



Advancing conservation strategies for the endangered Galapagos plant *Lecocarpus lecocarpoides*: insights from *ex situ* propagation

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ABSTRACT: The Galapagos Islands, renowned for their unique biodiversity, face a growing crisis, with over half of their endemic vascular plants under threat of extinction. Among the threatened flora, *Lecocarpus lecocarpoides* is classified as endangered, but is one of the least studied species. We present novel insights into the biology of *L. lecocarpoides*, acquired during the propagation of individuals for subsequent transplantation into their native habitat. The study encompasses seed viability, germination, growth, phenology, and morphology. Herbarium seeds were shown to remain viable for over 2 decades, highlighting the potential for seed bank conservation. In our germination experiment, which began with just 50 seeds, seed scarification resulted in a high germination rate of 75%. The resulting seedlings were planted in 2 sizes of pots and nurtured as seed producers. The plants in larger pots grew on average twice as tall and with nearly double the stem diameter of those in smaller pots. They yielded over 8000 seeds for conservation efforts, providing information on the development and fruit production capacity of *L. lecocarpoides*. We show differences in diaspore spine length between *L. lecocarpoides* populations, supporting the distinction of 2 *L. lecocarpoides* subspecies. Our findings provide essential data to inform conservation strategies for this endemic Galapagos species.

KEY WORDS: Biodiversity conservation · Seed viability · Seed scarification · Germination · Phenology · Morphology · Asteraceae · Galapagos flora

1. INTRODUCTION

The global flora is currently in crisis, with 39% of all vascular plants estimated to be threatened with extinction (Nic Lughadha et al. 2020). This predicament also affects the unique biodiversity of the Galapagos Islands, where 60% of the 168 vascular plants endemic to the archipelago are categorized as threatened (Tye 2007, 2011). The challenges to the Galapagos flora primarily arise from the introduction of non-native species and land use change (Adersen 1989, Mauchamp et al. 1998). To address these challenges, it is important to implement effective recovery actions informed by sound research.

One group of species requiring conservation attention is the genus *Lecocarpus*, belonging to the family Asteraceae. *Lecocarpus* is one of 6 plant genera endemic to the Galapagos (Tye 2011, McMullen 2018). It comprises 4 species of evergreen shrubs, characterized by their yellow composite flowers, opposite leaves divided into lobes, and fruits equipped with spines, hooks, or wings (Eliasson 1971). Each species is restricted to a single main island, in one case with its neighboring islets: *L. pinnatifidus* is found on Floreana Island, *L. leptolobus* in the southwest of San Cristobal, *L. darwinii* in the northeast of San Cristóbal, and *L. lecocarpoides* on Española Island and 4 islets in Gardner Bay (Sønderberg-Brok & Adersen 2007, Jaramillo et

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al. 2018, Tye & Jaramillo 2022). *Lecocarpus* species are usually found on open windswept slopes, ridges, or lava flows within the arid zone at lower altitudes and occasionally at higher altitudes at 310 to 390 m.a.s.l. (Sønderberg-Brok & Adersen 2007). Among the 3 *Lecocarpus* species evaluated for extinction risk, *L. pinatifidus* has been categorized as critically endangered, and *L. darwinii* and *L. lecocarpoides* have been categorized as endangered, while the status of *L. leptolobus* is yet to be evaluated (León-Yáñez et al. 2011).

L. lecocarpoides can be distinguished from other *Lecocarpus* species by its fruits, which possess straight, sturdy spines (Tye & Jaramillo 2022). This species is present in 5 populations, all of which fluctuate in size (León-Yáñez et al. 2011). On the main island of Española, it is restricted to a single population at Punta Manzanillo (Mauchamp et al. 1998, Atkinson et al. 2008). The former presence of goats on the island, possibly lasting 2 centuries, might have caused the extinction of other populations on Española. Instances of goats consuming *L. darwinii* have been reported in San Cristobal (Adersen 1989, Sønderberg-Brok & Adersen 2007). Despite the eradication of goats on Española in 1978, the population of *L. lecocarpoides* there did not expand (Mauchamp et al. 1998, Atkinson 2007). In 2007, only 1 individual was found in Punta Manzanillo, raising concerns over a possible local extinction (Atkinson et al. 2008). No plants were recorded between 2012 to 2020 at Punta Manzanillo nor elsewhere on the island (CDF 2023). In November 2020, members of the Galapagos Verde 2050 (GV2050), an ecological restoration program of the Charles Darwin Foundation (Jaramillo et al. 2020), found 24 *L. lecocarpoides* plants at Punta Manzanillo (CDF 2023). Approximately 50 seeds were collected during this expedition and transported to the Charles Darwin Research Station (CDRS) for further investigation (DPNG 2023).

Previous research on the *Lecocarpus* genus has included taxonomic revisions, drawing from fieldwork and laboratory investigations of morphological variation among populations (Eliasson 1971, Sønderberg-Brok & Adersen 2007, Tye & Jaramillo 2022). A recent review recognized 2 distinct subspecies within *L. lecocarpoides* based on diaspore spine length but did not provide detailed data on the specimens analyzed (Tye & Jaramillo 2022). Subspecies *lecocarpoides*, characterized by longer-spined diaspores, is distributed on the main island of Española and possibly Oeste islet (hereafter Oeste), while subspecies *brachyceratus*, featuring shorter-spined diaspores, is restricted to Gardner-by-Española, Osborn and Xarifa islets (hereafter Gardner, Osborn, and Xarifa) (Tye & Jaramillo 2022). Although this species has been integrated into assess-

ments of the conservation status of Galapagos threatened flora (Adersen 1989, Mauchamp et al. 1998, León-Yáñez et al. 2011) and featured in reports concerning advancements in its conservation (Atkinson et al. 2008), there have been no biological studies dedicated exclusively to *L. lecocarpoides*.

Addressing such knowledge gaps regarding rare plants in the Galapagos is recognized as a high priority (Tye 2007, Simbaña & Tye 2009). Understanding the life history of *L. lecocarpoides*, specifically the micro-climatic conditions that favor its germination, is essential for developing pragmatic conservation plans for this species (Atkinson et al. 2008). Identifying germination requirements can inform the establishment of new populations through seed sowing or other propagation techniques. Similarly, knowing the phenology of a species can help determine the optimal timing for various conservation actions, such as seed collection, invasive species removal, and population enhancement (Buisson et al. 2017). The lack of information regarding these critical aspects of *Lecocarpus lecocarpoides*' biology limits the development of an effective conservation strategy and informed decision-making regarding the species' management.

The present study had 2 objectives. The first was to propagate the *L. lecocarpoides* seeds collected in 2020 into adult plants for further seed production and subsequent transplantation to Punta Manzanillo, to increase the population size of this species. The second objective was to determine critical aspects of the species' biology that could inform management actions. These included seed viability and germination, growth, phenology, and morphology. In the case of seed viability and morphology, we used herbarium samples in addition to plants propagated at the CDRS to compare between populations and subspecies of *L. lecocarpoides*. Due to the species' remote location, this would have been difficult to do in the wild, whereas cultivating the plants *ex situ* enabled observation of important features under controlled conditions. The findings of the study make an important contribution to the conservation of *L. lecocarpoides* on Española.

2. MATERIALS AND METHODS

2.1. Study site

The study was conducted at the CDRS on Santa Cruz Island (0° 44' 33.29" S, 90° 18' 12.71" W). Herbarium samples from various *Lecocarpus lecocarpoides* populations deposited at the Charles Darwin

Station (CDS) herbarium, located within the CDRS, were included in this study. Fig. 1 shows the geographical distribution of each *L. lecocarpoides* population and subspecies. The seeds utilized for propagation were collected at Punta Manzanillo, located on the north coast of Española Island ($1^{\circ} 20' 47.94''$ S, $89^{\circ} 41' 57.86''$ W). After the study was completed, the plants produced were introduced to their original habitat at Punta Manzanillo.

