

Final Project Report

Project Title:

Integrating reintroduction and science communication to conserve the Vulnerable *Cattleya crispa* in Brazil

Grant Recipient: **Samantha Koehler**

Funding Organization: **San Diego County Orchid Society**

Team:

- Samantha Koehler (Coordinator, State University of Campinas, Campinas/Brazil)
- Maria Fernanda Calió (Coordination of educational activities, State University of Campinas, Campinas/Brazil)
- Thomas Berg, Alexandre Antonelli (logistic support for field activities)
- Karine Bresolin (field data collection)
- Nelson Barbosa Machado Neto (Technical support in orchid cultivation and propagation, University of Western São Paulo, Presidente Prudente/Brazil)
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1. Executive Summary

This project integrated orchid propagation and science communication to support the conservation of *Cattleya crispa*, a Vulnerable orchid species endemic to the Brazilian Atlantic Forest. Through careful field monitoring, seed collection, and laboratory propagation, we successfully produced 1,000 seedlings via asymbiotic germination and initiated complementary studies on native orchid mycorrhizal fungi, identifying at least one fungal strain capable of promoting faster symbiotic germination. In parallel, we implemented a broad outreach program - including workshops, educational exhibits, and courses - that engaged nearly 1,000 participants, ranging from schoolchildren to older adults, while training undergraduate and graduate students in conservation-focused science communication. Together, these actions strengthened both the biological basis for future reintroduction efforts (planned for 2026–2027) and public awareness of orchid

conservation, demonstrating the value of integrating research, capacity building, and community engagement in biodiversity conservation initiatives.

2. Background and Rationale

Over the past two years, the San Diego County Orchid Society has supported our efforts to conserve the orchid *Cattleya crispa* at the Alto da Figueira Natural Reserve, located in the municipality of Nova Friburgo, in the state of Rio de Janeiro, Brazil.

This is a highly ornamental orchid, reaching up to 50 cm in height and producing long inflorescences bearing up to ten whitish-purple flowers. The petals and sepals have distinctly wavy and ruffled margins. This species naturally occurs in lower-elevation areas of the Brazilian Atlantic Forest, up to approximately 1,000 m above sea level, where it grows as an epiphyte on large trees or as a rupicolous species on rocky substrates. It is currently threatened by habitat destruction and illegal collection for commercial purposes.

Our initial objective was to expand the population of this orchid through asymbiotic seed propagation and to reintroduce the resulting seedlings into the reserve. To achieve this goal, we also proposed training undergraduate and graduate students in orchid propagation techniques and in science communication aimed at the general public. Below, we report how these objectives were achieved.

3. Project Objectives

The aim of this project was to expand the population of the threatened orchid *Cattleya crispa* and engage the undergraduate and graduate students in science communication for orchid conservation.

- (1) Asymbiotically seed propagate 1000 individuals of *Cattleya crispa* and reintroduce them in the RPPN Alto da Figueira and preserved areas around;
- (2) Train undergraduate and graduate students to develop workshops to the public community members to communicate the fragility of orchids and empower them to take an active role in conservation.

4. Activities developed

Initial Phase: Seed Collection and Germination

One of the greatest challenges in orchid conservation is obtaining healthy seeds for the propagation of new plants. Vegetative propagation can be helpful, but it does not ensure high genetic variability, which is essential for the long-term survival of plant populations in natural environments across multiple generations.

The Alto da Figueira Natural Reserve harbors several very large individuals of *C. crispa*, estimated to be at least 20–30 years old (Figure 1A–E). These exceptionally beautiful plants flower naturally in the reserve at the beginning of the year, during the rainy summer season of the Brazilian Atlantic Forest, and are naturally pollinated. The fruits produced are quite large, measuring approximately 10 cm in length (Figure 1D), and therefore have the capacity to produce a very large number of seeds. However, the seeds only become viable — that is, ready to germinate — shortly before the fruit opens. If fruits are collected at the wrong time, the seeds will not germinate, and it becomes necessary to wait for the following year's fruiting season. This is what happened in 2023; however, persistence is an integral part of science, and in 2024 we successfully collected 11 beautiful fruits thanks to careful monitoring carried out by Karine and Rafael Ouverney.

To store the seeds prior to germination, they must be dried as thoroughly as possible to prevent the proliferation of microorganisms. The fruits are carefully opened using a sterilized scalpel, and the seeds are placed into paper envelopes. These envelopes are then stored at room temperature in sealed containers with silica gel for approximately two weeks. After this period, the seeds are transferred to refrigeration or freezer storage.

For this project, the seeds were sent to partner laboratories for viability testing and propagation. These partner laboratories provided training to Karine and Leonardo in orchid propagation, enabling the development of these activities both at the Reserve itself and at the State University of Campinas, where Samantha's laboratory is located; she is the coordinator of this project.

