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Article Title: **Germination characteristics of *Gymnocalycium monvillei* (Cactaceae) along its entire altitudinal range**

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Abstract: Germination characteristics are important to understand how species copes with environmental variation. The aims of this work were to analyze the effect of different temperatures (25 and 32 °C), water potentials (0, -0.2, -0.4 and -0.6 MPa) and light conditions (light vs. darkness) on the germination of five populations of the cactus *Gymnocalycium monvillei* along its entire altitudinal distribution. The experiments to assess the effects of temperature, water potential and light conditions were performed in germination chambers, and total germination (%) and mean germination time (T_{50}) were recorded. Germination decreased from higher to lower altitudinal provenances and the effect was much pronounced under 32 °C. For all altitudinal provenances, germination decreased with lower water potential, with this effect being more pronounced at 32 °C. On the other hand, lower altitudinal provenances were less affected by lower water potentials than higher provenances. All altitudinal provenances showed very low germination under dark conditions. T_{50} did not vary among altitudinal provenances at a temperature of 25 °C but at 32 °C, germination was slower at intermediate altitudes. Our results show that germination characteristics differ considerably among altitudinal provenances and seem to be important in determining the capacity of the species to inhabit such a broad gradient.

Keywords: Altitudinal gradients, Intraspecific variability, Germination traits, Cactaceae, *Gymnocalycium monvillei*.

Introduction

In mountain environments, the altitudinal gradient is the most important factor in determining the distribution of vegetation (Körner 1999). Even though altitude determines clear limits of vegetation belts, with most species presenting rather low altitudinal distributions or ranges (Gurvich et al. 2014; Chiapella and Demaio 2015), some plant species can present very wide altitudinal ranges and even occur in different ecological zones. Reproductive biology, particularly germination characteristics, is an important aspect in a plant life cycle (Baskin and Baskin 2014), as well as in determining the ability of a species to inhabit these environments (Pluess et al. 2005; Guo et al. 2010; Bauk et al. 2015).

Understanding these characteristics may be also important for predicting species responses to global changes (Gurvich et al. 2002). Succulent species, particularly cacti in the Americas, present their highest diversity in mountains (Mourelle and Ezcurra 1996) and some species have wide altitudinal ranges (Guerrero et al. 2011; Gurvich et al. 2014; Bauk et al. 2015), being ideal models to test questions about the effects of altitudinal gradients on germination characteristics in relation to different factors, such as temperature, water potential and light.

Total germination is a good indicator of seed quality and has direct effects on species fitness (Reed and Frankham 2003; Long et al. 2015). Increased germinability and decreased germination speed is generally associated with the following environmental conditions during seed development: high temperatures, short days, red light, drought, and high nitrogen levels (Fenner 1991; Gutterman 1994; Baskin and Baskin 2014). Maximum seed germination is expected to be achieved at the optimal conditions of a species (e.g. where species reach their highest abundance; Holm 1994; Giménez-Benavides et al. 2007). For instance, for *Silene ciliate* (Caryophyllaceae), a high mountain Mediterranean plant, seed germination is higher at the center of its altitudinal range than at the boundaries (Giménez-Benavides et al. 2007). In the Córdoba Mountains, *Gymnocalycium monvillei* (Lem.) Britton & Rose (Cactaceae) presents the highest abundance at intermediate altitudes along its range (1900 m a.s.l.; Bauk 2014), decreasing at both extremes of the gradient (2200-800 m a.s.l.); therefore, total seed germination and mean germination time are expected to follow this pattern.

