

Review

Germination Strategies and Seed Quality of *Colobanthus quitensis*: Implications for Sustainable Antarctic Ecosystems and Ex Situ Plant Conservation

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Abstract: The conservation of the Antarctic ecosystem is linked to scientific and tourism activities. *The Colección Activa de Plantas Vasculares Antárticas* at the Universidad de Concepción aims to help conserve the region's vascular flora to support scientific research while minimizing anthropogenic pressure on natural ecosystems. *Colobanthus quitensis* is the only native dicotyledonous plant native to Antarctica and, as an extremophilic plant, is capable of withstanding extreme abiotic conditions. This species has potentially important use as a biological model to study the physiological deterioration of dormant seeds and other traits, some of which may contribute to vegetation development in Antarctica. Although studies have addressed the reproduction and germination of *C. quitensis*, there is no recent synthesis of information regarding its reproductive biology and seed traits that could support its conservation. This work synthesizes and critically analyzes the current knowledge of *C. quitensis* reproduction in natural and controlled environments, identifying factors that contribute to the decline in seed quality. We propose that growth conditions, seed morphology, and interactions with endophytic fungi influence germination and seedling establishment. We also highlight critical knowledge gaps that must be addressed to better understand the species' phenology, the impact of biotic and abiotic factors on its development, strategies for seed conservation, and biotechnological applications.

Keywords: Antarctic ecosystem; extremophilic plant; reproduction; seed physiological quality; seed conservation

1. Introduction

Few regions of the world are characterized by environmental conditions as extreme for vegetation development as those of Antarctica. Since the Antarctic Treaty came into force in 1961, its ecosystems have received varying degrees of protection, with the entire region declared a natural reserve dedicated to peace and science [1]. However, human activities, including industrial fishing, tourism, and scientific research, have contributed

to environmental pollution [2], as well as the introduction and propagation of exotic species [3,4]. These activities threaten the fragile ecosystems of Antarctica, highlighting the importance of conservation efforts and sustainable research practices.

Colobanthus quitensis (Kunth) Bartl. (Caryophyllaceae) is the only native dicotyledonous plant present in Antarctica and has a broad geographic distribution [5–7]. Its Antarctic populations are found along the western coast of the Antarctic Peninsula, as well as in the South Shetland and South Orkney Islands (known collectively as the maritime Antarctic) [7,8]. Some of these populations are located within Antarctic Specially Protected Areas (ASPAs), where strict conservation measures aim to prevent disturbances caused by human activities (Figure 1) [9]. However, there is a notable lack of germplasm banks that could provide an alternative to field samples of this species for research purposes. For this reason, in 2009, the Laboratorio de Biotecnología y Estudios Ambientales (LABEA) created the *Colección Activa de Plantas Vasculares Antárticas* (220.418.012-INV) at the Universidad de Concepción, Campus Los Ángeles. Here, different populations of *C. quitensis* are grown from seed under controlled common garden conditions or *in vitro* from germinated seeds or vegetative segments (explants) [10]. The common garden conditions maintain plants in growth chambers at 14 ± 1 °C with a light intensity of $100\text{--}120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, photoperiod of 16/8 h light/dark, and relative humidity between 75 and 80%, using a leaf–peat–perlite (3:2:1)-based substrate and with fertilization every two weeks [9]. This collection aims to generate scientific knowledge that contributes to Antarctic research while reducing the negative impacts of human presence in the region. Therefore, the collection supports the long-term sustainability of the Antarctic ecosystem.



Figure 1. Map showing the geographic locations of populations of *Colobanthus quitensis* analyzed in the articles identified in this review. (a) Geographic location of South American populations. (b) Drake

Passage region with (c) enlargement of the South Shetland Islands archipelago indicating King George Island and Livingston Island. The location of the populations indicated with red dots are Conguillío (pC), La Vega (pV), Sector Laredo (pL), La Marisma (pPA), Omora (pO), Arctowski (pA), Hannah Point (pH), and Byers Peninsula (pBy). Map modified from [11].

The growing interest in understanding plant responses to extreme environmental conditions has led researchers to focus on unconventional biological models like extremophilic plants. Such species can contribute to fields as wide as genetic improvement in crops and ecosystem sustainability [12,13]. *Colobanthus quitensis* provides a promising model for studying the adverse effects of abiotic stress on plant development, particularly in the context of climate change, and for exploring the role of endophytic and rhizospheric microorganisms in these processes [3,14–18]. A model organism for scientific studies should facilitate intensive and extensive investigation of biological phenomena, providing a deeper understanding of these processes in similar organisms as well as in more complex ones [19]. It should also be easy to cultivate and maintain in large numbers under laboratory conditions [20]. Advances in understanding the genetic characteristics of *C. quitensis* [9,11,21] and the development of protocols for *in vitro* cultivation and growth under common garden conditions [10,22,23] further support its use as a biological model species.

