ISSN 1409-3871

LANKESTERIANA

VOL. 25, No. 3 DECEMBER 2025

INTERNATIONAL JOURNAL ON ORCHIDOLOGY

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VOL. 25, No. 3 **December 2025**

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International Journal on Orchidology

Effective publication dates ISSN 2215-2067 (electronic): October 16 – December 19, 2025 (specific dates recorded on the title page of each individual paper)
Effective publication date ISSN 1409-3871 (printed): December 22, 2025

Layout: Jardín Botánico Lankester.
Cover: Flower of *Pleurothallis trimeroglossa*. Photograph by Mario A. Sierra-Ariza.
Printer: MasterLitho.
Printed copies: 25

Printed in Costa Rica / Impreso en Costa Rica

R Lankesteriana / International Journal on Orchidology
No. 1 (2001)-- . -- San José, Costa Rica: Editorial Universidad de Costa Rica, 2001--
v.
ISSN-1409-3871

1. Botánica - Publicaciones periódicas, 2. Publicaciones periódicas costarricenses

OBITUARY

MARGARET ANN DIX
(MAY 19TH, 1939 – JUNE 2ND, 2025)

Award of the "Medal Ricardo Bressani" with Michael W. Dix, at the UVG, 2017. Modified from the personal archive.

Margaret was born on the island of Jersey in the English Channel, at La Planque, her family's farm. She earned a bachelor's degree with first-class honors in zoology, with a minor in limnology, from the University of London, and later completed a master's degree in zoology at Mount Holyoke College in Massachusetts. Under the guidance of Edward O. Wilson, she studied ecology and entomology at Harvard University. There she met her colleague and life partner, biologist Michael W. Dix, with whom she began a closely intertwined professional and personal journey starting in 1966. Their life together began as an adventure and left a lasting impact on their family, students, colleagues, and the field of Neotropical biological science.

In the late 1960s, Margaret and Michael selected Central America and Mesoamerica, respectively, for their doctoral fieldwork. In 1972, after receiving a Latin American Teaching Fellowship from Tufts University, they chose Guatemala as their destination. This decision marked the start of a lifetime dedicated to teaching, research, and conservation in the country. Partnering with the *Fundación Universidad del Valle de Guatemala* (FUVG), they developed the curriculum for the country's first Licentiate degree in Biology by the end of 1972, working together with Mario Dary-Rivera, who was developing the curriculum at the University of San Carlos de Guatemala, the country's public university. In the early years of the program, Margaret and Michael taught all the biology courses at both universities, training the first generation of biologists in Guatemala, including Margarita Palmieri and Ana Maria de Merida. The foundation laid by Margaret and Michael not only thrived in academia but also left a lasting impact on Guatemala, its people, and natural resources.

Margaret left a lasting impact on her students, not only through her classes but also through hands-on laboratories and fieldwork. Along with Michael, the

Fieldwork at Agua Blanca, Jutiapa with Ximena Leiva, 2014. Photo by M.L. Maldonado.

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University's academic community, and students, she helped lead the reforestation of the area around the American School of Guatemala (CAG for its Spanish acronym) and the University of the Valley of Guatemala (UVG). The ravine reserve area at the central campus later became the Botanical Garden and Biological Collections, which were named in honor of Margaret and Michael Dix in 2024. After the February 1976 earthquake in Guatemala, construction started on the pond in the lower part of the reserve, where Margaret introduced her students to freshwater ecology. That same year, the Science Building was inaugurated, with laboratories designed by Margaret and Michael. These spaces are still used today to teach the new generation of biology and chemistry students.

In 1978, with the arrival of the first scientific equipment from abroad, the Center for Environmental Studies and Biodiversity (CEAB for its Spanish acronym) was established. The first project was led by Michael Dix in collaboration with the National Forest Institute (INAFOR) in 1977. It focused on identifying and eradicating the pine weevil, Dendroctonus spp., in pine-oak forests. The project ended due to the impact of Guatemala's internal armed conflict. From 1977 to 2001, Margaret served as Director of the Department of Biology at UVG. Whenever a curious and interested student approached, she enthusiastically shared her experience, knowledge, and ideas for future research to promote their curiosity and critical thinking skills. Margaret and Michael worked closely as scientific advisors with the teams from the Ministry of Environment and later the National Commission for Protected Areas.

Between 1997 and 2002, Margaret, along with Michael, launched the Master's Degree in Environmental Studies, developed with support from the Ministry of Energy and Mines of Guatemala (MEM for its Spanish acronym), to enhance the professional quality of its graduates. Her interdisciplinary approach and passion for knowledge were evident in every class, project, and mentorship. Over the years, Margaret taught courses for undergraduate and master's students in general biology, sociobiology, terrestrial and aquatic ecology, vertebrate and invertebrate anatomy and physiology, and water resources management. The variety of courses she taught demonstrated that she was a multidisciplinary academic who could integrate knowledge across fields and share her curiosity and enthusiasm for learning with others.

At lake Atitlán, 2010. Photo by Hugo Villavicencio.

Since 1973, Margaret has actively participated in the Guatemalan Orchid Association (AGO for its Spanish acronym). She believed that science should be accessible to everyone, so at the AGO, she promoted citizen science before it became widely popular. Her collaborative work with Michael led to the publication "Orchids of Guatemala: A Revised Annotated Checklist" in 2000, as well as the taxonomic review of the genus *Lycaste* in the compendium "Flora Mesoamericana", both edited by the Missouri Botanical Garden, making it an essential reference for Guatemalan botany. Additionally, she authored several scientific articles on aquatic ecology and epiphyte diversity; those about orchids were published in this journal.

Margaret, together with Michael, served as a vital link between Guatemala and the international community of orchid researchers, generously paving the way for scientific collaboration and exchange at a time when such connections were difficult. Through her expertise, hospitality, and intellectual rigor, she connected foreign specialists with Guatemalan orchids and landscapes, fostering lasting relationships with figures such as Carlyle A. Luer and Calaway Dodson of the Marie Selby Botanical

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With Milena Montúfar and Claudia Santizo at the UVG ecology laboratory, circa 1987. Photographer unknown.

Checking the biological collections at classroom C-102 A from the UVG, circa 1990. Photographer unknown.

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With Gerardo Salazar, in Mataquescuintla, 2008. Photo by M.L. Maldonado.

Gardens; Carl Withner of the Brooklyn Botanic Garden; Mark Whitten from the Florida Museum of Natural History; Joseph Arditti of the University of California, Irvine; Gustavo Romero of the Oakes Ames Orchid Herbarium at Harvard University; Miguel Angel Soto Arenas and Gerardo Salazar of the Mexican Association of Orchidology and UNAM; Robert L. Dressler of the Lankester Botanical Garden and the Missouri Botanical Garden; and Henry Oakeley of the Royal Botanic Gardens, Kew. These, along with many other colleagues, regarded Margaret as a trusted partner and collaborator, recognizing her as a prominent Neotropical orchidologist devoted to the exploration, study, and conservation of Guatemalan flora. The scope of her interdisciplinary research and teaching exceeds what can be captured in a few pages. Her collaborative spirit led to the creation of multiple research programs at UVG, including the CDC (Centers for Disease Control and Prevention) malaria program, which had a multiplying effect on university research. With funding from the National Academy of Sciences, she established biological control projects targeting the malaria vector (*Salvinia* spp.).