Viability tests were conducted at Nelson's laboratory. Orchid seed viability can be readily assessed using the tetrazolium salt test. This test indirectly measures respiratory activity in the cells of the seed embryo, as the tetrazolium salt reacts with enzymes

involved in cellular respiration, producing a red-colored solution. A small sample of seeds is used for this test, and seeds that stain intensely red indicate living embryos and, therefore, potential viability (Figure 2A). All 11 collected fruits contained viable seeds, although the percentage of viable seeds varied considerably among fruits (Table 1). This variation can result from several factors, such as premature fruit collection and genetic incompatibilities arising from pollen–ovule crosses during fruit formation.

Table 1. Seed viability percentages of the orchid *Cattleya crispa* obtained from the tetrazolium salt test. All fruits were collected at the Alto da Figueira Reserve.

Fruit	% Viability
1	97
2	96
3	41
4	80
5	90
6	35
7	90
8	51
9	91
10	95
11	62

Since all fruits contained viable seeds, most of them were used to produce seedlings through asymbiotic germination, that is, in the absence of specific mycorrhizal fungi and instead using culture media that provide all the nutrients required for germination and early development. Asymbiotic germination was carried out in Virginia's laboratory according to the protocol described below.

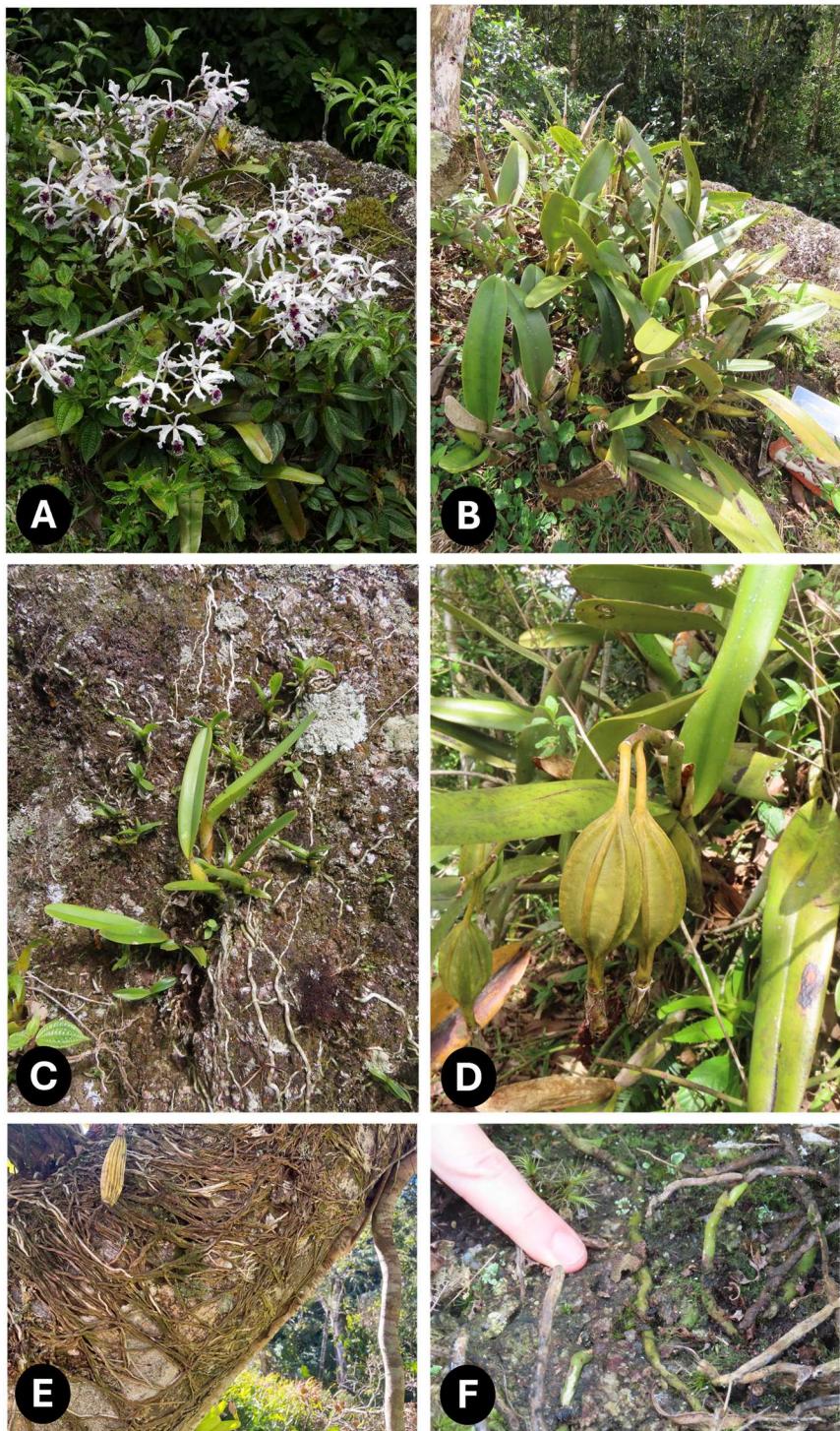


Figure 1. Individuals of the orchid *Cattleya crispa* growing at the Alto da Figueira Natural Reserve (Rio de Janeiro/Brazil). (A) Flowering individuals. (B) Individuals growing on granitic rock. (C) Individuals of different ages growing on granitic rock near the mother plant. Note the length of the roots. (D) Fruits 8–9-month-old. (E) Roots of an old individual growing as an epiphyte. (D) Young roots suitable for sampling endophytic fungi.