The CDRS and Punta Manzanillo are both located near sea level and exhibit similar temperature patterns which are consistent with the typical seasonal climate of the Galapagos (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/n054p443_supp.pdf). The warm season occurs from January to May and is marked by elevated temperatures and variable precipitation. The cool season spans from June to December, with cooler temperatures and minimal rainfall in the lowlands, where these 2 sites are located (Trueman & D'Ozouville 2010).

2.2. Seed viability

We conducted tetrazolium tests to investigate the decline in *L. lecocarpoides* seed viability over time. We used 8 herbarium samples collected from 4 populations over 20 years from 2001 to 2021 (Table S1). Thirty seeds of each sample were tested using a 1.0% 2,3,5-triphenyl tetrazolium chloride solution following the methodology of Baskin & Baskin (2014) and Santos de Souza et al. (2019). This number was selected based on the smallest sample size among the 8 samples.

The CDS herbarium is maintained at approximately $21\text{--}23^{\circ}\text{C}$ and $<50\%$ relative humidity (Mahtani-Williams & Jaramillo 2023).

Interpretation of staining was based on AOSA-SCST (Miller & Peter 2010), with viability indicated by uniform coloring throughout the embryo, signifying the presence of healthy tissues. Minor damage to the radicle tip was considered still viable, as was up to half of the cotyledon area unstained as long as this was restricted to regions away from the hypocotyl/cotyledon attachment point (Fig. S2).

2.3. Germination

Forty *L. lecocarpoides* seeds that showed no signs of damage, herbivory, or immaturity were selected from the seeds collected in November 2020. After being collected, the seeds were stored in air-tight containers at the CDS herbarium for 6 mo, with storage conditions as previously described (Mahtani-Williams & Jaramillo 2023). Atkinson et al. (2008) described *L. lecocarpoides* seeds germinating after 2 d if the outer seed coat layer was broken. Therefore, to enhance their germination, we subjected the seeds to a pre-germinative treatment that involved seed soaking and mechanical scarification.

Due to the limited number of *L. lecocarpoides* seeds and the very few individuals found in the wild, a control group with seeds not scarified was not included in this study. The absence of a control group is a limitation of the study, but it was a necessary compromise given the rarity of the species and the need to

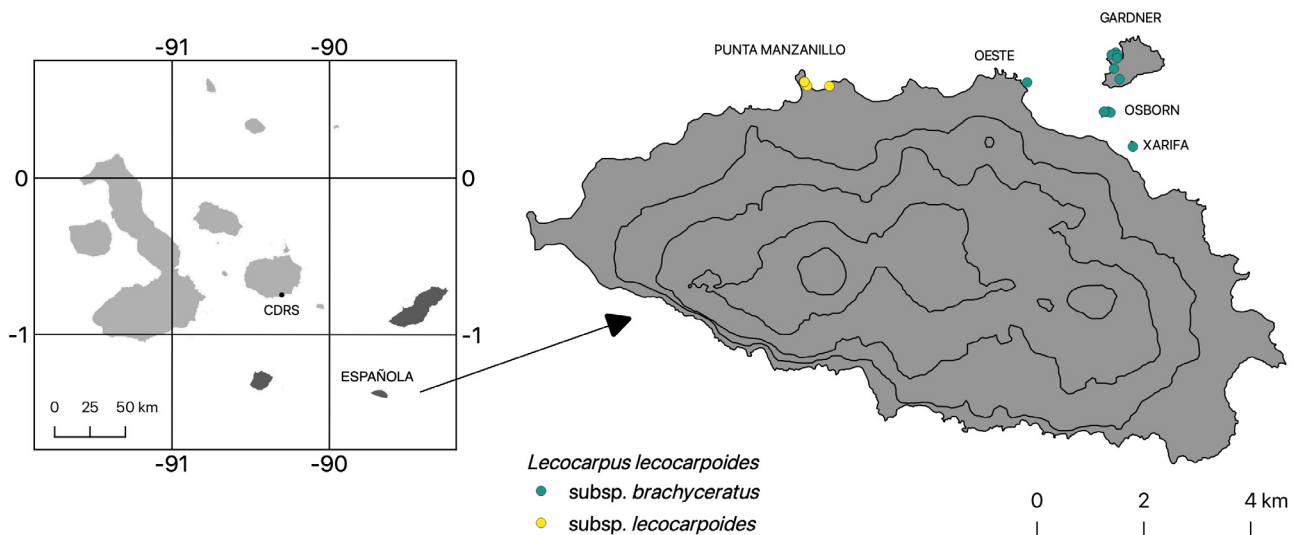


Fig. 1. Distribution of *Lecocarpus lecocarpoides*. Names on the map correspond to populations, while points represent collection sites of herbarium specimens. Contour lines show changes in altitude of 40 m. Data source: CDF (2023)

maximize seedling production while generating valuable information on *L. lecocarpoides* germination.

Soaking consisted of submerging the seeds in distilled water for 1 wk, with water changes every 2 d, to stimulate germination (Luna et al. 2014) and soften the external coat layers, making them easier to manipulate and cut. Scarification was then performed by making an incision in the seed coat using a scalpel under a stereoscope without physically damaging the embryo. Seed structures were identified using Jaramillo et al. (2021) before making the incisions.

The scarified seeds were sown 1 cm deep in a germination tray containing a 1:1 mix of commercial germinating medium (BM2; Berger) and gravel in April 2021. The timing was selected to match the approximate germination time in the wild. A prior study noted regeneration in March 1977 and a lot of regeneration in January 1998 at Punta Manzanillo (Sønderberg-Brok & Adersen 2007), coinciding with the warm-rainy season, suggesting that germination typically occurs during this period. A visit to Punta Manzanillo in June 2021 revealed 67 seedlings that were not present during a previous visit in January of the same year, confirming that the timing for the experiment was similar to the germination period in the wild.

The experiment was conducted at the CDRS in a facility adapted for plant propagation with access to natural light and external air to simulate natural settings. The temperature and humidity conditions at the CDRS during the germination period were $26.00 \pm 0.81^\circ\text{C}$ (mean \pm SD) and $90.85 \pm 2.60\%$ (mean \pm SD) (CDF 2024). The germinating seeds were counted daily. The tray was watered twice a week with sufficient distilled water to maintain soil moisture. While this regular watering schedule may differ from field conditions, it was implemented to maintain consistent soil moisture levels, promoting optimal conditions for germination. The experiment concluded after 3 mo with no further germinations recorded. The outcome was expressed as the percentage of germination achieved.

2.4. Growth

Germinated seedlings were transplanted to larger containers after the emergence of the first true leaves. To evaluate the impact of pot size on plant growth, 19 of the plants were transplanted into pots with an approximate volume of 5.9 l, while the remaining 11 plants were placed in pots with an approximate volume of 1.6 l.

Upon transplantation, we began recording growth measurements. Plant height was measured as the length of the longest stem from soil surface to tip,

including leaves but without lifting or extending them, and stem diameter was measured with a caliper at the base of each plant. These measurements were recorded 1–4 times per month, depending on time availability, and this monitoring phase continued until the plants were taken to Española in September 2022. From that point, we recorded plant mortality in the field every 3 mo.

