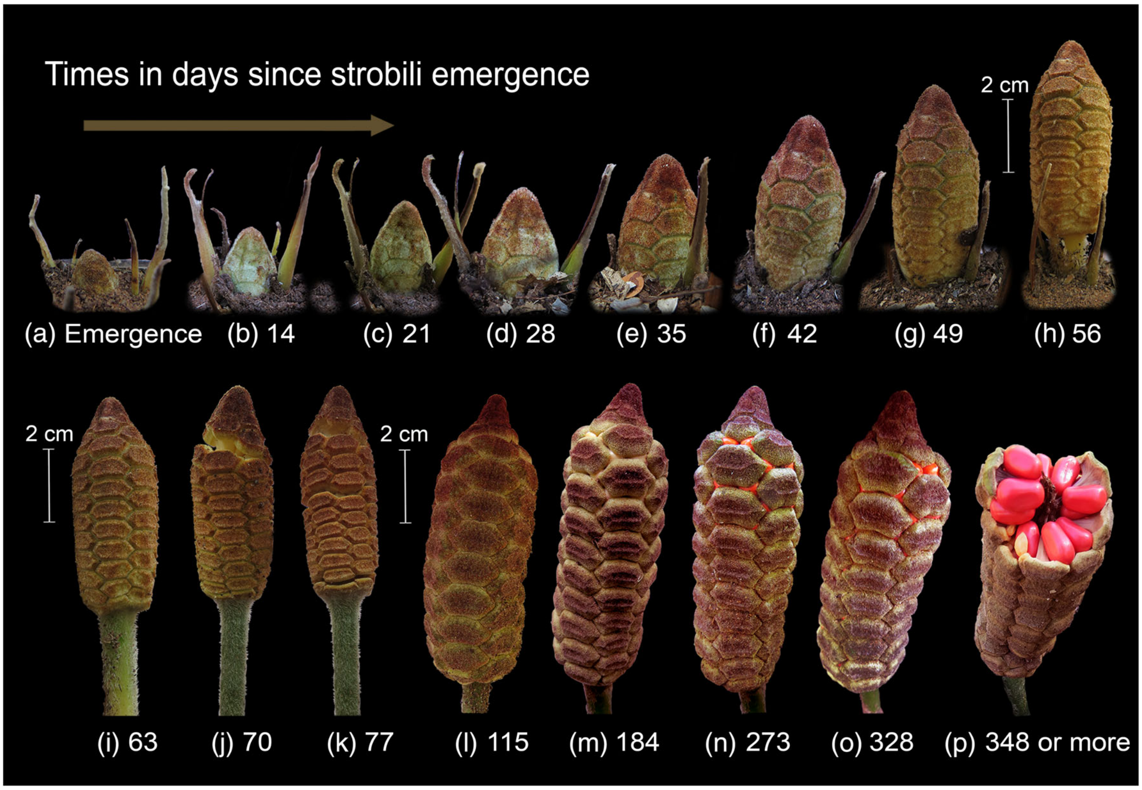


FIGURE 3 Biology of ovuliferous strobili of *Zamia boliviana*. Morphology and lifespan of megastrobili. Phases: (a,b) emergence/presence of sterile apex (7–14 days); (c–h) emergence from the ground (15–60 days); (i) fully emerged; prereceptive (60–70 days); (j–k) open and receptive (70–80 days); (l) closed postreceptive (80–90 days); (m–o) seed development (90–330 days); and (p) seed dehiscence (330 days or more)

up to 345° = December, in 30° intervals. We visually verified that the data distribution was unimodal and tested for the circular distribution of von Misses using the Watson test (Zar, 2010). The following parameters were estimated: (1) mean angle, (2) angular standard deviation, and (3) length of the vector r. The vector r is a measure of dispersion and ranges from 0 (the phenophases are equally distributed around the circle) to 1 (all phenophases occur on the same date). We tested the significance of the mean angle for each phenophase in each year using the Rayleigh test (z) (Zar, 2010). When the mean angle is significant, r indicates the concentration or degree of seasonality or synchrony of a given phenophase (Morellato et al., 2000, 2010). Additionally, to test whether each phenophase had a similar seasonality (intensity and activity index) between polliniferous and ovuliferous individuals and among years, we compared the significant angles using the Watson–Williams (F) test for activity and the Watson–Wheeler test for intensity, according to the premises of circular statistics (Zar, 2010). We considered only the PR and open/receptive (OR) phenophases, as they are critical to pollination. Tests were performed using the *rayleigh.test* and *watson.test* functions, and for the comparisons, we used the *wallraff.test* function, all available in the *circular* package for R (Agostinelli & Lund, 2017; R Core Team, 2013).

3 | RESULTS

Although *Z. boliviana* reproductive structures begin their development underground, most development occurs aboveground. Polliniferous and ovuliferous individuals were of similar size, but there were differences in the number of strobili produced by each sex. Ovuliferous plants produced only one strobilus per xylopodium, while polliniferous plants produced 1–5 strobili per xylopodium or branch (apical meristem) of the xylopodium. In the studied populations, we found 197 adult individuals in the VB plot, of which only 105 (53.30%) produced reproductive structures, including 60 (57.14%) polliniferous and 45 (42.86%) ovuliferous plants. In the VC plot, we registered 216 adult plants, of which 127 (58.79%) produced reproductive structures, including 79 (62.20%) polliniferous and 48 (37.80%) ovuliferous plants.