For orchid seed asymbiotic we used a modified oatmeal agar with $\frac{1}{4}$ strength MS salts (Orchid Maintenance Medium, OMM agar, PhytoTech Labs). Four grams of oats were boiled in 1L of distilled water for 10 minutes. The solution was filtered. The following components were then added: 100mL of MS macronutrient stock solution (10 \times), 10mL of MS micronutrient stock solution (100 \times), 10mL of White's vitamin stock solution (100 \times), 10mL of EDTA-Fe stock solution, 100mL of citric acid stock solution (10 \times), 1mL of IAA stock solution (1000 \times), 1mL of IBA stock solution (1000 \times), 1mL of NAA stock solution (1000 \times), 0.1g of inositol, and 20 g/L of sucrose. The final volume was adjusted to 4L; to obtain $\frac{1}{4}$ strength MS salts. Agar was added at 10 g/L, and the pH was adjusted to 5.7. The medium was autoclaved and poured into plates under a laminar flow hood. The protocols for the preparation of all solutions used here are described in Appendix 1.

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We successfully produced the planned 1,000 seedlings. However, they are not yet large enough for transplantation. Their development began on November 29, 2024, and in December 2025 they were replanted once again to continue growing for an additional 12 months, until they reach a size suitable for reintroduction into their natural habitat (Figure 2B). Therefore, the reintroduction phase is expected to take place between 2026 and 2027, due to the slow growth rate of the seedlings. We will keep you updated!

Isolation of Endophytic Fungi

In parallel with seed germination and seedling propagation for reintroduction, we carried out the isolation of endophytic fungi (fungi that live within plant roots). Some studies have shown that symbiotically germinated seeds produce plants that are more resilient and/or develop more rapidly. We tested this premise using the orchid *C. crispa*.

Field collections were conducted in October 2025 by Samantha, with assistance from Rafael. Root samples from adult plants, seedlings, and protocorms (the developmental stage of orchids prior to seedling formation; Figure 2C) were collected in the field, both directly from the substrate and using seed baiting traps. Seed baiting traps consist of nylon mesh envelopes containing seeds (Figure 2D). The mesh size is small enough to prevent the seeds from escaping the envelope, while still allowing the entry of mycorrhizal fungi. The envelope is securely tied around the mother plant and collected approximately one year later, when protocorms have already formed.

Field-collected samples were stored in sterilized containers moistened with cotton. In Samantha's laboratory, samples were washed under running water and then surface-sterilized in 70% ethanol and 2% sodium hypochlorite for 1 minute each, followed by rinsing in distilled water for 3 minutes. One-centimeter root segments and protocorms were macerated with a sterile scalpel on sterilized plates, followed by the addition of a low-carbohydrate fungal culture medium (½ FIM medium; Appendix 2). Fungal growth was monitored daily for up to 72 hours, and isolates with characteristics typical of orchid mycorrhizal fungi were subcultured onto new plates containing a carbohydrate-rich medium (PDA; Appendix 3).

A total of 40 endophytic fungi were isolated from roots, seedlings, and protocorms occurring directly in the substrate, and an additional eight isolates were obtained from protocorms recovered from seed baiting traps. The fungi are currently being identified using DNA sequencing, and two potentially mycorrhizal fungal species belonging to the genus *Tulasnella* have already been identified as new species.

Symbiotic Germination Tests

To evaluate the ability of the isolated *Tulasnella* species (*Tulasnella* sp. nov., strain D and strain G) to promote seed germination of *C. crispa*, symbiotic germination tests were conducted.

Symbiotic germination tests consist of placing orchid seeds on a culture medium rich in complex carbohydrates, such as oats (OMA medium; Appendix 4), which cannot be digested by the orchid itself, but only by the fungus. Thus, the orchid depends on the fungus to break down these carbohydrates into smaller molecules and transfer them to the plant. Therefore, if the orchid seed continues its development to seedling formation, it can be concluded that the fungus has mycorrhizal potential to enable the germination and development of that particular orchid species. In the case of *C. crispa*, only *Tulasnella* strain D demonstrated mycorrhizal potential (Figure 2E). Figure 3 illustrates the symbiotic development of seedlings grown on OMA medium containing this fungus.

With the aim of developing more efficient propagation protocols, we compared the early development of *C. crispa* under symbiotic conditions (with *Tulasnella* strain D) and asymbiotic conditions (½ MS culture medium, traditionally used for orchid propagation). Seeds under the symbiotic treatment (week 15) reached stage 7 earlier than seeds under the asymbiotic treatment (week 18) (Figure 4).

