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ORIGINAL ARTICLE

Germination requirements of the endangered cliff-dwelling *Primula palinuri* Petagna and implications for conservation

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Abstract

Primula palinuri, an endangered plant species strictly confined to the coastal limestone cliffs of southern Italy, represents a key Mediterranean endemic threatened by habitat loss and environmental stressors. This study investigated the effects of temperature, salinity, water stress, and burial depth on its seed germination. Germination was favoured by cool temperatures, with an optimum at 15 °C. Rates significantly declined at both 5 °C and 25 °C. Increasing salinity markedly inhibited germination, confirming the species' sensitivity to salt stress. Water stress experiments demonstrated that *P. palinuri* requires high soil moisture for successful germination, with total inhibition under moderate (- 0.4 MPa) to severe drought conditions (- 1.5 MPa). Burial depth negatively affected seedling emergence, likely due to the small seed size and light-dependency. These results underline the species' narrow ecological requirements, emphasising the need for careful habitat selection in conservation actions, favouring moist, shady, and salt-protected microsites with shallow seed placement.

Keywords Conservation biology · Endemic species · Ex situ · In situ · Regeneration niche

Introduction

Plant biodiversity faces significant challenges globally, with the number of endangered species increasing alarmingly (Fao 2019). Recent assessments reveal that 12 102 plant species are currently threatened with extinction (IUCN Red List 2024; link: https://www.iucnredlist.org). The main threats include habitat loss and fragmentation, climate change, overexploitation, pollution, and the spread of invasive alien species, all of which are intensifying at alarming rates (IUCN Red List 2024; link: https://www.iucnredlist.org).

Maintaining healthy ecosystems is of vital importance for both human and other living organisms' livelihoods, as they ensure the provision of ecosystem services that mitigate the effects of natural disasters and climate change (Gann et al. 2019). Native plants play a critical role in maintaining healthy ecosystems, and their rapid decline underscores the urgent need for conservation strategies that address the complex and multifactorial drivers that put them at risk.

Ex-situ conservation is a fundamental strategy for the conservation of endangered species. It encompasses a range of methods, including seed banking and cultivating living collections in botanical gardens, which collectively serve as reservoirs of genetic diversity and as a source for population replenishment (Silveira et al. 2018). When seeds are collected from wild populations, their storage in seed banks enables future reintroductions and habitat restoration initiatives. Hence, knowledge on the management and propagation of these genetic resources is vital to effectively store and then use them in conservation projects; however, the seed biology of many wild plant species is still unknown (e.g. Ladouceur et al. 2017), limiting the implementation and success of practical conservation initiatives.

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The germination niche, described as the range of environmental conditions that allows the transition from seed to seedling (Grubb 1977), is driven by abiotic and biotic cues such as temperature, humidity, light and chemical compounds (Baskin and Baskin 2004; Baskin and Baskin 2014), the perception and response to which are regulated by the physiological dormancy state of the seed (Vleeshouwers et al. 1995). When the ecological requirements for regeneration are satisfied both inside and beyond the current range, species can either maintain their presence *in-situ* or expand into new areas, thereby shaping interactions with other organisms through competition and facilitation (Grubb 1977; Dobrowski et al. 2015; Kroiss et al. 2015). Investigating seed germination requirements is essential to identify optimal propagation conditions and germination niche, thereby informing both *in-situ* and *ex-situ* conservation strategies (Ladouceur et al. 2017).

The plant species that populate coastal cliffs are exposed to extreme abiotic conditions that strongly influence the germination niche and the survival of seedlings: for example, seasonal and daily temperature fluctuations, with cold winters, hot summers and marked variations between day and night, can both promote and inhibit germination (Thanos et al. 1989; Rosbakh and Poschlod 2015; Fernández-Pascual et al. 2017); water stress, due to the poor water retention of the substrate and the salinity brought by the sea spray, further limit plant establishment (Rodwell 1991; Ciccarelli et al. 2016; Fernández-Pascual et al. 2017); substrate characteristics, including shallow depth of soil and partial burial of seeds, can alter access to the light and moisture needed to break dormancy (Balestri and Cinelli 2004; Fernández-Pascual et al. 2017). Together, these factors act as ecological filters that favour germination during periods with suitable conditions, preventing establishment during seasons of high stress such as the Mediterranean summer (Baskin and Baskin 2014; Jurado and Flores 2005; Fernández-Pascual et al. 2017).

The present study focuses on the germination ecology of *Primula palinuri*, an endemic species that inhabits the coastal cliffs of southern Italy and is adapted to these challenging environments.

According to the IUCN Red List*, P. palinuri* is classified as endangered, with population declines primarily driven by habitat loss and insufficient recruitment (Silvestro et al. 2020). The unique ecological niche it occupies, characterised by harsh abiotic conditions, makes it a critical species for studying biodiversity in cliff ecosystems, which are known to host relict flora (De Micco and Aronne 2012b). The conservation of *P. palinuri* is essential not only for maintaining local biodiversity but also for understanding the evolutionary adaptations of plants in extreme environments (Aronne et al. 2017).

The study of the germination requirements of *P. palinuri* is critical to support effective propagation and conservation strategies to enhance recruitment success and for informing optimal translocation practices. Recruitment failure has been identified as a key bottleneck for the species. This suggests that specific germination conditions must be met for the successful population regeneration (Silvestro et al. 2020).

The main goal of this study was to investigate the seed germination requirements of *P. palinuri* by examining the effects of key abiotic factors: light, temperature, salinity, drought, and burial depth.