3.1 | Strobili's biology: morphology, lifespan, and growth dynamics

The polliniferous strobili passed through four distinct stages of development over their lifespan, while ovuliferous strobili went through eight stages, with morphological and

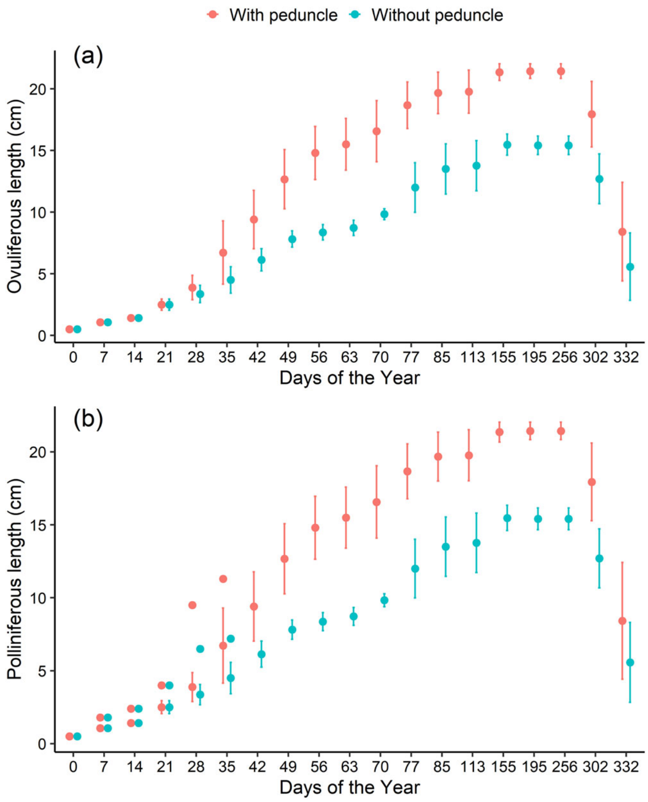


FIGURE 4 Growth dynamics of *Zamia boliviana* strobili. (a) Growth dynamics of polliniferous strobili ($n = 6$); (b) growth dynamics of ovuliferous strobili ($n = 6$)

chronological variation inherent to each reproductive organ (Table 1, Figures 2 and 3) and clear distinctions from the postreceptivity phase onward. Both polliniferous and ovuliferous individuals of *Z. boliviana* underwent a single strobili emergence period that lasted 4 months, mainly during dry season (June–October). In the VB and VC populations, soil emergence of polliniferous and ovuliferous strobili began in July.

On average, the lifespan of polliniferous strobili was 60 days and ovuliferous strobili 330 days, with seed dispersal beginning in April and lasting through August (Table 1, Figure 4a,b). (Figure 4a,b). Polliniferous and ovuliferous strobili differed from each other in the growth dynamics of their structures and, therefore, in peduncle sizes and in the structure of the strobilus itself. In polliniferous strobili, PR began around 45 days after emergence from the soil (Figure 2), followed by a short phase of sporophyll distension (1–2 days), sometimes concomitant with the PR phase (2–4 days). Finally, post-PR (2 days) and senescence phases occurred over 1 day or more, depending on climatic conditions and the activity of the organisms interacting with the polliniferous strobili (Segalla, Pinheiro, & Morellato, 2021) (Figure 2). In ovuliferous strobili, the cone required an average time of 60–70 days from soil emergence to the receptive phase, and subsequently exhibited open cracks (receptive phase) for 7–15 days, rarely longer. The seed development phase lasted 270–300 days and was followed by the seed dehiscence phase, which started 330 days after strobili emergence (Figure 3) and lasted for up to 30 days, depending on the biotic (seed consumers and predators) and abiotic factors (temperature, relative humidity) involved. The period of PR and the receptive phase of the polliniferous and ovuliferous strobili, respectively, overlapped for at least 30 days.

3.2 | Frequency of reproductive cycles

Over the 36 months of phenological observations on 105 plants sampled in VB, all polliniferous plants, and 58% of the 45 ovuliferous plants reproduced at least once (2017–2019). Of the 60 polliniferous plants, 28% reproduced in more than one reproductive cohort, while the ovuliferous plants produced strobili only once in the 3-year period. In VC, 89% of the 79 polliniferous plants reproduced, and 71% of the 48 ovuliferous plants were reproductive during the 3 years of monitoring. However, while 28% of the reproductive polliniferous plants reproduced in two or three uninterrupted cohorts, ovuliferous plants produced strobili in just one breeding season. Figure 5 illustrates the frequency of the reproductive cycles of polliniferous and ovuliferous individuals in the VB and VC populations.

3.3 | Synchrony of phenological intensity and activity of the populations

In general, our analyses indicated seasonality in the production of strobili within populations and for both sexes; emergence of new strobili started in June and lasted until October (Figure 5). Considering the 3 years of phenological data, we observed synchrony within the two populations (VB and VC) between OR and PR (Tables S1 and S2). The peak activity of OR and PR phases varied slightly between study years in the two *Z. boliviana* populations, with the highest activity rates concentrated between August and October. Phenographs showed an overlap in the reproductive phases for both polliniferous and ovuliferous plants and presented similar angular distance for the two populations studied (Figure 6a-d; Tables S1 and S2).

When comparing the Fournier's intensity index for PR and OR phases in polliniferous and ovuliferous individuals over the 3 years of observation (Tables S4 and S5; Figure 6a–d and Figure 7a–d), we observed the highest average intensity of the PR phase in polliniferous