These results are highly encouraging. At present, we are preparing data for publication so that other researchers can evaluate whether orchid propagation using their native mycorrhizal fungi is more efficient for other orchid species. Further studies are needed to assess whether orchids associated with their mycorrhizal fungi are also more resilient to diseases and climate change.

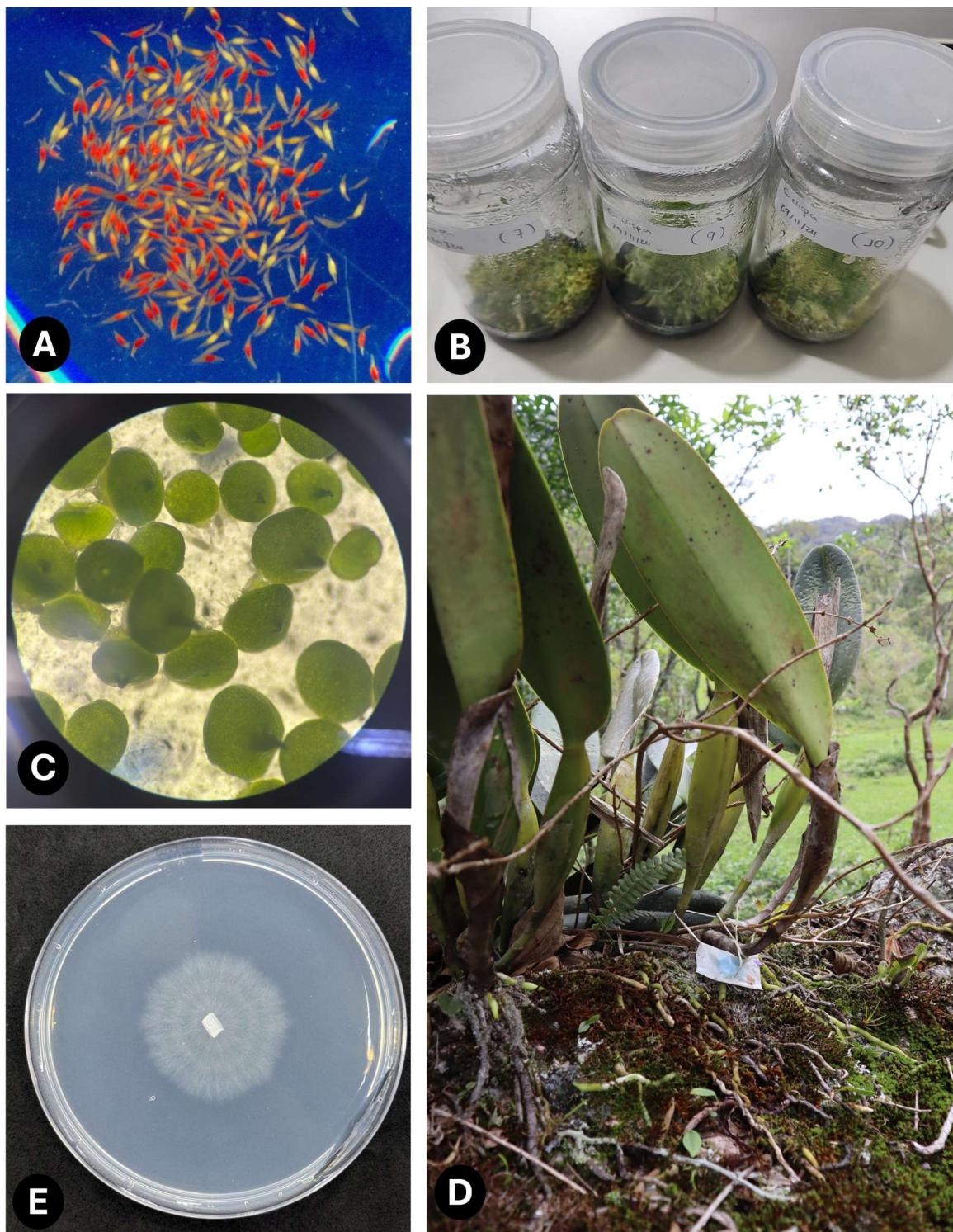


Figure 2. Studies on symbiotic and asymbiotic propagation of individuals of the orchid *Cattleya crispa* from Alto da Figueira Natural Reserve (Rio de Janeiro/Brazil). (A) Results of the tetrazolium salt test used to assess seed viability. (B) One-year-old seedlings propagated under asymbiotic conditions. (C) Protocorms propagated symbiotically. (D) Seed baiting trap attached to adult individual. (E) *Tulasnella* strain D isolated from seedlings.

