The First Ex-Situ Germination and Dispersal Mechanisms of the Rare, Critically Endangered Tree, Pleodendron costaricense

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The First *Ex-Situ* Germination and Dispersal Mechanisms of the Rare, Critically Endangered Tree, *Pleodendron costaricense*

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Abstract

Background and Research aims: The extinction of relict and rare tree species is accelerated by habitat loss and climate change. *Pleodendron costaricense* is a critically endangered tree, with only four mature individuals known in Southern Pacific Costa Rica. With the discovery of three additional trees, we set out to learn more about *P. costaricense's* natural history and attempt the first successful germination.

Methods: We collected fruits from two trees and carried out preliminary germination trials in a nursery at the study site. We also used camera traps in one of the fruiting mother-trees to understand natural dispersal mechanisms of the species.

Results: Although plagued by excessive levels of invertebrate predation, we were able to germinate and produce 59 saplings ready for restoration planting. Five mammal species were detected on the camera traps feeding on the fruits, along with one primate potentially using the fruits as a topical medicine.

Conclusion: *P. costaricense* can be propagated *ex-situ*, potentially with greater success using stimulating hormones. To improve production rates, future efforts should focus on the protection of germinating seeds and saplings from seed predators. We also identified numerous potential natural mammalian seed dispersers, mostly in the family Procyonidae.

Implications for Conservation: Given the propagation knowledge we have developed, the active restoration efforts of the saplings by Osa Conservation to help increase population numbers, and the strict protection of the two fruiting mother trees, there is now the possibility to attain a positive conservation outcome for this critically endangered species.

Keywords

Canellaceae, germination, Osa Peninsula, kinkajou, olingo, seed dispersal, camera traps, zoopharmacognosy, green list

Introduction

Relict and rare tree species represent evolutionary heritage for plant conservation (Pardo et al., 2018; Tang et al., 2017). Often taxonomically isolated, they provide a unique opportunity to understand both past and recent biogeographical and evolutionary process (Kozlowski et al., 2012), as for millions of years these plants have been able to cope under changing environments on earth (Garfì et al., 2011; Tang et al., 2011). In addition to their scientific and phylogenetic value, relict tree species can also have high ethnobotanical value to both people and wildlife (e.g., Fernandez et al., 2005; Senkoro et al., 2020).

However, recently described, or undescribed species—many of which are rare—are those at the greatest risk of

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extinction (Liu et al., 2022). This is mostly due to major habitat loss and the rapid rate of climate change (Botanic Gardens Conservation International, 2021; Enquist et al., 2019; Kozlowski et al., 2012; Vincent et al., 2020). To address this risk for plants and trees, the Global Strategy for Plant Conservation (GSPC) aims to protect 75% of threatened plants *in-situ* and 75% in *ex-situ* conservation collections, preferable in countries of origin (see the Convention on Biological Diversity, 2012). This accompanies the target to ensure that at least 20% of threatened plant species are available for recovery in restoration programs.

This is challenging when the most basic ecology, distributional knowledge, and propagation techniques are poorly known for rare species—and so broader conservation strategies are challenging to implement. Knowledge of the reproductive ecology, for example, is critical to predict potential population survival ability and implement viable conservation efforts. Effective seed dispersal is essential for species to survive the threats of habitat loss, fragmentation, and climate change—to enable gene flow between existing populations and colonization of new habitat (Di Musciano et al., 2020; Renton et al., 2012).

Canellaceae is one of the most threatened angiosperm families in which several species are endangered and at risk of extinction. Of these, *Pleodendron costaricense* is an extremely rare, lowland rainforest tree, known only from two sites along the southern Pacific Costa Rica (Hammel & Zamora, 2005). This *living fossil* (termed as such by Hammel and Zamora, 2005) belongs to the earliest angiosperms. It was first collected in 1998 and kept botanists puzzled until collections of better material and DNA analysis placed the plant within the Canellaceae, and in 2005 it was described to species. *Pleodendron costaricense* is disjunct by about 2000 km from its presumed closest relative, *P. macranthum* in Puerto Rico (Hammel & Zamora, 2005). Although this alone is insufficient to reveal the biogeographical history of this species, the pollen of *Pleodendron* has been reported from the middle-Oligocene of Puerto Rico.

Until our work, only four large trees and one sapling were known in the wild. Due to the rarity and small population size, *P. costaricense* has been categorized as Critically Endangered by the International Union for Conservation of Nature (IUCN) red list (Rivers, 2019). Only two of the first four adult individuals produced fruits, and *ex-situ* germination attempts have so far been unsuccessful. All individuals were found at the edge of a road, outside of National Parks, and it is currently absent from any botanical gardens or arboretum collection. There is also a lack of any evidence of natural regeneration, the reason for which is unknown. Like most tropical forest tree species, there is no information about the seed natural history, ecology, or dispersal mechanisms.