Colobanthus quitensis primarily reproduces sexually through seeds [6,24], and its high tolerance to extreme environmental conditions enables it to establish reproductive populations in the <0.4% of ice-free land available in Antarctica [7,8]. However, the harsh conditions in Antarctica affect the species' physiology from the early stages of its development [25–28]. For collections such as the *Colección Activa de Plantas Vasculares Antárticas*, it is essential to preserve seeds with optimal physiological quality to ensure their long-term viability and thereby contribute to the conservation of *C. quitensis*. Seed quality is the result of a combination of factors, including viability, longevity, germination success, and vigor, all of which are influenced by environmental, phytosanitary, morphological, physiological, and genetic factors [29–31]. Studying seed quality allows the establishment of storage protocols that reduce physiological deterioration [32]. However, most research in this field has prioritized species of agricultural or economic significance [33], often overlooking species with no immediate commercial value that produce small, dormant seeds. These traits present additional challenges for conducting comprehensive physiological seed quality assessments. *Colobanthus quitensis*, as an extremophilic species, produces small seeds ranging from 0.5 to 0.85 mm in size [34], which undergo dormancy [35] and are prone to physiological deterioration when stored at 4 °C [22], the standard temperature used for seed storage across various species [36]. This review aims to highlight how *C. quitensis* can provide new insights into the physiological seed quality of extremophilic species, and what the potential applications of this knowledge are for economically significant crops and ecological studies. Consequently, identifying current knowledge gaps regarding its reproduction and the physiological quality of its seeds, based on the methodologies and conditions used in these studies, is crucial for advancing research in this field.

2. Reproductive Strategies of *Colobanthus quitensis*

In natural ecosystems, *C. quitensis* produces reproductive structures after the second or third year of development, when the diameter of its vegetative cushion ranges between 0.2 and 1 cm [37]. Under controlled conditions, the specific cushion size or plant age at which flowering occurs has not been reported. However, laboratory observations have documented the presence of floral buds in small plants grown both in common garden settings (Figure 2C) and *in vitro* cultures (Figure 3D) (Table 1). Seed propagation is the main form of plant establishment in the natural environment, although asexual reproduction through cushion fragmentation has been recorded [6,24]. Successful sexual reproduction is associated with increased temperature during the spring and summer months [6,38,39]. The average air temperature faced by plants near Arctowski station on King George Island South Shetland Islands (Figure 1) during their growth period can be highly variable,

generally above 0.9 °C and below 3.7 °C [40]. However, mature seeds are produced even in the southernmost known population in northern Alexander Island (69 °S) [7] (Table 1).

The number of flowers produced by *C. quitensis* ranges from 1 to 28 per plant and depends on the age of the plant, the size of the plant cushion, and the soil temperature and moisture conditions during the growth stage, as well as its geographic location [21,39,41]. However, even though flowers are produced, they do not always successfully develop seeds [6]. In Chilean Antarctic expeditions conducted during the summer of 2021–2022 in Byers Peninsula (Livingston Island) and Arctowski, as well as during the summer of 2023–2024 in Arctowski (Figure 1), numerous flowers were observed on plant cushions of various sizes (Figure 2A,B, Table 1). The onset of flowering on Signy Island (South Orkney Islands) in 2023/24 was also delayed compared to observations from previous Antarctic expeditions [42]. It is known that flowering in any given year is influenced by both the climatic conditions of that year and by the climate and resources dedicated to reproduction in the previous year. In species from cold ecosystems, such as Arctic and alpine regions, annual variability in flowering is associated with the timing of snowmelt and seasonal temperatures. Earlier snowmelt can increase exposure to freezing conditions and alter temperatures during the flowering period, thereby influencing flower abundance and overall plant fitness [43,44]. As a result, the balance between flowering in one year and the following year can offset the resources allocated to flowering in the current year [45]. However, increases in temperatures in the maritime Antarctic in recent years have been linked with increased frequency of successful sexual reproduction by *C. quitensis*, along with considerable expansion of some populations [6,17,24,46,47]. This may be due to an increase in the number of flowers per plant, in the number of seeds matured per flower, or in germination success. These observations have received support from studies where the effects of changing environmental conditions on the growth, reproduction, and seed germination of this species have been simulated [29,41,48,49].

In the ‘common garden’ facility at the Universidad de Concepción, where *C. quitensis* is grown at photosynthetically optimal temperatures (14 ± 1 °C), the species has successfully reproduced for several years. Growing *C. quitensis* in a common garden alters the characteristics of its leaves and flowers, as evidenced by a larger flower peduncle (Figure 2C) compared to plants grown in the field where the flower peduncle and flower capsules do not usually exceed the height of the plant cushion (Figure 2A,B, Table 1).

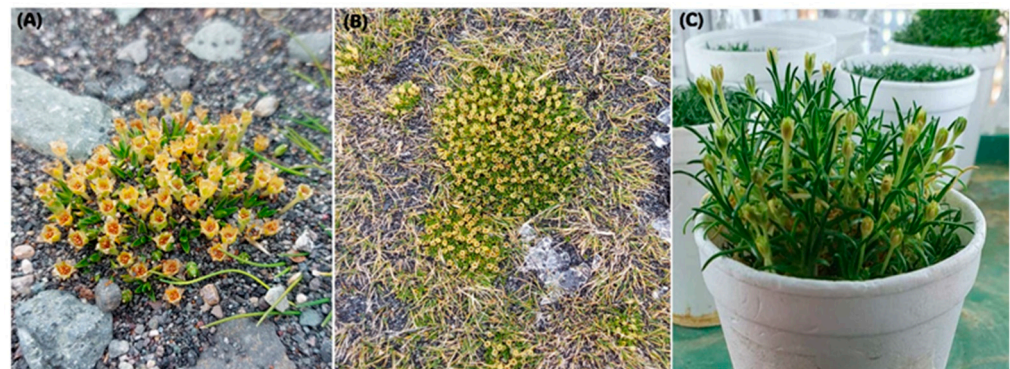


Figure 2. Reproduction by *Colobanthus quitensis* plants. (A) A small cushion in Antarctica showing open seed capsules (summer 2023/24, ≈ 0 °C *). (B) Large and small cushions with seed capsules in Antarctica (summer 2023/24, ≈ 0 °C *). (C) Plants flowering under common garden growing conditions (summer 2019/20, 13 ± 2 °C **). * Average temperature in the vicinity of the plants during the reproductive period [50]; available information at https://www.meteoblue.com/en/weather/history/climate/weatherarchive/henryk-arctowski-polish-antarctic-station_antarctica_6620757, accessed on 21 October 2024. ** Growing temperature under common garden conditions.