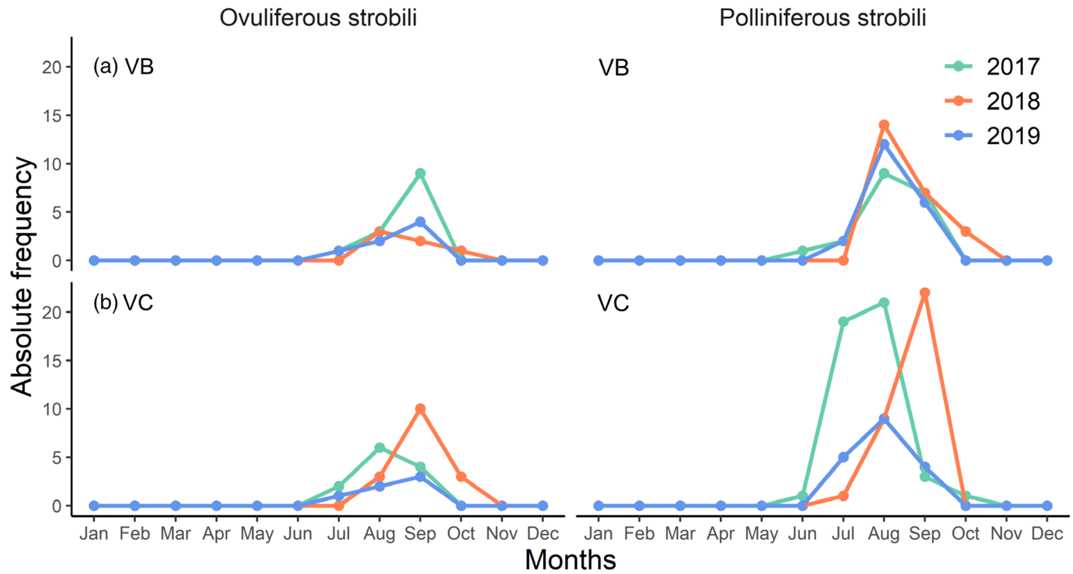
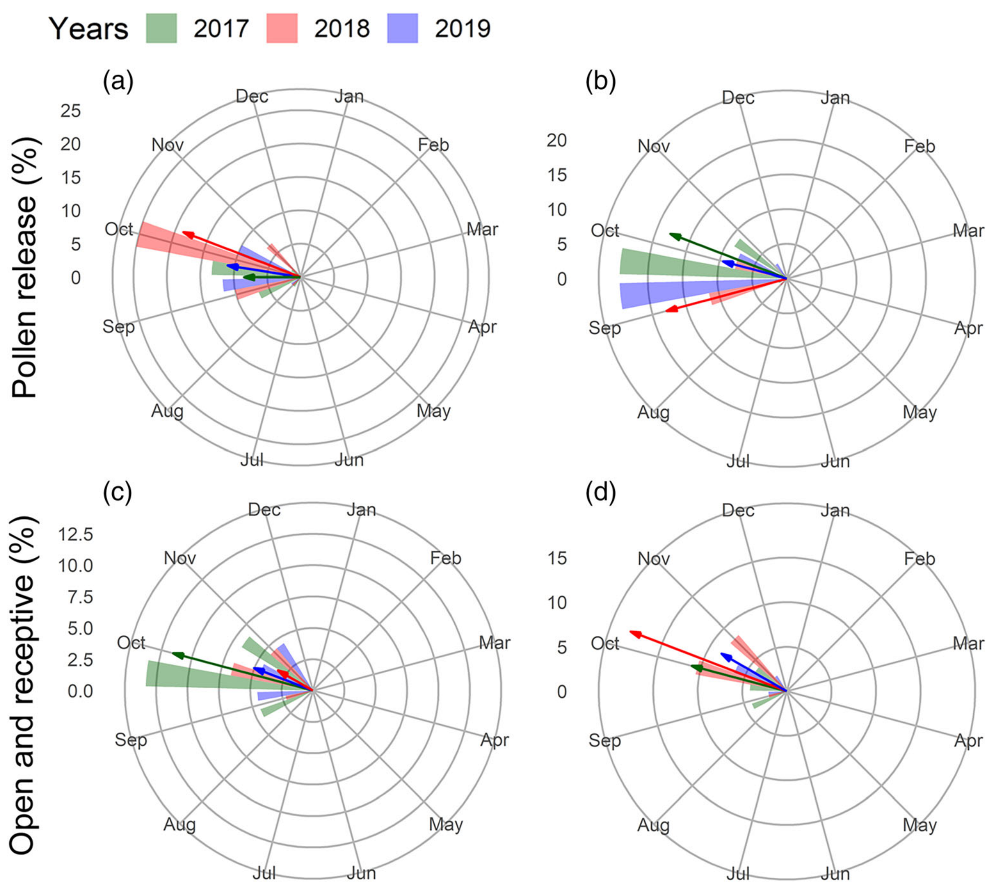


FIGURE 5 Frequency of reproductive cycles of megasporangiate (ovuliferous) and microsporangiate (polliniferous) *Zamia boliviana* individuals in two populations (January 2017 to December 2019). (a) Vale Bonito (VB), (b) Vale do Chapadão (VC), Glória do Oeste and Cáceres, respectively, State of Mato Grosso, Brazil

FIGURE 6 Reproductive phenology (% of open and receptive [OR] and pollen release [PR] events) of two populations of *Zamia boliviana* (Vale Bonito [VB] and Vale do Chapadão [VC] in the State of Mato Grosso, Brazil. Polliniferous strobili – PR: (a) VB and (b) VC. Open and receptive ovuliferous strobili: (c) VB and (d) VC. The arrows indicate the mean angles of phenophases; the arrow length indicates the *r* value or phenophase concentration around mean



individuals from August to November, peaking in August in the VB population (August: 65.43%) and in November (November: 68.95%) in the VC population. In contrast, ovuliferous individuals from both populations showed high Fournier's intensity values for the OR phenophase between September and November, peaking in November and October in the VB and VC populations (81.05% and 84.57%, respectively).