Based on previous knowledge of the ecology of cliff plants, we hypothesised that, for *P. palinuri*:

i) Germination is favoured by moderate temperatures and the presence of light, indicators of favourable periods; ii) increased salinity and water stress inhibit the germination process; iii) an increase in burial depth reduces final germination, while shallow burial facilitates seedling emergence by ensuring adequate access to light and moisture. Through this investigation, we aimed to provide essential knowledge to guide future conservation and management strategies. These insights will be pivotal for both *in-situ* conservation and the development of effective propagation.

Materials and methods

Study species

P. palinuri is situated within the broader context of the *Primula* genus, which encompasses approximately 500 species globally, with a significant concentration in the Himalayas and temperate regions of the Northern Hemisphere (Ren et al. 2016), and it is classified under the section *Auricula*, which includes several species adapted to high-altitude environments and characterized by unique morphological traits (Zhang and Kadereit 2004; Crema et al. 2013). *P. palinuri* is a rhizomatous geophytic herbaceous plant with fleshy, obovate-spatulate, leathery leaves, gathered in rosettes either adhering to the rock or at the apex of long pendulous rhizomes. The inflorescence comprises 10–15 golden-yellow flowers with a floury white calyx and tubular corolla. The fruit is a globose dehiscent capsule with numerous seeds (Pignatti 2018).

The current distribution of the species reflects the effects of geological changes and coastal retreat, which have resulted in the persistence of only a few populations (Ricciardi 1973). Genetic and demographic studies indicate a scarcity of young individuals throughout its distribution area signalling a lack of regeneration of the species, raising concerns about its long-term survival (De Micco and Aronne 2012a, b).

P. palinuri is adapted to rocky environments and it benefits from microclimates with higher humidity and more stable temperatures, conditions that are essential for its life cycle (Pesaresi et al. 2014).

The primary threats to this endemic species include natural processes, such as landslides associated with coastal erosion, as well as human-related activities like rock stabilization works, leisure activities, collection of plant species for ornamental or private purposes, road construction, and urban development (IUCN:https://www.iucnredlist.org, accessed on April 12, 2025).

Seed collection

Seeds of *P. palinuri* were collected in July 2023 from the wild population located in Palinuro Cape (PAL, SAC IT8050008), within the Cilento, Vallo di Diano, and Alburni National Park, in the Campania region, Italy, following the guidelines provided by Bacchetta et al. (2006).

Seeds were collected on a costal cliff, at an altitude ranging between 14 and 40 m a.s.l., from a population estimated to consist of 500 to 1000 plants, with seeds sampled from 50 individuals; particularly, we collected two mature capsules per individual. We estimated the parameters of the collected seed lot: average moisture content (%MC ± SD), average number of capsules per infructescence, and average number of seeds per capsule.

Due to logistical constraints and limited accessibility, the collection did not cover the entire Palinuro Cape population. Many individuals grow in small soil pockets on the rocky cliffs, which can only be accessed by boat and climbing equipment. Therefore, sampling focused on the more accessible portion of the population, which hosts the highest concentration of individuals (Fig. 1).

Seed moisture content

Moisture content (MC, %) was analysed at the Germplasm bank of the Botanical Garden of Rome (Sapienza University of Rome, Italy).

Fresh mass (g) was determined using four randomly selected samples, each containing 50 seeds. The same samples were then oven-dried at 103 °C for 17 h. After drying, they were cooled to room temperature for 15 min (Bacchetta et al. 2006) before measuring dry mass. All measurements