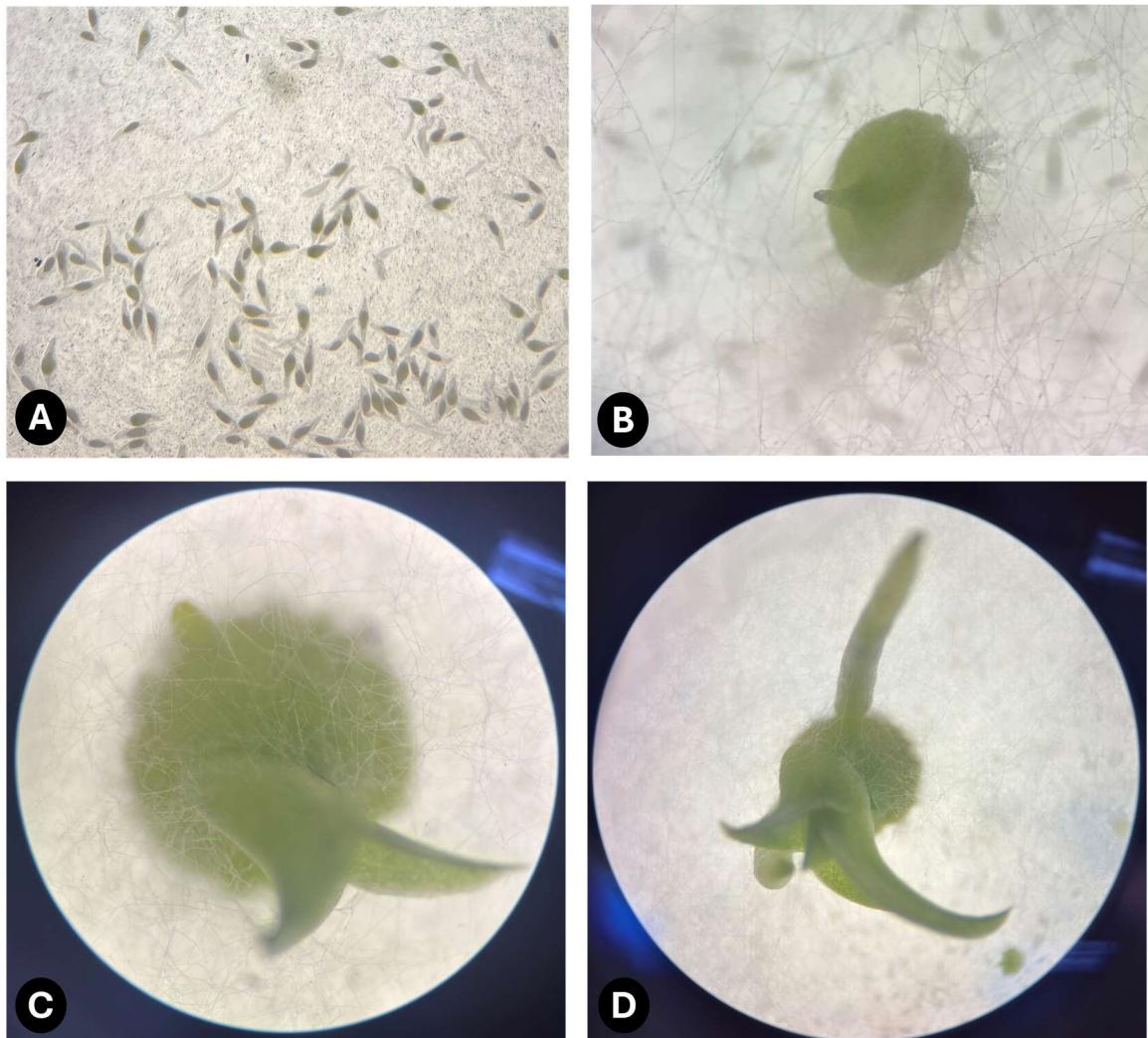


Figure 3. Developmental stages of the orchid *Cattleya crispa* in symbiotic germination studies with fungus *Tulasnella* sp.nov. strain D. (A) Embryo increases in volume within seed coat. (B) Protocorm with rizoids. (C) Seedling with first leaves developed. (D) Seedling with leaves and roots.