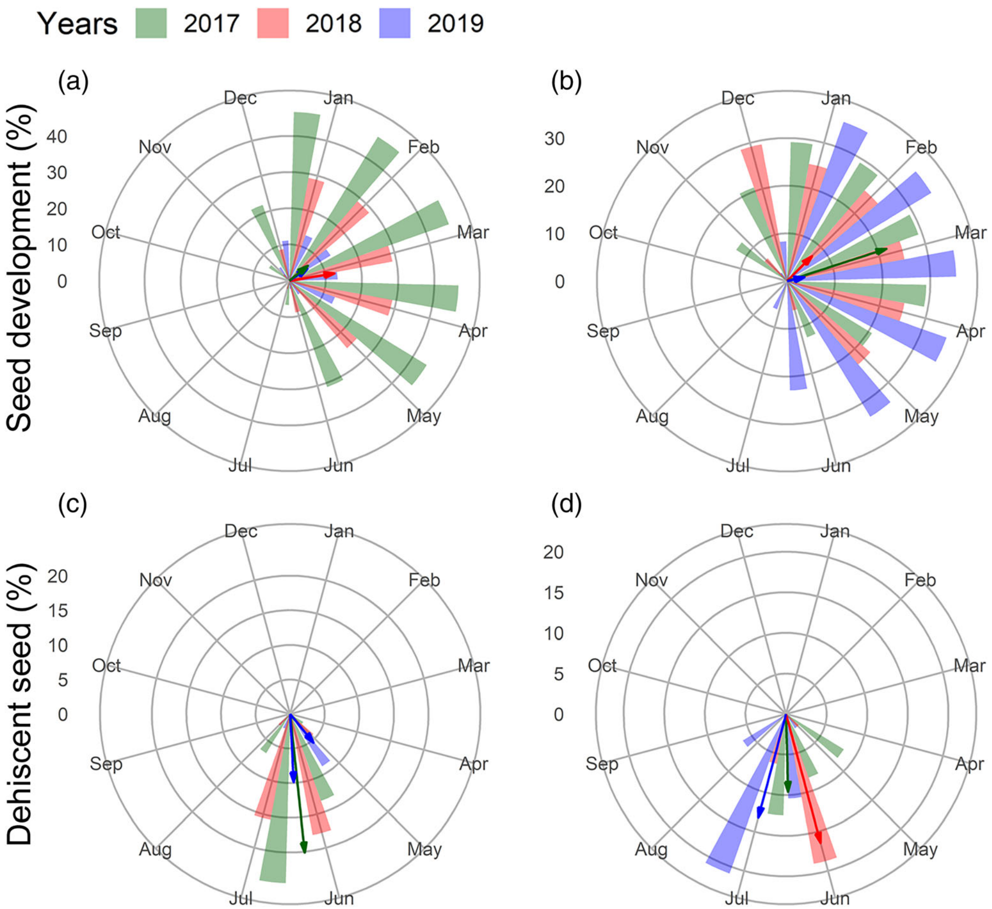


FIGURE 7 Phenology of two *Zamia boliviana* populations' seed development and dispersal (% of open and receptive [OR] events) in the State of Mato Grosso, Brazil. Seed in development: (a) Vale Bonito (VB) and (b) Vale do Chapadão (VC). Seed dehiscence: (c) VB and (d) VC. The arrows indicate the mean angles of phenophase; the arrow length indicates the r value or phenophase concentration around mean

Regarding the phenological activity of the two populations, of the polliniferous individuals that produced strobili, 13.33% (2017), 25.00% (2018), and 11.66% (2019) in the VB population and 24.05% (2017), 24.05% (2018), and 11.39% (2019) in the VC population exhibited PR phase between August and October. Of the ovuliferous individuals that produced strobili, 13.3% (2017), 6.6% (2018), and 4.4% (2019) in the VB population and 4.16% (2017), 10.41% (2018), and 6.25% (2019) in the VC population were open and receptive between September and November (Tables S3 and S4).

No statistical differences in the Fournier intensity index were found for the strobili emergence phase when comparing the 3 years at both locations, neither for the polliniferous (VB: K $= 2.35$, $p = 0.67$; VC: $K = 2.63$, $p = 0.62$) nor the ovuliferous plants (VB: $K = 1.12$, $p = 0.89$; VC: $K = 0.78$, $p = 0.94$). Conversely, the phenological activity index for the strobili emergence phase showed differences when comparing the different years in one of the locations studied (VC), both for the polliniferous (VB: $F = 0.25$, $df = 2$, 102, $p = 0.78$; VC: $F = 33.99$, $df = 2$, 116, $p < 0.001$) and ovuliferous plants (VB: $F = 0.49$, $df = 2$, 51, $p = 0.614$; VC: $F = 7.32$, $df = 2$, 65, $p = 0.001$). Therefore, in at least one of the studied populations, the timing of strobili emergence, but not its intensity, varied across the 3 years. Vector r was similar for the immature emerging (IE) phenophase for both polliniferous and ovuliferous individuals and across all years (Tables S1 and S5).

In general, we found no statistical differences in the production of strobili between polliniferous and ovuliferous individuals and populations during the time of our study (Tables S3–S5). We also found no significant differences between the PR and OR phases for phenological intensity and activity in 2017–2019 when applying the Wallraff rank sum test of angular distance for VB and VC populations (phenological intensity: PR versus OR/VB – 2017, 2018, 2019, $p = 0.24$, 0.24, 0.67, respectively, and PR versus OR/VC, $p = 0.92$, 0.89, 0.89, respectively; phenological activity: PR versus OR/VB – 2017, 2018, 2019, $p = 0.21$, 0.07, 0.17, respectively, and PR versus OR/VC, $p = 0.16$, 0.00, 0.27, respectively). Surprisingly, there was a significant difference between the angular dispersion of phenophases PR and OR for the VC population in 2018. Angular dispersion also varied in the VB population, although the difference was not statistically significant ($p = 0.07$). Table S5 summarizes the intersexual differences and associated statistics of two populations of *Z. boliviana*. The intensity and activity of PR of polliniferous strobili was synchronous with the intensity and activity of receptivity to pollen of ovuliferous strobili in both study populations.

4 | DISCUSSION

4.1 | Strobili biology: morphology, lifespan, and growth dynamics

Our study represents the longest series of systematic phenological data for a cycad species in South America. We demonstrated a high degree of synchrony between polliniferous and ovuliferous plants and a potential association with rainfall patterns in the highly seasonally dry Cerrado woodlands. These results corroborate other studies related to the phenology and population structure of cycads (e.g., Clark & Clark, 1987, 1988; Clugston et al., 2016; Griffith et al., 2012; Grobbelaar et al., 1989; Lopez-Gallego & O'Neil, 2010; Martínez-Domínguez et al., 2018; Negrón-Ortiz & Breckon, 1989; Ornduff, 1987; Ornduff, 1992; Pérez-Farrera & Vovides, 2004) and additionally confirm that the timing of reproduction closely follows local climatic patterns as observed for other plant species (Marcelo et al., 2021). The emergence of reproductive structures occurred during the dry season, with the ovuliferous strobili development continuing well beyond the end of the polliniferous strobili lifespan, as expected.

The absence of a perfect match in the duration of the PR and OR phases demonstrates the concept of the different reproductive costs between the sexes in dioecious systems (Calonje et al., 2011; Delph, 1999; Tang, 1990). The two structures have different purposes, that is, the production of pollen (polliniferous strobili) and ovules and, subsequently, seeds (ovuliferous) that expend less or more of the plant's resources, respectively. This differential leads to more frequent and abundant reproduction in polliniferous plants compared with ovuliferous plants, as reported by Jones (2002). Polliniferous and ovuliferous *Z. boliviana* strobili also differ in terms of morphology, with differences in color and appearance, a characteristic frequently found in *Zamia* spp. (Jones, 2002). The postreceptive phase (late egg) is markedly longer in ovuliferous strobili (270–300 days) as also noted in *Z. splendens* Schutzman (Haynes, 2004) and *Z. integrifolia* L.f. The latter requires 9–12 months for seed maturation (Tang, 1990). According to Jones (2002), both sexes have a similar period of development until pollination, after which the lifespan of polliniferous strobili ends, whereas ovuliferous strobili continue developing for several more months. The observed disparity in coning frequency between the sexes is expected because ovuliferous plants produce larger strobili with longer lifespans, causing a considerable depletion of their storage reserves and, consequently, longer intervals between the formation of strobili in ovuliferous than in polliniferous plants (Jones, 2002; Tang, 1990).