2.5. Phenology

Phenology was assessed for each germinated individual while still at the CDRS by observing and counting flowers and immature and mature fruiting heads. Flower buds (Fig. S3A) were excluded from this study because their small size and resemblance to emerging leaves made obtaining precise counts challenging. Capitula with fully expanded ray florets were counted as flowers (Fig. S3B). Once ray florets withered and ovary enlargement occurred, they were counted as 'immature fruiting heads' (Fig. S3C). When the fruiting heads turned completely brown, they were counted as 'mature fruiting heads' (Fig. S3D). Phenology data were collected at the same time as growth data (1–4 times per month) for a duration of 17 mo.

Following each monitoring session, mature fruiting heads were collected and given a dry heat treatment to eliminate seed pathogens and lower their seed moisture content. This involved subjecting them to temperatures of 35–45°C in an oven for 2–3 d (Bridson & Forman 1998). While these temperatures may seem high, they are still within what is naturally encountered in the Galapagos. According to meteorological data from the CDRS, there were 2 days in March 2022 that reached temperatures above 35°C (CDF 2024). Once dried, the seeds were stored in airtight glass containers with pouches of indicator silica gel to maintain low-moisture storage conditions (Devitis et al. 2020). The labeled containers were kept in the fruit collection of the CDS herbarium. This methodology is used for the preservation of herbarium specimens and is not necessarily suitable for seed storage. However, it was the closest available alternative to seed banking conditions since there are no seed banking facilities in the Galapagos.

2.6. Morphology

For a deeper understanding of the structure of fruits and seeds of *L. lecocarpoides*, transverse and longitudinal sections were made on seeds obtained from

the germinated individuals. The identification of seed structures followed AOSA-SCST (Miller & Peter 2010). A Lankester composite dissection plate (LCDP) was used to document the morphology of various plant parts such as flowers, leaves, and reproductive structures.

We documented the same morphological traits as Tye & Jaramillo (2022) for the seedlings germinated as part of this study, including information on the capitula, diaspores, and outer phyllaries (Table S2). Then, we compared mean diaspore spine length between *L. lecocarpoides* populations using diaspores from herbarium samples and from individuals germinated at the CDRS.

To assess differences in mean spine length, we employed an ANOVA using R v.4.2.1 (R Core Team 2023) followed by a Tukey's HSD test to identify pairwise differences among populations. Values were reported as mean \pm SD.

We examined 73 herbarium samples of *L. lecocarpoides*, of which 42 contained seeds (Table S3). From these samples, we measured 70 diaspores from each population, excluding that from Oeste due to a lack of seed samples. For the germinated seedlings from Punta Manzanillo, we measured 70 diaspores from the 22 individuals that successfully reached the reproductive stage.

We assessed assumptions of normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. The Shapiro-Wilk test indicated normal distributions for the Xarifa, Osborn, Punta Manzanillo, and *ex situ* propagated data ($p > 0.01$), whereas the Gardner data showed non-normality ($p < 0.01$). Additionally, Levene's test revealed violations of the homogeneity of variances assumption ($F_{4,345} = 7.7419$, $p < 0.001$). Despite these deviations from assumptions, we proceeded with the 1-way ANOVA analysis, relying on the robustness of ANOVA for balanced designs and large sample sizes (Sawyer 2009).

3. RESULTS

3.1. Seed viability

Tetrazolium viability tests on herbarium seeds indicated a seed viability of approximately 65% in seed lots collected between 2019 and 2021, while those collected in 2001 (the oldest *Lecocarpus lecocarpoides* samples stored in the CDS Herbarium) showed a viability range of 0 to 33.33% (Table 1). We found

that seeds whose tetrazolium coloration pattern indicated they were viable stained similarly (almost completely) irrespective of the year of collection (Fig. S4).

Longitudinal sectioning of seeds classed as viable revealed a consistent red pigmentation in all parts of the embryo except the cotyledons. The entire plumule, hypocotyl, radicle, and at least half of the cotyledons were consistently stained red.

3.2. Germination

Out of the 40 *L. lecocarpoides* seeds sown, 30 (75%) successfully germinated, with a median germination time (T50) of 10.5 d (Fig. 2). The first seedlings were observed after 5 d, and germination continued for 42 d. Some of the emerging seedlings retained the seed coat attached to them upon breaking through the soil surface (Fig. S5).

3.3. Growth

Plant height and stem diameter exhibited similar growth patterns, characterized by a period of slow growth from April to July, followed by faster growth from August to November, and then another period of slow growth from December to August of the next year (Fig. 3). On average, plants in larger pots grew twice as high (mean \pm SE = 75.2 \pm 16.8 cm, $n = 19$) as those in smaller pots (38.3 \pm 7.6 cm, $n = 11$). Similarly, plants in larger pots reached a mean (\pm SE) stem diameter of 8.3 \pm 1.6 mm, while plants in smaller pots measured 4.4 \pm 0.6 mm. Despite seasonal changes in air temperature (Fig. S6), we did not observe any relationship between growth and temperature.

Of the initial 30 germinated plants, 24 survived at the CDRS until September 2022 at ~15 mo old. After this date, 19 plants were repatriated to Punta Manza-

Table 1. Results of tetrazolium seed viability tests performed on *Lecocarpus lecocarpoides* seed samples from the Charles Darwin Station herbarium

Population	Subspecies	Year collected	Seeds viable / seeds tested	Viability (%)
Punta Manzanillo	<i>lecocarpoides</i>	2001	0/30	0
Gardner	<i>brachyceratus</i>	2001	10/30	33.33
Osborn	<i>brachyceratus</i>	2001	7/30	23.33
Oeste	<i>brachyceratus</i>	2005	2/30	6.66
Punta Manzanillo	<i>lecocarpoides</i>	2005	1/30	3.33
Gardner	<i>brachyceratus</i>	2007	4/30	13.33
Xarifa	<i>brachyceratus</i>	2019	20/30	66.66
Oeste	<i>brachyceratus</i>	2021	19/30	63.33

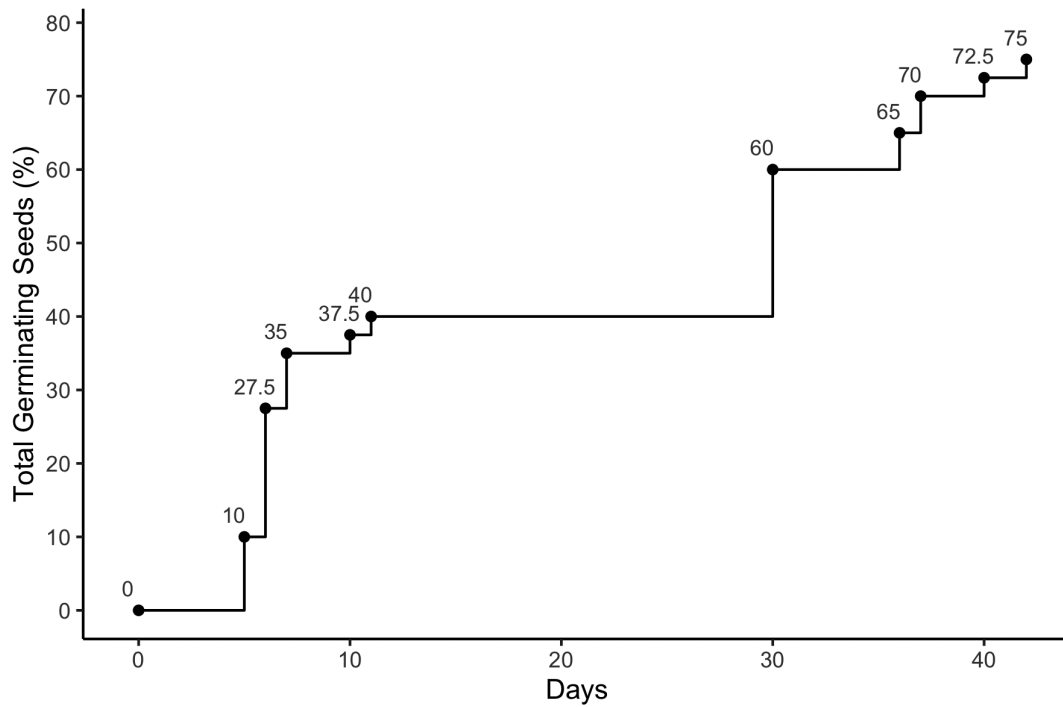


Fig. 2. Germination rate of *Lecocarpus lecocarpoides* seeds from Punta Manzanillo over time. Seeds underwent a soaking and scarification treatment before sowing