Seed germination is related to temperature (Shimono and Kudo 2005; Baskin and Baskin 1998, 2014). Seeds of a given species could require different temperatures to germinate in relation to their

provenance: high or low altitudes (Cavieres and Arroyo 2000; Giménez-Benavides et al. 2005). For cactus seeds, the favorable temperature is about 25 °C (Rojas-Aréchiga and Vázquez-Yanes 2000; Cheib and Garcia 2012). However, this value can vary depending on the environmental characteristics where species occur (Gurvich et al. 2008). Climate change scenarios forecast increases in mean and extreme temperatures (IPCC 2014). Extreme increases in temperature have negative effects on seed germination (decreasing seed germination and increasing mean germination time) of some species, but also have neutral or even positive effects on other species (Bell et al. 1987; Pérez-Sánchez et al. 2011). However, little is known about how germination, in terms of both germinability and speed, of populations growing at different altitudes could respond to increases in temperatures. It is expected that populations from lower sites will be less affected by increased temperatures than populations from higher sites.

Soil water potential is another important factor determining germination. In general, species germination percentage decreases and mean germination time increases with decreasing water potential, but some species can maintain a relatively high germination percentage even at quite low water potential (Flores and Briones 2001; Guillén et al. 2011). For example, seeds of some cactus species are adapted to germinate at high soil humidity, but not necessarily at the soil field water capacity (Flores and Briones 2001; Guillén et al. 2011); for other species, maximum germination has been obtained in distilled water (0 MPa) and germination percentage decreased with decreasing water potential (De la Barrera and Nobel 2003; Meiado et al. 2010; De la Rosa-Manzano and Briones 2010; Guillén et al. 2011; Mazzola et al. 2013). To our knowledge, cactus species have not been studied in terms of intraspecific variations in germination at different water potentials along altitudinal gradients. It would be expected that populations from lower altitudes, where soil humidity is lower due to either lower precipitation or higher temperatures, would perform better at lower water potentials than populations from higher altitudes.

Cactus seeds can be positively photoblastic, i.e. seeds require light to germinate, or neutrally photoblastic, i.e. seeds can germinate under light or dark conditions (Rojas-Aréchiga et al. 1997; Flores et al. 2006, 2011, 2015). The influence of light on seed germination has been associated with seed size in many plant species (Milberg et al. 2000) including cacti species (Gurvich et al. 2008; Flores et al. 2006, 2011, 2015) and plant and seedling size (seeds from shorter plants have a stronger light requirement for germination than those from taller plants; Rojas-Aréchiga et al. 1997; Flores et al. 2011). It has been

suggested that photoblastism in cacti could be related to seedling size and shape (Gurvich et al. 2008; Flores et al. 2011), because big seedlings could have greater capacity to reach the soil surface than small seedlings when buried (Flores et al. 2015). However, no studies have analyzed possible intraspecific variation due to the influence of light on cactus seed germination. In *G.monvillei*, seedlings from higher altitudes are more columnar than those from lower altitudes (under more humid conditions, at higher altitudes, a columnar shape would be more advantageous increasing seedling growth; Bauk et al. 2015), and would therefore have higher capacity to reach light if germination occurs belowground. If positive photoblastism in cacti is associated with the capacity to reach light during the germination process (Flores et al. 2011), it is expected that seeds from plants at higher altitudes (which are more columnar, Bauk et al. 2015) will have higher capacity to germinate under darkness than those from lower altitudes.

Gymnocalycium monvillei is a globose cactus endemic to Córdoba Mountains, central Argentina, and presents a very wide altitudinal distribution (Charles 2009; Gurvich et al. 2014). A previous study in this species, but involving a single population at a low altitude, showed that its germination is relatively low (40%) and negatively photoblastic (Gurvich et al. 2008). Along the altitudinal gradient, there are important differences in irradiation, temperature and soil moisture, generating very different environmental conditions that could affect germination and establishment (García-Pérez et al. 2007; Bauk et al. 2015). Studies of regenerative traits along gradients may help to understand how species cope with environmental conditions and are necessary to predict the effect of climatic change in abundance and distribution of species (Butler et al. 2012; Dávila et al. 2013; Aragón-Gastelum et al. 2016). The aims of this work were to analyze germination, in terms of total germination and speed (T_{50}), in *G. monvillei* along its entire altitudinal distribution, and in relation to different temperatures, water potentials, and light/darkness conditions.