Differences in the growth dynamics of polliniferous and ovuliferous structures, such as the sizes of peduncles and strobili in *Z. boliviana*, seem to be inherent to cycads (Jones, 2002) and may reflect the microconditions present in the plants' habitats (e.g., variation in soil fertility or shading). In *Z. boliviana*, the PR phase per strobilus is relatively short, between 2 and 4 days, but the dehiscence period can extend for more than a week, depending on the number of strobili per plant and its growth and maturation dynamics. Pollen viability is also an important variable that may be associated with this process, but few studies on the longevity and storage of cycad pollen have been conducted (see Calonje et al., 2011).

4.2 | Frequency of reproductive cycles

The low reproductive frequency, especially in ovuliferous plants, over the three reproductive seasons is consistent with other Zamiaceae species. Notably, in long-lived dioecious populations such as cycads, ovuliferous plants usually produce only one strobilus per season per stem. This is in contrast with polliniferous plants that often produce more than one strobilus per season per stem (Grobbelaar, 2002). Generally, polliniferous strobili are produced more frequently and in greater abundance than ovuliferous strobili because they drain less of the plant's resources (Jones, 2002; Tang, 1990).

4.3 | Synchrony of phenological intensity and activity in *Z. boliviana* populations

Application of the activity index and Fournier's intensity index, combined with statistical tests and graphical representations of the data revealed similar seasonality and high levels of synchrony in the reproductive phenology of the studied *Z. boliviana* populations. Our analyses revealed a clear overlap in strobili emergence, PR, and receptivity between polliniferous and ovuliferous individuals, populations, and subsequent years. Differences in the angular dispersion of PR and OR in the VC population in 2018, with time disparities between phenophases, can reveal reproductive seasons that previously failed (Jones, 2002; Käfer et al., 2016). This slight difference likely reflects the timing of receptivity of ovuliferous individuals, as the process of opening and closing of the cracks is slower in ovuliferous individuals compared with the dehiscence time of polliniferous individuals.

In general, the synchronous phenological pattern observed for *Z. boliviana* is in line with what has been described for other cycad species, at least in tropical regions. Martínez-Domínguez et al. (2018) found a similar pattern for *Ceratozamia tenuis* (Dyer) D.W.Stev and Vovides, which is endemic to Mexico, reporting congruence between the ontogenesis of both sexes and slightly disjunctive pollen production, preceding or extending beyond the peak of ovuliferous plants. Therefore, this reproductive pattern appears to be a recurring pattern among Zamiaceae species. Although the emergence of strobili occurred over 4 months in *Z. boliviana*, there was a peak of activity in the PR and OR phenophases, confirming that the reproductive process occurs synchronously across the polliniferous and ovuliferous plants, as expected and as has been reported for most other *Zamia* spp. (Clugston et al., 2016; Martínez-Domínguez et al., 2018). In dioecious populations, polliniferous individuals tend to slightly anticipate or postpone their reproductive events (Forero-Montaña & Zimmerman, 2010; Morellato, 2004). This strategy helps ensure greater success in the pollination of ovuliferous plants, according to Escobedo-Sarti and Mondragón (2016) and Morellato (2004). The reproductive biology of the *Zamia* spp. is characterized by cross-pollination and predominantly obligatory and specialized mutualistic interactions between pollinators and their host plants, in some cases described as a cycad brood-site pollination mutualism (Segalla et al., 2019; Segalla, Pinheiro, & Morellato, 2021). If pollinators are present, even with low synchrony, successful pollination can be achieved, as highlighted by Escobedo-Sarti and Mondragón (2016). The shorter flowering period of ovuliferous plants can also reduce competition between them; if fewer ovuliferous flowers emerged simultaneously, each is more likely to be visited by pollinators (Escobedo-Sarti & Mondragón, 2016).

The understanding of reproductive biology, and more specifically phenology, supports ex situ preservation of cycads, playing a fundamental role in conservation by maintaining genetic reserves of wild populations (Calonje et al., 2011; Clugston et al., 2016; Segalla et al., 2019; Terry et al., 2012). As plant populations become more fragmented due to human activities, it is crucial to understand patterns of gene exchange and demographic changes over time (Martel et al., 2021). Knowledge about the timing of polliniferous and ovuliferous development can guide targeted pollen collection and assisted pollination efforts to improve chances of successful reproduction in botanical collections and in the wild. (Calonje et al., 2011; Clugston et al., 2016; Terry et al., 2012). From a conservation perspective, phenological research serves as a baseline for a variety of research studies, including the establishment of a calendar for collection of seeds and other plant resources for in situ or ex situ conservation and informing conservation plans that take into account not only target species but also their ecological interactions (Morellato et al., 2016). Systematic phenological data are important for explaining ecological patterns, predicting the effects of climate change, and addressing applied environmental and conservation issues (Morellato et al., 2016). In fact, phenological research can help evaluate, manage, and mitigate the consequences of land use change and other natural and anthropogenic disturbances (Morellato et al., 2016), all key factors in accelerating the decline of *Zamia* spp. in South America (Segalla et al., 2019; Segalla, Pinheiro, Barônio, & Morellato, 2021). Thus, understanding the processes that influence individual phenology and interactions within populations is critical not only to ensure the viability of these plant populations but also for the conservation of communities and ecosystems (Morellato et al., 2016; Segalla et al., 2019).

5 | CONCLUSIONS AND REMARKS

Our study provides relevant and new biological data for two *Z. boliviana* populations in their natural environment, demonstrating the temporal distinction between the development of polliniferous and ovuliferous strobili and showing the necessary overlap between PR and receptive phases of this dioecious species. The reproductive events of *Z. boliviana* were seasonal, with sex-to-sex synchrony in terms of strobili production, peak intensity, and activity, and subsequent dehiscence of seeds coincided with the dry season. The reproductive success of this cycad undoubtedly depends on reproductive synchrony, in addition to other biotic and abiotic factors (e.g., number of reproductive adults, pollen availability, effective pollination, seed dispersal, adaptation to seasonal niches) (Gutiérrez-Ortega et al., 2021; Mora et al., 2013; Morellato, 2004; Octavio-Aguilar, Iglesias-Andreu, et al., 2017; Pérez-Farrera & Vovides, 2004; Velasco García et al., 2016). Overall, our study not only allows the acquisition of basic information but is also a contribution to the knowledge of cycads and *Zamia* spp. by providing a valuable dataset for future studies and conservation efforts, including restoration and conservation of *Zamia* in South America.