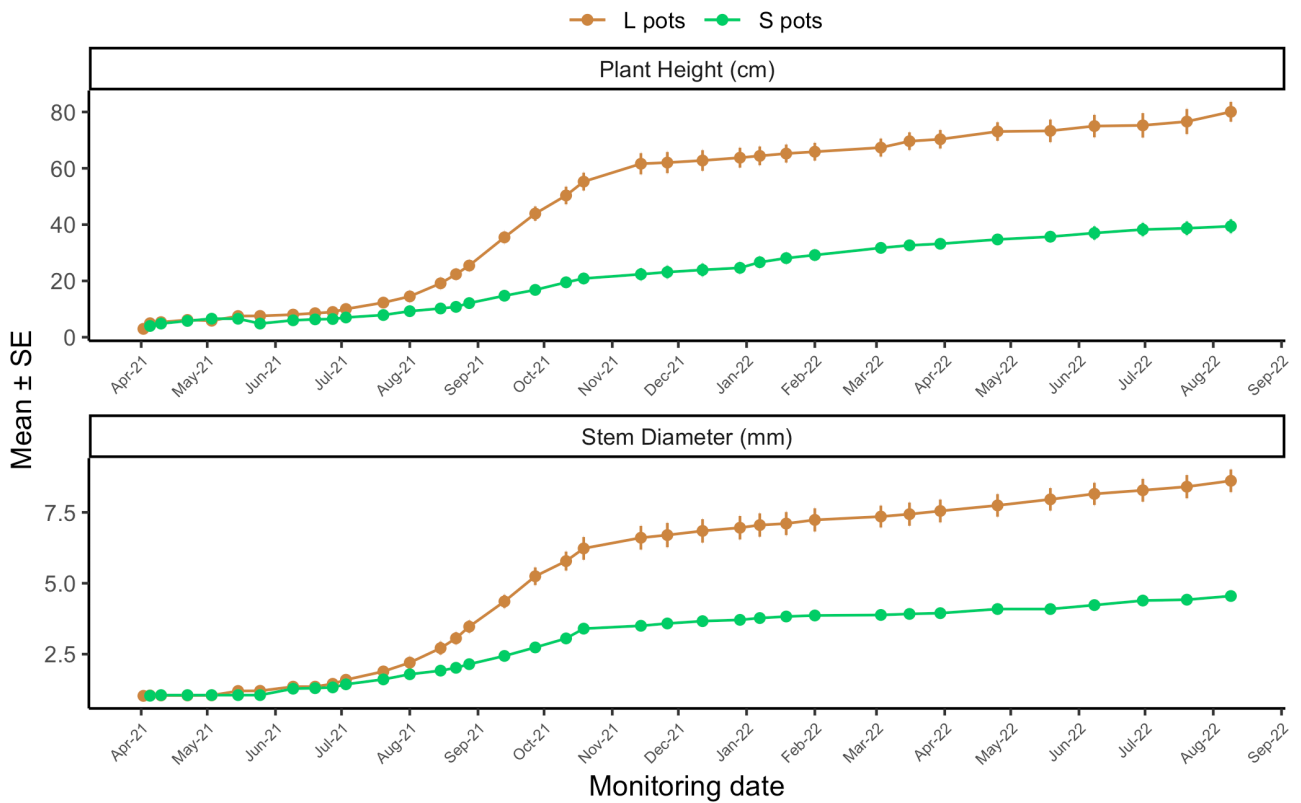


Fig. 3. Mean plant height and basal stem diameter of propagated *Lecocarpus lecocarpoides* plants from Punta Manzanillo through time, cultivated in small (S) or large (L) pots. Data are mean \pm SE

nillo; of these, at least 10 survived until a monitoring session carried out in Dec. 2022 (~20 mo old). The remaining 5 plants were kept at the CDRS to register their mortality date. They died between June and October 2023 (~26 to 30 mo old).

3.4. Phenology

L. lecocarpoides individuals began producing flowers (capitula with fully extended ray florets) in August, approximately 4–5 mo after the first germinations. The peak flowering time, characterized by maximum number of flowers (mean \pm SE = 13.5 ± 2.5 flowers plant⁻¹, n = 28), was reached in October, about 6–7 mo after the first germinations. Flowering then gradually declined and persisted at a low level until the plants were taken to Española (Fig. 4). The production of immature fruiting heads began approximately 2–3 mo after the first flowers and peaked in November (mean \pm SE = 14.5 ± 4.5 new immature fruiting heads plant⁻¹, n = 28). Mature fruits were present from about a month after the first immature fruiting heads and gradually increased until a peak (9.5 ± 1.9 new mature fruiting heads plant⁻¹, n = 28) in April (Fig. 4).

There was a substantial difference in fruit production between *L. lecocarpoides* plants in large and small pots. Plants grown in large pots yielded a mean \pm SE of 114.4 ± 19.9 fruiting heads plant⁻¹ (n = 19), whereas plants in small pots only produced 16.4 ± 2.0 fruiting heads plant⁻¹ (n = 11) (Fig. 5). In total, the 28 individuals that reached reproductive maturity produced 1927 fruiting heads (8102 individual fruits) until September 2022.

3.5. Morphology

The transverse and longitudinal sections of *L. lecocarpoides* fruits revealed a single seed surrounded by a pericarp composed of several layers with varying thicknesses and hardnesses (Fig. 6). The outermost layer of the fruit is hydrophilic and produces mucilage when soaked in distilled water.

All the germinated individuals of *L. lecocarpoides* from Punta Manzanillo displayed deeply pinnatifid leaves, characterized by incisions that extended to roughly half the distance towards the central vein, and often exhibited secondary lobes (Fig. 7). Approximately 89% of the individuals had very narrowly winged petioles, 93% had dentated bracts, and 91% had diaspores with spines longer than or equal to the diaspore body.

Fig. 8 presents distributions and means for all the quantitative morphological traits that were measured. The comparison between spine and diaspore length was assessed qualitatively (spine length > diaspore length, spine length < diaspore length, or spine length = diaspore length), and therefore it was not included in Fig. 8.

The ANOVA revealed a significant effect of population on mean diaspore spine length ($F = 147.7$, $p < 0.001$). Post-hoc Tukey multiple comparisons indicated significant differences in mean spine length between all pairs of populations ($p < 0.05$), except for the comparison between Punta Manzanillo and *ex situ* propagated populations ($p = 0.978$) (Fig. 9). The mean (\pm SD) spine length in decreasing order for each population is as follows: *ex situ* propagated (4.64 ± 0.95 mm), Punta Manzanillo (4.74 ± 1.19 mm), Xarifa (2.83 ± 1.06 mm), Gardner (2.15 ± 1.14 mm), and Osborn (1.55 ± 0.48 mm). The difference in diaspore spine length is apparent in field photographs of the various populations (Fig. S7).