Given the known existence of just four adult individuals in the wild, of which only two are mature, the absence of any known natural regeneration, and the vulnerable location outside of strict protected areas, there is a need to act quickly to prevent this relict tree from soon appearing on the list of extinct tree species. The production of baseline information, including a thorough understanding of the distribution, threats, germination techniques, and ecology of *P. costaricense*, and other highly threatened tree species generally, can enable the development and implementation of conservation actions. This can shift the thinking from the context as a rare and Critically Endangered red listed species on the cusp of extinction, toward an optimistic green list framework, where conservation actions and a positive conservation legacy can be attained (see Akçakaya et al., 2018).

Here we leverage the discovery of three additional individuals (two mature and fruit-bearing), all within a private protected reserve, to report and provide key information on the following: 1. The first preliminary successful germination *ex-situ* of this rare and threatened species. 2. Different pre-germination treatments. 3. The potential dispersal mechanism of *P. costaricense*. And 4. We discuss potential threats that might be limiting the trees recovery but look toward how the learning developed here can be leveraged to implement conservation actions that could result in a positive conservation legacy for *P. costaricense*.

Methods

Study site

The Osa Peninsula in southwest Costa Rica is home to the largest remaining tract of Pacific lowland rainforest in Mesoamerica (Holdridge, 1967) and hosts four protected areas: Piedras Blancas and Corcovado National Parks, the Reserva Forestal Golfo Dulce, and the Terraba del Sierpe Wetland (see Figure 1). The small but megadiverse peninsula is home to approximately 455 species of native trees, of which ~4.8% are endemic to the Osa Peninsula and the adjacent mainland of Costa Rica (see Cornejo et al., 2012).