Along with Michael, she was part of the scientific team that helped establish the Maya and Sierra de las Minas Biosphere Reserves, inspiring students to conduct basic research, which is documented in numerous theses under her mentorship. Meanwhile, she supported Michael in successfully advocating for the university's acceptance of the donation of the FUVG Farm in Alta Verapaz and the creation of the Quetzal Refuge Reserve in Suchitepéquez, on the Atitlán volcano. Both sites became important locations for future research at the Center for Environmental Studies and Biodiversity.

Her passion for limnology motivated Margaret to dedicate herself to saving important bodies of water in Guatemala, including Lake Izabal and Lake Atitlán. Her love for Lake Atitlán prompted her to support the Atitlán Study Center (CEA for its Spanish acronym) at UVG's Altiplano campus, both in person and remotely through May 2025. By that time, she had left behind

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At the scientific station in the Reserve " Refugio del Quetzal", Atitlán Volcano, Suchitepéquez, 2003. Family archive.

the draft of her final paper, which highlights a decade of work on the lake.

Beyond her remarkable scientific career, Margaret was a devoted mother who involved her children in her various academic and field expeditions. Alumni remember her for her strength, warmth, and intellectual generosity. Gerda Huertas, one of her students shared: *Among my most valuable memories are the field trips. What impressed me was that Margaret often traveled with Michael, accompanied by their three children, including the youngest, who was still a baby. On my first trip with them in 1983, while we did our summer fieldwork in ecology, Michael was shipwrecked in the Golfete of Lake Izabal, having stayed behind with the field supplies and luggage boat to ensure all students left safely for the field site wearing life vests and in daylight. As Margaret tended to her children and worried about Michael after the last boatload of students arrived without him until the early hours of the morning, she continued to attend to the students. I marveled at her strength in difficult moments. Her and Michael's support led me to improve my writing in Spanish, my licentiate thesis on waste from African palm refining, and later projects such as the economic importance of tillandsias in Guatemala. She was my academic mother and someone I could trust and whose example I would emulate in my own life.*

For her part, Ximena Leiva, a colleague on biodiversity issues, mentioned: *I got closer to Margaret working as a professional on biodiversity issues. I have a few pictures of her, but many of her hands, always patiently showing me something new.*

Mayra L. Maldonado also shared one of many treasured memories: *I will forever miss the few quiet afternoons in the Department of Biology at UVG, when she would sit with me in the orchid herbarium, with an identification guide in hand and the drawing stereoscope, helping me identify difficult specimens, and talking about how we could fix the world.*

Margaret Dix's legacy spans multiple generations. Her life exemplified a dedication to science,

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On the road to Agua Blanca, Jutiapa, 2014. Photo by M.L. Maldonado.

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education, conservation, and humanity. Guatemala and the scientific community of the Neotropics are deeply thankful for her contributions. Today, many of her former students—who trained with discipline, dedication, and vision—lead environmental organizations, shape public policies, and promote sustainable development through influential roles in government, civil society, and academia. In each of them and their achievements, a part of her lives on legacy.

At the XLIV National Orchid Show by the AGO, in the venue "José Mariano Arzú Castillo", Guatemala, 2018. Photo by Maria Cristina de Bianchi.

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LANKESTERIANA 25(3): 151–166. 2025. doi: http://dx.doi.org/10.15517/zzdx7h51

A NEW SPECIES OF *PLEUROTHALLIS* (PLEUROTHALLIDINAE) FROM THE *P. TALPINARIA* COMPLEX, WITH NOTES ON COMPARATIVE FLORAL MORPHOLOGY AND POLLINATION ECOLOGY

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Abstract. A new species of *Pleurothallis* subgenus *Talpinaria*, discovered in the department of Tolima, Central Andes of Colombia, is described, illustrated, and discussed. The new species, *Pleurothallis vallejoi* is compared with *P. talpinaria* from Colombia and Venezuela, *P. trimeroglossa* from Peru and Ecuador, *P. jostii*, presumed to occur in Ecuador, and *P. gracilicolumna* from Colombia. The new species is distinguished from the previous ones by a lip with subquadrate lateral lobes, with narrowly elliptical basal auricles, and a callus that is elongated, thin, and slightly elevated, with the median lobe occupying almost half of the total lip length. Additionally, the pollination mechanism of the *P. talpinaria* complex is described and discussed.

Resumen. Se describe, ilustra y discute una nueva especie de *Pleurothallis* subgénero *Talpinaria*, descubierta en el departamento del Tolima, en los Andes Centrales de Colombia. La nueva especie se compara con *Pleurothallis talpinaria* de Colombia y Venezuela, *P. trimeroglossa* de Perú y Ecuador, *P. jostii*, presumiblemente presente en Ecuador, y *P. gracilicolumna* de Colombia. La nueva especie se distingue de las anteriores por su labelo con lóbulos laterales subcuadrados, con aurículas basales estrechamente elípticas y un callo alargado, delgado y ligeramente elevado, con el lóbulo medio ocupando casi la mitad de la longitud total del labelo. Además, se describe y discute el mecanismo de polinización del complejo *P. talpinaria*.

Keywords / Palabras clave: Andean orchids, Diptera, orquídeas andinas, *Pleurothallis gracilicolumna*, *Pleurothallis jostii*, *Pleurothallis talpinaria*, *Pleurothallis trimeroglossa*, *Sylvicola*

Introduction. *Pleurothallis* R.Br. is a neotropical genus widely distributed from southern Mexico to South America, including the Antilles (Ackerman *et al.*, 2014). It is the fourth most diverse genus within the subtribe Pleurothallidinae, preceded by *Lepanthes* Sw., *Stelis* Sw., and *Masdevallia* Ruiz & Pav. (Karremans, 2016; Karremans & Vieira-Uribe, 2020). Currently, the diversity of the genus exceeds 550 species with the inclusion of recently published new species (Damián-Parizaca *et al.*, 2025; Moreno *et al.*, 2023; Pupulin *et al.*, 2021; Sierra-Ariza, 2023, 2024; Sierra-Ariza *et al.*, 2022; Wilson *et al.*, 2022). Colombia stands out as the country with the highest number of species recorded in the genus, with 247 documented species (Karremans *et al.*, 2023).

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Received 5 May 2025; accepted for publication 26 August 2025. First published online: 00 October 2025.

Pleurothallis subgen. *Talpinaria* is morphologically characterized by a petiolate leaf and a single successive flower produced subapically, emerging from the annulus located very close to the apex of the stem, with a conspicuous spathaceous bract near the apex of the ramicaul. The dorsal sepals are ovate and free from the lateral sepals, which are fully connate into an ovate synsepal, dorsal sepal and synsepal appear to be similar to each other in shape and size, and petals are narrowly acute (Luer, 1998).

The first known species of this subgenus was described in 1859 by the German botanist Gustav Karl Wilhelm Hermann Karsten as *Talpinaria bivalvis* H.Karst., based on a collection from the Eastern Cordillera of Colombia. However, the name *T. bivalvis* did not last long. In 1886, Heinrich Gustav Reichenbach transferred the species to the genus *Pleurothallis*, renaming it *Pleurothallis talpinaria* Rchb.f., due the previous existence of *Pleurothallis bivalvis* Lindl.