Under low-temperature and windy conditions, the flowers are cleistogamous and self-fertilizing, opening only when the seeds are ripe or have almost completed their maturation process (Figure 1a,b) [39,51]. Even in the common garden, flowers tend to open only when the seeds are ripe (Figure 3A). However, in field plants that develop between rocks where the influence of wind is less, flowers can open before generating seeds [52]; in these conditions, fertilization is likely to occur with the help of raindrops. In the common garden, it has been observed that warmer temperatures stimulate flower opening, exposing reproductive structures, which may promote cross-pollination, thus facilitating genetic exchange (Figure 3B, Table 1). The germination success of cleistogamic seeds is expected to be higher compared to chasmogamic seeds due to reduced contamination from pathogens present in air and water [53]. However, no studies are available on the temperature ranges in which *C. quitensis* flowers self-pollinate or cross-pollinate, the role of soil fertility in seed production and quality, or the frequency of pathogen occurrence. The final stage of reproduction of *C. quitensis* occurs when the capsules open and the mature seeds are exposed [54]. Seed size varies between the different *C. quitensis* populations held in the *Colección Activa de Plantas Vasculares Antárticas* at the LABEA (Figure 3C). It is also likely that the morphological characteristics of seeds will vary because of their growth conditions, even within the same population. The implications of these factors for the establishment of new individuals are yet to be studied. Under natural conditions, permanent seed banks form in the soil in the immediate vicinity of the parent plants, although some seeds may be dispersed further by wind, rain, or during snowmelt [38,55]. Under *in vitro* culture conditions, the occasional presence of viviparism has been detected (Figure 3D,E). Viviparism in flowering plants refers to the ability of some seeds to generate seedlings while still attached to their mother plant. This phenomenon has been reported in 0.1% of flowering plant families [56] and is a potentially valuable strategy for species in extreme environments because it shortens the period required for the establishment of progeny [57]. In *C. apetalus*, a species closely related to *C. quitensis*, viviparism has been detected under controlled growth conditions with an air humidity of 75% [52]. Some *C. quitensis* seeds cultivated *in vitro*, where ambient humidity is high, have shown vivipary (Figure 3D,E, Table 1), a phenomenon previously detected in plants with orthodox seeds [58,59]; however, to date, this phenomenon has not been recorded in their natural environment.

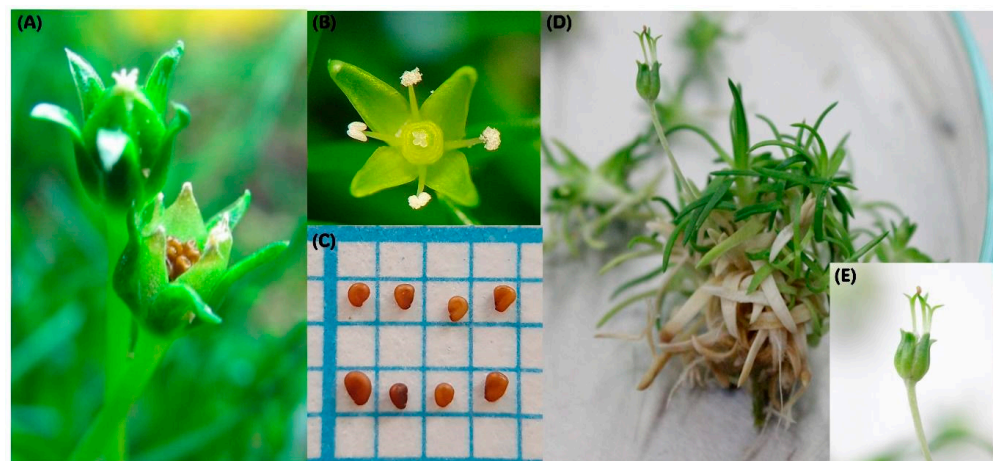


Figure 3. *Colobanthus quitensis* capsule and seeds obtained under laboratory conditions. (A) Closed flower bud and open capsule showing seeds. (B) Open flower from a common garden showing the stigma and stamens. (C) Example seeds from different populations: from left to right, top: Hannah Point, Byers Peninsula, Arctowski, and Omora Park; bottom: La Marisma, Laredo, La Vega, and Conguillío Park (the square measures 1 mm per side). (D) Plant from *in vitro* culture showing vivipary. (E) Close-up showing the seed attached to the cotyledon of a new seedling generated by vivipary.

Recently, the presence of seed color heteromorphism has been detected in different populations of *C. quitensis*, with light and dark brown seed forms; however, only the influence of seed form on tolerance to salinity has been studied, with some populations identified as being tolerant to this stress [60,61]. The environmental, metabolic, physiological, or genetic causes underlying heteromorphism in *C. quitensis* and its possible influence on other aspects of the phenology of the species require further research (Table 1).