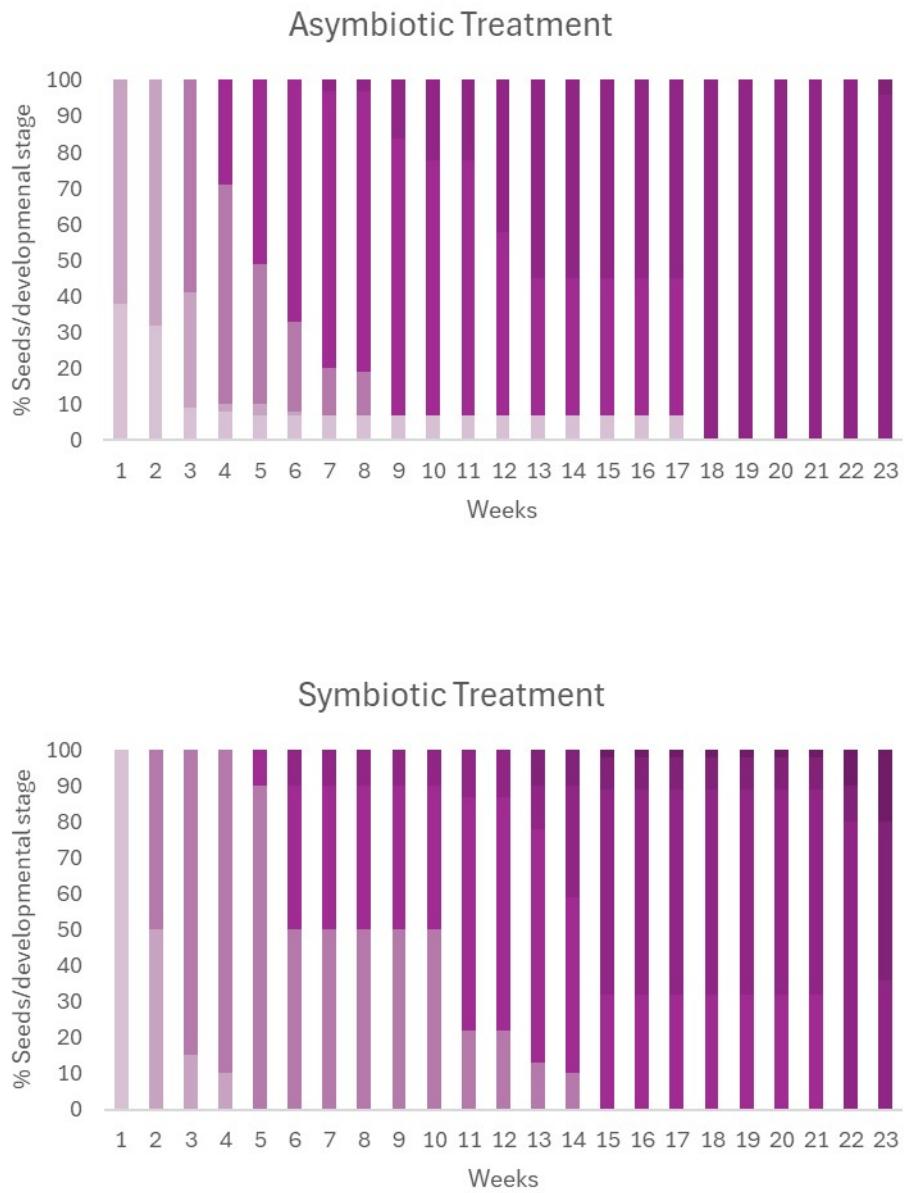


Figure 4. Percentage of seeds in each of the seven developmental stages (according to Seaton & Ramsey 2005)¹ over the 23 weeks of observation. The darker the color, the later the developmental stage. The developmental stages are: (1) seed with embryo (ungerminated); (2) swollen seed indicating initial imbibition; (3) seed coat broken, establishment of protocorm; (4) protocorm with rhizoids; (5) protocorm with emerging shoot initial; (6) seedling with first leaves; (7) seedling with leaves and root.

¹ Seaton, P. and Ramsay, M.M (2005) Growing Orchids from Seed. Kew: Royal Botanic Gardens, Kew 96pp.

Orchid Conservation Workshops

Throughout the development of the project, we implemented three types of outreach and science communication activities aimed at raising awareness about the importance of conserving Brazilian orchids: *workshops, educational exhibits, and courses on orchid cultivation and conservation*. These activities were developed by Samantha and Maria Fernanda, with the participation of undergraduate and graduate students from different programs at the State University of Campinas.

The *workshops* consisted of one-hour sessions. In the first stage, a brief presentation was given to introduce basic information on orchid and fungal morphology using live specimens (Figure 5), as well as to explain the dependence of orchids on specific fungi for germination and successful establishment in natural environments (Figure 5).

In the second stage, participants were invited to learn how scientists extract fungi from inside orchid roots for study. Each participant received a small piece of root placed in a mortar containing a small amount of water, which they manually macerated until an aqueous solution was obtained. This solution was then aspirated using a disposable pipette and transferred to a Petri dish containing a culture medium prepared with culinary agar to simulate a fungal growth medium (Figure 5). Subsequently, the presenter provided Petri dishes containing fully developed orchid mycorrhizal fungi that had been previously isolated and grown in the laboratory. Participants were then invited to observe the fungi using stereoscopic and compound microscopes (Figure 5).

To conclude the activity, the presenter highlighted the importance of studying orchid mycorrhizal fungi and how this knowledge can contribute to the propagation of more resilient orchid seedlings. At this stage, the team specifically mentioned the *Cattleya crispa* project funded by the San Diego County Orchid Society (Figure 5).

During 2024 and 2025, a total of 6 workshops were conducted, reaching 160 participants across a range of ages 5-17.

The *educational exhibits* consisted of events in which the external public - primarily school groups - visited the university. Unlike the workshops, visitors circulated among exhibition tables designed to highlight the importance of science and universities for society.

Our exhibit was titled “*Did You Know That Fungi Are Friends of Orchids?*” and covered the same core content as the workshops: what orchids are, what fungi are, the dependence of orchids on specific fungi for development, and how this knowledge is applied to the conservation of threatened orchids such as *Cattleya crispa*. To adapt the content to this more concise format, we assembled an exhibition featuring live plants, fungal samples, microscopes to observe fungi hyphae, and photographs (Figures 5). Between 2024 and 2025, we conducted two educational exhibits, which together reached approximately 800 visitors, primarily students between 12 and 17 years of age.