In 1921, Friedrich Richard Rudolf Schlechter described the second species in this subgenus, *Pleurothallis trimeroglossa* Schltr., based on a collection made by Weberbauer near Huancayo, Junín, Peru (Schlechter, 1921). Although Schlechter did not compare it with *P. talpinaria*, later Schweinfurth (1942) and Luer (1998) concluded that both entities belonged to the same species. It is believed that the holotype of *P. trimeroglossa* was destroyed in Berlin during World War II. Fortunately, before its destruction, Gordon Dillon drew the lip and column of the type in great detail, and the drawing was deposited in the AMES Herbarium at Harvard University, allowing for future morphological analysis. The name *P. trimeroglossa* was ultimately accepted as a distinct species by Wilson *et al.* (2017).

During the reorganization of the genus *Pleurothallis*, Luer (1986) established the subgenus *Talpinaria*, designating *P. talpinaria* as the type species. He also included *P. hitchcockii* Ames, *P. punctulata* Rolfe, and *P. sandemanii* Luer in this subgenus. Although this author noted that the flowers of the subgenus differ significantly from each other, leading to an uneasy alliance, Luer (2004) reinstated the genus *Talpinaria* for these four species.

Phylogenetic studies, such as those conducted by Pridgeon, Solano & Chase (2001), as well as by Wilson *et al.* (2011, 2013), confirmed that *P. talpinaria* belongs to a clade within *Pleurothallis*, supporting Reichenbach's transfer of *T. bivalvis* to *Pleurothallis*. These studies also clarify that subgenus *Talpinaria* is polyphyletic, corroborating Luer's previous observations of the uneasy morphological alliances within the subgenus and confirming the morphological diversity noted by Luer.

Wilson *et al.* (2017) described a third species related to *P. talpinaria*, named *P. jostii* Mark Wilson & J.Portilla. A year later, Wilson (2018) described a fourth species, *P. gracilicolumna* Mark Wilson, which added significant morphological complexity to this group.

Despite the accumulated knowledge about the morphology and systematics of *Pleurothallis*, little is known about its reproductive ecology. Specifically, pollination in species of *Pleurothallis sensu stricto* remains poorly understood, with most available records limited to observations of floral visitors without conclusive evidence of pollination. Dipterans, particularly from the family Drosophilidae, have been frequently reported as visitors to these flowers (Pridgeon, 2005). A fly of the genus *Lycoria* Meigen (Sciaridae) was observed transporting pollinia of *Pleurothallis monocardia* Rchb.f.; visits of Drosophilidae to *Pleurothallis xanthochlora* Rchb.f. were also recorded, although without evidence of pollination (Dodson, 1962). A species of *Drosophila* Fallén was identified as an effective pollinator of *Pleurothallis ruscifolia* (Jacq.) R.Br. and *Pleurothallis eumecocaulon* Schltr. (Dodson, 1965).

Additionally, a high diversity of dipteran visitors has been documented in species of the genus, including representatives from Bibionidae, Drosophilidae, Empididae, Mycetophilidae, Sciaridae, and Tachinidae; beetles (Curculionidae, Chrysomelidae) carrying pollinia have also been observed, as well as wasps (Vespidae, Braconidae) visiting flowers of *Pleurothallis* sp. (Duque, 1993). Furthermore, phenomena such as autogamy in *P. ruscifolia* (Catling, 1990) and cleistogamy in *Pleurothallis cleistogama* Luer have been documented. A more recent well-documented case is that of *Pleurothallis helleri* A.D.Hawkes, which attracts various arthropods (flies, beetles, butterflies, and even spiders), but only the tiny flies of the family Ceratopogonidae have been identified as effective pollinators (Karremans, 2023). Regarding *Pleurothallis* subgen. *Talpinaria* no reports have been found concerning its pollinators or even floral visitors.

The following describes, illustrates, and discusses a new species of the genus *Pleurothallis* subgen. *Talpinaria*, from the Central Andes of Colombia. It is morphologically compared with *P. gracilicolumna* from Colombia*, P. jostii* presumably from Ecuador, *P. talpinaria* from Colombia and Venezuela, and *P. trimeroglossa* from Peru and Ecuador. The pollination mechanism within the *P. talpinaria* complex is also described and discussed.

Materials and methods. *Plant material, taxonomic and morphological comparisons.*— Between 2019 and 2024, several expeditions were carried out in the municipality of Ibagué, Tolima, where the new species was discovered. During these explorations, various populations of the species were identified in their natural environment. The specimens were photographed to document their morphological characteristics, using a D5300 camera with a NIKKOR AF 105mm f/2.8 D Micro lens.

The specimens were preserved in newspaper soaked with 75% ethanol, and the floral structures were stored in a 50% glycerol mixture (equal parts glycerin and 70% alcohol). The collected material was dried in an electric oven at 75°C for 14 h and then incorporated into the TOLI Herbarium collection. The floral structures were analyzed under a Motic SMZ 168 Led stereoscope.

To verify the identity of the new species, the available literature on the genus was reviewed (Luer, 1988, 2005; Wilson, 2018; Wilson *et al.*, 2017). Additionally, specimens were examined online from the AMES Herbarium (Harvard University, 2025) and KEW Herbarium (The Kew Data Portal, 2025), as well as from the national herbaria TOLI, HPUJ, JBB, and COL (www.biovirtual.unal.edu.co/es/colecciones/búsqueda/plantas/). Lankester composite digital plates (LCDP) were created using Adobe Photoshop® 2024 (25.3.1), where the vegetative and reproductive characteristics of each species were illustrated, showing the floral structures from different views in order to highlight their diagnostic features, thus facilitating comparison and delimitation among the studied taxa.

Scanning electron microscopy of lip.— Recently opened flowers of species of the *P. talpinaria* complex (*P. talpinaria*, *P. gracilicolumna, P. jostii* and *P.* cf. *trimeroglossa*) were harvested from plants in the Colorado College living collection. Lips of the flowers were removed by excision from the column base and preserved in Kew Mix (Wilson *et al.*, 2016). Flowers were prepared for and examined by scanning electron microscopy using methods described previously (Wilson *et al.*, 2016, 2018).

Taxonomic treatment

Pleurothallis vallejoi Sierra-Ariza, J. Alvarez-Diaz & Mark Wilson, ***sp. nov.*** (Fig. 1).

TYPE: COLOMBIA. Tolima: Municipio de Ibagué, corregimiento San Juan de La China, 2048 m. 10 March 2024, *M.A. Sierra-Ariza, J. Alvarez-Diaz & Fernando Tinoco 491* (Holotype: TOLI).

Diagnosis: *P. vallejoi* is morphologically most similar to *P. trimeroglossa* (Fig. 2)*,* from which it differs in the sub-quadrate lateral lobes of the lip, 1.7–2.0 × 1.2–1.5 mm (*vs*. transversely oblong, 1.8–2.1 × 1.1–1.3 mm), basal auricles that are narrowly elliptical, 1.7–2.0 × 0.6–0.8 mm (*vs*. narrowly oblong, 2.0–2.3 × 0.4–0.6 mm), a callus that is elongated, thin, slightly elevated, and inconspicuous (*vs*. dome-shaped, flattened, and notably visible), and a median lobe of 4.1–4.3 mm long (*vs*. 4.8–5.1 mm).