ACKNOWLEDGMENTS

RS thanks the Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso for allowing the development of the doctoral thesis in the "Programa de Pós-graduação em Biologia Vegetal, UNESP – Rio Claro." This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001 and by the National Council for Scientific and Technological Development (CNPq). The authors thank Pró-Reitoria de Pesquisa – UNICAMP – for the language services provided. LPCM receives a research productivity fellowship from CNPq, and the Phenology Lab facilities are supported by the São Paulo Research Foundation (FAPESP) grant to LPCM. GJB is grateful for a postdoctoral fellowship awarded by CAPES/PNPD/UFVJM (process nº 88887.352134/2019-00) and CPNq/PDJ process nº 165548/2020-7.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Rosane Segalla: conceptualization, methodology, field data collection, data curation and analysis, writing original draft. Leonor Patrícia Cerdeira Morellato: conceptualization, methodology, supervision, writing, reviewing, editing, validation. Fábio Pinheiro: writing, reviewing, editing. Gudryan J. Baronio: software, data analysis, validation. All authors reviewed and approved the final version of the manuscript.

ORCID

Rosane Segalla https://orcid.org/0000-0002-2821-687X
Fábio Pinheiro https://orcid.org/0000-0003-3243-2652
Gudryan J. Baronio https://orcid.org/0000-0003-2988-9168
Leonor Patrícia Cerdeira Morellato https://orcid.org/0000-0001-5265-8988

REFERENCES

Agostinelli, C., & Lund, U., (2017). *R package 'circular': Circular statistics, version 0.4-93*. https://r-forge.r-project.org/projects/circular/

Bencke, C. S. C., & Morellato, L. P. C. (2002). Estudo comparativo da fenologia de nove espécies arbóreas em três tipos de floresta atlântica no sudeste do Brasil. *Revista Brasileira de Botânica*, *25*, 237–248. https://doi.org/10.1590/S0100-84042002000200012

Calonje, M., Kay, J., & Griffith, P. M. (2011). Propagation of cycad collections from seed: Applied reproductive biology for conservation. *Sibbaldia*, *9*, 77–96.

Calonje M, Stevenson DW, Osborne R (2013–2021) *The world list of cycads*. http://www.cycadlist.org

Clark, D. A., & Clark, D. B. (1987). Temporal and environmental patterns of reproduction in *Zamia skinneri*, a tropical rain forest cycad. *Journal of Ecology*, *75*, 135–149. https://doi.org/10.2307/2260540

Clark, D. B., & Clark, D. A. (1988). Leaf production and the cost of reproduction in the Neotropical rain forest cycad, *Zamia skinneri*. *Journal of Ecology*, *76*, 1153–1163. http://www.jstor.org/stable/2260640

Clugston, J. A. R., Griffith, M. P., Kenicer, G. J., Husby, C. E., Calonje, M. A., Stevenson, D. W., & Little, D. P. (2016). *Zamia* (Zamiaceae) phenology in a phylogenetic context: Does in situ reproductive timing correlate with ancestry? *Edinburgh Journal of Botany*, *73*, 345–370. https://doi.org/10.1017/s096042861600007x

Delph, L. F. (1999). Sexual dimorphism in life history. In M. Geber, T. E. Dawson, & L. F. Delph (Eds.), *Gender and sexual dimorphism in flowering plants* (pp. 149–173). Springer-Verlag. https://doi.org/10.1007/978-3-662-03908-3_6

Escobedo-Sarti, J., & Mondragón, D. (2016). Flowering phenology of *Catopsis compacta* (Bromeliaceae), a dioecious epiphyte in an oak forest. *Botanical Sciences*, *94*, 729–736. https://doi.org/10.17129/botsci.669

Forero-Montaña, J., & Zimmerman, J. K. (2010). Sexual dimorphism in the timing of flowering in two dioecious trees in a subtropical wet forest, Puerto Rico. *Caribbean Journal of Science*, *46*, 88–95. https://doi.org/10.18475/cjos.v46i1.a11

Fournier, L. A. (1974). Un método cuantitativo para la medición de características fenológicas en árboles. *Turrialba*, *24*, 422–423.

Funamoto, D., & Sugiura, S. (2021). Relative importance of diurnal and nocturnal pollinators for reproduction in the early spring flowering shrub *Stachyurus praecox* (Stachyuraceae). *Plant Species Biology*, *36*, 94–101. https://doi.org/10.1111/1442-1984.12299

Gorelick, R., & Marler, T. E. (2012). Evolutionary developmental biology in cycad phenology. *Communicative & Integrative Biology*, *5*, 272–274. https://doi.org/10.4161/cib.19428

Griffith, M. P., Calonje, M. A., Stevenson, D. W., Husby, C. E., & Little, D. P. (2012). Time, place, and relationships: Cycad phenology in a phylogenetic and biogeographic context. *Memoirs of the New York Botanical Garden*, *106*, 59–81.

Grobbelaar, N. (2002). *Cycads: With special reference to the southern African species*. South Africa.

Grobbelaar, N., Meyer, J., & Burchmore, J. (1989). Coning and sex ratio of *Encephalartos transvenosus* at the Modjadji nature reserve. *South African Journal of Botany*, *55*, 79–82.

Gutiérrez-Ortega, J. S., Martínez, J. F., Molina-Freaner, F., Pérez-Farrera, M. A., Vovides, A. P., Hernández-López, A., Tezuka, A., Nagano, A. J., Watano, Y., Takahashi, Y., & Murakami, M. (2021). Speciation along a latitudinal gradient: The origin of the Neotropical cycad sister pair *Dioon sonorense-D. vovidesii* (Zamiaceae). *Ecology and Evolution*, *11* (11), 6962–6976.