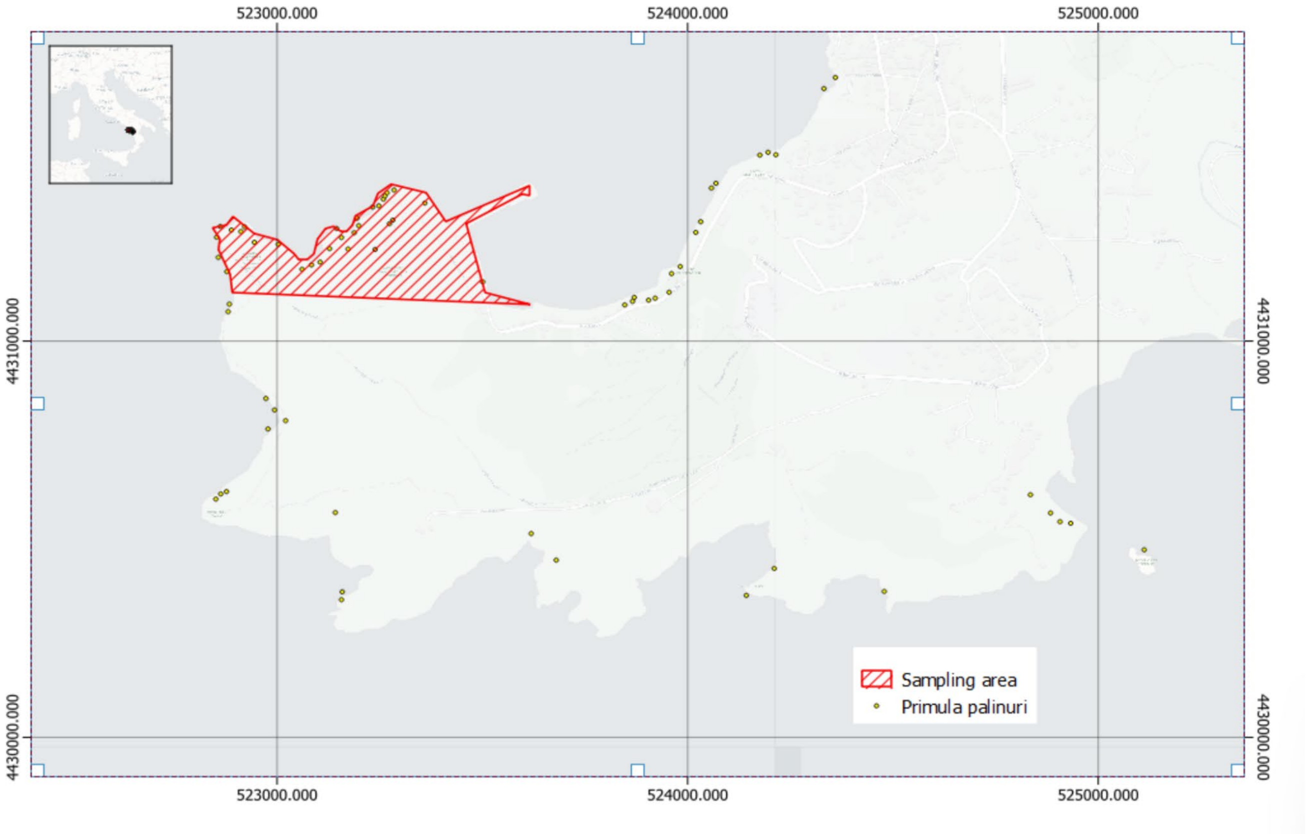


Fig. 1 Sampling area for Primula palinuri seeds. The yellow points represent l *Primula palinuri* plants, while the red lines highlight the sampling area

were performed using a precision balance (Gilbertini E42S) with 0.1 mg accuracy.

According to Roberts (1973), seed water content is a useful parameter to distinguish between orthodox and recalcitrant seeds; orthodox seeds typically have a moisture content of 15% or lower. To determine internal seed moisture, the formula provided by ISTA (2005, Eq. 1) was used.

$$Water\ content\ (MC,\%) = [fresh\ weight - dry\ weight)/fresh\ weight] * 100 \tag{1}$$

Seed yield

The number of capsules per infructescence was recorded in the field by counting the capsules on each sampled plant. The number of seeds per capsule was determined by counting the seeds contained in each collected capsule. Based on these data, the average seed production per individual rosette was calculated by multiplying the number of capsules produced by each plant by the number of seeds per capsule. Following Bacchetta et al. (2006), the seed samples were stored at 20 °C and 15% relative humidity for 15 days to allow for post-maturation and ensure uniform seed maturity levels before starting the laboratory trials.

Temperature, light, salt and drought effects on germination

In all germination experiments, four replicates of 25 seeds were sown on Petri dishes containing either agar or filter paper. To prevent evaporation, the edges of the Petri dishes were tightly sealed with parafilm. Germination was defined as the visible appearance of a 2 mm radicle. The cabinets were programmed to alternate between 12 h of darkness and 12 h of light (photoperiod 12/12) to replicate natural day-night cycles, with the 12-h light period coincided with the highest temperature. To replicate the influence of light on seed germination, Petri dishes were positioned beneath cold white, fluorescent tubes. These tubes emitted a photon flux density of 80 µmol (photon)–(m^{-2} s^{-1}), providing an adequate light source for the germination process. At the end of the experiments, a cut-test was performed on the non-germinated seeds to assess embryo presence and integrity. The final germination percentage was then adjusted based on the number of full (non-empty) seeds.

To study the germination adaptations and requirements of *P. palinuri* seeds to temperature, we conducted experiments on 1% agar in germination cabinets set at three alternating temperature ranges (15–6 °C, 20–10 °C, and 25–15 °C) and five constant temperatures (5, 10, 15, 20, 25, and 30 °C). These temperatures were chosen because, for many Mediterranean coastal species, the optimal range for germination is between 5 and 15 °C, with germination decreasing as temperatures increase (Thanos et al. 1989; Thanos et al. 1995; Kadis and Georghiou 2010; Santo et al. 2019; Salmeri et al. 2024).

To evaluate the germination capacity of *P. palinuri* seeds under salt stress, we exposed seeds to five NaCl concentrations on three layers of Whatman No. 1 filter paper: 0 mM (control), 50 mM, 100 mM, 150 mM, and 200 mM. The salt solutions were applied to filter papers, initially moistened with 6 ml of the corresponding solution, and subsequently supplemented weekly with 1 ml to maintain moisture levels and prevent dilution. The experiment was conducted at a constant temperature of 15 °C.

Drought stress was tested by placing the seeds on three layers of Whatman No. 1 filter paper moistened with solutions of different osmotic potentials (0, –0.4 MPa, –0.8 MPa, –1.2 MPa, and –1.5 MPa) at 15 °C, following the method outlined by Michel and Kaufmann (1973). These values were obtained by dissolving polyethylene glycol (PEG 8000) in distilled water. The filter papers were initially wetted with 6 ml of the respective PEG solution, and moisture was maintained by supplying an additional 1 ml of the same solution at weekly intervals.

To assess the species' response to light conditions, we tested three treatments at 15 °C using seeds stored for one year at 15 °C and 15% RH: In Petri dishes filled whit agar 1%, seeds were exposed to a 12 h light/12 h dark cycle; dishes kept in complete darkness and monitored weekly; and dishes were kept in complete darkness and checked only at the end of the experiment. The latter two treatments allowed us to evaluate whether brief light exposure during periodic monitoring could influence germination under dark conditions.

Seed burials

The seeds used in this experiment were stored for one year at 5 °C. Before the experiment, their germinability was tested at 15 °C to confirm that they remained viable after storage.