At low temperatures, *C. quitensis* allocates more resources to vegetative growth than to sexual reproduction [6,38], whereas under warmer temperatures and higher water availability, both the growth and reproduction of the species are favored [21,38,62]. However, the detailed relationship between environmental conditions and seed production and quality has not been analyzed. In agricultural crops, nutritional deficiencies can affect pollen fertility as well as seed production and germination success [63]. Under natural conditions, larger and more extensive populations of *C. quitensis* have been recorded in areas where the soil is more fertile and there is high enzymatic activity of rhizosphere microorganisms [64]. However, research has yet to address whether there is any relationship between soil nutrient availability and the nutritional reserves of seeds produced by *C. quitensis*.

Table 1. Summary of reproductive features of *Colobanthus quitensis* grown under natural or controlled laboratory conditions.

Reproduction Features	Natural Conditions	Controlled Conditions	References
Reproduction type	Sexual reproduction is the main form of establishment. Asexual reproduction is also recorded	Plants can be established either in the common garden or <i>in vitro</i> from seeds (sexual reproduction) or explants (asexual reproduction).	[6,24]
Reproduction time	Successful reproduction occurs with increased temperature and water availability during spring and summer.	The environmental conditions remain constant. Flowering is detected throughout most of the year, although it is concentrated in spring and summer months.	[6,38,39]
Plant size for sexual reproduction	Flowering is detected in small plant cushions, whose minimum diameter varies from 0.2 to 1 cm, or in cushions of several centimeters in diameter.	No published reports, although small plants (<2 cm) have been observed at LABEA producing viable flowers and seeds.	[6,24,37] Figure 2A,B
Reproductive strategies	In cold and windy conditions, the flowers remain cleistogamous and self-fertilize.	Cleistogamous (higher frequency) and chasmogamous (lower frequency) flowers have been detected, so there may be self-fertilization or cross-fertilization.	[39,51,52] Figure 3A,B
Flower morphology	The flower stalk does not usually exceed the height of the plant cushion.	The flower stalk tends to exceed the height of the plant cushion.	[39,51] Figure 2
Seed viability	Not all flowers produce viable seeds successfully. The presence of dormant seeds has also been described.	Viable seeds are produced, some of which are dormant.	[6,35,39,41]
Seed heteromorphism	No seed color differences have been noted.	Seed color heteromorphism has been detected, with light and dark brown seeds.	[60,61]
Viviparism	No viviparism has been reported.	Viviparism has been observed in some seeds grown <i>in vitro</i> under high relative humidity conditions.	Figure 3D,E

3. Specific Features of *C. quitensis* Seed Production

Colobanthus quitensis produces small, triangular seeds that are wider towards the cotyledon area (Figure 3C), and their inability to germinate in some studies has been associated with the presence of deep primary dormancy [35,39]. Dormancy is a defense mechanism against environmental stress that, in the case of *C. quitensis*, could prevent germination late in the summer season in Antarctica, thereby avoiding seedling mortality [55,65]. It is still unclear whether the trigger for dormancy is an environmental condition or a physiological characteristic of the seeds, although laboratory evidence indicates that there is a genetic component influencing the presence of dormancy in *C. quitensis* populations, which is associated with their origin [66]. It is important to note that physiological dormancy is classified as primary when seeds are dispersed in a dormant state directly from the mother plant or secondary when it develops in response to specific environmental conditions that inhibit germination [67]. Laboratory studies have shown significant differences in *in vitro* germination between seeds from Antarctic and southern Chilean populations [9,22], which could indicate the presence of dormancy in some populations but not in others. Therefore, we hypothesize that the clear climatic differences between the regions where this species occurs (maritime Antarctic, sub-Antarctic, and throughout the Andes, including tropical latitudes at high altitudes) will exert different levels of stress that influence the expression of dormancy and, consequently, have an additional impact on seed quality. Given the observed differences in germination among populations, we believe that a more thorough study on dormancy is necessary.

Since dormancy may be related to embryo and seed coat characteristics, it affects seed longevity, water absorption rate, water content, resistance to mechanical damage, germination ability, and speed [68,69]. The inability of *C. quitensis* seeds to germinate under controlled conditions, despite embryo viability, as observed with the tetrazolium test [39,51], is related to dormancy [22] or the use of suboptimal protocols to stimulate this process. The germination capacity of seeds under certain temperature conditions will also be influenced by the conditions under which the mother plant developed, thus suggesting that maternal influence is fundamental to the success of germination [29].

Colobanthus quitensis seeds consist of a seed coat, perisperm, sparse endosperm, and an embryo [35]. The endosperm is involved in embryo nutrition, seed longevity, seed protection, and growth control during development and germination [30,70]. Some very small seeds of plant species that accumulate very limited nutritional reserves, such as orchids, have insufficient energy to germinate [71]. In such cases, natural associations with mycorrhizal fungi of genera such as *Ceratobasidium*, *Tulasnella*, *Rhizoctonia*, *Epulorhiza*, or *Ceratorhiza* provide the nutrients necessary for germination and initial seedling development [72]. Further, association with non-mycorrhizal endophytic fungi can also increase endogenous production of indoleacetic acid and gibberellins, which encourage germination [73]. It is known that *C. quitensis* establishes symbiotic relationships with endophytic fungi, which benefit growth under environmentally stressful conditions [74–76]. Therefore, it is likely that some of these fungi may also influence seed germination.