Tree Discovery

During a series of botanical expeditions and standardized surveys in March to June 2020, no individuals of *P. costaricense* were detected (see Text S1 for details). However, while incidentally exploring the Cerro Osa area between expeditions in April 2020 (part of the Osa National Wildlife Refuge managed by NGO Osa Conservation), co-author and Costa Rican botanist Leonardo Álvarez-Alcázar located a single mature individual of *P. costaricense*, in flower (named tree #1; see Figure 2 for more detail of the specimen found). Later, in August, two more individuals were found by searching the area nearby, one of which was in peak fruit production (named tree #2). The third tree was still young and not of fruit-bearing age. The three individuals discovered are located close together (within 50m) and growing along a stream in a sloping old-growth riparian forest. At one time, this riparian patch was surrounded cattle pasture in the 1960s but is in the process of recovery and now covered by secondary forest (see Whitworth et al., 2018, 2021 for greater detail).
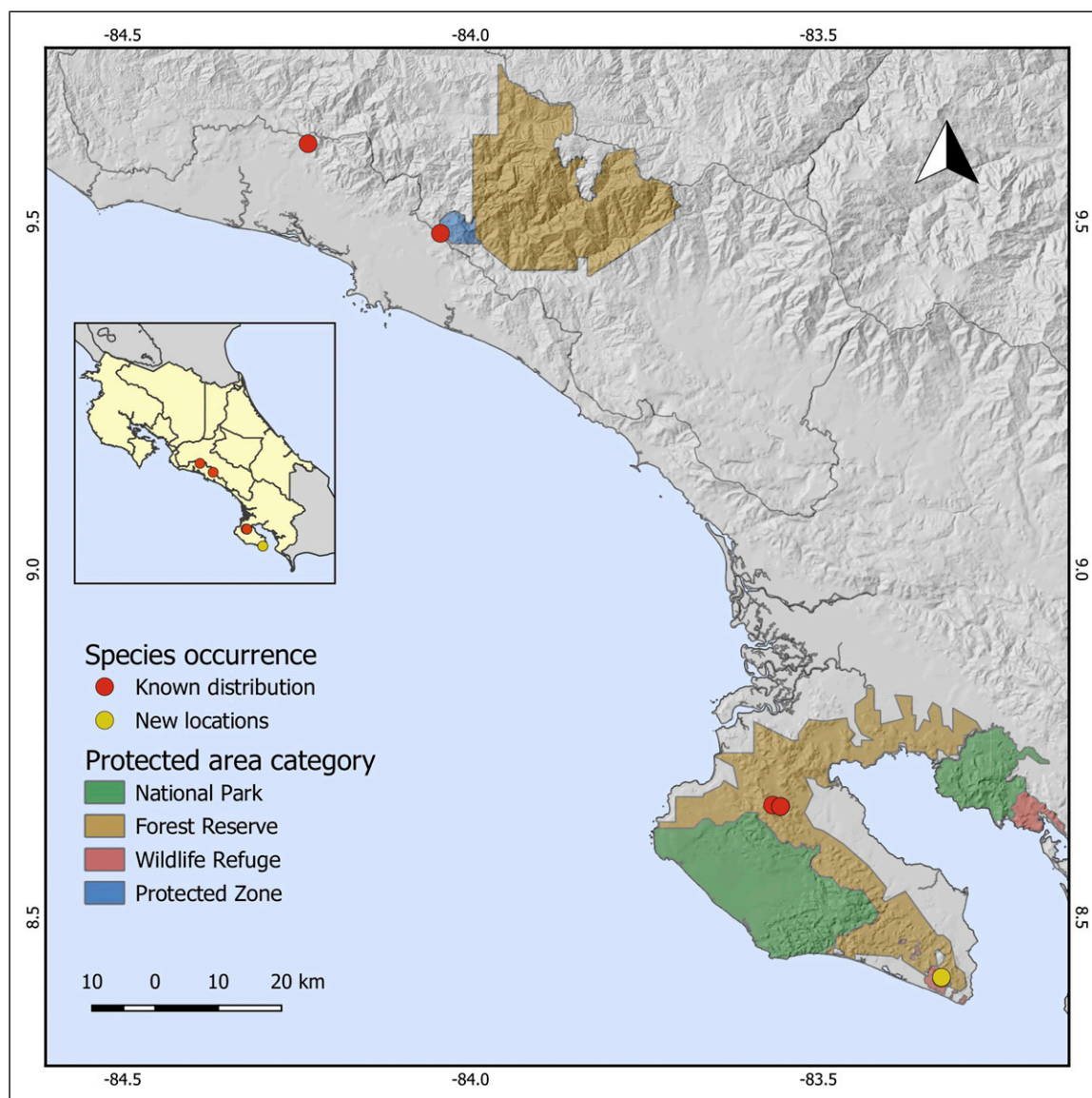
Figure 1. Study site and location of individuals of *Pleodendron costaricense* recorded in the southern Pacific.
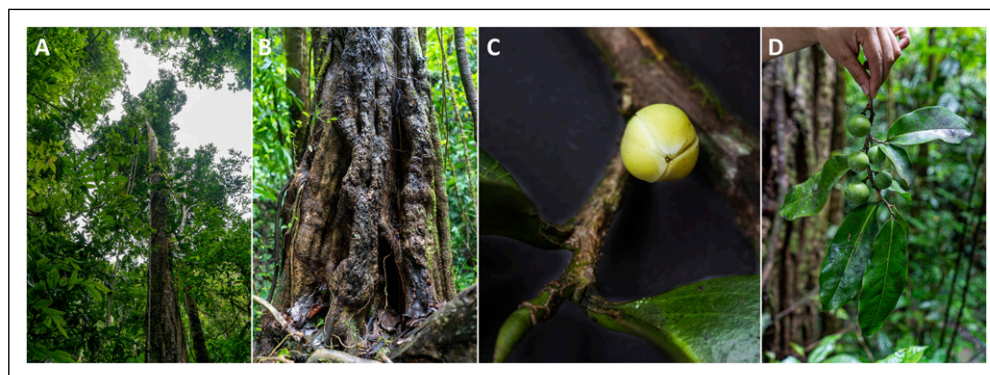