Hall, J. A., Walter, G. H., Bergstrom, D. M., & Machin, P. (2004). Pollination ecology of the Australian cycad *Lepidozamia peroffskyana* (Zamiaceae). *Australian Journal of Botany*, *52*, 333–343. https://doi.org/10.1071/BT03159

Haynes, J. (2004). Phenology of *Zamia splendens* in South Florida. *Cycad Newsletter*, *27*, 11.

Heilbuth, J. C. (2000). Lower species richness in dioecious clades. *American Naturalist*, *156*, 221–241. http://www.jstor.org/stable/10.1086/303389

Jones, D. L. (2002). *Cycads of the world*. Smithsonian Institution Press.

Käfer, J., Marais, G. A. B., & Pannell, J. R. (2016). On the rarity of dioecy in flowering plants. *Molecular Ecology*, *26*, 1225–1241. https://doi.org/10.1111/mec.14020

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., Zimmermann, N., Linder, H. P., & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, *4*, 170–122. https://doi.org/10.1038/sdata.2017.122

Köppen, W. (1918). Klassification der klimate nach temperatur, niederschlag and jahreslauf. *Petermanns Geographische Mitteilungen*, *64*(193–203), 243–248.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*, 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Krieg, C., Watkins, J. E., Chambers, S., & Husby, C. E. (2017). Sex-specific differences in functional traits and resource acquisition in five cycad species. *AoB Plants*, *9*, plx013. https://doi.org/10.1093/aobpla/plx013

Labouche, A.-M., & Pannell, J. R. (2016). A test of the size-constraint hypothesis for a limit to sexual dimorphism in plants. *Oecologia*, *181*, 873–884. https://doi.org/10.1007/s00442-016-3616-3

Laidlaw, M., & Forster, P. (2012). Climate predictions accelerate decline for threatened *Macrozamia* cycads from Queensland. *Biology*, *1*, 880–894. https://doi.org/10.3390/biology1030880

Lazcano-Lara, J. C., & Ackerman, J. D. (2018). Best in the company of nearby males: Female success in the threatened cycad, *Zamia portoricensis*. *Plant Biology*, *6*, e5252. https://doi.org/10.7717/peerj.5252

Lopez-Gallego, C., (2007). *Effects of habitat degradation on the evolutionary dynamics of populations in a rainforest cycad (Gymnospermae)* [Ph.D. dissertation]. New Orleans, University of New Orleans.

Lopez-Gallego, C. (2015). *Plan de acción para la conservación de las Zamias de Colombia*. Ministerio de Ambiente y Desarrollo, Sostenible: Universidad de Antioquia.

Lopez-Gallego, C., & O'Neil, P. (2010). Life-history variation following habitat degradation associated with differing fine-scale spatial genetic structure in a rainforest cycad. *Population Ecology*, *52*, 191–201. https://doi.org/10.1007/s10144-009-0171-3

Marcelo, V. G., de Brito, V. L., Vallejo-Marín, M., & Consolaro, H. (2021). Andromonoecy in *Solanum lycocarpum* A. St.-Hil. (Solanaceae): Floral attributes, visitors and variation in sexual expression over time. *Plant Species Biology*, *36*, 308–321. https://doi.org/10.1111/1442-1984.12316

Martel, C., Scopece, G., Cozzolino, S., Ayasse, M., Pinheiro, F., & Cafasso, D. (2021). Genetic diversity in natural populations of the endangered Neotropical orchid *Telipogon peruvianus*. *Plant Species Biology*, *36*, 6–16. https://doi.org/10.1111/1442-1984.12291

Martínez-Domínguez, L., Nicolalde-Morejón, F., Stevenson, D. W., & Santiago-Jiménez, Q. (2018). Taxonomic concepts, phenology and epiphytism: The case of *Ceratozamia tenuis* (Zamiaceae). *Revista Mexicana de Biodiversidad*, *89*, 331–339. https://doi.org/10.22201/ib.20078706e.2018.2.2357

Mendoza, I., Peres, C. A., & Morellato, L. P. C. (2017). Continental-scale patterns and climatic drivers of fruiting phenology: A quantitative neotropical review. *Global and Planetary Change*, *148*, 227–241. https://doi.org/10.1016/j.gloplacha.2016.12.001

Mora, R., Yáñez-Espinosa, L., Flores, J., & Nava-Zárate, N. (2013). Strobili and seed production of *Dioon* edule (Zamiaceae) in a population with low seedlings density in San Luis Potosí, Mexico. *Tropical Conservation Science*, *6*, 268–282. https://doi.org/10.1177/194008291300600208

Morellato, L. (2004). Phenology, sex ratio, and spatial distribution among dioecious species of *Trichilia* (Meliaceae). *Plant Biology*, *6*, 491–497.

Morellato, L. P. C., Alberti, L., & Hudson, I. L. (2010). Applications of circular statistics in plant phenology: A case studies approach. In I. Hudson & M. Keatley (Eds.), *Phenological research*. Springer. https://doi.org/10.1007/978-90-481-3335-2_16

Morellato, L. P. C., Alberton, B., Alvarado, S. T., Borges, B., Buisson, E., Camargo, M. G. G., & Peres, C. A. (2016). Linking plant phenology to conservation biology. *Biological Conservation*, *195*, 60–72. https://doi.org/10.1016/j.biocon.2015.12.033

Morellato, L. P. C., Camargo, M. G. G., & Gressler, E. (2013). A review of plant phenology in South and Central America. In M. Schwartz (Ed.), *Phenology: An integrative environmental science*. Springer. https://doi.org/10.1007/978-94-007-6925-0_6

Morellato, P. L., Talora, C., Takahasi, D. C., Bencke, C. C. A., Romera, E. C., & Zipparro, V. B. (2000). Phenology of Atlantic rain Forest trees: A comparative study. *Biotropica*, *32*, 811–823. https://doi.org/10.1646/0006-3606(2000)032[0811:POARFT]2.0.CO;2

Munguia-Rosas, M. A., Ollerton, J., & Parra-Tabla, V. (2011). Phenotypic selection on flowering phenology and size in two dioecious plant species with different pollen vectors. *Plant Species Biology*, *26*, 205–212. https://doi.org/10.1111/j.1442-1984.2011.00320.x

Negrón-Ortiz, V., & Breckon, G. J. (1989). Population structure in *Zamia debilis* (Zamiaceae). I. Size classes, leaf phenology, and leaf turnover. *American Journal of Botany*, *76*, 891–900. http://www.jstor.org/stable/2444545

Norstog, K. J., & Nicholls, T. J. (1997). *The biology of the cycads*. Cornell University Press.