Materials and Methods

Study species, study area characteristics and measured variables

Gymnocalycium monvillei inhabits rocky outcrops from 880 to 2200 m a.s.l. (Demaio et al. 2011; Gurvich et al. 2014). We studied five populations (thereafter altitudinal provenances) located along an altitudinal gradient between the localities of Cuesta Blanca (31° 28' S, 64° 34' W) and Pampa de Achala

(31° 41' S, 64° 50' W), at 878, 1250, 1555, 1940 and 2230 m a.s.l. (Fig. 1). Mean annual temperature and precipitation varied from 16.5 °C and 680mm to 10.3 °C and 790 mm, between the lowest and the highest sites of this altitudinal gradient, respectively (De Fina 1992). Mean temperature of the warmer months are 15 and 24 °C for the extreme sites. Vegetation varied from subtropical dry forest at lower altitudes to cold-temperate grasslands at higher ones (Giorgis et al. 2011). Frost can occur all year round and snow events are common during winter at sites above 1900 m a.s.l. Plants produce between 1 and 7 fruits per season, which bears between 200 and 4000 seeds (Giorgis et al. 2015). Seeds are light (0.24 mg) and do not vary in size or weight with altitude (Bauk et al. 2015). The main dispersal is mirmechory. Species abundance is greatest at the 1940m a.s.l. site, with 1.5 individuals per square meter, and lower at both extremes where its density is of 0.5 individuals per square meter (Karen Bauk, unpublished data).

Mature fruits from 20 individuals of similar size (about 10 cm in diameter) were collected from individuals of each altitudinal provenance between December and February 2013-14 (when fruits are mature and dispersal occurs). Seeds were air-dried and stored in the laboratory at ambient conditions (25 °C and 25 % air relative humidity) until the start of experiments, which were performed during the year after seed collection.

Germination experiments: effect of temperature, water potentials and light

Three experiments were performed to evaluate the effect of temperature, water potential, and light conditions on germination and germination speed (T_{50}) of seeds from the altitudinal provenances. A factorial experimental design was used for all experiments. All germination assays were performed in Petri dishes (5.5cm in diameter), using sterile filter paper with 10 ml distilled water, except in the water potential treatments, which had filter paper but with PEG-6000 (polyethylene glycol). The filter paper could retain PEG and thus change the water potential in the dishes, but for studies that use the same germination substrate for all PEG-6000 treatments, errors in water potential measurements would have no effect on relative ranking

of water stress between treatments (Hardegree and Emmerich 1990). For all experiments, five Petri dishes (replicates) per altitudinal provenance were used, containing 20 seeds per replicate. Seeds were placed under a photoperiod of 12/12 h (light/darkness). Light condition was provided by cool white fluorescent light tubes (400-700 nm), with a photon flux density of $38 \mu\text{mol m}^{-2} \text{s}^{-1}$. In all experiments, seed germination was recorded until day 30, and radicle protrusion was the criterion for considering seed germination (ISTA 2003). The evaluated response variables were final germination percentage and mean germination time (T_{50} ; Soltani et al. 2015). T_{50} is the time to reach 50% of germination of the total number of germinated seeds. T_{50} was calculated as:

$$T_{50} = \frac{\sum n_i t_i}{\sum n_i}$$

where: n_i is the number of seeds newly germinated at time i , and t_i is the period elapsed from the beginning of the germination test, expressed as number of days.

In the temperature effect experiment seeds from the five altitudinal provenances were evaluated for germination at 25 °C and 32 °C constant temperatures; these temperature values were selected because 25 °C is the optimal germination temperature for the species and 32 °C is to simulate an important increase in temperature at noon (Funes et al. 2009; Sosa Pivatto et al. 2014).