Epiphytic, caespitose *herb*, suberect to inclined, small, to 18 cm tall. *Roots* slender, 1.2 mm in diameter. *Ramicauls* reddish, 4.0–9.5 cm long, with a tubular sheath in the middle zone or in the lower third and two other sheaths at the base, papyraceous, fibrous, light brown. *Leaf* light green, cuneate, coriaceous to fleshy, oblong-lanceolate, acute, 5.4–8.0 × 1.2–16.0 cm, the base cuneate, older leaves glaucous and waxy. *Inflorescence* a fascicle of successive flowers, with generally one flower open at a time, erect, enclosed at the base by a spathaceous bract, conduplicate, fibrous, oblong, *ca*. 12 mm long; peduncle terete, green, 8 mm long; floral bract tubular, papyraceous, acute, 3 mm long. *Ovary* pale pink spotted with purple, papillate, pedicellate, cylindrical, longitudinal sulcate, curved, 9 mm long. *Flowers* resupinate; *sepals* pale pink with light purple spots on adaxial side and pink with brown spots on abaxial side, membranaceous, papillate, concave, 7-veined. *Dorsal sepal* ovate, rounded, 9.5–12.0

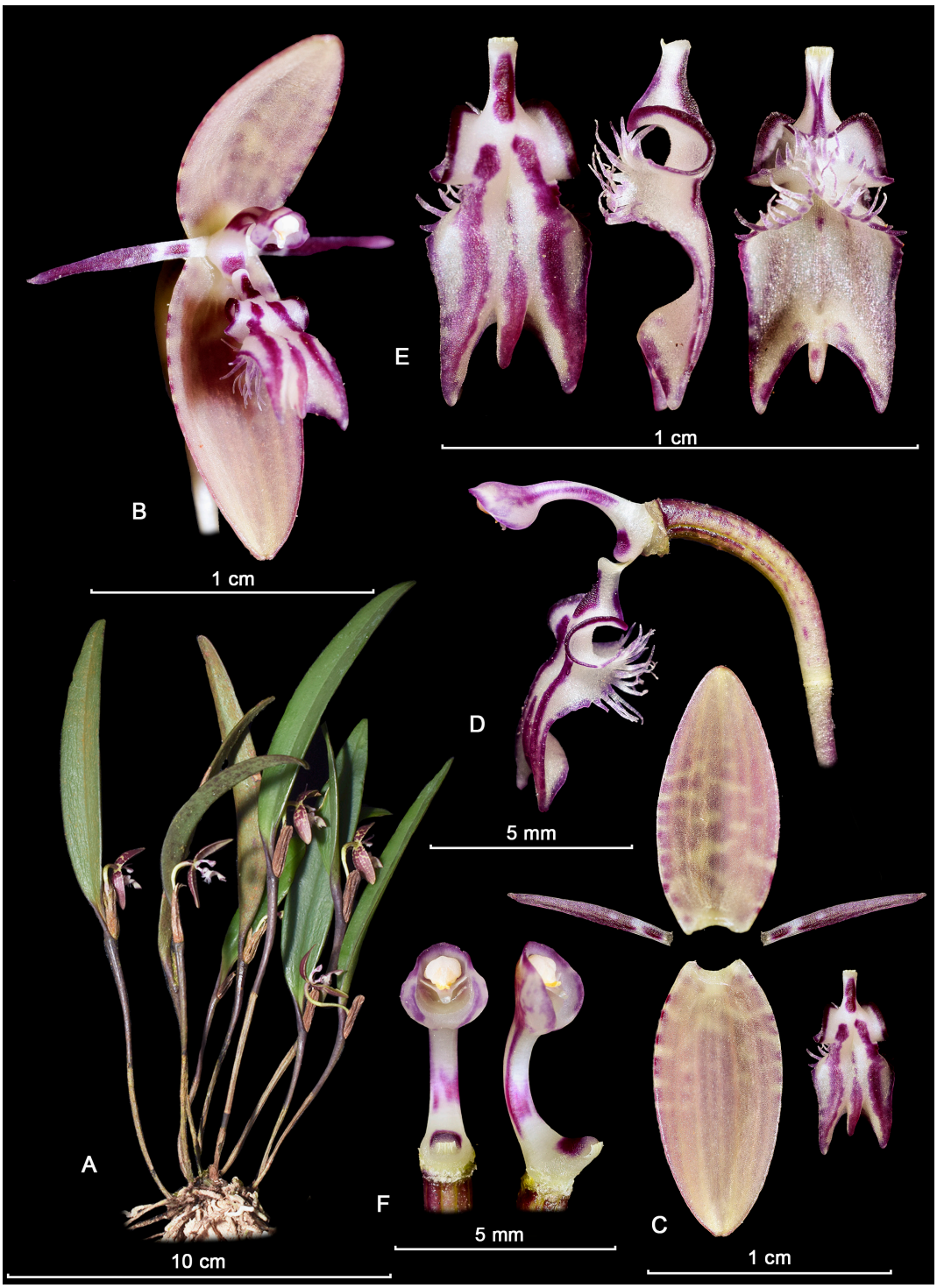

FIGURE 1. Lankester composite digital plate of *Pleurothallis vallejoi*. **A**. Habit. **B**. Flower. **C**. Dissected perianth. **D**. Lip and column lateral view. **E**. Lip. **F**. Column. LCDP by M. A. Sierra-Ariza based on *M.A. Sierra-Ariza, J. Alvarez-Diaz & Fernando Tinoco 491* (Holotipo: TOLI).