Five replicates of 10 seeds were placed on Whatman no. 1 filter paper (55 mm diameter) and buried at various depths in five black plastic boxes (200 × 110 × 64 mm) with dark edges but transparent lids. Each box was filled with soil and moistened with 200 ml of distilled water. The seeds were buried at depths of 0, 0.1, 0.2, 0.5, 1.0, and 4.0 cm.

The boxes were incubated at a constant temperature of 15 °C with a 12-h photoperiod and checked every two days to monitor the emergence of seedlings above the soil surface. Emerging seedlings were removed. After 10 consecutive days with no seedlings emerging, the replicates were exhumed, and the number of germinated but non-emerged seeds was recorded. The viability of non-germinated seeds was assessed through a cut-test.

Data analysis

For the number of capsules per individual, the number of seeds per capsule and for moisture content (MC, %), mean and standard deviation values were calculated. The germination data were analysed fitting, to the germination curves, the log-logistic model, which is based on time-to-event analysis (Onofri et al. 2010, 2022). This approach accounts for censored data, allowing for inferences on germination events that did not occur at the specific evaluation time but during the interval between evaluations. The model was fitted to the pooled data from the four replicates of each treatment in each experiment, ensuring consistency in the analysis of germination dynamics across experimental conditions.

The model is expressed by Eq. 2.

Equation 2 Model function. F(t) is the fraction of seeds germinated in the time interval t, d represents the total germination (i.e., the upper asymptote of the function), b is the slope of the curve at the inflection point (i.e., usually described as seed uniformity) and e is the time required to achieve 50% seed germination (T50 or ED50), used here as an indicator of the germination rate.

$$F(t) = 1 + exp(-b(t - e))d \tag{2}$$

The analyses were performed using R and RStudio (R Core Team 2023). The *drcte* package was utilized for this purpose (Onofri et al. 2022).

The germination models were calculated using the *drmte* function, which adapts time-to-event models for seed science. The parameter “b” corresponds to the slope of the logistic model at the inflexion point (germination speed). The parameter “d” corresponds to the upper asymptote and represents the maximum of the germination process (final germination). The parameter “e” represents the days needed to reach a percentage of germinated seeds equal to the half of d (T_{50}) (Ritz et al. 2019). Since both “b” and “e” describe the dynamics of germination speed, we chose to report “e” (T_{50}), as it is a widely adopted and interpretable parameter in seed science literature. Differences in the germination parameters (b, d, and e) between the two treatments were tested using the *compCDF* function, which compares time-to-event curves. A difference was considered statistically significant if the p-value was less than 0.05 (Trotta et al. 2024).

Results

Seed moisture content

Moisture content (MC, %) per seed had a mean value of 5.8 ± 1.3%. The variability was moderate, as indicated by the interquartile range (IQR, 5–7.2%), and no significant outliers were observed in the distribution. This suggests relatively consistent moisture levels across the samples analysed (Fig. 2a).

Seed yield

The number of capsules per infructescence averaged 7.8 ± 1.1. The median and interquartile range suggest that