In the water potential effect experiment, the effect of water potential was evaluated for the altitudinal provenances 2230, 1555 and 878 m a.s.l, at 25 and 32°C. Water potential treatments (-0.20, -0.40 and -0.60 MPa) were simulated using solutions of polyethylene glycol (PEG) 6000 for each temperature, following Villela et al. (1991). For the 25°C and -0.20, -0.40 and -0.60 MPa solutions we added 119.57, 178.34 and 223.66 g of PEG 6000/1 L of distilled water, while for the 32°C and -0.20, -0.40 and -0.60 MPa solutions we added 131.34, 192.38 and 239.32 g of PEG 6000/1 L of distilled water. The water potential values chosen are in the range of those reported during the rainy season for different soil types in semiarid ecosystems (i.e., from 0.34 to -0.80 MPa; Ramírez-Padilla and Valverde 2005). These treatments were compared with a control (0 MPa) prepared with distilled water. The different temperature treatments were performed in different germination chambers during the same period. PEG 6000 was dissolved in distilled water and placed in a shaker bed at 20°C for 16 hours. Each Petri dish included a PEG 6000 solution that is harmless to seeds and mimics variations in soil moisture (Van den

Berg and Zeng 2006; Zeng et al. 2010). Each dish at 0 MPa was prepared with 2 ml distilled water. The water potentials of PEG solutions at each temperature were verified using a dew point micro-voltmeter (HR33T; Wescor Inc., Logan, UT, USA). All dishes were sealed with adherent transparent paper to prevent evaporation.

In the light effect experiment, we evaluated seed germination under two conditions for all altitudinal provenances: 12/12 h (light/darkness) photoperiod and continuous darkness. To provide darkness conditions Petri dishes were wrapped in double layer of aluminum foils as suggested by Baskin and Baskin (2014). Petri dishes were maintained at 25 °C. For each altitudinal provenance, the observed number of germinated seeds of both treatments were used to evaluate the relative light germination (RLG) according to the formula:

$$RLG = \frac{G_l}{G_d + G_l}$$

Where G_l = germination percentage in light, and G_d = mean germination percentage in darkness (Milberg et al. 2000; Flores et al. 2011, 2015). In the calculations, we used the mean of the germination in darkness of each provenance and combined it with germination in light of each sample, to get five different values of RLG per provenance site. RLG represents a range of values from 0 (germination only in the dark) to 1 (germination only under light conditions), and is relatively unaffected by dormancy (Milberg et al. 2000). In this experiment T_{50} was not evaluated.

Statistical analyses

To analyze the percentage of germinated seeds in the three experiments, we performed generalized linear models, assuming binomial error distribution and using Logit link function. In the first experiment, we evaluated the effect of altitudinal provenance, temperature and their interaction; in the second experiment, we analyzed the effect altitudinal provenance, temperature, water potential and all of their interactions. For T_{50} , we performed linear models based on the factorial design of the first and second experiments. When errors did not fulfill homoscedasticity assumptions we added a variance

function to the model (Di Rienzo et al. 2011). The replicates without germinated seeds were discarded because T_{50} cannot be measured without germination. For this reason, in the water potential experiment, we evaluated altitude provenance, water potential and their interaction effect on T_{50} just at 25 °C, and not at 32 °C. To explore differences among treatments in the analysis described here, we performed a post hoc LSD-Fisher test involving all analyses that had significant effects of factors or interactions. Finally, to evaluate the effect of altitude on RLG, we performed a non parametric analysis of variance (Kruskall-Wallis test) with provenance as factor. To explore differences among treatments we made pairwise comparisons among rank mean of treatments (see Balzarini et al. 2008 for analysis details). All statistical analyses were performed using Infostat v. 2015 (<http://www.infostat.com.ar>).

Results

Effect of temperature on seed germination of different *G. monvillei* provenances

We found a significant effect of altitudinal provenance × temperature interaction on seed germination percentage (p -value = 0.0171, Deviance = 12.0317, d.f = 4; Fig. 2). At 25 °C, seed germination decreased with decreasing altitude, from 2230 m a.s.l. and increased at 878 m. a.s.l. (Fig. 2). Seeds germinated at 32 °C exhibited a similar response, but with less pronounced differences among altitudinal provenances (Fig. 2). For all altitudinal provenances, germination was higher at 25 than at 32 °C, with the exception of the population at 1250 m. a.s.l., whose percentage of germinated seeds did not differ significantly between temperature treatments.