Figure 2. Tree, trunk, flowers, and fruits of *Pleodendron costaricense*. (a) Tree, 15–35 m tall; (b) external bark gray and white, weakly fissured and scaly. (c) Flowers solitary, or in fasciculate clusters, axillary or slightly supra-axillary. (d) Leaves distichous; fruit a berry, oblate, green, smooth, and slightly glaucous.
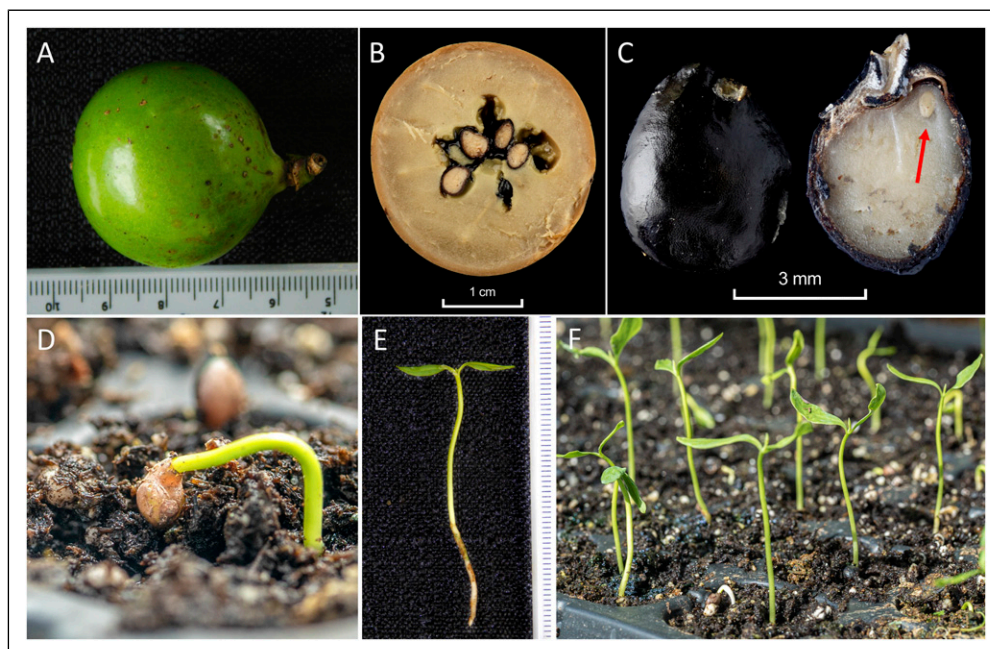
Figure 3. Fruits and germination process of *Pleodendron costaricense*. (a) Fruit; (b) cross-section of fruits showing the disposition of the seeds; (c) median longitudinal section of a seed, the red arrow points the undeveloped embryo; (d) epigeal germination; (e) seedling morphology; and (f) seedlings in a tray—those with gibberellic acid as pregerminated treatment.

Sample collections and seed processing

Once identified and located, we started monitoring the phenology of the trees by visiting weekly. At the beginning of May, tree #1 began to drop immature fruits. In June–August, the fruits were ripe (note that we only discovered tree #2 when in full fruit in August). Samples with fertile material were obtained from the fruit-bearing tree #1, and deposited in the National Herbarium of Costa Rica (Herbarium codes = R. Pillco & W. Whitworth 25 and L. Alvarez & M. Lopez 303). Fruit samples were sent to the Lankester Botanical Garden to take high-resolution photographs and details of the seeds (see Figure 3). We collected mature fruits by climbing the trees. Ripe fruits were easily recognizable by their larger size and change from green to yellowish coloration and becoming soft to the touch.

Seeds were extracted by squeezing fruits and dropping the pulp with the seeds in a container with water, and later separated from the pulp using mesh. We discarded some of the non-viable seeds from the overall collected seeds with a simple pre-screening—which involved discarding floating seeds in water (Di Sacco et al., 2018). In June, with the seeds collected from tree #1 and in July with the seeds from tree #2, we carried out a seed quality test. This test consisted of sectioning a total of 337 seeds with a scalpel (263 seeds from tree #1 and 74 seeds from tree #2) and examining under a magnifying glass. The seed embryo was evaluated, and the number of full, empty, and infested seeds was recorded. The proportion of filled seeds corresponds to the proportion of potentially viable seeds within the collected sample (proportion of filled seeds = # of filled seeds/# of cut seeds; see Di Sacco et al., 2018).

Seed germination trials

Preliminary germination trails with 300 seeds were established in the greenhouse at the Osa Biological Station (8.40388 *N*, 83.33,661 W; located approx. 3.7 km from the trees). The seeds were planted in trays, maintained in the greenhouse under a plastic transparent roof, which permitted exposure to natural light. The greenhouse had an ambient temperature of 33°C and 1500–2000 lux sunlight. Trays were watered automatically once a day for 4 minutes to keep the substrate sufficiently humid, but not saturated or waterlogged.

In addition to the basic standard water–soil germination efforts, we began trialing and testing different substrates, phytohormones and chemical compounds. At the family level, Canellaceae is known to display dormancy (C. Baskin & J. Baskin, 2004, 2005). Studies attempting to understand seed germination and dormancy have been carried out for other threatened species, such as *Warburgia salutaris*. Another example involved the use of gibberellic acid (GA_3) as pre-germination treatment for *Drimys granadensis* (Winteraceae), which produced promising germination

results of ~41.3% (see Castañeda-Garzón & Pérez-Martínez, 2017).

Therefore, we use pre-treatments to facilitate germination. The soaking elements we had readily available and on-hand to trial were gibberellic acid (GA_3), sulphuric acid (H_2SO_4), and hydrogen peroxide (H_2O_2), each of which has proven useful in initializing germination and breaking seed dormancy (Gupta & Chakrabarty, 2013; Luna et al., 2014; Morais et al., 2014; Wojtyla et al., 2016). We also use two commonly used household cleaning and disinfecting agents, vinegar and bleach, commonly used to disinfect seeds, but which have also proven effective in breaking seed dormancy (Elezz & Ahmed, 2021). We also wanted to test as many as possible substrates in these field trials to determine if any might have an obvious effect, these were compost (CO), soil with chopped grass (SG), vermicompost (VC), riverbed sand (RS), and forest mulch (FM).