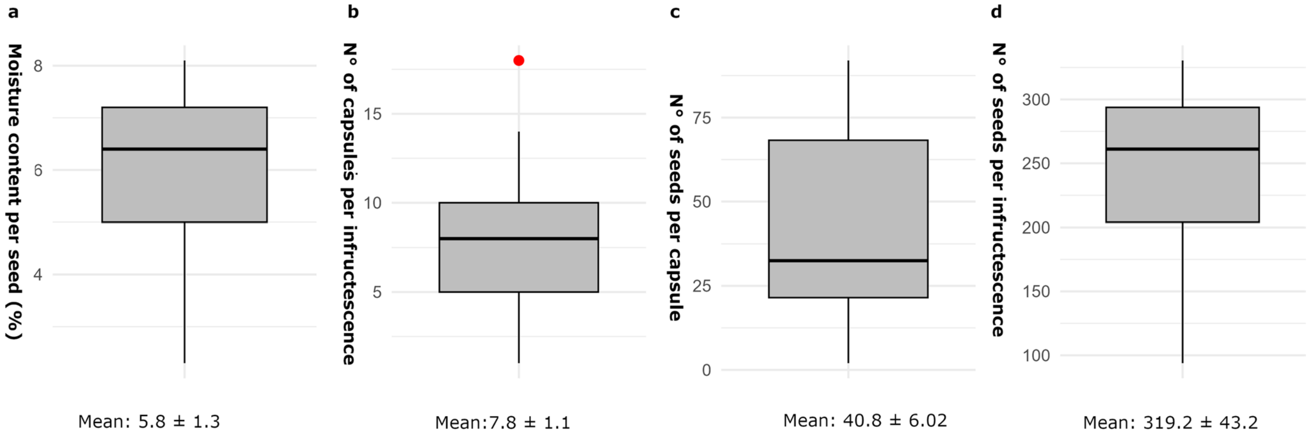
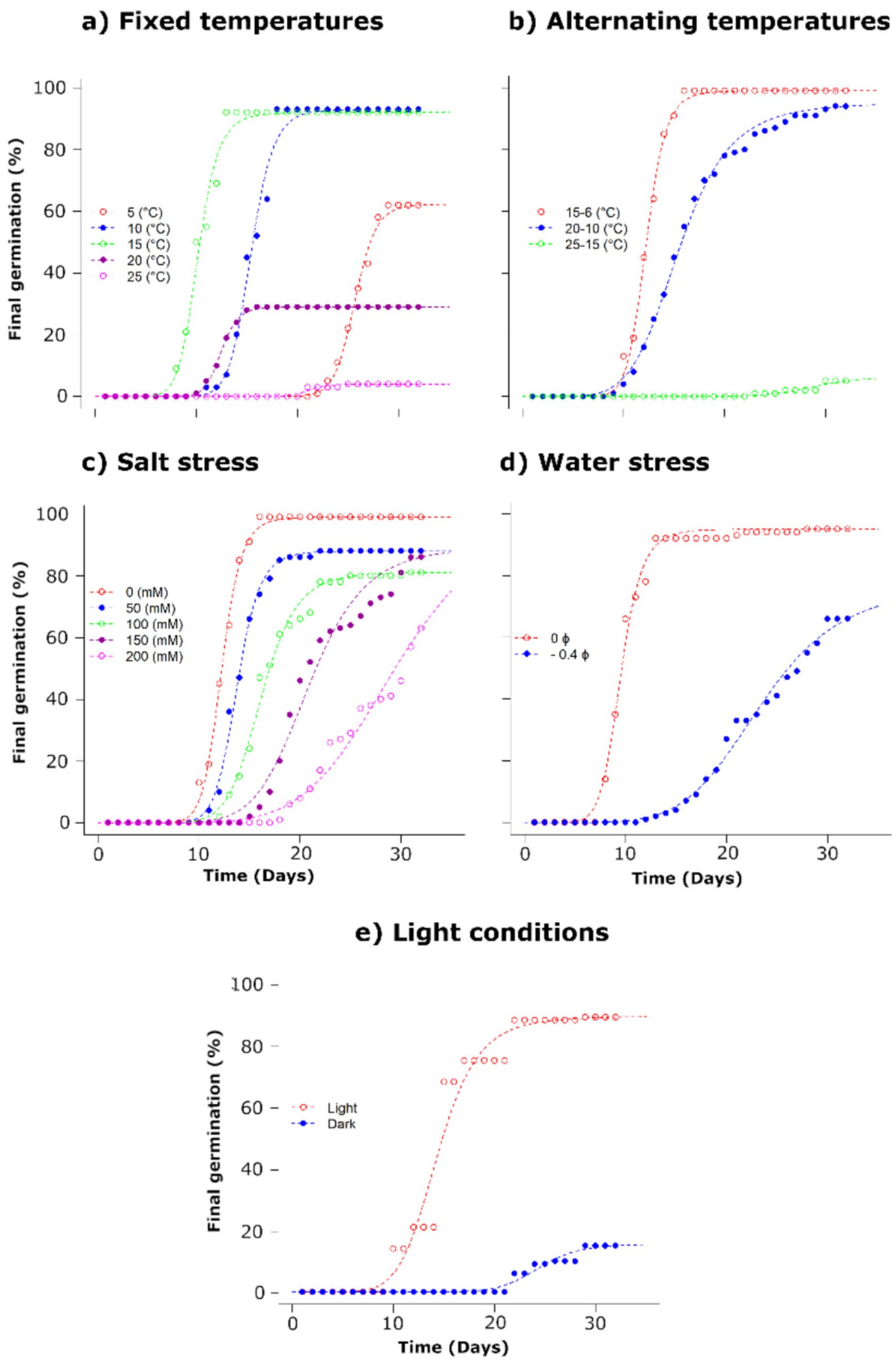


Fig. 2 Boxplot representing different variables. **a** distribution of moisture content per seed (MC per seed, %); **b** number of capsules per infructescence; **c** number of seeds per capsule; **d** number of seeds per infructescence. In all panels, the boxplots show the median as a black horizontal line within the interquartile range (IQR), with whiskers extending to indicate variability beyond the quartiles. Outliers are highlighted where present (red dots)


a) Fixed temperatures
b) Alternating temperatures
c) Salt stress
d) Water stress
e) Light conditions
Final germination (%)
Time (Days)
0
20
40
60
80
100
0
10
20
30
5 (°C)
10 (°C)
15 (°C)
20 (°C)
25 (°C)
15-6 (°C)
20-10 (°C)
25-15 (°C)
0 (mM)
50 (mM)
100 (mM)
150 (mM)
200 (mM)
0 ϕ
- 0.4 ϕ
Light
Dark

◂**Fig. 3** Cumulative probability of seed germination under different environmental conditions. **a** Germination at constant temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C); **b** Germination at alternating temperatures (15–6 °C, 20–10 °C, 25–15 °C); **c** Effect of salinity on germination at varying concentrations (0 mM, 50 mM, 100 mM, 150 mM, 200 mM); **d** Effect of water potential on germination (0, −0.4 MPa); **e** Effect of Dark and Light exposure on germination (Day/Light Cycle and Total dark exposure). Effect of Dark and Light exposure on germination (Day/Light Cycle and Total dark exposure)

most samples clustered around the mean, with limited variability (Fig. 2b).

The number of seeds per capsule showed a mean value of 40.8 ± 6.02. The wide interquartile range and long whiskers suggest a wider distribution of seed production among individuals. Despite this variability, no significant outliers were observed, and the median (32.5 seeds) was slightly below the mean, suggesting a slight positive skewness of the data (Fig. 2c).