We also found a significant effect of altitudinal provenance × temperature interaction on T_{50} ($p < 0.0001$; Fig. 3). At 25°C, there were no significant differences among altitudinal provenances from 2230 to 1250 m a.s.l. in T_{50} , whereas the 878 m a.s.l. altitudinal provenance showed an increase in T_{50} . For seeds germinated at 32°C, the higher altitudes (2230, 1940 and 1555 m.a.s.l.) presented higher T_{50} values than the lower altitudes (1250 and 878 m a.s.l.). Two altitudinal provenances (2230 and 1250 ma.s.l.) showed no significant differences in T_{50} between temperatures. Altitudinal provenances 1940 and 1555 m a.s.l. showed significantly higher T_{50} at 32 °C than at 25 °C, and 878 m a.s.l. altitudinal provenance showed the opposite pattern (Fig. 3).

Combined effect of water potential and temperature on seed germination of different *G. monvillei* provenances

We found a significant effect of the triple interaction on seed germination percentage ($p = 0.0008$; Fig. 4). Temperature increase led to a reduction of germination in all altitudinal provenances, as we found in the previous experiment. Water potential effect depended on elevation and temperature: in general, water potential led to a reduction of germination, but this effect was more pronounced at 32 °C than at 25 °C as well as in the highest altitudinal provenance. At 32 °C, no individuals of any altitudinal provenance germinated at water potentials of -0.4 or -0.6 MPa. Within each altitudinal provenance, seed germination was higher at the combined treatment of 25 °C and water potential of 0.0 MPa (control). Only at the intermediate altitude (1555 m a.s.l.) germination at -0.2 and 25 °C did not differ from the control.

T_{50} was analyzed for effect of altitude provenance, water potential and their interaction only at 25 °C because germination was low or null at 32 °C.—There was a significant effect of the interaction for T_{50} ($F = 4.25$, d.f. = 6, $p = 0.0019$; Fig. 5). At 2230 m a.s.l. altitudinal provenance, water potentials of -0.2 and -0.4 MPa yielded significantly higher T_{50} than 0 and -0.6 MPa water potentials. The 1555 m a.s.l. altitudinal provenance presented significantly lower T_{50} at 0.0 MPa water potential than at the other potentials, which did not differ among them. The 878 m a.s.l. altitudinal provenance did not show differences in T_{50} among water potentials.

Effect of light on seed germination of different *G. monvillei* altitudinal provenances

In general terms RLG was high for all altitudinal provenances, indicating that *G. monvillei* is positively photoblastic (Fig. 6). However, there was a significant difference among treatments ($p = 0.0005$). Comparisons indicate that the main difference is among the lowest altitudinal provenance (878 m a.s.l) which presented a lower RLG, and the three highest altitudes (Fig. 6).

Discussion

Our findings show that seed germination of *Gymnocalycium monvillei* is influenced by altitude, temperature, light, and water availability, and that there is an important interaction among these factors. In addition, the effects found were not always in agreement with our expectations, highlighting the complex plant - environment interactions that occur in mountain environments (Wali and Krajina 1973; Körner 2007; Albert et al. 2010).

We expected that total germination would follow the species abundance patterns; however, we found that maximum germination occurred at the highest altitudinal provenance, where the species is not particularly abundant. With respect to temperatures, we found that for most altitudinal provenances germination decreased as temperature increased, and we did not find the expected results that the lower altitudinal provenances would be less affected by the higher temperatures. For most altitudinal provenances (except 1250 m), germination at 32 °C was lower than at 25 °C, indicating that thermoinhibition could be occurring (Hills and Staden 2003). This result agrees with previous reports where 25 °C was suggested to be the optimal temperature for cactus seed germination (Nobel 2003; Rojas-Aréchiga and Vázquez-Yanes 2000). Furthermore, T_{50} did not vary among altitudinal provenances at 25 °C but it did vary at 32 °C, with a slower germination at the two lower altitudes. We observed important differences in total germination among altitudinal provenances. Few studies in cacti have analyzed intraspecific differences in germination (Arredondo-Gómez and Camacho-Morfín 1995; Bárcenas-Argüello et al. 2013). These results highlight that researchers should be cautious in the use of only one or a few populations to characterize the germination characteristics of a species.