4. DISCUSSION

This study has documented the germination, growth, phenology, and morphology of *Lecocarpus lecocarpoides* plants grown at the CDRS. Additionally, we assessed the viability of seed samples from CDS herbarium specimens and compared the diaspores of *L. lecocarpoides* populations.

The finding that *L. lecocarpoides* seeds can remain viable for over 20 yr in herbarium conditions suggests that this species has orthodox seeds well-suited for storage in seed banks. Seed banks are invaluable tools for *ex situ* conservation, particularly in the face of catastrophic events or rapid population declines, and they serve as a source of genetically diverse material for ecological restoration (Liu et al. 2020). Currently, there are no long-term seed storage facilities in the Galapagos. Creating one would significantly contribute to achieving (even if late) Target 8 of the Global Strategy for Plant Conservation (<https://www.cbd.int/gspc>), which recommends having

'at least 75 percent of threatened plant species in *ex situ* collections, preferably in the country of origin, and at least 20 percent available for recovery and restoration programs by 2020'

Such a facility could significantly support conservation and restoration efforts in the Galapagos Islands.

Variation in seed viability between populations and collection years may have been influenced by several factors, including seed maturity at the time of collec-

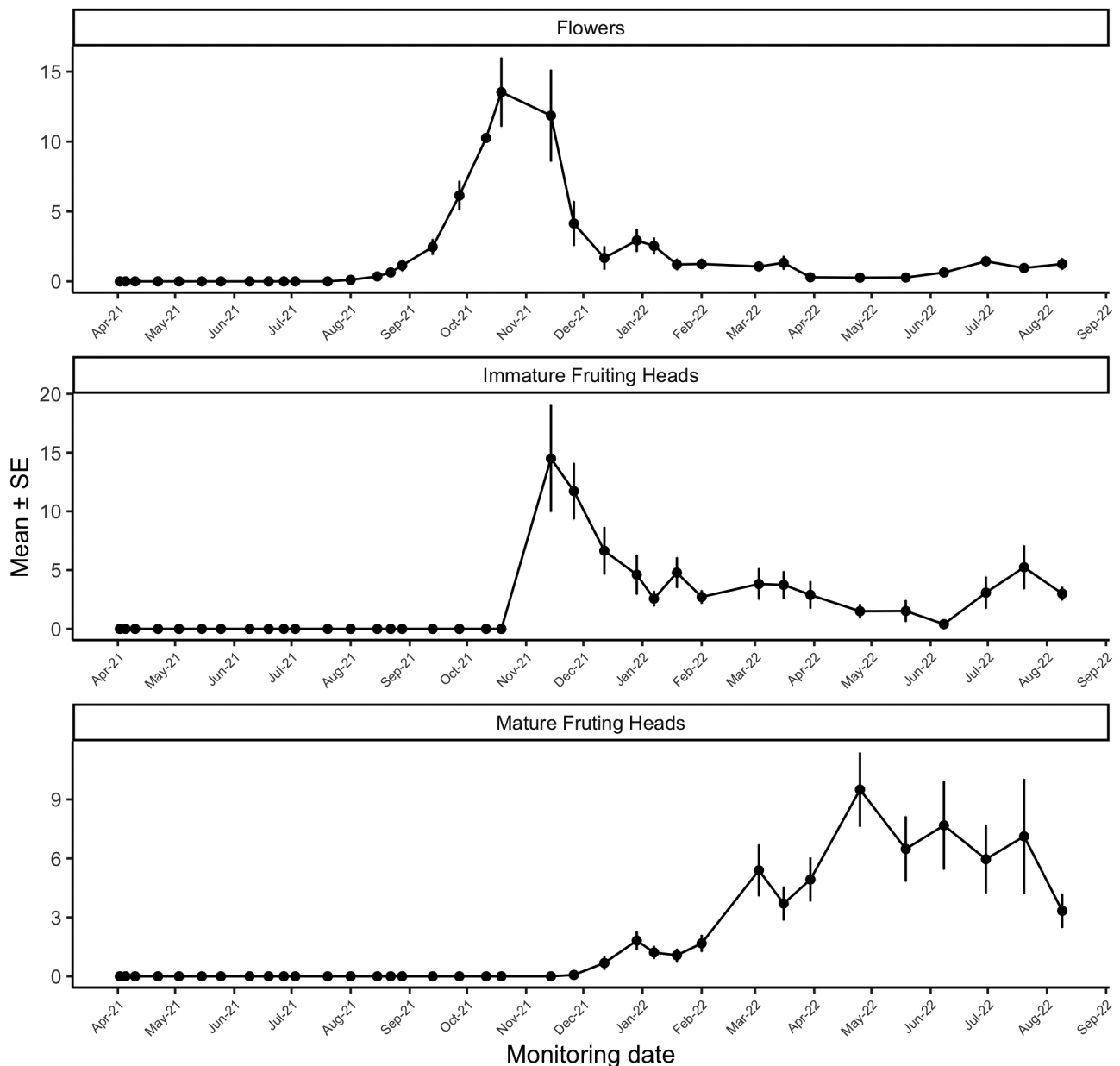


Fig. 4. Phenological patterns of propagated *Lecocarpus lecocarpoides* from Punta Manzanillo, showing number of flowers, immature fruiting heads, and new mature fruiting heads per plant through time. Data are mean \pm SE

tion and herbarium processing protocols (De-Vitis et al. 2020). Particularly, the uniformity of collection and storage protocols over the past 21 yr cannot be fully assured. However, we have no evidence of systematic differences between populations in these factors.

The germination percentage of *L. lecocarpoides* seeds in our study was similar to the results of viability tests, indicating that our seed scarification technique results in the germination of the majority of viable seeds. While we lack seed viability data for our 2020 Punta Manzanillo seed sample, we have reported

viabilities from other studies as well as our viability results for similarly aged seeds from other populations. Atkinson et al. (2008) previously reported a seed viability of approximately 80% for *L. lecocarpoides* populations excluding Punta Manzanillo (Atkinson et al. 2008). We found a seed viability of 67% for 2019 Xarifa seeds and 63% for 2021 Oeste seeds. We obtained a 75% germination rate with the 2020 Punta Manzanillo seeds, suggesting that if the seed viability resembled that of other populations, then most or all viable seeds germinated. Thus, the scarification