The estimated average seed production per rosette was 319.2 ± 43.2

Temperature, light, salt, drought and light/dark cycle effects on germination

Germination was observed predominantly at intermediate temperatures, showing higher success under alternating regimes (Tab. S1-S2). At 10 °C and 15 °C (Fig. 3, panel a), germination was high (d = 93 and 92%) and with low T_{50} values (e = 15.42 and 10.18 days, respectively). At 5 °C, germination declined (62%) and was slower ($T_{50} = 25.71$ days). At higher temperatures (20 and 25 °C) final germination dropped sharply (d = 29 and 4%, respectively), with variable germination speeds (e = 12.46 and 21.11 days). Alternating temperatures of 15–6 °C promoted both rapid ($T_{50} = 12.23 \pm 0.17$ days) and high germination ($d = 99\% \pm 1$), similarly to constant 15 °C. In contrast, 25–15 °C led to delayed germination ($T_{50} = 28.95 \pm 3.36$ days) and a markedly lower final germination (d = 6%). The 20–10 °C regime showed intermediate results, with $T_{50} = 15.48$ days and d = 95 ± 2% (Fig. 3, panel b). Significant differences were found in germination speed (T_{50}) across treatments, whereas final germination was comparable only between 15–6 °C and 20–10 °C ($p > 0.05$).

The rate of seed germination at different salt concentrations shows that quick germination is enhanced by the absence of salts in the control (0 mM) (Fig. 3, panel c). An increase in salt concentration consistently reduces germination rate and percentage (Tab. S3).

At 0 mM, seeds germinated rapidly ($T_{50} = 12.23 \pm 0.17$ days) and almost completely (99% ± 1). Similar values were recorded at 50 and 100 mM ($T_{50} = 13.75 \pm 0.21$ and 16.16 ± 0.34 days; final germination = 88 ± 3% and 81 ± 4%, respectively). At 150 mM, germination was delayed ($T_{50} = 20.92 \pm 0.58$ days), but still relatively high (89 ± 4%). At 200 mM germination was strongly inhibited ($T_{50} = 29.55 \pm 3.10$ days, 1 ± 3%). Statistical analyses indicated no significant difference between 50 mM, 100 mM, 150 mM treatments (p-value > 0.05).

Germination was strongly affected by water stress, with significant differences between the control condition ($\phi = 0$) and the moderate stress condition ($-0.4\ \phi$) (Fig. 3, panel d).

Under control conditions ($\phi = 0$), germination was rapid (T_{50} of 9.52 ± 0.19 days), while at $-0.4\ \phi$ it was slower (23.58 ± 1.22 days; Tab S4). Final germination also declined, from 95% ± 2 in the control conditions to 77% ± 7 at–0.4 ϕ. These differences were significant ($p < 0.05$). No germination occurred at more severe stress levels (−0.8, −1.2, and −1.5 ϕ), which are therefore not shown in the figure or statistical analysis.

Germination was influenced by light regime, occurring only under alternating light–dark conditions and not in continuous darkness (Fig. 3, panel e). Under light conditions, T_{50} is 14.38, indicating rapid germination (Tab. S5).

The final germination percentage reached 89% under light conditions, indicating a high germination success. Under constant dark conditions, the value of “d” drops to 16%, suggesting a significantly lower final germination percentage. For T_{50} and final germination percentage, the differences between light and dark conditions are significant ($p < 0.05$), indicating that light has a distinct and relevant effect on germination.

Four capsules were kept in complete darkness for the entire duration of the experiment and were not opened at any point to avoid any light exposure. At the end of the experiment, when these capsules were finally opened, a total of 8 seeds out of 100 had germinated across the 4 capsules, corresponding to an average of 2.25 ± 1.26 germinated seeds per capsule. Exposure to light, even when minimal and brief, resulted in final germination rates up to eight times higher than in complete darkness.

Seed burial

The highest percentage of seedling emergence (90 ± 4%) occurred at 0 cm level, followed by 0.1 cm depth, where 47 ± 1% the seeds emerged successfully (Fig. 4a). However, at this depth, a significant percentage of seeds (22%) germinated but never emerged (Fig. 4b). At