Twenty-two germination treatments were established in the greenhouse. In each seed cohort, germination was checked every week until no seedling emergence appeared 4 weeks after the last seedling count was made. At the end of the final counting day, we inspected non-germinating seeds for viability by cutting them in half and checking them for the presence of the endosperm in viable seeds. Given the large percentage of predation, only the number of seeds left after the seed predation events were considered to calculate the germination percentage. The final percentage of germination (%G = # of germinated seeds/number of seeds sown), the germination period, and the difference in the number of days between initial and final emergence were recorded (see Bhardwaj, 2013).

Identification of seed dispersers

To monitor potential seed dispersers of *P. costaricense*, five camera traps (models; Reconyx Ultrafire XR6, Bushnell Trophy Cam and Bushnell Core No Glow) were placed at tree #1, from the lower to the upper canopy, facing branches covered in fruits to increase detection likelihood. All cameras were programmed to take 20s video with a 10s resting period to maximize battery life (Bowler et al., 2017; Meek et al., 2014; Montagna et al., 2018; Whitworth et al., 2019). To record terrestrial dispersers, 30 ripe fruits were collected from the ground and piled under the target tree's crown (tree #1), and a camera trap was installed to focus on the fruit pile (programming identical to that in the canopy). Cameras were set in May 2020, when many unripe fruits were still present in the crown, until August 2020, when all fruits were gone. The camera trap encounter rate was calculated as the number of videos/100 camera trap days for each given species. Videos of the same species at the same location within 30 minutes of a previous detection were excluded from the calculation, as to capture separate

independent encounter events (see Arévalo-Sandi et al., 2018; Day et al., 2016; Michalski et al., 2015).

Results

Seed processing

On average, *P. costaricense* contained 25 seeds per fruit (min. 15, max. 39). The approximate size of the seeds was 4–5 x 4 mm, ovate, somewhat reniform, black, shiny, with the funicular scar printed and rounded. The seeds presented an underdeveloped (small) embryo (see Figure 4), and the proportion of potential viable seeds was ~0.57 (337 seeds dissected, 95% CI 0.52–0.62, SE 0.027).

Seed germination trials

We experienced an extremely high percentage of predation in the treatments carried out. The preliminary field germination trials with 300 seeds resulted in zero germination after 4 weeks, shortly after which all seeds were predated (see Text S2 for details on predation observations).

Due to these extremely high and unexpected predation rates by invertebrates (>95% for 11 treatments), of the 22 treatments only 10 had at least 10 seeds left none-predated. Eight other species of trees were also in the germination house at this time, all of which remained completely intact and free from seed predation. The pre-germination treatments with sufficient none-predated seeds to be tested for germination success can be observed in Table 1 (see Table S1 for the full list including those with excessive predation).

The highest germination percentage and fastest germination period were obtained in seeds soaked in Gibberellic acid for 24 hours (T4, 91% germination—see Table 1). This was followed by seeds soaked in H_2O_2 x10 min (T8, 43.5%), and seeds soaked in water x12h (T17,19.9%). Germination started 28 days after sowing seeds soaked in GA_3 (T4), H_2O_2 (T8), and for two seeds soaked in a wet towel with water (T22). Seeds soaked directly in the water germinated on day 56 and day 126 (see Table 2). Germination continued until days 42, 49, 84, and 161 in T4, T8, T18, and T17, respectively. Although the second GA_3 treatment (T5) had 23 seeds that failed to germinate at all, subsequent opening of the seeds showed that they were empty and likely non-viable. Due to the predation levels, it was not notable as to any obvious effect of substrate on germination rates (additional controlled trials would be necessary).

Identification of seed dispersers

In total, six species of mammals were recorded on the arboreal camera traps, capturing 247 independent records (>30 mins apart) over 198 camera-trap nights. The recorded species were: *Potos flavus*, *Bassaricyon gabbii*, *Cebus imitator*, *Nasua narica*, *Caluromys derbianus*, and an

Figure 4. Snapshots from camera trap of visitors and seed dispersal of *Pleodendron costaricense*. (a) *Potos flavus* (kinkajou); (b) *Bassaricyon gabbii* (olingo); (c) *Cebus imitator* (white-faced capuchin), (d) *Caluromys derbianus* (Central American woolly opossum), (e); *Nasua narica* (White-nosed Coati), and (f) *Didelphis marsupialis* (Common opossum).

unidentified rodent. From these recorded species, only three species interacted directly with the fruits.