Colobanthus quitensis plants produce between 12 and 43 seeds per capsule, with the lowest production reported in populations growing under more extreme environmental conditions [5,6,9,29,38,54], along with lower germination success [6,28]. In an ecological context, even when the germination percentage is low, it may still be sufficient for the maintenance of populations [77]. Harsh environmental conditions generally limit seed production and quality in agricultural settings [78]. The climatic conditions to which *C. quitensis* plants are subjected in Antarctic and high Andean ecosystems are more intense than those faced by agricultural species, albeit the species shows considerable stress adaptation and resistance [25,47,75,79], which may affect not only seed production and germination success but also the plant's ability to develop seed nutritional reserves, testa thickness, and the expression of dormancy.

The coloration of the seed coat varies within *C. quitensis* populations, and the germination percentages differ between light and dark brown seeds when exposed to different

NaCl concentrations [60,61]. Seed heteromorphism, not only in testa coloration but also in morphology, has been reported in other Caryophyllaceae species [80,81]. This phenomenon is influenced by genetic factors, the position of the seeds on the plant, and their distance from the plant's vascular tissues [82]. Flower position determines seed filling, its hormonal gradient, seed coat structure, and composition which, combined, influence both the germination process and dormancy [83]. However, studies have not addressed whether *C. quitensis* seed color variation is due to asynchronous seed maturation in flowers, or to biochemical, molecular, or nutritional factors; it is also unknown whether seed coloration is related to other indicators of seed physiological quality. Identifying such correlates of seed quality would help inform conservation decisions.

The long-term storage of seeds under dry air conditions generally depends on their ability to tolerate desiccation and exposure to low temperatures [84]. The ambient moisture content and temperature range employed during seed preservation are critical to maintaining seed viability [32] as some reserve substances such as storage triacylglycerides can crystallize at temperatures of $-20\text{ }^{\circ}\text{C}$ [85]. Although *C. quitensis* has shown a rapid loss of germination capacity with storage time at $4\text{ }^{\circ}\text{C}$, sharing characteristics of recalcitrant seeds [86], the Royal Botanic Gardens informs that it is a species that produces orthodox seeds that can tolerate freezing at $-20\text{ }^{\circ}\text{C}$ and show 100% germination when using gibberellic acid (GA3) in *in vitro* culture [87]. This classification is supported by the fact that the species can maintain permanent seed banks in the soil [51], which maintain some degree of viability for periods of between two and four years [39,54]. However, no studies have been conducted evaluating different methods of seed preservation.

4. Characteristics of *C. quitensis* Seed Bank Formation

Colobanthus quitensis populations generate permanent seed banks, providing a mechanism of adaptation to extreme environmental conditions [51,54,55], implicitly assuming an extended length of time that the seeds will remain viable and contributing to the persistence of the population over time [88]. Such a mechanism could be particularly important in the extreme environmental conditions of Antarctica. Three types of seed banks are generally recognized, transient, short-term persistent, and long-term persistent, with the latter having longevity of more than five years [88].

Once seeds enter the soil seed bank, their persistence depends on their ability to remain viable by neutralizing reactive oxygen species, which can cause physiological damage [67]. As a result, each non-lethal stress condition encountered by the seeds in the soil seed bank triggers a phenotypic response that acts as priming, allowing the seeds to respond more effectively to any future event [89]. It is therefore believed that every seedling that emerges carries the memory of the parent plants from the time of seed formation and their subsequent entry into the soil seed bank. In this way, both genetic and epigenetic factors—viewed as memory mechanisms—contribute to the variability found in soil seed banks [67].

The available studies on the seed banks of *C. quitensis* have had a maximum duration of four years [34], so it is not yet possible to confirm which type of seed bank the species generates or if there are any differences between the seed banks of maritime Antarctic, sub-Antarctic, and lower latitude populations. Recently, there has been recognition of the importance of seed dormancy [90] and its relationship with longevity [91], temperature variation [92], and soil characteristics, which together influence the dynamics of soil seed banks [93].

Small, light, round seeds are more likely to penetrate more deeply into the soil profile and generate permanent banks [88]. *Colobanthus quitensis* seeds typically measure between 0.5 and 0.85 mm [34] and weigh approximately 0.05 mg [35], features that could facilitate the formation of a permanent soil seed bank. The ease of burial due to the small size of seeds and positive photoblastism are characteristics that contribute to the formation of seed banks in the soil [94]. Regarding the ability of *C. quitensis* seeds to germinate in the presence of light or darkness, the literature includes studies where seeds are placed to germinate

under photoperiods of 24 h light [54] or 24 h darkness [29], as well as in photoperiods that combine 12 h light/12 h darkness [28] or 16 h light/8 h darkness [22]. This indicates that the species can germinate under all daylength conditions; however, no single study has compared germination success under different lighting conditions to classify *C. quitensis* seeds as positively photoblastic, negatively photoblastic, or neutral, although available evidence suggests that the species is neutral to light conditions for germination.

Plant growth conditions influence the characteristics of the seeds produced and their subsequent persistence in soil, with Antarctic populations producing heavier seeds than those growing in greenhouses [35,51,95]. Longer seeds generally have larger embryos and more endosperms, factors that facilitate germination [70]. Therefore, we hypothesize that differences in seed size among populations of *C. quitensis* may influence various germination indicators, such as germination percentage or speed, and that these differences will be maintained even when parent plants are grown under common garden conditions. Consistent with this hypothesis, differences in morphology, genetic factors, and the expression of mechanisms of tolerance to environmental conditions between *C. quitensis* populations have been reported under common garden conditions [9,11,96].