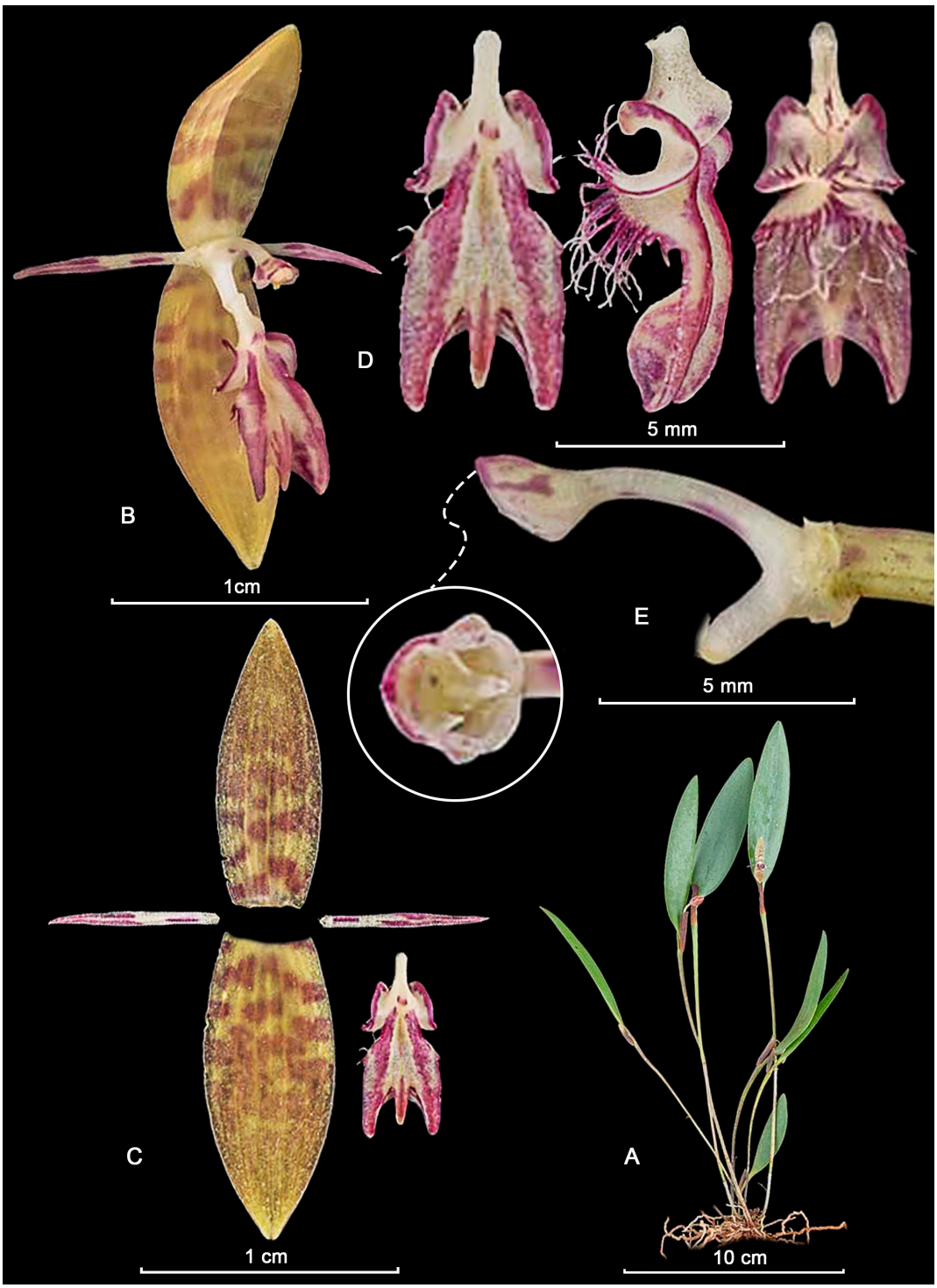

Figure 2. Lankester composite digital plate of *Pleurothallis trimeroglossa*. **A**. Habit. **B**. Flower. **C**. Dissected perianth. **D**. Lip. **E**. Column. Photographs by A. Goicochea. LCDP by M. A. Sierra-Ariza.

× 5.0–6.5 mm. *Lateral sepals* connate into a lanceolate, bifid synsepal, 9.5–13.0 × 5–6 mm. *Petals* deep purple, whitish at the base, fleshy, linear, acuminate, slightly oblique, papillose, 7–9 × 0.7–0.9 mm, 1–veined. *Lip* white with purple spots, protuberant, trilobed, 7.2–8.0 × 3.0–3.5 mm; prolonged base, oblong, 1.0–1.3 × 0.5–0.7 mm; subquadrate, revolute, fimbriated lateral lobes, 1.7–2.0 × 1.2–1.5 mm, forming two basal auricles, narrowly elliptical when viewed from above, 1.7–2.0 × 0.6–0.8 mm; with a callus located at the intersection of the base and the lateral lobes, elongated, thin, and slightly elevated, almost imperceptible to the naked eye, at the insertion of the lateral lobes and the middle lobe, a waist of 2.0–2.5 mm in width is formed, where a median sulcus of moderate depth is located; middle lobe tridentate, teeth separated, lateral teeth longer than the central, subtriangular, straight, rounded, 1.7–2.0 × 0.8–1.0 mm, middle tooth oblong, rounded, 1.0–1.3 × 0.3–0.5 mm. *Column* white spotted purple, slender, terete, arching, strongly dilated at the apex, 6.5–7.2 × 1.8–2.2 mm, pronounced column foot, 1.2 mm long. *Anther* cap white, subapical, rounded, papillose, 1.0–1.3 mm. *Pollinia* 2, yellow, rounded. *Capsule* not seen.

Eponymy: Named in honor of Dr. Gustavo Adolfo Vallejo, a distinguished researcher from Tolima, recently recognized as a member of the *Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, the first from that university to receive such distinction. This dedication highlights his significant contributions to tropical parasitology, particularly in identifying American trypanosomes affecting vulnerable populations, as well as his commitment to training new researchers and his lasting human and scientific legacy.

Additional specimen examined: COLOMBIA. Tolima: Municipio de Ibagué, corregimiento San Juan de La China, 2070 m. 10 March 2024, *J. Alvarez-Diaz, Fernando Tinoco* & *M.A. Sierra-Ariza 21* (JBB).

Phenology: The species exhibits continuous flowering throughout the year, with peak records between February and September. However, this cycle is influenced by climatic variables, with flowering being more frequent during rainy seasons and in areas with higher light exposure.

Distribution, Habitat and Ecology: The new species has been recorded in the Andes, on the eastern slope of the Central Cordillera, in various localities in the department of Tolima, mainly in the mountains surrounding the city of Ibagué. Its habitat is located in the transition zone between premontane and lower montane forests, at elevations ranging from 1800 to 2200 m. The ecosystem where it grows is subject to strong human intervention, which has reduced the natural areas to small fragments of forest and secondary vegetation, with a landscape dominated by open areas and pastures. In these zones, land use is mainly devoted to agricultural crops and livestock activities.

The species shows tolerance to environmental changes within its ecological niche, establishing itself along roadsides and forest fragments, where it occurs in the lower vertical strata and on the lower branches of trees. Most individuals were found on hosts with fissured bark, with principal phorophytes including species of the genera *Weinmannia* L. (Cunoniaceae), *Vismia* Vand. (Hypericaceae), *Ladenbergia* Klotzsch (Rubiaceae) and, to a lesser extent, some species of the family Lauraceae.

In addition, it is worth noting that the flowers of this species exhibit intraspecific color variation, with shades ranging from creamy white to light yellow, and with spots varying from light purple to red.

Conservation status: Data Deficient (DD). So far, *Pleurothallis vallejoi* has only been recorded in some forest remnants in the municipality of Ibagué, Tolima, Colombia. The area where it grows is highly degraded due to changes in land use for livestock and agricultural crops. Given the level of disturbance in its habitat and the low number of observed individuals, it is recommended to assess the conservation status of the species based on an evaluation of habitat availability and quality, as well the study of its known populations.

Discussion. *Taxonomy.— Pleurothallis vallejoi* (Fig. 1) belongs to the *P. talpinaria* complex, together with *P. trimeroglossa* (Fig. 2), *P. jostii* (Fig. 3), *P. talpinaria* (Fig. 4), and *P. gracilicolumna* (Fig. 5), but is distinguished by its narrowly elliptic auricles of intermediate size and subquadrate lateral lobes. The middle lobe is comparatively shorter than in allied species, while the lateral teeth are conspicuously longer than the cen-