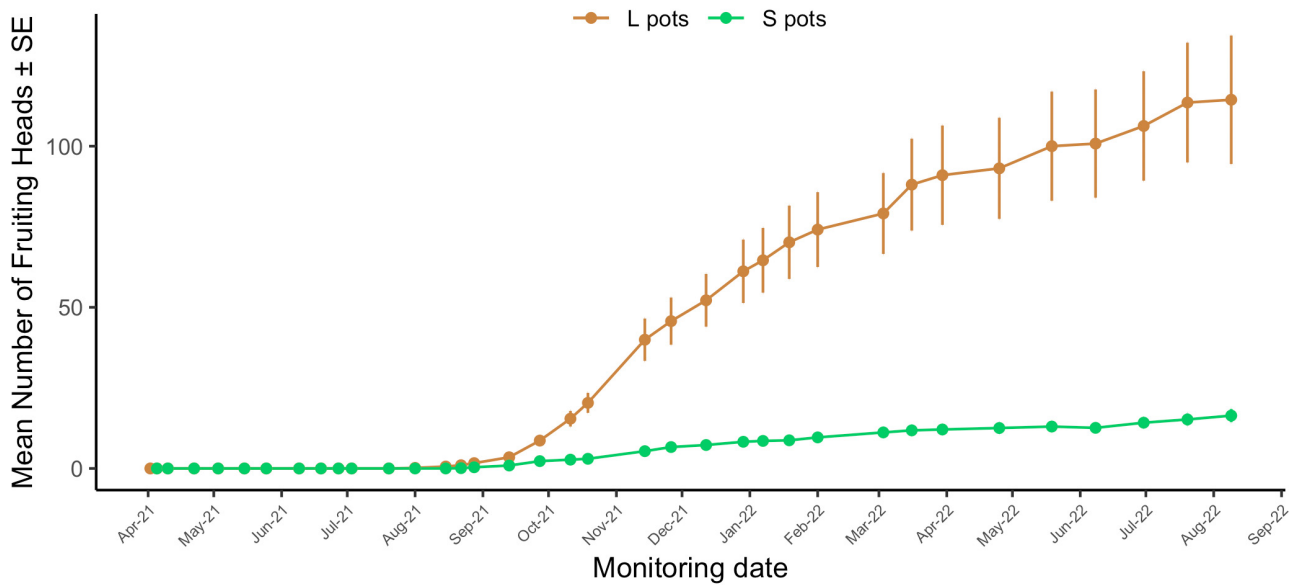


Fig. 5. Cumulative number of fruiting heads produced by propagated *Lecocarpus lecocarpoides* plants from Punta Manzanillo, cultivated in small (S) or large (L) pots. Data are mean \pm SE

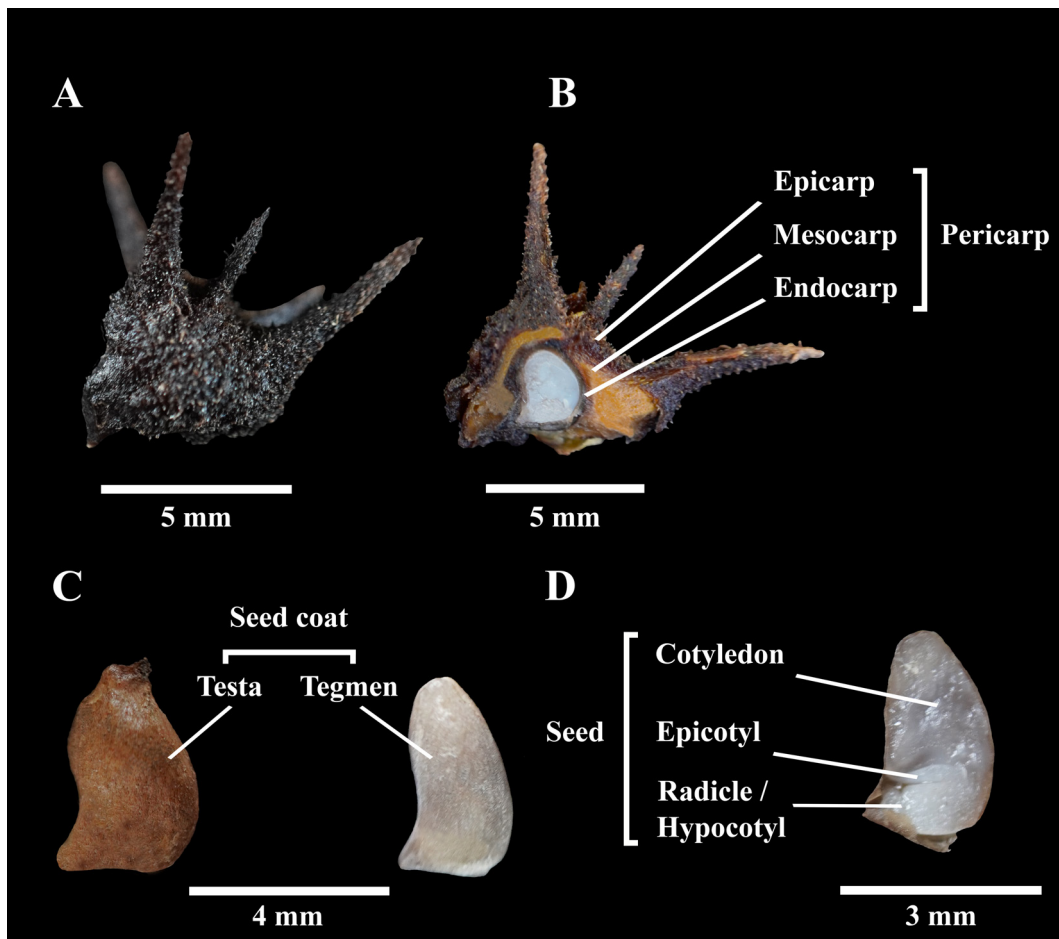


Fig. 6. *Lecocarpus lecocarpoides* fruits and seeds. (A) Lateral view of the whole fruit showing external morphology. (B) Longitudinal dissection of the fruit highlighting the pericarp layers: epicarp, mesocarp, and endocarp. (C) Seed coat components: testa and tegmen. (D) Seed inner anatomy showing cotyledon, epicotyl, and radicle/hypocotyl