Two strictly arboreal mammal species were recorded feeding on *P. costaricense*; *Potos flavus* (kinkajou, Procyonidae, LC) and *Bassaricyon gabbii* (olingo, Procyonidae, LC; see Figure 4 & Appendix video S1). From the 247 independent detections, 82% belonged to these two nocturnal mammal species, kinkajou (182) and olingo (35). Until mid-July, both kinkajous and olingos spend most of their time searching for ripe fruits. This behavior is evident by the fact that these two nocturnal mammals visit different fruits repeatedly and only eat them when they are mature and soft.

Table 1. Germination and predation summary of the treatment trials of *P. costaricense* seeds. Rows in bold indicate the five treatments that displayed germination of at least some seeds.

Treatment	Tree Source	Substrate	Soaking Element	Soaking Duration	Seed Quantity	Predated Seeds	Remaining Seeds	Germinated Seeds	Not Germinated Empty Seed	Not Germinated Full Seed	Germination %	Germination Period
T2	2	SG + VC	Control	na	252	238	14	0	4	10	0	na
T4	**2**	**SG + CO**	**GA3 (1000 pmm)**	**24 hrs**	**266**	**196**	**70**	**64**	**5**	**1**	**91**	**14**
T5	1	SG + FM	GA3 (1000 pmm)	24 hrs	71	48	23	0	23	0	0	na
T8	**2**	**SG + CO**	**H2O2**	**10 min**	**408**	**385**	**23**	**10**	**10**	**3**	**43.5**	**21**
T9	2	SG + VC	H2O2	10 min	196	181	15	0	10	5	0	na
T12	1	SG	Vinegar (5%)	24 hrs	294	266	28	0	10	18	0	na
T15	1	CO	Water	12 hrs	243	220	23	0	21	2	0	na
T17	**1**	**RS + FM**	**Water**	**12 hrs**	**500**	**359**	**141**	**28**	**108**	**5**	**19.9**	**35**
T18	**1**	**SG**	**Water**	**12 hrs**	**528**	**490**	**38**	**3**	**30**	**5**	**7.9**	**28**
T22	**2**	**SG + VC**	**Wet towel with water**	**7 days**	**72**	**60**	**12**	**2**	**7**	**3**	**16.7**	**na**

*CO (compost), SG (soil with chopped grass), VC (vermicompost), RS (river sand), FM (forest mulch), GA_3 (Gibberellic acid), H_2SO_4 (Sulphuric acid), H_2O_2 (Hydrogen peroxide).

Table 2. Seed germination times for the five treatments that each had some germinating seeds. Seed time indicates the date that the seeds were put to germinate. Surviving seedlings relates to the number surviving after 4 weeks, following the predation events by snails and other mortality events.

Treatment	Tree Source	Soaking Element	Seedtime	28	35	42	49	56	84	126	140	154	161	Germinated Seeds	Survival Seedlings
T4	2	GA3 (1000 pmm)	8/12/2020	1	45	18	0	0	0	0	0	0	0	64	30
T8	2	H2O2	8/12/2020	3	4	0	3	0	0	0	0	0	0	10	7
T17	1	Water	6/29/2020	0	0	0	0	0	0	22	4	1	1	28	21
T18	1	Water	6/29/2020	0	0	0	0	1	2	0	0	0	0	3	1
T22	2	Wet towel with water	8/12/2020	2	0	0	0	0	0	0	0	0	0	2	0
Total														107	59

They bite the fruit, swallow down the pulp and seeds, and throw away the peels. When climbing we found some fruits empty but still attached to the branches. The capture rate from the cameras (independent records per 100 days) peaked in mid-July and started decreasing by mid-August. Olingo capture rate increased toward mid-August, while kinkajou capture rates increased towards mid-July (see Appendix Figure S1).

When the fruits were still immature (mid-June), an individual capuchin monkey (*Cebus imitator*—White-faced capuchin, Cebidae, LC) collected fruits, bit them, and rubbed its body with the fruit. Immediately, four more individuals

joined the activity and started to rub each other in a group frenzy (see Appendix video S2). After 14 minutes, the monkeys left the branch one by one with their fur doused with the fruits' essential oils. After this event, there were four more similar events involving just a single individual performing the same dousing to the fur.

Terrestrial camera traps captured 92 independent events over 82 camera-trap nights of 10 species of bird, and small to medium sized mammals. Only three species consumed the fallen fruits of *P. costaricense*: *Nasua narica* (White-nosed Coati, Procyonidae, LC), *Didelphis marsupialis* (Common opossum, Didelphidae, LC), and *Caluromys derbianus* (Central American woolly opossum, Didelphidae, LC). Each of these three species was only observed feeding on the fruits on a single occasion.