Local soil characteristics are likely to influence the development of seed banks [6,55,97], not least as the presence of an apparently healthy reproducing population does not automatically lead to the existence of a seed bank [55]. Further studies are required to elucidate the relationship between soil characteristics and the persistence of seeds in the soil. A detailed study of the microbial community is also required since fungi present in the soil can interact with seeds and establish both symbiotic relationships that favor germination [98] and pathogenic relationships that will reduce viability. Recent temperature increases in Antarctica are expected to favor the activity of some soil microorganisms, including in the plant rhizosphere, increasing the availability of nutrients in the soil [64,99,100] and, in turn, the establishment and survival of *C. quitensis*, which could favor the growth, reproduction, and seed production of this species.

A seed bank potentially includes different soil strata related to annual reproductive cycles [88], so seed quality might be expected to vary in relation to position. Applying this concept to *C. quitensis*, the shallowest soil layers have been associated with the youngest seeds [34]. However, the most recently produced seeds do not always show the best germination rates due to the presence of dormancy [54]. To date, studies have focused on the seed density, germination capacity, and survival of samples collected in the first 10 cm of the soil profile [34,55]. Most of the soils have been influenced by permafrost and extreme cold, with the presence of volcanic and granitic types primarily in the Antarctic Peninsula. They typically lack or have very few of the strata commonly identified elsewhere, resulting in soil depths that rarely exceed a few centimeters, where the organic matter content is often scarce [101–103]. At this time, there is no research available on *C. quitensis* that indicates the depth of its seed banks, how many seeds are introduced each year into the soil strata, or the rate at which soil depth increases over time.

5. The Relationship of *C. quitensis* with Endophytic Fungi, with Specific Emphasis on Seeds

Most plants establish symbiotic relationships with endophytic fungi, whose influence promotes plant growth in the presence of biotic and abiotic stresses [74,104,105]. Endophytic fungi are often found in roots, rather than leaves, although they may be present in both [106]. The presence of these fungi leads to increased seed mass and enhances the accumulation of soluble sugars, auxins, and gibberellins, which lead to increased germination success [73,105–108]. The diversity of endophytic fungi differs among plant species and is also influenced by the characteristics of the environment in which they develop, including soil and climatic factors [53]. These fungi can be vertically transmitted to plant offspring [107]; however, their presence in the parent plant does not necessarily result in transfer to the offspring [98]. Vertical transmission depends on the species involved, genotype, resource availability, environmental stress, and timing of seed initiation [107,109,110].

Endophytic fungi including *Alternaria* sp., *Eupenicillium osmophilum*, *Penicillium chrysogenum*, *P. brevicompactum*, and *Phaeosphaeria* sp. have been identified in the roots of *C. quitensis*, with a frequency of occurrence greater than 1% of the total fungal community [75]. These have beneficial effects on plant growth and flowering [75,76]. On the other hand, endophytic fungi belonging to the genera *Aspergillus*, *Cadophora*, *Davidiella*, *Entrophospora*, *Fusarium*, *Geomyces*, *Gyoeffjella*, *Microdochium*, *Mycocentrospora*, and *Phaeosphaeria* have also been identified in leaves of *C. quitensis*, which are believed to confer resistance against freezing and ultraviolet radiation [111]. *Fusarium* and *Penicillium* species positively influence orchid seed germination by producing hormones and metabolites that stimulate both germination and seedling growth [112,113]. However, studies have yet to address the transmission of endophytic fungi associated with *C. quitensis* or the effect of these fungi on seed germination under field conditions.

Plant domestication reduces their associated microbial diversity relative to that found under natural conditions [114]. However, no studies comparing the diversity of endophytic fungi associated with *C. quitensis* plants in natural and cultivated conditions have yet been carried out. Plants collected from the field and propagated in the laboratory for more than five years produce seeds that rapidly lose their germination capacity with storage at 4 °C, with even seeds collected recently showing poor germination [115]. Factors underlying this loss of germination capacity potentially include the loss of these endophytic fungi over time or the establishment of new symbiotic relationships that do not favor seed germination. A simulated climate change study showed that in the face of increased temperature and water availability, there was a reduction in the number and functionality of endophytic fungi associated with *C. quitensis* [41]. This suggests that multiple factors, including illumination, temperature, irrigation, soil type, and fertility, used in laboratory studies may lead to important modifications in the microbial community associated with this species.

6. Interpretation of Physiological Quality of Seeds

Seed storage is a frequent practice in the agricultural industry and in germplasm banks [32]. There is a clear need to perfect the process of seed conservation, as suboptimal storage conditions cause and accelerate physiological deterioration in seeds, leading to partial or total loss of germination and resulting in economic losses [36].

Seed quality can be assessed in terms of the integrity of cellular structures, physiological processes, and morphological and phenotypic characteristics [31,116,117] that guarantee high germination, viability, longevity, emergence, and vigor. The study of these parameters allows for the characterization of species, varieties, and ecotypes [32]. In some species, the maximum physiological potential is reached at seed maturation, after which deterioration begins [33], evidenced by the accumulation of free radicals and lipid peroxidation that generate damage to the integrity of membranes, DNA, RNA, protein synthesis, and metabolic processes [30]. Understanding the process of seed quality loss and its underlying causative factors is critical for maximizing the success of ex situ seed bank conservation [118,119].