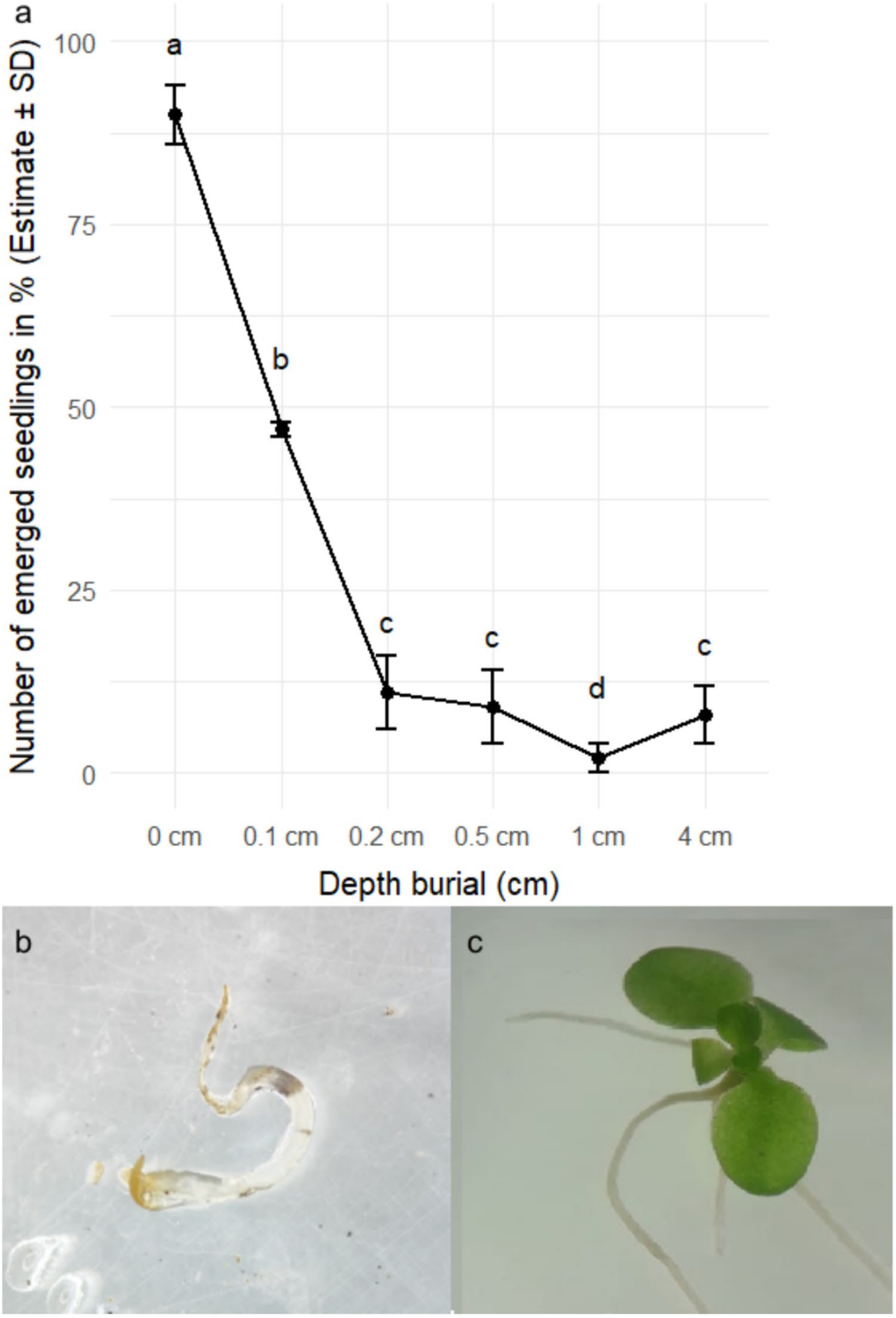


Fig. 4 **a** Effect of burial depth (0 cm,0.1 cm, 0.2 cm, 0.5 cm, 1 cm, 4 cm) on the number of emerged seedlings (estimate ± SD). Different letters above the dots indicate statistically significant differences between the treatments ($p < 0.05$). **b** seed germinated in the soil but not emerged; **c** healthy-looking seedling emerged on the surface and developed green cotyledons

depths greater than 0.1 cm, seedling emergence was significantly reduced: at 0.2 cm, only 11 ± 5% of the seeds germinated and emerged, while 16% germinated without emerging; at 0.5 cm, 9 ± 5% seedlings germinated and emerged (Fig. 4c); beyond 4 cm, germination dropped to 8% and the seedlings in the soil were transparent in colour.

Discussions

This study investigated for the first time the effects of several environmental factors on the seed germination of *P. palinuri*, a cliff-dwelling endangered species, endemic to southern Italy.

The seeds of *P. palinuri* exhibit orthodox behaviour, as confirmed by experimental results from Seed Information

Database (SID, Society for Ecological Restoration, International Network for Seed Based Restoration and Royal Botanic Gardens Kew 2023), which reported 100% viability after drying to a moisture content in equilibrium with 15% relative humidity and subsequent freezing at 20 °C for four weeks. Our findings on the species' seed moisture content (5.8 ± 1.3%) are in agreement with those from Seed Information Database (SID, Society for Ecological Restoration, International Network for Seed Based Restoration and Royal Botanic Gardens Kew 2023), as the values we observed fall well within the threshold values considered suitable for long-term storage through cryopreservation. Moisture content (MC %) is a key parameter in seed conservation, as it directly influences respiration rates and the speed of metabolic processes, thereby affecting seed longevity (Bacchetta et al. 2006).

For the field seed yield components, it was possible to compare them with those previously reported by Aronne et al. (2014), as they were measured on the same population after 13 years. The average number of capsules per infructescence in our study (7.8 ± 1.1) was similar to the 2011 value (8.8 ± 1.92), while the number of seeds per capsule appears lower (40.8 ± 6.02 vs. > 50 in the earlier study).

Our results indicate that *P. palinuri* germinates more successfully under cool temperatures, consistent with autumn or early spring conditions. Germination peaked at 15 °C and decreased at both lower (5 °C) and higher (25 °C, 25–15 °C) temperature suggesting that *P. palinuri* seeds are adapted to germinate under mild temperatures typically occurring in late winter or early spring. These findings align with the germination patterns of other Mediterranean coastal species, which typically germinate during the autumn when average temperatures range between 10–15 °C (Del Vecchio et al. 2012; De Vitis et al. 2014; Fernández-Pascual et al. 2017; Santo et al. 2019; Cruz-Tejada et al. 2024). Alternating temperatures markedly enhanced germination, leading to very high percentages, close to 100%. This strong response is consistent with the small seed size of *P. palinuri*, as species with smaller seeds are generally more responsive to thermal fluctuations (Pearson et al. 2002; Mattana et al. 2010). In *P. palinuri*, as in many other Mediterranean species, summer and winter temperatures may function as environmental signals to delay germination, helping avoid seedling establishment during periods with low survival chances. This temperature-dependent dormancy strategy likely reflects an evolutionary adaptation to the Mediterranean climate.