Obeso, J. R. (2002). The costs of reproduction in plants. *New Phytologist*, *155*, 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x

Octavio-Aguilar, P., Iglesias-Andreu, L. G., Núñez de Cáceres-González, F. F., & Galván-Hernández, D. M. (2017). Fine-scale genetic structure of *Zamia furfuracea*: Variation with life-cycle stages. *International Journal of Plant Sciences*, *178*, 57–66. https://doi.org/10.1086/689200

Octavio-Aguilar, P., Rivera-Fernández, A., Iglesias-Andreu, L. G., Vovides, P. A., & de Cáceres-González, F. F. N. (2017). Extinction risk of *Zamia inermis*: A demographic study in its single natural population. *Biodiversity and Conservation*, *26*, 787–800. https://doi.org/10.1007/s10531-016-1270-z

Okubamichael, D. Y., Jack, S., De Wet Bösenberg, J., Timm Hoffman, M., & Donaldson, J. S. (2016). Repeat photography confirms alarming decline in south African cycads. *Biodiversity and Conservation*, *25*, 2153–2170. https://doi.org/10.1007/s10531-016-1183-x

Ornduff, R. (1987). Sex ratios and coning frequency of the cycad *Zamia pumila* L. (Zamiaceae) in The Dominican Republic. *Biotropica*, *19*, 361–364. http://www.jstor.org/stable/2388634

Ornduff, R. (1992). Features of coning and foliar phenology, size classes, and insect associates of *Cycas armstrongii* (Cycadaceae) in the Northern Territory, Australia. *Bulletin of the Torrey Botanical Club*, *119*, 39–43. http://www.jstor.org/stable/2996918

Pérez-Farrera, M. A., & Vovides, A. P. (2004). Spatial distribution, population structure, and fecundity of *Ceratozamia matudai* Lundell (Zamiaceae) in El Triunfo Biosphere Reserve, Chiapas, Mexico. *Botanical Review*, *70*, 299–311. https://doi.org/10.1663/0006-8101(2004)070[0299:SDPSAF]2.0.CO;2

R Core Team. (2013). *R: A language and environment for statistical computing, version 3.0.1*. R Foundation for Statistical Computing. https://www.R-project.org/

Reed, T. E., Jenouvrier, S., & Visser, M. E. (2012). Phenological mismatch strongly affects individual fitness but not population demography in a woodland passerine. *Journal of Animal Ecology*, *82*, 131–144. https://doi.org/10.1111/j.1365-2656.2012.02020.x

Schneider, D., Wink, M., Sporer, F., & Lounibos, P. (2002). Cycads: Their evolution, toxins, herbivores and insect pollinators. *Naturwissenschaften*, *89*, 281–294. https://doi.org/10.1007/s00114-002-0330-2

Segalla, R., Pinheiro, F., Barônio, G. J., & Morellato, L. P. C. (2021). Male-biased effective sex ratio across populations of the threatened *Zamia boliviana* (Zamiaceae). *Plant Ecology*, *222*, 587–602. https://doi.org/10.1007/s11258-021-01127-3

Segalla, R., Pinheiro, F., & Morellato, L. P. C. (2021). Reproductive biology of the South American cycad *Zamia boliviana*, involving brood-site pollination. *Plant Species Biology*, *36*, 1–13. https://doi.org/10.1111/1442-1984.12322

Segalla, R., Telles, F. J., Pinheiro, F., & Morellato, P. (2019). A review of current knowledge of Zamiaceae, with emphasis on *Zamia* from South America. *Tropical Conservation Science*, *12*, 1–21. https://doi.org/10.1177/1940082919877479

SEPLAN (Secretaria de Estado de Planejamento) (2000). *Aspectos geomorfológicos da folha vila Guarita –MIR-300 (SC.21-Z-B) - Memória Técnica*. Portal de Dados e Metadados Geográficos do Estado de Mato Grosso. http://metadados.seplan.mt.gov.br/metadados/srv/por/catalog.search#/home

Skelley, P. E., & Segalla, R. (2019). A new species of *Pharaxonotha* Reitter (Coleoptera: Erotylidae) from central South America. *Zootaxa*, *4590*, 184–190. https://doi.org/10.11646/zootaxa.4590.1.9

Stevenson, D. W. (2004). Zamiaceae of Bolivia, Ecuador and Peru. In T. Walters & R. Osborne (Eds.), *Cycad classification concepts and recommendations* (pp. 173–194). CAB International.

Tang, W. (1990). Reproduction in the cycad *Zamia pumila* in a fire-climax habitat: An eight-year study. *Bulletin of the Torrey Botanical Club*, *117*, 368–374. http://www.jstor.org/stable/2996834

Terry, I., Tang, W., Taylor, A. S. B., Donaldson, J. S., Singh, R., Vovides, A. P., & Cibrián-Jaramillo, A. (2012). An overview of cycad pollination studies. *Memoirs of the New York Botanical Garden*, *106*, 352–394.

Velasco García, M. V., Valdez-Hernández, J. I., Ramírez-Herrera, C., Hernández-Hernández, M. L., López-Upton, J., López-Mata, L., & López-Sánchez, H. (2016). Estructura, heterogeneidad de estadios y patrón de dispersión espacial de em *Dioon holmgrenii* (Zamiaceae). *Botanical Sciences*, *94*, 75–87. https://doi.org/10.17129/botsci.258

Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Prentice-Hall/Pearson.

Zhang, X., Zhang, C., & Zhao, X. (2014). Effect of sex ratio, habitat factors and neighborhood competition on stem growth in the dioecious tree *Fraxinus mandshurica*. *Ecological Research*, *29*, 309–317. https://doi.org/10.1007/s11284-013-1125-y

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Segalla, R., Pinheiro, F., Baronio, G. J., & Morellato, L. P. C. (2022). Phenology of *Zamia boliviana* (Zamiaceae), a threatened species from a seasonally dry biodiversity hotspot in South America. *Plant Species Biology*, *37*(1), 118–131. https://doi.org/10.1111/1442-1984.12360