Germination testing allows for discrimination between batches of seeds of the same species tested under optimal conditions, along with assessing the appearance of abnormal growth structures [120]. This approach does not consider any influence of environmental stress or evaluate other growth parameters, and the optimal conditions will differ between species and populations [33]. For example, in *C. quitensis*, the optimal germination temperature depends on the conditions under which the parent plant grows, as it has been shown that plants grown at 5 °C produce seeds capable of germinating between 5 and 15 °C, whereas plants grown at 11 °C allow for an extended germination temperature range up to 25 °C [29]. Germination tests typically last between 7 and 28 days [120]; however, the time required for germination also depends on the degree of seed deterioration [121] and the presence of dormancy [68].

Longevity refers to the period over which seed germination remains above a critical level under constant storage conditions. Longevity is affected by genotype and pre- and post-harvest environmental conditions, although the integration of these components

is poorly understood [30,122]. Mathematical models based on biochemical studies to determine seed longevity [117,123] have not yet been applied in studies of *C. quitensis*.

Seed vigor and longevity are polygenetic traits, the products of interactions between physiological, biochemical, physical, and genetic properties, and contribute to the rapid and uniform emergence of seedlings with normal development [124,125]. From an ecological perspective, considering that the reproductive process in Antarctica is concentrated within the four summer months, flower production and the opening of seed capsules with mature seeds can take up to two months, and the germination and initial seedling establishment process in soil seed bank studies can take up to 45 days [115]. Rapid germination can contribute to successful establishment while conditions remain optimal for plant growth before the onset of winter. From a conservation standpoint in plant collections, achieving uniform and rapid germination reduces study time and resource consumption, facilitating the development of research. Multiple tests of vigor exist based on biochemical parameters, the imposition of stress conditions, and seedling growth [33]. However, since vigor includes multiple parameters, none of these approaches fully explain the properties involved in quality assessment or are applicable to all species. It is therefore recommended that several vigor tests should be performed to try and ensure the inclusion of all possible parameters [126].

7. Studies Related to Seed Quality in *C. quitensis*

Seed germination success relates to the imposed conditions [119], nutritional reserves [70], testa permeability, and the presence of dormancy [22,30]. Understanding each component is necessary to analyze all factors implicated in seed quality [31].

Dormancy extends longevity at the expense of germination [30]. In *C. quitensis*, factors influencing dormancy such as testa structural characteristics or seed hormone content are not completely understood, although it is known that the amounts of calcium, hemicellulose, and lignin in the testa determine water permeability, which limits germination [30]. In some crop plants, a lower lipid content in seeds has been associated with reduced longevity [32] as lipids help protect seeds from oxidative damage caused by reactive oxygen species, which affects the metabolic capacity of the seeds, preventing them from germinating [127,128]. There remain many gaps in knowledge about *C. quitensis* reproduction, seed production, factors causing seed deterioration, and the techniques required to analyze or control this process (Figure 4).

A flotation test is a very simple method of analyzing the physiological quality of seeds that distinguishes between viable and non-viable seeds according to whether they float in water, where seeds that float are assumed to be non-viable [129]. Air cavities that limit viability and affect germination have been detected in pepper (*Piper nigrum*) seeds [31]. Such cavities could underlie the success of the flotation test. This technique has been used in the analysis of *C. quitensis* seeds [9]. However, it is not 100% effective for *C. quitensis* as germination tests subsequently performed on seeds classified as non-viable showed that some could still germinate [115]. It is possible that *C. quitensis* does not generate these cavities inside its seeds, but this is unknown. Alternatively, because of the dormancy present in the species, the rate of water absorption by the seeds may be too slow for this technique to be effective. However, there is evidence that seeds floating in water have a greater dispersal capacity [130], which could form a dispersal strategy that the species uses under natural conditions.

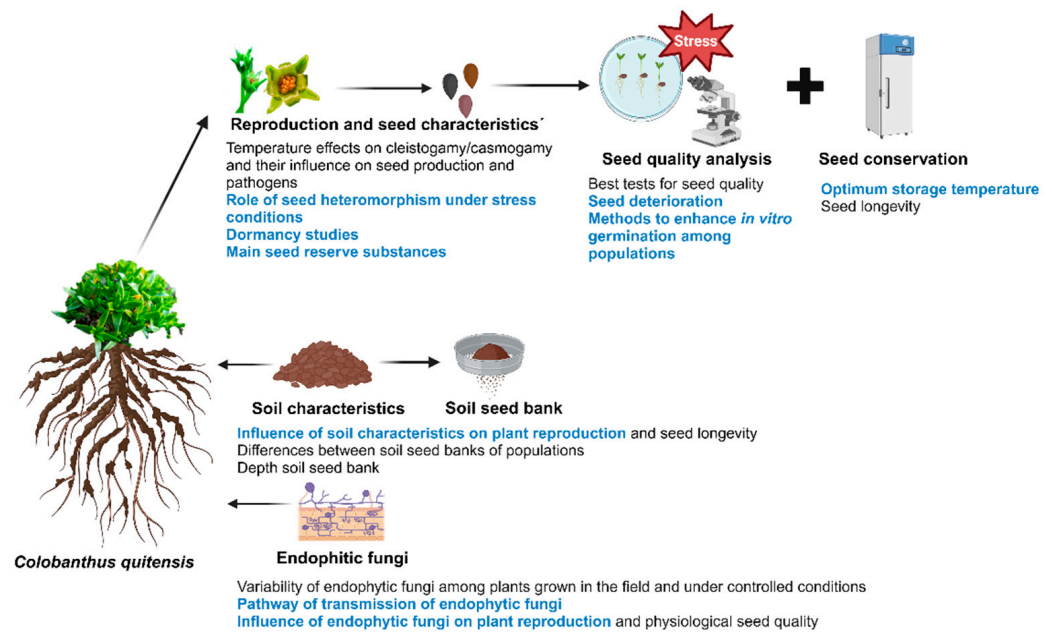


Figure 4. Summary of the main information gaps identified in this review relating to *Colobanthus quitensis* reproduction, seed production, and physiological quality. In blue are the topics that are being investigated at the Laboratorio de Biotecnología y Estudios Ambientales at Universidad de Concepción.