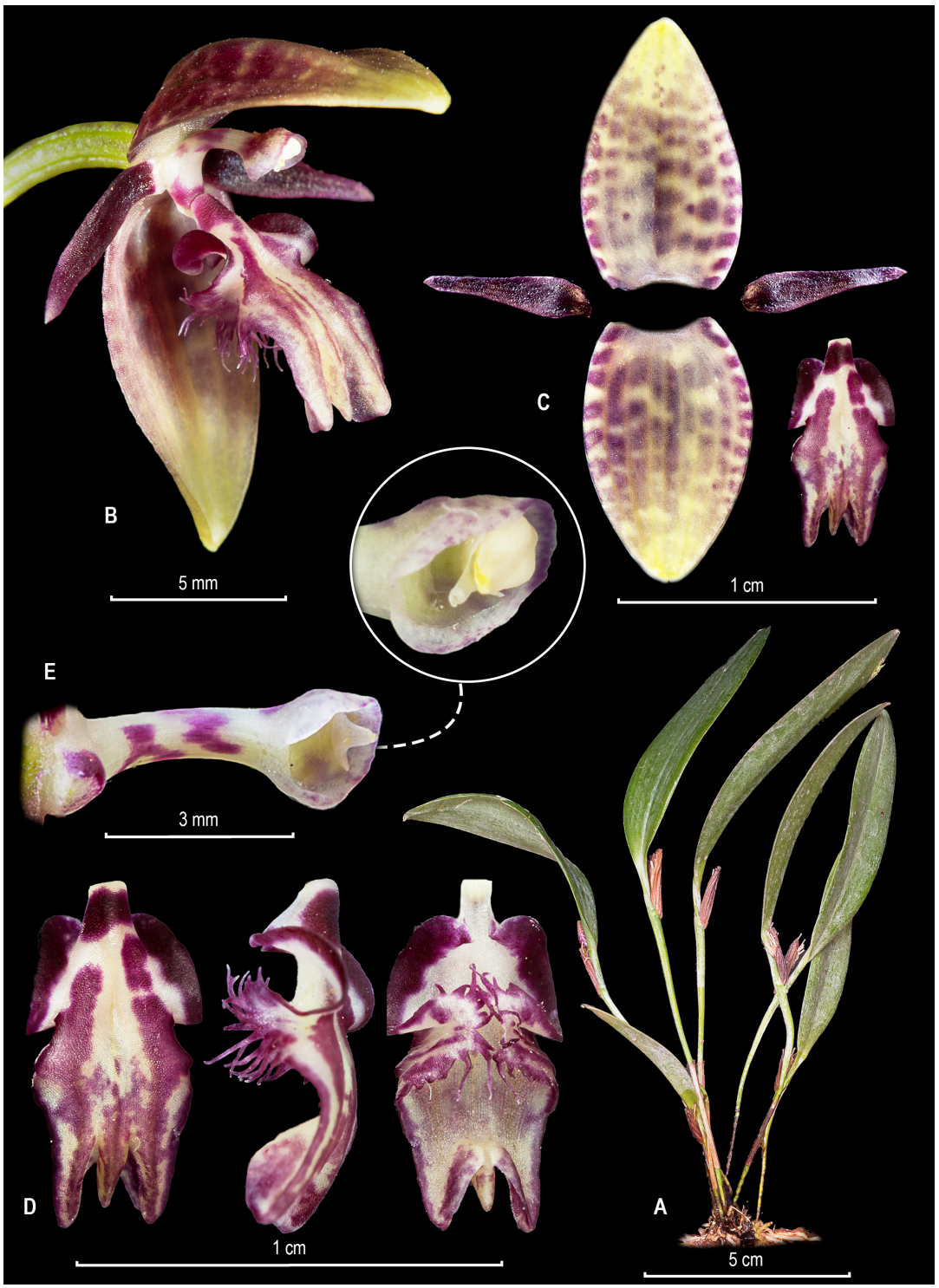

Figure 3. Lankester composite digital plate of *Pleurothallis jostii*. **A**. Habit. **B**. Flower. **C**. Dissected perianth. **D**. Lip. **E**. Column. Photographs by M. Wilson. LCDP by M. A. Sierra-Ariza.

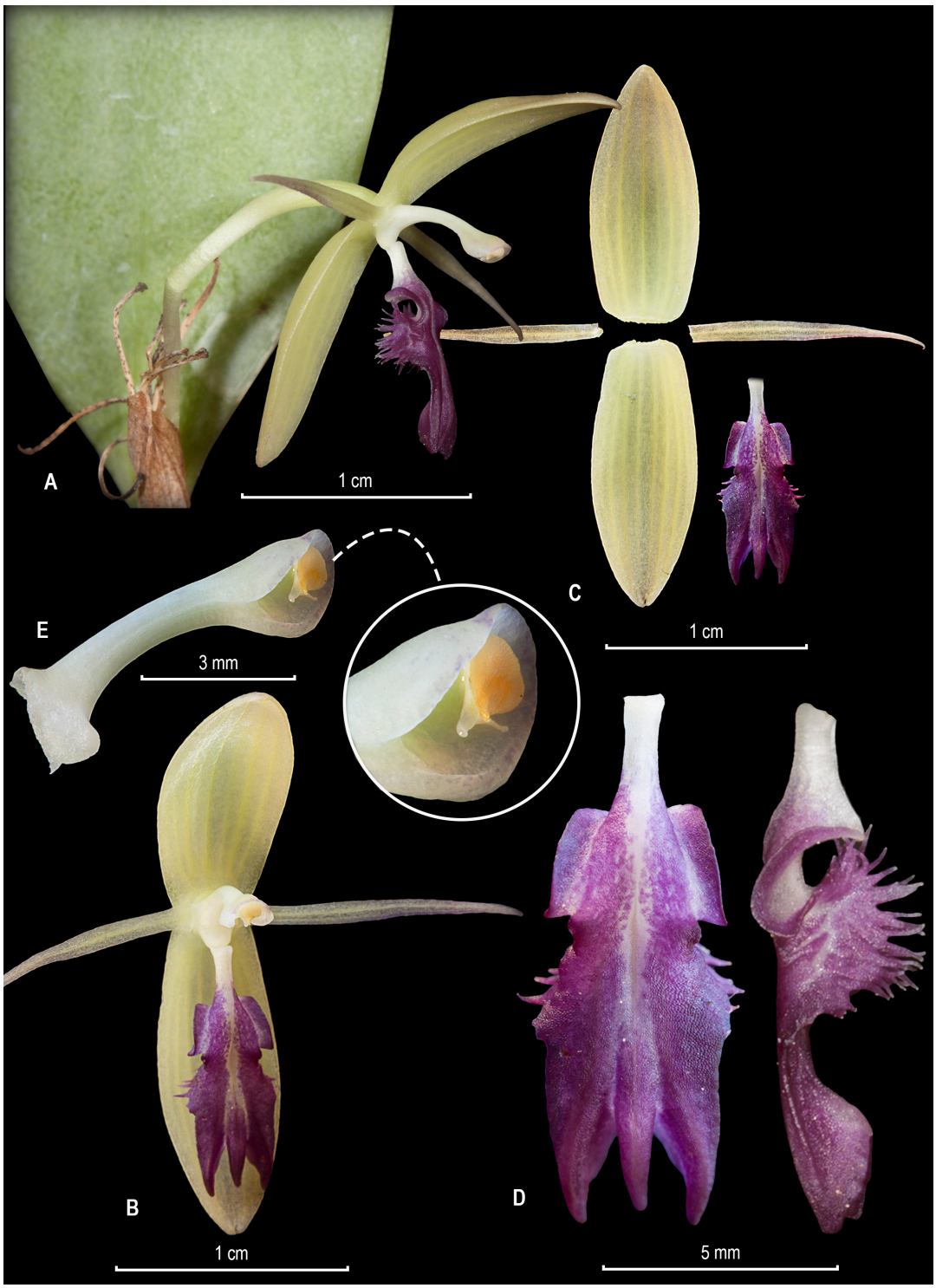

FIGURE 4. Lankester composite digital plate of *Pleurothallis talpinaria*. **A**. Flower, ovary, pedicel, and spathaceous bract. **B**. Flower. **C**. Dissected perianth. **D**. Lip. **E**. Column. Photographs by M. Wilson. LCDP by M. A. Sierra-Ariza.