Despite being typically associated with coastal cliffs, *P. palinuri* exhibited a pronounced sensitivity to increased salinity. Germination rates decreased dramatically with higher salt concentrations, confirming results from Strumia et al. (2020), who observed a germination drop from 100% (control) to 15% under full seawater exposure. *P. palinuri* tends to grow at slightly higher altitudes, which may offer some protection from direct exposure to salt spray. This suggests that the species has limited physiological adaptation to salinity, in line with its closer phylogenetic affinity to mountain *Primula* species (Aronne et al. 2015; Crema et al. 2013; Strumia et al. 2020). Consequently, the species is not halophytic and does not exhibit significant physiological mechanisms for salt tolerance, a pattern common among many chasmophytic species that, despite exposure to salt, do not evolve halophytic traits due to insufficient selective pressure (Santo et al. 2017; Walsh et al. 2023). On the other hand, variability in salt tolerance among individuals within the same population might reflect microhabitat differences, suggesting possible local adaptation. Seeds from more salt-exposed soil pockets could potentially develop higher tolerance than those from sheltered niches (Rejili et al. 2010; Cardona et al. 2021; Panero et al. 2024).

However, despite this limited salt tolerance*, P. palinuri* has evolved morphological and physiological traits that allow it to cope with the warmer and more arid conditions typical of Mediterranean coastal cliffs. This likely reflects adaptations driven by past climate changes (Aronne et al. 2015).

Germination in *P. palinuri* occurred rapidly and almost completely under optimal moisture (0 ϕ) but was increasingly delayed and ultimately inhibited as water stress intensified.

These findings are consistent with prior observations on *P. palinuri* (Aronne et al. 2010) and other Mediterranean species, which tend to synchronize the germination with periods of high soil moisture (Aronne et al. 2010; Umebese and Bankole 2013).

The necessity for water aligns with the ecological requirements of the species for seed release; in fact, it has been highlighted that it is a serotinous species (Silvestro et al. 2020), with seed release from the capsule occurring only in response to moisture cycles following periods of drought (Silvestro et al. 2020). This mechanism ensures that germination starts during favourable conditions, avoiding erratic wet periods or summer drought.

The results of the light-darkness trials, which showed high germination under light–dark cycles and a strong reduction under continuous darkness, indicate that *P. palinuri* seeds require light to germinate; even brief light exposure markedly increased germination, consistent with the seed-burying experiment. This pattern aligns with the general tendency of small-seeded species to require light for germination (Pearson et al. 2002). The seed-burying experiment revealed a marked decline in seedling emergence with increasing depth. Highest emergence was observed at lower depths (≤ 0.5 cm), likely due to optimal light and moisture conditions (Thanos et al. 1989; Castillo et al. 2023). As burial depth increased, seedling emergence declined significantly, as shown by studies highlighting a negative correlation between burial depth and emergence rates (Hu et al. 2013; Singh et al. 2012). Small seeds like those of *P. palinuri* may lack the energy reserves to emerge from deeper soil layers (Bond et al. 1999). Moreover, increasing burial depth reduces light availability, and since our data indicate that *P. palinuri* seeds are photosensitive, darkness at greater depths may further inhibit germination. According to the Seed Information Database (Society for Ecological Restoration, International Network for Seed Based Restoration, Royal Botanic Gardens Kew 2023), *P. palinuri* seeds (0.354 mg; Bond et al. 1999) showed maximum emergence at the soil surface, suggesting a light-dependent germination strategy typical of cliff species. Our findings are consistent with this evidence, as we also observed the highest emergence at 0 cm and a sharp decline with increasing burial depth, confirming the light requirement of the species.

Light is a crucial germination signal for *Primula* species (Peng et al. 2021). Deep burial hampers light perception and increases soil pressure, limiting root growth (Benvenuti et al. 2001; Benvenuti 2003; Benvenuti 2007). While our trials relied on stored seeds, future studies could attempt similar investigations on freshly harvested seeds to verify whether such identified behaviours are confirmed under natural dormancy conditions (Hitchmough et al. 2011).

Conlusion

This study highlights the intricate interplay of abiotic factors shaping the seed germination of *Primula palinuri*, offering valuable insights into the species' adaptive strategies for survival in harsh cliff environments. The findings demonstrate that temperature cues, light availability, moisture conditions, and shallow burial depth critically influence germination success. The presence of non-deep physiological dormancy, responsive to seasonal environmental triggers, suggests that *P. palinuri* is finely attuned to the climatic and ecological features of its Mediterranean habitat.

In the context of accelerating climate change and ongoing habitat alteration, understanding these environmental influences on the germination dynamics is essential for developing informed conservation and management strategies. Our results provide clear practical guidelines for translocation or reinforcement actions: sites should replicate natural habitat conditions, avoiding areas with high salinity or strong marine aerosol, and prioritising shady slopes or protected rock crevices with well-drained soils that can retain moisture without waterlogging. These microsites should also guarantee moderate daily temperature fluctuations and shelter from extreme heat or cold, conditions often ensured by topography and surrounding vegetation.

Sowing protocols should carefully consider the species' sensitivity to burial depth, with seeds placed no deeper than 0.5 cm to ensure light perception and successful emergence. Identifying translocation sites that closely mimic natural conditions, particularly in terms of moisture retention, temperature variability, and reduced salinity, is key to enhancing seedling establishment and long-term population persistence of *P. palinuri*.

Further research should further examine the soil properties of *P. palinuri* habitats and assess seed longevity through storage and seed ageing tests, supporting long-term *ex-situ* conservation strategies.

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Data availability The datasets analyzed during the current study are not publicly available but are available from the corresponding author on request.

Declarations

Ethical approval Our study brings together authors based in the country where the study was carried out. All authors were engaged with the research and study design to ensure that the diverse perspectives they represent were considered. Where relevant, literature published by scientists from the region was cited.

Informed consent No identifiable personal information or images were collected or used during the study. Therefore, it was not necessary to obtain informed consent for publication purposes, by international ethical guidelines on the protection of personal data and the use of non-human material.

Conflict of interest The authors declare no conflict of interest.

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