Finally, we also offered a *course on orchid cultivation and conservation* for adults aged 50 and older, consisting of a single four-hour session. The course covered basic concepts of orchid morphology and taxonomy, practical tips on cultivation and pest control, and an overview of how our research at the university contributes to the conservation of threatened species through the study of *Cattleya crispa*. *The course was attended by 33 participants in 2024.*

5. Results and Outputs

Students involved

This project involved the direct participation of four university professors from three different Brazilian universities. The data collected supported the development of part of Leonardo Souza de Andrade’s doctoral dissertation and an undergraduate research project conducted by Arthur Fernandes. In addition, the project involved two technical fellows, Milena Martins and Thomas Regueira, as well as five undergraduate work-study fellows: Jacqueline Costa De Lima (02/26/2024–12/02/2024); Ediléia Milena Sousa Pereira (03/01/2024–03/01/2025); Mileny Santos Pereira (10/15/2025–09/22/2025); Thelma Eleuteio Ataide (09/18/2025–12/01/2025); and Lisandra Das Graças Dias Campos (01/31/2025–12/31/2025).

Audience reached

Overall, our science communication activities reached nearly 1,000 people (993 records).

Institutions involved

- Antonelli Foundation for Biodiversity Research and Conservation (Nova Friburgo, Rio de Janeiro)
- State University of Campinas (Campinas, São Paulo)
- University of Western São Paulo (Presidente Prudente, São Paulo)
- North Fluminense State University (Campos, Rio de Janeiro)

Participation in an International Scientific Event

Koehler S., Andrade L.S., Calió M.F., Zandoná L., Bresolin K., Berg T., Neto N.B.M., Carvalho V.S. **CAN ORCHID MYCORRHIZAL FUNGI IMPROVE CONSERVATION OF THE VULNERABLE CATTLEYA CRISPA IN BRAZIL?** 8th International Orchid Conservation Congress, Perth, Australia. 3 – 6 September, 2024. (poster) (Appendix 4)

6. Financial Summary

- Training of Karine Bresolin and Leonardo de Souza Andrade in asymbiotic orchid propagation and cultivation techniques. Costs included transportation, accommodation, and meals for 4 nights and 5 days. Amount: R\$ 2,500.00 / USD 430
- Laboratory supplies (reagents for fungal culture and orchid propagation, Petri dishes, glass flasks, gloves): R\$ 3,000.00 / USD 520
- Orchid cultivation materials (pots, substrates). Amount: R\$ 500.00 / USD 100
- Materials for the development of educational activities (storage boxes, stationery, printing services). Amount: R\$ 1,000.00 / USD 200
- Four field trips for sample collection (fruits, seedlings, and roots of Cattleya crispa) (route Campinas/SP – Nova Friburgo/RJ – Campinas/SP; 1,200 km) (fuel, tolls, car rental, meals, accommodation): R\$ 10,000.00 / USD 2,000
- Workshops (rental of tables, chairs, tents, meals, transportation). Amount: R\$ 4,300.00 / USD 750

7. Acknowledgments

We would like to sincerely thank the Society for its support in the development of these activities. We remain fully available to provide photographs, texts, and any additional information that may be of interest to the Society.

We sincerely thank Rafael Ouverney and Rafael da Silva for all the support during fieldwork.



Figure 5. Educational activities developed as part of this project. (A) Orchids and fungi available for hands-on manipulation. (B) Materials used to simulate the process of isolating endophytic fungi from roots. (C) Student macerating the root. (D) Students observing fungal growth on a Petri dish. (E) Student observing microscope slides containing fungal hyphae. (F) Samantha explaining the importance of conserving *Cattleya crispa*.

Appendix 1. Stock solutions for OMM Medium for asymbiotic seed germination

Reagents

1. MS Macronutrient Stock Solution 10× (full strength)

(100 mL yields 1L)

19 g/L potassium nitrate

16.5 g/L ammonium nitrate

1.8 g/L magnesium sulfate

1.7 g/L potassium phosphate

3.3 g/L calcium chloride

2. MS Micronutrient Stock Solution 100× (full strength)

(10 mL yields 1L)

0.62 g/L boric acid

0.0025 g/L cobalt chloride hexahydrate

0.0025 g/L copper sulfate pentahydrate

1.69 g/L manganese sulfate monohydrate

0.0213 g/L molybdic acid

0.083 g/L potassium iodide

0.86 g/L Zinc sulfate heptahydrate

White's Vitamin Stock Solution 100× (full strength)

(10 mL yields 1L)

1 g/L thiamine HCl

0.05 g/L pyridoxine

0.05 g/L nicotinic acid

0.2 g/L glycine

Citric Acid Stock Solution 10×

(100 mL yields 1L)

1.5 g citric acid in 1L of distilled water

IAA Stock Solution 1000×

(1 mL yields 1L)

0.3 g IAA in 1 L of distilled water

IBA Stock Solution 1000×

(1 mL yields 1L)

1.75 g IBA in 1 L of distilled water

NAA Stock Solution 1000×

(1 mL yields 1L)

1.75 g NAA in 1 L of distilled water

Appendix 2. Fungi Isolation Medium (FIM) Protocol

(Clements et al. 1986)²

Reagents

0.5 g calcium nitrate

0.2 g monobasic potassium phosphate

0.1 g potassium chloride

0.1 g magnesium sulfate

0.1 g yeast extract

5 g sucrose

10 g agar

Preparation

Add all reagents to a 1 L Erlenmeyer flask.