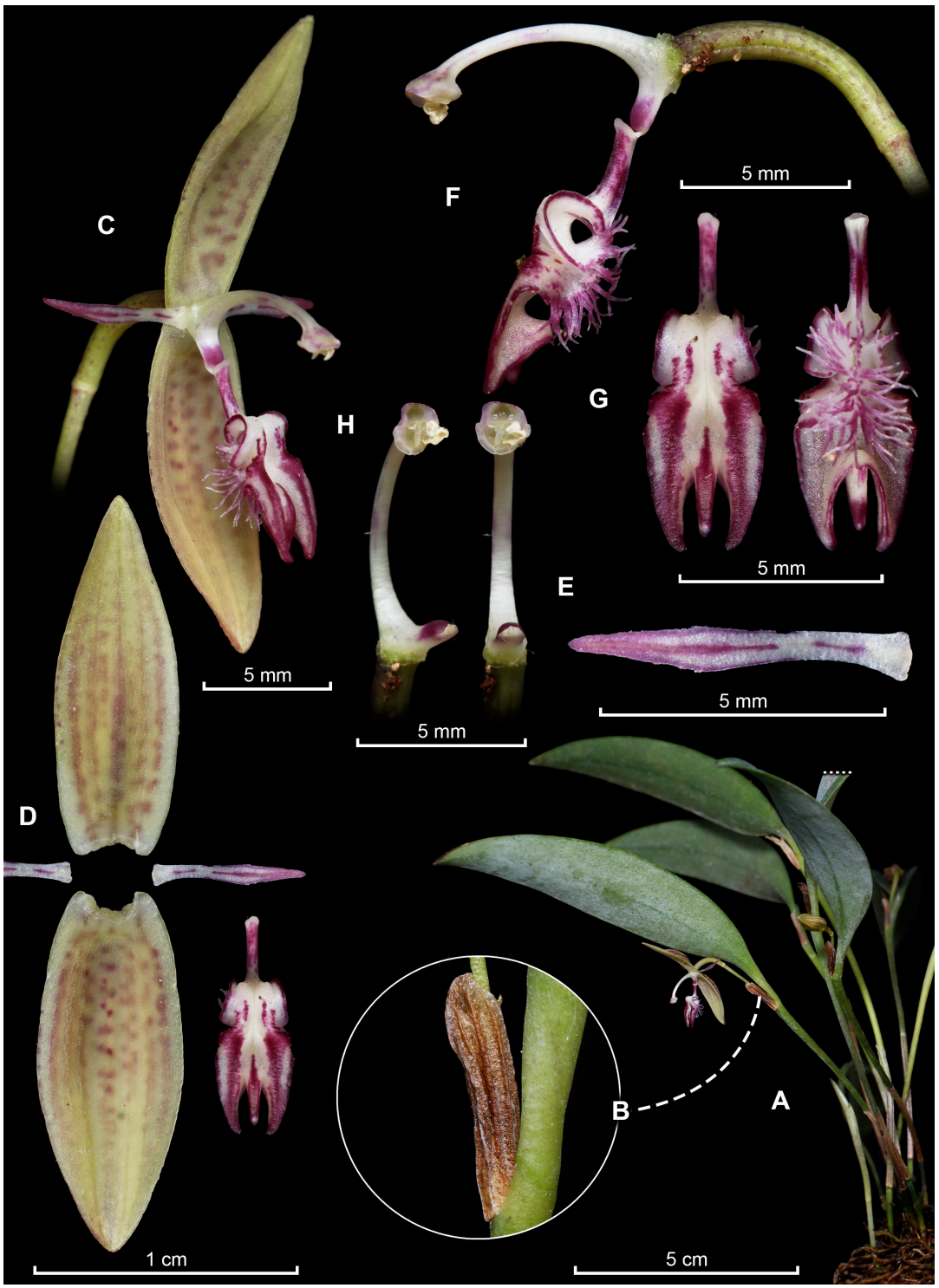

Figure 5. Lankester composite digital plate of *Pleurothallis gracilicolumna*. **A**. Habit. **B**. Spathaceous bract. **C**. Flower. **D**. Dissected perianth. **E**. Petal. **F**. Lip and column, lateral view. **G**. Lip. **H**. Column. LCDP by E. Restrepo.

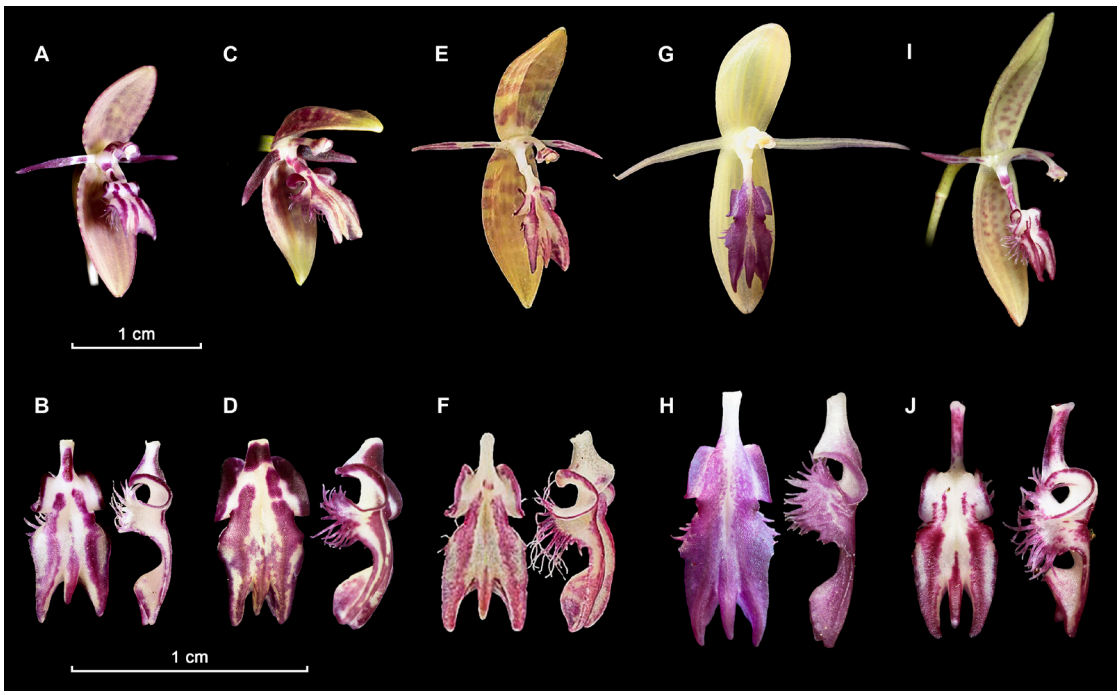

FIGURE 6. Comparison between flowers and labella of species belonging to the *Pleurothallis talpinaria* complex: *Pleurothallis vallejoi* (A, B), *Pleurothallis jostii* (C, D), *Pleurothallis trimeroglossa* (E, F), *Pleurothallis talpinaria* (G, H), and *Pleurothallis gracilicolumna* (I, J). Photographs by M. A. Sierra-Ariza (A, B), M. Wilson (C, D, G, H), A. Goicochea (E, F), and E. Restrepo (I, J). Plate by M. A. Sierra-Ariza and E. Restrepo.

tral one. The callus is minute, elongated, and barely perceptible, contrasting with the conspicuous dome-shaped or crest-like calli of related taxa, or its absence. Differentiating the members of this complex requires detailed analysis of lip morphology, particularly the shape and relative proportions of each part, which in some cases can be recognized by the percentage they occupy relative to the total lip length (Fig. 6, Table 1).