Discussion

We present the first successful *ex-situ* germination of the rare, critically endangered tree, *Pleodendron costaricense*—producing 59 saplings that reached a suitable size for restoration planting in 2021. We discovered three new adult trees, the first *P. costaricense* located within a strictly protected area, taking the known adult population of the species from four to seven trees. We uncover probable seed dispersal mechanisms of the species, determine important information regarding the susceptibility to seed predators, and deliver preliminary information on effective germination techniques. Finally, we discuss how ongoing conservation actions and knowledge generation can now lead to a positive conservation legacy—for a species that likely would have gone extinct with the death of the existing adult trees.

Despite an intensive search for *P. costaricense* along 40 km of transect carried out across different locations, forest types, and elevations, the absence of individuals during these standardized surveys showcases the extremely low abundance and patchy distribution of the species. This is a major challenge for threatened tree conservationists—to locate scarce individuals, which requires substantial field time to carry out botanical searches, often in remote and difficult to access locations. Such plant finding missions play an important role in preventing the extinction of endangered species, by allowing for the subsequent development and implementation of ongoing research and applied conservation efforts (Gillson et al., 2020). Only once located can regular monitoring of their phenology for the collection of seeds take place, and for protection of adult trees be prioritized.

Although we experienced extremely high levels of predation, the germination success that we obtained with gibberellic acid and hydrogen peroxide treatments indicates that the seeds of *P. costaricense* present some degree of physiological dormancy. Gibberellic acid appeared to stimulate high germination rates of *P. costaricense* seeds (91%) and speeds up germination when compared with simple water application. It has been shown elsewhere that GA_3 treatments can break physiological dormancy, inducing seedling germination

(Finch-Savage & Leubner-Metzger, 2006). The seeds soaked in hydrogen peroxide also germinated relatively well (43.5%) and began to germinate in a short period (first germination 28 days). Seeds treated only with water germinated, but with lower rates. In one of the water-based treatments, the first seed germinated at day 28, the other at 56 days, and the other at 126 days. However, the high predation rates destroyed many of our treatments completely, and a standardized and controlled experiment under controlled settings would be useful in future work to consolidate our preliminary findings.

Our seed dissection also suggests that *Pleodendron* may present morphophysiological dormancy (MPD), dormancy evident in seeds with underdeveloped (small) embryos like the ones we detected here dissection (C. Baskin & Baskin, 2014; J. Baskin & Baskin, 2004). If *P. costaricense* displays MPD, and can germinate at a low rate under simple wet natural conditions, then why the near-complete absence of seedlings under the mother trees? We propose that this is a potential case of the Janzen–Connell effect. Of all the fruits that fell to the ground from the two *Pleodendron* trees, our weekly checks detected only one seedling that managed to germinate naturally, *in-situ*, under the canopy of tree #1. This seedling died soon after.

This absence of regeneration beneath the parent trees is likely due to the high predation of seeds by ants, beetles, and cockroaches as we experienced in our trials. The high predation rates we noticed by insects suggests that *P. costaricense* seeds are a rich food source that makes them vulnerable to invertebrate seed predators. According to Hana et al., 2020, the detection probability of seeds by predators could be influenced by the physical property of the coat, or by the level of inhibition of the seeds since inhibited seeds release different amounts of volatile components. For example, in granivore beetles, preferences are driven by the structural properties of the seeds and not chemical properties (Hana et al., 2020). Thus, the chemical properties of *P. costaricense* seeds may be serving as attractants to predators. Seed predators, particularly the ants, may also act as dispersal agents of unconsumed seeds, a well-known mechanism for other tropical forest species when moved to storage sites by ants (Arnan et al., 2012; Handel & Beattie, 1990; Levey & Byrne, 1993). *Labidus coecus*, the species we observed here, appears to have the most widespread diet of all the Ecitoninae. Although *L. coecus* feeds on ants and other arthropods, it has also been observed under fruiting trees (for almost 2 months), consuming fallen fruits, and partially or entirely burying the seeds (Powell & Baker, 2008). If seed fate is so low around these parent trees, then *P. costaricense* likely depends on dispersal agents to move seeds farther from the parents.

The use of arboreal camera trapping—a burgeoning non-invasive and cost-effective methodology—allowed us to uncover these plant-frugivore interactions of this rare species (Zhu et al., 2021). In just 3 months, we detected five species of mammals feeding on *P. costaricense* fruits and one primate potentially using the fruits as a topical medicine-repellent. We

were able to determine that *P. costaricense* has an endo-zoochory seed dispersal mechanism, specifically with Procyonids as the main seed dispersal agents.