The pattern of higher germination at higher altitude was also observed in *Polylepis australis* (Rosaceae, a high altitude tree) in the Córdoba Mountains (Marcora et al. 2008). An explanation for this pattern is that seed ripening at colder sites is slower, so the longer filling period could positively affect seed quality (Fenner and Thompson 2005).

We expected that lower altitudinal provenances would perform better at lower water potentials than higher altitudinal provenances (Tilki and Dirik 2007). This hypothesis was confirmed because the decrease of water potential reduced seed germination percentage, especially at the highest altitude (2230 m a.s.l.). Highest germination with high water availability (0 MPa) is consistent with findings for many cactus species (De la Barrera and Nobel 2003; Meiado et al. 2010; De la Rosa-Manzano and Briones

2010; Guillén et al. 2011; Mazzola et al. 2013), although the threshold (-0.1, -0.2, -0.4) below which germination decreases varies among species. We also found a strong interaction of the effects of water potential and temperature, since at 32 °C, germination strongly decreased mainly in the higher altitudinal provenances. These results suggest that a combination of high temperatures and low soil moisture could negatively affect germination and likely recruitment (Flores and Briones 2001).

Seeds from the elevated and intermediate altitudinal provenances germinated more slowly with less water availability (-0.2 and -0.4 MPa) than control seeds, and seeds from the lowest altitude did not show differences in T_{50} between water potentials. This suggests that seeds from lower altitudes have the capacity to germinate faster with less water availability as a response to the more xeric conditions at these sites. These results coincide with findings for cactus species adapted to germinate at high soil humidity, but not necessarily at the soil field water capacity (Flores and Briones 2001; Guillén et al. 2011). The last hypothesis was that seeds from higher altitudinal provenances, which present more columnar seedlings, have higher capacity to germinate under darkness than those from lower altitudes. However, RLG was similar, and even higher at the highest altitudinal provenance.

In this study we observed considerable variation in germination responses among altitudinal provenances, and that the responses do not always show a clear pattern regarding altitude. Another important outcome of the present study is that evaluating only one population per species may not be enough to characterize the germination response of the species because, as we demonstrated, local environmental conditions could affect seeds. The observed variation in germination response to temperature, water potential and light appears to be of adaptive significance, but a genetic basis for patterns of intraspecific variation in germination response in this species has still not been demonstrated.

In conclusion, *G. monvillei* seed germination responded favorably under environmental conditions of 12 h photoperiod and high soil water availability, and temperatures near 25°C, with the highest germination in the seeds of populations growing at the highest elevations. These results highlight the importance of reproductive traits in explaining the wide altitudinal distribution of this species (Bauk et al. 2015). The obtained responses for water availability and

temperature suggest adaptive mechanisms of *G. monvillei* seeds to tolerate abiotic stresses, which is also relevant in terms of future climate changes (Gurvich et al. 2002). Taking together the responses to high temperature and low water potentials, our results suggest that an increase in temperature (which also would affect humidity since there is a higher level of evapotranspiration) would decrease germination for all altitudinal provenances, but the lower ones would be less affected. Overall, this article contributes to the knowledge of the *G. monvillei* ecophysiology, which could be used for *in situ* and *ex situ* conservation plans (Flores et al. 2011; Mazzola et al. 2013) and for predictions of the species response to environmental changes (Gurvich et al. 2002). This is the first study focusing on germination characteristics of a cactus species along an altitudinal gradient, which is very important because local adaptation studies in altitudinal gradients are very scarce (Cavieres and Arroyo 2000; Angert and Schemske 2005; Walder and Erschbamer 2015).