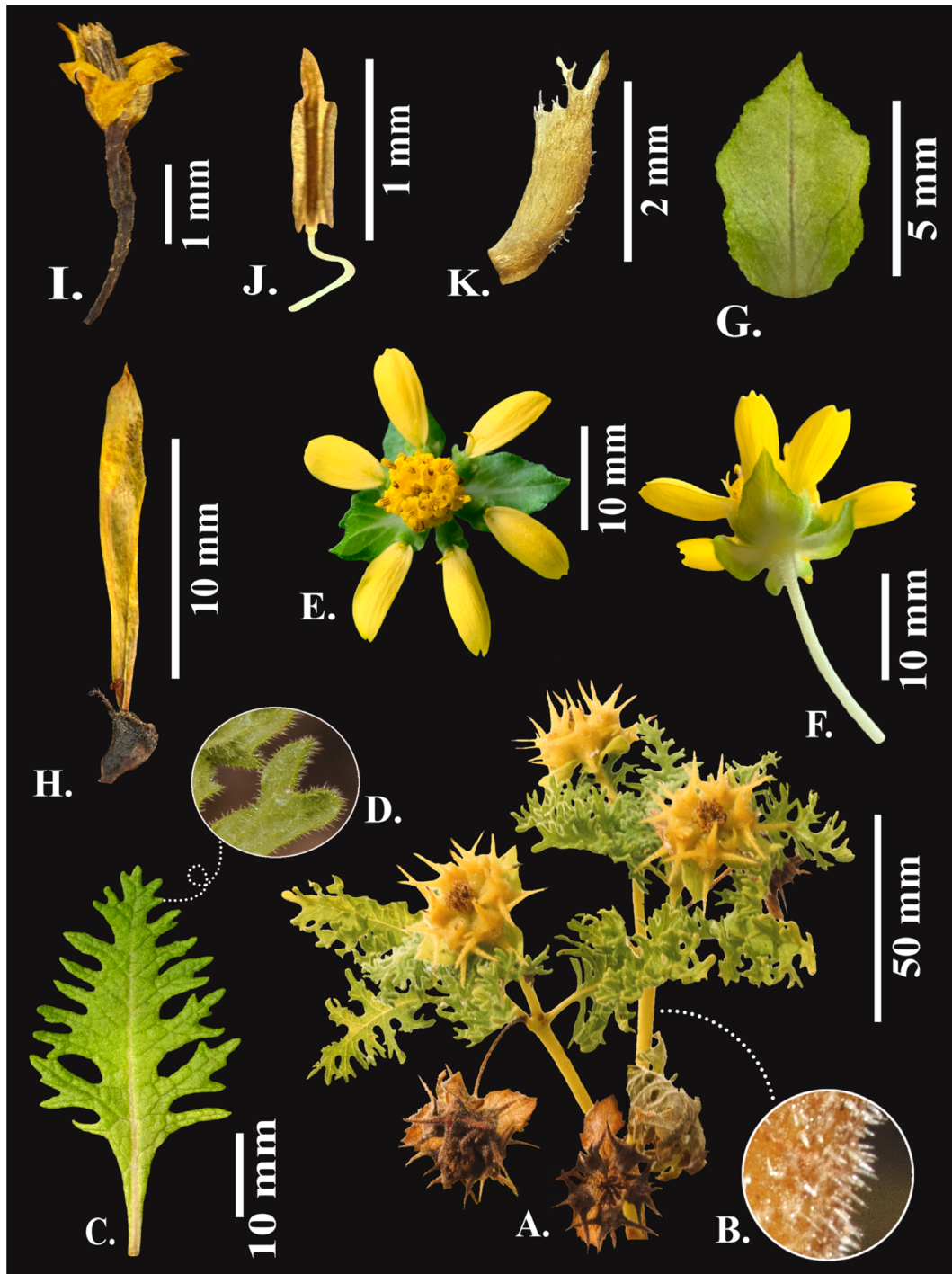


Fig. 7. Lankester composite dissection plate of *Lecocarpus lecocarpoides* subsp. *lecocarpoides*. (A) Branchlet, showing some seeds. (B) Stem, trichomes. (C) Leaf, adaxial surface. (D) Leaf, abaxial surface showing trichomes. (E) Inflorescence, front view. (F) Inflorescence, back view. (G) Bract, adaxial surface. (H) Ray flower. (I) Disk flower. (J) Anther, inner side. (K) Palea, lateral view. Note: Rehydrated herbarium material from specimen Jaramillo, P. 6872, CDS 58953A (H–K). Created by N. Espinosa-Ortega

technique employed is likely advantageous, but additional experiments are needed to determine whether it is necessary. If successful, direct seeding in the field could eliminate the risk of accidentally

transporting organisms from other islands, reduce the high costs associated with *ex situ* propagation, and allow more flexibility in the timing of interventions (Lázaro-González et al. 2023).

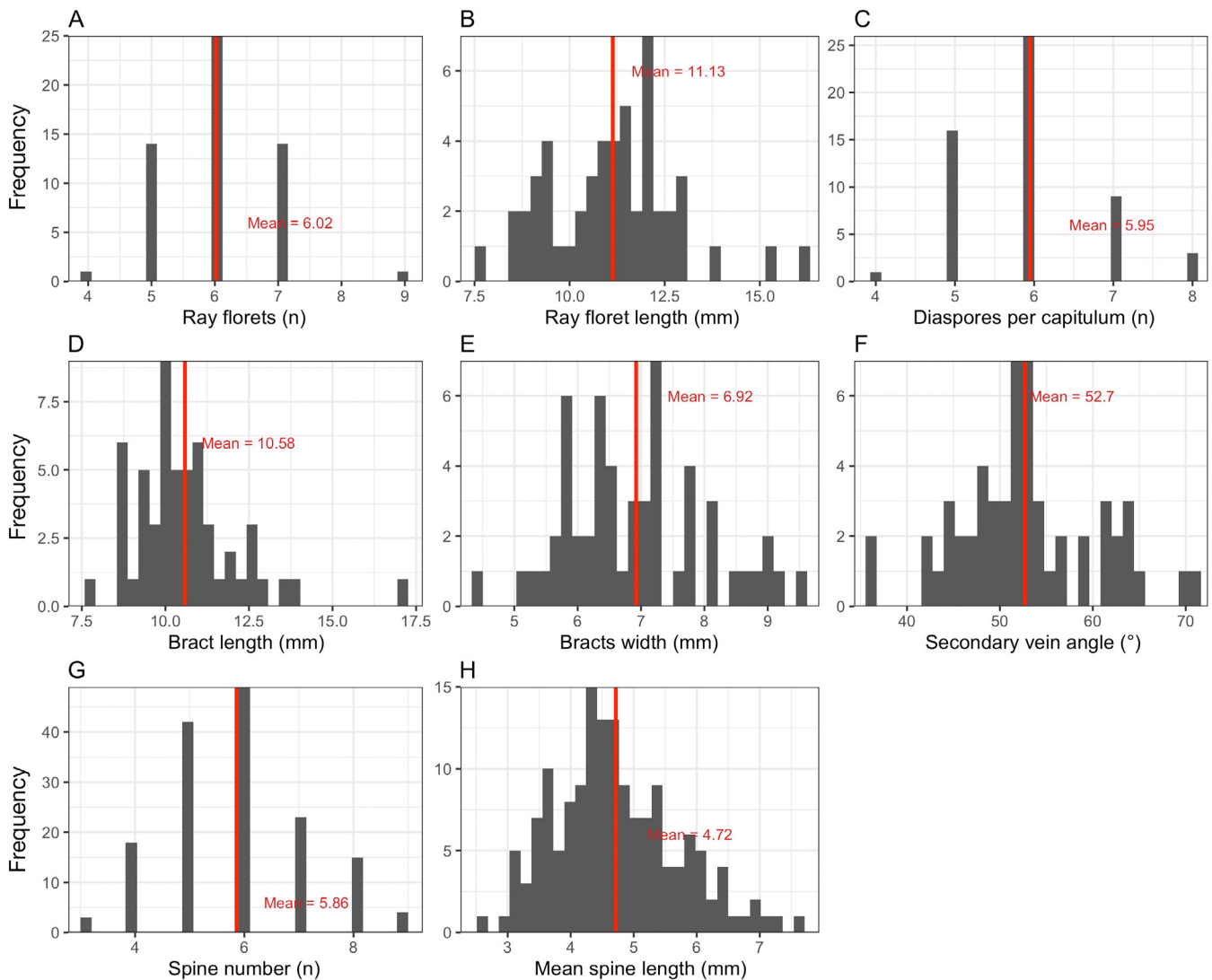


Fig. 8. Distribution of morphological traits of *Lecocarpus lecocarpoides* plants from Punta Manzanillo

An unexpected finding was the longevity of some of the germinated plants, surpassing the 2 yr mark while continually reproducing until senescence. We observed a similar occurrence in Punta Manzanillo, with a wild plant that exhibited flowers and mature fruits when we encountered it in November 2021. By March 2023 it seemed to have recently died but it still had many fruits attached. This observation challenges the characterization of this species as annual (Adersen 1989, Atkinson 2007). It is more accurately classified as a short-lived species with a lifespan of up to 2 yr. For annual plants, conservation efforts may focus primarily on seed collection and annual reintroductions. However, for a species with a longer lifespan, increasing the survival of established plants provides another strategy to maintain and enhance populations over the long term.