Add distilled water to a final volume of 1L.

Add the reagents to a 1L Erlenmeyer flask.

Add distilled water to a final volume of 1L.

Divide the solution into two 1L Erlenmeyer flasks (500 mL each).

Autoclave.

After autoclaving and cooling, add 500 μ L of 5 g of streptomycin sulfate (antibiotic) per liter of medium.

² Clements, M. A., Muir, H., & Cribb, P. J. (1986). A preliminary report on the symbiotic germination of European terrestrial orchids. *Kew Bulletin*, 437-445.

Appendix 3. Potato Dextrose Agar (PDA) Medium Protocol

Reagents

39 g of commercial PDA (potato dextrose agar)

1 L of distilled water

Preparation

Add the reagents to a 1L Erlenmeyer flask.

Add distilled water to a final volume of 1L.

Divide the solution into two 1L Erlenmeyer flasks (500 mL each).

Autoclave.

Pour 25 mL (approximately half a plate) into autoclaved Petri dishes inside a laminar flow hood.

Appendix 4. Oat Medium Agar (OMA) Protocol

Reagents

4 g oat flakes

1L of distilled water

Preparation

Boil the oats in distilled water for 10 minutes

Filter the solution

Add 10 g of agar

Adjust the pH to 5.2

Autoclave

Appendix 4. Poster presented at the International Orchid Conservation Congress, in Perth, Australia, 3 – 8 September/2024.

Can orchid mycorrhizal fungi improve conservation of the Vulnerable *Cattleya crispa* in Brazil?

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BACKGROUND

- Nearly 10% of all orchid species are found in Brazil, with ca. 1,500 species occurring in the Atlantic Forest biome (AF) – a degraded biome, with approximately 70% of the country's population residing within its boundaries.
- Cattleya crispa* (Fig. 1) is one of the most charismatic and ornamental orchids of the AF. This species mainly occurs in coastal areas, which face tremendous pressure from real estate development. Its populations have been declining for the past 40 years, primarily due to intense collection pressure driven by cultivation and commercialization purposes². To aggravate this scenario, the plant has slow growth, with a generation time estimated to be around ten years³. The National Center for Flora Conservation in Brazil predicts that *C. crispa* will experience a decline of at least 30% in the total number of individuals over the next 30 years.
- Orchid conservation projects worldwide succeeded by creating pilot projects that initiate grassroots action and combine science, education and horticulture⁴. Symbiotic orchid seed germination is a promising alternative for orchid propagation and reintroductions, as it showed to improve seedling growth and greenhouse acclimatization.

GOALS

- To isolate and identify potential orchid mycorrhizal fungi associated with *Cattleya crispa* roots.
- To train undergraduate and graduate students to develop workshops to the community members to communicate the fragility of orchids and empower them to take an active role in conservation.

LOCATION

RPPN Alto da Figueira, a privately-owned 120-hectare reserve covered with primary rainforest, near Nova Friburgo (Rio de Janeiro, Brazil).



Cattleya crispa (A) Flower, (B) Fruits.

METHODS

- We isolated fungi from young orchid roots in 1/4 FIM medium following Zettler & Corey (2018). Colonies were further subcultured in PDA and OMA. Endophytic fungi were submitted to molecular identification using primers ITS-F-ITS4.
- Seeds were initially germinated aseptically in Murashige & Skoog (MS) medium at 25 °C with a 12-hour light/dark cycle. Symbiotic germination trials are pending confirmation of fungal identification.
- For educational activities, we prepared two types of events. For students aged 12-18, we developed an interactive activity introducing them to the symbiotic relationship between orchids and fungi, showing how scientists isolate fungi from orchid roots for study. For adults, we organized courses on orchid biology, emphasizing the threats they face and the importance of preserving their natural habitats. We also created content for dissemination on our Instagram channel, @diveg_unicamp.

RESULTS

- We isolated 24 endophytic fungal strains.
- A total of 100 aseptically germinated young plants, now 24 months old, are under cultivation for future reintroduction.
- In 2024, our team presented three workshops, attended by 1000 people, including students aged 12-18 and adults.



NEXT STEPS

- DNA sequencing for barcode identification.
- Perform symbiotic germination trials.
- Prepare downloadable orchid conservation materials for educators and students.

Funding:
FAPESP/BIOTA 22/12497-6
San Diego Orchid County Society