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Figure Legends

Fig. 1. Location of the five collection sites (altitudinal provenances: 878, 1250, 1555, 1940 and 2230 m a.s.l.) of seeds of *Gymnocalycium monvillei* along an altitudinal gradient in Córdoba Mountains (Córdoba Province, Argentina). Map obtained with DIVA-GIS 2 (Hijmans et al. 2002).

Fig. 2. Germination percentage (\pm standard error) for each combined treatment of each altitudinal provenances (878, 1250, 1555, 1940 and 2230 m a.s.l.) and temperature (25 and 32 °C) of *Gymnocalycium monvillei*. Different letters indicate differences between treatments (LSD-Fisher test, $p < 0.05$).

Fig. 3. Mean germination time (T_{50}) (\pm standard error) for each combined treatment of altitudinal provenances (878, 1250, 1555, 1940 and 2230 m a.s.l.) and temperatures (25 and 32 °C) of *Gymnocalycium monvillei*. Different letters indicate significant differences among treatments (LSD-Fisher test, $p < 0.05$).

Fig. 4. Germination percentage (\pm standard error) for each combined treatment of altitudinal provenances (878, 1555 and 2230 m a.s.l.) x temperature (25 and 32 °C) x water potentials (0, -0.2, -0.4 and -0.6 MPa) of *Gymnocalycium monvillei*. Different letters indicate significant differences among treatments (LSD-Fisher test, $p < 0.05$).

Fig. 5. Mean germination time (T_{50}) (\pm standard error) for each combined treatment of altitudinal provenances (878, 1555 and 2230 m a.s.l.) and water potentials (0, -0.2, -0.4 and -0.6 MPa) of *Gymnocalycium monvillei*. Different letters indicate differences between treatments (LSD test, $p < 0.05$).

Fig. 6. Relative light germination (RLG) (\pm standard deviation) of different altitudinal provenances (878, 1250, 1555, 1940 and 2230 m a.s.l.) of *Gymnocalycium monvillei*. Different letters indicate differences between treatments (Pairwise comparison between treatment ranks, $p < 0.05$).

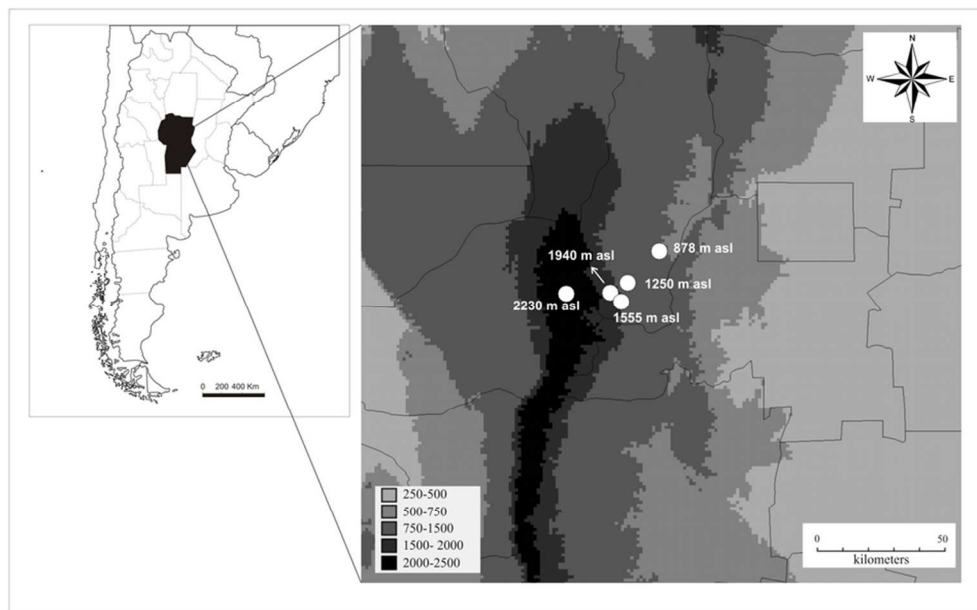


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