The digestive retention time of kinkajou (2.5 h—range 0.7–5.6 h) is known to be similar to that of Ateles, the largest arboreal Neotropical seed dispersing animal (Kays, 1999; Lambert et al., 2014), known to disperse Neotropical seeds up to and over 1.3 km away (Fuzessy et al., 2017; Link & Di Fiore, 2006). In a study by Kays, 2000, it was reported that no seeds ingested by olingos were damaged by ingestion, and a study done in French Guiana on two free-ranging kinkajous found that transit through the kinkajou's gut differentially affected germination time and survival rates of seeds. Two of the five tree species displayed increased germination rates, one species reduced germination time, and one species displayed greater seedling survival (see Julien-Laferriere, 2001). Seeds were moved an average of 200m from the source of the tree, and up to a maximum distance of 340 m (Julien-Laferriere, 2001). Additional studies such as gut passage experimentation with captive animals and post-monitoring of germination efficacy of passed seeds would help verify the dispersal link between Procyonids and *P. costaricense*.

While primates are typically among the most important taxonomic group or arboreal seed dispersers in tropical forests (Bufalo et al., 2016; Chapman & Dunham, 2018; Clark et al., 2001), we found that white-fronted capuchins (one of four primate species in the region) appear to use *Pleodendron* fruits as medicine rather than food. There is a growing body of evidence documenting the selective use of medicinal plants by animals—known as zoopharmacognosy animals (Campbell, 2000; Huffman & Pebsworth, 2018; Laska et al., 2007; Morrogh-Bernard, 2008). Most other animals simply scratch or bite themselves or rub against stationary objects to get relief from irritating arthropods. Primates, particularly capuchin monkeys, have more so-phisticated responses known as fur rubbing. Previously they have been reported to use citrus plants, *Piper*, *Clematis,* and seeds pods of *Sloanea* (Baker, 1996; DeJoseph et al., 2002). It is known that these plant genera contain several secondary compounds that have medicinal uses, such as anethole, apiole, carvone, cineole, dillapiole, and phenylpropanoids. Some species of the Canellaceae family are highly used in Africa for both rural and urban populations, including the tree *Warburgia ugandensis*. This tree species is used for several skin and parasite diseases due to its anti-fungal and anti-bacterial properties (Akwatulira, 2011; de Castro Oliveira et al., 2019; Kowalski & Staden, 2001). Although research on *Pleodendron* is currently limited, initial chemical research on *P. costaricense* leaves has detected secondary compounds with antifungal properties (Amiguet et al., 2006)—a plausible explanation as to why white-fronted capuchins douse their fur in the oils of *P. costaricense*. Additional chemical analyses of fruits would be useful to learn more.

Implications for Conservation

Determining the historic natural distribution of a rare and relic species such as *P. costaricense* is extremely challenging, likely impossible, yet a requirement for green list frameworks (Akçakaya et al., 2018). However, we can monitor change from the baseline state of *P. costaricense* prior to our work. This represents just four adult individuals, all located outside of any strict protected area, no known case of *ex-situ* germination success or population restoration, and a complete lack of knowledge around dispersal mechanisms and whether existing natural dispersers of fruits and seeds exist. As such, the conservation outlook for the species could be considered bleak, with extinction when this current generation of adult trees dies, probable.

However, our discovery of three new adult trees in a landscape of strict conservation protection, two of which are fruit-bearing and produce viable seeds that can successfully be germinated, and the knowledge that natural dispersers of the seeds do exist, leads to a far more positive conservation legacy for *P. costaricense.* In terms of the ongoing conservation efforts of *P. costaricense*, we are pleased to report that the saplings produced from this investigation have been planted in the 2021 restoration efforts by on-the-ground local NGO, Osa Conservation. Of the 59 saplings planted in 2021, 42 remain alive (as of May 2022), and have an average height of 41.14 cm (ranging from 12 to 63 cm); one sapling is planted within the grounds of the British Embassy in San Jose, and the rest are within the protected reserve of Osa Conservation. Another 80 seeds have been germinated in 2021, once again using gibberellic acid, and in 2022 the Osa Conservation botanical team aims to produce numbers of saplings into the 100s.

In addition to active restoration efforts, before we can feel comfortable that a successful recovery can be made, there is much more work to do in terms of monitoring the growth and ongoing survival of saplings, further research to provide a better understanding as to the efficacy of natural dispersal mechanisms, the discovery of additional adult trees, and ensuring their ongoing protection. The genetic diversity of the Costa Rican *P. costaricense* population is also of concern with such a small existing known number of mature individuals (Cobo-Simón et al., 2020), and further work should be done to better understand this status.

However, given the propagation knowledge developed here to boost population numbers, the out-planting efforts of saplings in restoration efforts, and the strict protection of at least two mature fruiting *P. costaricense* trees, there is a now a strong possibility for a positive conservation legacy for this critically endangered species.

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Supplemental Material

Supplemental material for this article is available online.

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