Seed physiological quality testing based on stress conditions, where seeds are subjected to high humidity and extreme temperature conditions (accelerated and cold aging test), has allowed the evaluation of seed physiological quality, vigor, longevity, storability, and field germination in species such as rice, quinoa, and maize [131–133]. The germination capacity of *C. quitensis* seeds has been studied using stress tests based on the effect of low and high temperatures [29], different concentrations of sodium chloride [25,60], sodium fluoride [28], Fe^{3+} [134], copper [26], methanesulfonic acid [135], and a marine air component involved in the sulfur cycle [136], as well as elevated salt concentrations due to their proximity to the coast [101,102]. However, none of these analyses had the primary objective of evaluating the physiological quality of the seeds, but rather the resistance of the species to extreme environmental conditions.

It is challenging to study *C. quitensis* seeds biochemically due to the large number of samples required for this type of analysis; in addition, some seed quality analyses are also difficult to apply due to the size of the seeds. To some extent, this can be solved by using image processing software such as SVIS[®], Vigor-S, ImagJ, or Germinator, which allow more detailed analysis of the different structures resulting from germination [124,131,137]. In the analysis of small seeds, X-ray spectroscopy and attenuated total reflection Fourier transformed infrared spectroscopy can be used to identify changes in seed morphology at the level of the seed coat or deeper layers involving the embryo, allowing for the identification of anomalies in the embryo or detection of compounds that influence germination [30,133,137–139]. The application of these techniques in studies of *C. quitensis* will help to determine more precisely how seed quality loss manifests, without the need for destructive studies. A summary of the factors affecting the seed quality of *C. quitensis* and strategies to evaluate or counteract these is given in Table 2.

Table 2. Factors affecting *Colobanthus quitensis* seed quality and methodologies that can be used to study this phenomenon.

Factors Contributing to the Decline in Seed Quality	Change Origin	Evidence of Seed Quality Loss	Seed Quality or Vigor Evaluation Method	References
Environmental conditions	Environmental origin due to changes in temperature, relative humidity, amount of nutrients, or salinity.	Decreased germination and increased time required for germination. Changes in the structural integrity of the seed coat.	Germination tests under stress conditions. Image processing.	[25,29,60,101,102,124,131,137]
Conservation and storage conditions	Anthropogenic influence due to the imposition of suboptimal conditions of temperature and relative humidity. Possible presence of pathogens.	Decreased germination and increased time required for germination. Embryo death. Accumulation of reactive oxygen species and damage to the genetic material of the seed.	Germination tests under stress conditions. Image processing.	[32,36,120,124,131,137]
Dormancy	Possible physiological origin and/or influence of environmental factors.	Absence of germination until methods to interrupt dormancy are used.	Germination test using techniques to interrupt dormancy (scarification, stentification, mechanical rupture of the testa, or the use of hormones). Viability analysis using tetrazolium salt staining.	[30,68]
Physiological deterioration	Natural process that occurs in any seed once it has matured.	Germination is affected by oxidative stress generated by reactive oxygen species, depletion of nutritional reserves, changes in hormone profiles and/or damage to genetic material.	Germination test. Viability analysis using tetrazolium salt staining, Development of biochemical studies. Image processing.	[30,32,33,115,119,124,131,137]

8. Conclusions and Future Perspectives

This review highlights the relevance of studying the reproduction of *C. quitensis* and the ex situ conservation of its seeds as fundamental elements for the proper management of germplasm banks. These efforts are crucial for promoting scientific research on this unconventional model species and contributing to the preservation and sustainability of the protected ecosystems in which it thrives, thereby minimizing the negative impacts of human activity.

We identify several critical areas that require further research, including seed conservation and storage, quality analysis techniques, soil seed banks, and the interaction between microorganisms and the reproduction of *C. quitensis* (Figure 4). To fill these critical knowledge gaps, it is necessary to improve our understanding of the phenology of the species and the impact of biotic and abiotic factors on its development and evaluate strategies for seed protection and biotechnological applications. The reproductive success of the species is strongly influenced by the environmental conditions of both the previous and the current year. The formation of permanent seed banks represents an adaptive strategy that allows *C. quitensis* seeds to remain viable for extended periods. This is further facilitated by seed dormancy and Antarctic soil conditions, which favor the long-term viability of seeds. These characteristics can inform seed conservation, which serves as a preventive resource to support biodiversity conservation in ecosystems vulnerable to climate change.

New seed analysis tools, such as imaging techniques, can offer innovative solutions for assessing the physiological quality and longevity of species with small seeds like *C. quitensis*. However, the morphological, physiological, biochemical, and genetic factors influencing germination loss under controlled conditions remain largely unknown, complicating the development of efficient storage protocols and the understanding of dormancy in this species. Additionally, uncertainty persists regarding the role that microorganisms present in their natural habitats may play in germination and seedling establishment.

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