At the CDRS, *L. lecocarpoides* individuals in larger containers reached approximately twice the size of those in smaller pots, accompanied by an approximately 8-fold increase in fruit production. Mauchamp et al. (1998) reported adult plants in Punta Manzanillo with diameters of 6–16 mm and heights of 20–40 cm, aligning more closely with the dimensions of the small pot individuals in our study. Herbarium specimens and field observations further support the notion of Punta Manzanillo individuals being smaller. These observations suggest that enhancing growth conditions at Punta Manzanillo could lead to larger plants and increased fruit production, which could increase the soil seedbank, promoting population recovery there. Such enhancement could include treatments that improve soil conditions, such as biochar or the water-saving technologies tested by the Galapagos Verde

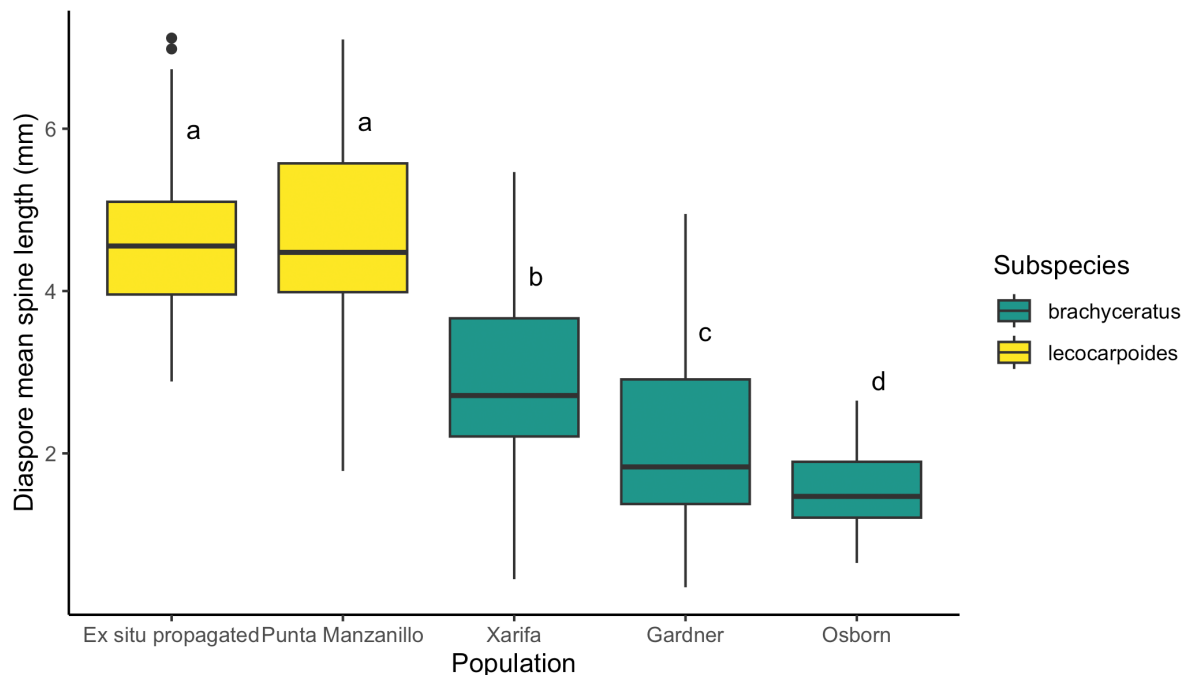


Fig. 9. Boxplot of diaspore mean spine length of *Lecocarpus lecocarpoides* by population. Horizontal lines represent the median, boxes indicate interquartile range, lower and upper boundaries representing the 1st and 3rd quartiles, respectively, minimum/maximum whisker values calculated as $Q1/Q3 \pm 1.5$ IQR, dots are outliers. Lowercase letters: significant differences between groups ($\alpha = 0.05$). Groups sharing a letter are not significantly different from each other

2050 Program (Tapia et al. 2019, Jaramillo et al. 2020, Jaramillo 2021, Negroita et al. 2021).

Moreover, counts of mature fruiting heads demonstrate the reproductive capacity of this species under favorable conditions. These data, together with the data on seed viability, germination and mortality can serve as a first step towards developing a population model for *L. lecocarpoides*. A next step would be to gather field data regarding its life stages in its natural habitat. Such a model would help predict population trends under different scenarios, including alternative conservation strategies (Vitt et al. 2009), and could be utilized to estimate species extinction risk for an updated Red List evaluation (IUCN 2017). This species currently appears as Vulnerable in the IUCN Red List (<https://www.iucnredlist.org/species/39145/10170322>) but as endangered in Ecuador's regional Red List (León-Yáñez et al. 2011). The global assessment lacks important details, such as a distribution map, population trend, and number of mature individuals. Therefore, an updated assessment that meets global IUCN criteria is needed to establish its conservation status, though this is outside the scope of the present study.

Based on the most recent taxonomic key for the *Lecocarpus* genus (Tye & Jaramillo 2022), the diagnostic traits that differentiate *L. lecocarpoides* from other species of *Lecocarpus* are the number of ray florets and

the length of the ray limb. Our findings support this regarding the number of ray florets, but not ray limb length, which seems rather to be influenced by environmental conditions. Sønderberg-Brok & Adersen (2007) suggested not employing corolla length in keys to the *Lecocarpus* genus, as it can be quite variable. Thus, number of ray florets is a more dependable diagnostic trait than ray limb length.

Tye & Jaramillo (2022) identified diaspore spine length as the key distinguishing trait between *L. lecocarpoides* subspecies. They characterized subsp. *lecocarpoides* as having mature diaspores with spines measuring up to 6 mm, while the spines of mature diaspores of subsp. *brachyceratus* are ≤ 3 mm in length. Our results correspond very closely to these descriptions. A molecular genetic study could provide more robust evidence for the degree of separation of the 2 subspecies and clarify evolutionary pathways. No genetic studies of evolution and speciation within the *Lecocarpus* genus have been undertaken, but 3 nucleotide sequences of *Lecocarpus* have been used for phylogenetic research on broader Asteraceae groups (Rauscher 2002, Blösch et al. 2009), suggesting existing potential for such research.

Atkinson (2007) recommends 2 sites for the establishment of new *L. lecocarpoides* populations, one near Punta Manzanillo and another opposite to Oeste Islet.

Establishing another population on Española could decrease the likelihood of local extinction caused by catastrophic events, habitat loss, or disease affecting the only existing population. However, it would be wise to determine the subspecies of the Oeste population before establishing a new population near it, as it could mix genetically distinct populations.

This study has shown that diaspore spine length differs between populations of *L. lecocarpoides*. However, the authors did not identify any patterns between the variation in diaspore spine length and ecogeographic features. Whether this difference is caused by adaptive radiation or genetic drift remains unclear. It is possible that the spines evolved as defense or dispersal mechanisms, conferring greater advantages in certain populations. For instance, *Tribulus cistoides*, another plant species found in the Galapagos with very similar diaspore morphology, was found to have increased mericarp depth on islands with vertebrate seed predation (Reyes-Corral et al. 2023). It is also possible that genetic drift, particularly influential in small and isolated populations, might have been a greater driver (Sønderberg-Brok & Adersen 2007). Again, obtaining data from the Oeste population could help clarify any trends.

5. CONCLUSIONS

We can draw the following conclusions from this study. (1) Seed scarification and soaking result in high germination rates in *Lecocarpus lecocarpoides* and may contribute to expanding populations of this endangered species. (2) *L. lecocarpoides* seeds stored in controlled herbarium conditions can remain viable for up to 20 yr according to tetrazolium viability tests. Therefore, seed banking could be important for conservation and restoration efforts with this species. (3) In *ex situ* plant propagation efforts, the growth and total fruit production of *L. lecocarpoides* can be greatly affected by pot size, an important consideration for securing fruits for restoration and research purposes. (4) Morphological data support the recognition of *L. lecocarpoides* subsp. *brachyceratus* as a separate subspecies, highlighting the need to consider the main island and Gardner Bay islet populations as 2 separate conservation units in future management plans. However, genetic studies are needed for a more robust assessment of taxonomic boundaries. (5) Establishing a second population of *L. lecocarpoides* on Española Island would be beneficial for its conservation. However, before establishing any populations near Oeste Islet, its subspecies needs to be determined to avoid mixing genetically distinct populations.

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