Ecology.— Relatively little is known about pollination in *Pleurothallis* (Table 1 in Karremans & Díaz-Morales, 2019) and nothing at all so far about pollination in subgenus *Talpinaria*. Notably, the labellum of the species in the *P. talpinaria* species complex lack a glenion, so prominent in other groups such as *Pleurothallis* subsection *Macrophyllae-Fasciculatae*, that is hypothesized to provide a nectar-like reward, resulting in correct positioning of the pollinator below the column during pollinarium acquisition and subsequent deposition (Wilson *et al.*, 2016, 2018).

Neither has any nectar-like reward ever been observed by the authors on the lips of these species, either *in situ* or under cultivated conditions. Understanding that secretion may occur *in situ* under certain conditions, but has not yet been seen, the lip of four species was examined by scanning electron microscopy to determine whether any cellular differentiation typical of secretory tissue could be observed (Fig. 7). Of the four species examined, *P. talpinaria*, *P. gracilicolumna*, *P. jostii* and *P.* cf. *trimeroglossa*, there was no discernible cellular differentiation on the adaxial surfaces. Preliminarily we conclude, therefore, that species in the *P. talpinaria* species complex are non-rewarding and are probably deceit pollinated (Karremans, 2023; Karremans & Díaz-Morales, 2019).

In addition to the lack of a glenion, any observed nectar-like secretions, or any cellular differentiation on the adaxial surface of the labellum indicating potential for secretion, the most unusual characteristic of the labellum of the *P. talpinaria* species complex is that it is motile. The labellum of most *Pleurothallis* species is hinged at the attachment to the column base and, for example in some species of subsection *Macrophyllae-Fasciculatae* Luer (2005), may

Table 1. Morphometric comparison and distribution of the *P. talpinaria* complex species. Measurements are given in milimeters (mm), based on the holotype, Wilson *et al.* (2017), and Wilson (2018).

	Pleurothallis gracilicolumna	***Pleurothallis jostii***	***Pleurothallis talpinaria***	***Pleurothallis trimeroglossa***	***Pleurothallis vallejoi***
Total flower length	28–30	16–19	23–27	22–24	19–24
Dorsal sepal	12.0–15.9 × 4.2–5.0	10.0–10.5 × 5.5–6.7	12.8–14.0 × 5.0–5.4	10.5–11.3 × 3.4–4.0	9.5–12.0 × 5.0–6.5
Synsepal	12.0–16.5 × 4–5	10 × 6.0–6.8	12.5–13.0 × 5.3–6.0	10.5–11.7 × 4.5–4.8	9.5–13.0 × 5–6
Petals	5.0–7.8 × 0.5	6–7 × 1.0–1.4	11–12 × 1.0	5.7–6.0 × 0.6	7–9 × 0.7–0.9
Lip size	8.2–10.0 × 3	7–8 × 3.3–3.9	11 × 4.0–4.2	7.0–8.5 × 2.8–3.0	7.2–8.0 × 3.0–3.5
Lip base	2.7–3.0 × 0.5	1.0 × 0.9	2.2 × 0.7	2.2 × 0.7	1.3–1.6 × 0.5–0.7
Basal auricles	2.1–2.3 × 0.4–0.6	2.9 × 1.1	2.5 × 0.6	2.0–2.3 × 0.4–0.6	1.7–2.0 × 0.6–0.8
Lateral lobes	2.1–2.4 × 3.5–3.8	1.9 × 2.3	2.5 × 2.8	1.8–2.1 × 1.1–1.3	1.7–2.0 × 1.2–1.5
Constriction (lateral + middle lobes)	2.8–3.0	2.8	2.6	1.8–2.0	2.0–2.5
Middle lobe	6.4–6.5 × 4.0–4.3	4.9 × 3.9	6.1 × 3.9	4.8–5.1	4.1–4.3 × 3.4–3.6
Lateral teeth	1.5–1.8 × 1.3–1.5	1.6 × 0.8	1.7 × 1.2	1.4–1.6 × 1.1–1.3	1.7–2.0 × 0.8–1.0
Central tooth	1.2–1.3 × 0.8–1.0	1.2 × 0.6	1.5 × 0.7	0.8–1.1 × 0.3–0.4	1.0–1.3 × 0.3–0.5
Sulcus	Deep	None	Moderate	Deep	Moderate
Column length	7.5–8.0	4.6–4.8	6.8	6	6.5–7.2 × 1.8–2.2
Distribution	Colombia	Ecuador?	Colombia, Venezuela	Bolivia, Ecuador, Perú	Colombia

be sufficiently loosely attached to depress from an elevated position to a depressed position, against the synsepal. However, in the *P. talpinaria* species complex, the connection between labellum and column is sufficiently loose that the lip is easily agitated by a small breeze or human breath. Whether this labellar motility plays any role in the hypothesized deceit pollination of the group, as it does in certain *Bulbophyllum* species for example (Borba & Semir, 1998), remains to be investigated. However, we do propose that given the open morphology of the flowers (Karremans & Díaz-Morales, 2019), rendering them accessible to visitors of various sizes, the lip may only remain in the 'horizontal' position for the pollinator, or smaller visitors, and become depressed by visitors of greater size and mass, thereby distancing them from the pollinarium.

An interesting addition to the possibility that these open flowers select the desired pollinator via labellum motility combined with distance of the labellum from the clinandrium, is the case of *P. trimeroglossa*. This species can be recognized by a pronounced

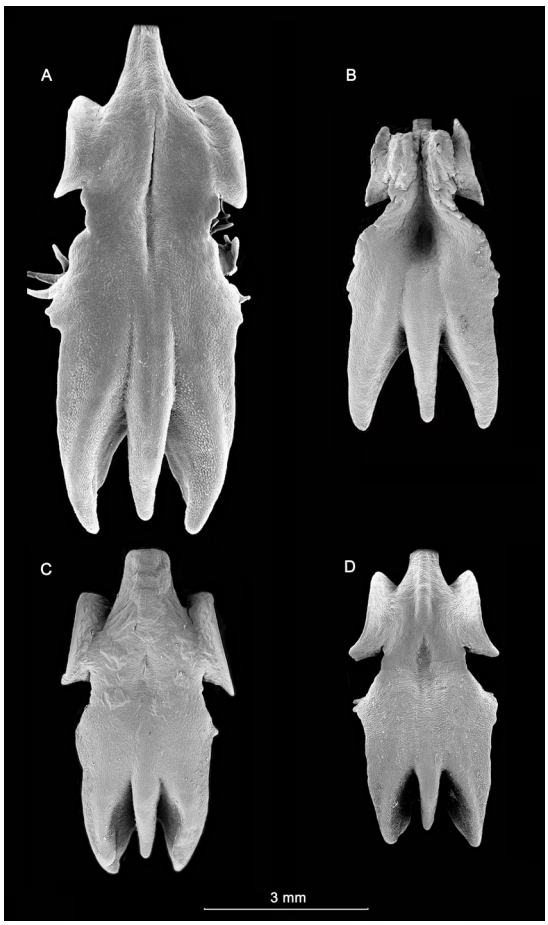

FIGURE 7. Scanning electron micrographs of labella of *P. talpinaria*-complex species illustrating absence of differentiated secretory tissue on the adaxial surfaces. **A**. *Pleurothallis talpinaria* PL0946. **B**. *Pleurothallis gracilicolumna* PL0942. **C**. *Pleurothallis jostii* PL0782. **D**. *Pleurothallis* cf. *trimeroglossa*. Scanning electron micrographs by Kehan Zhao. Composition by M. Wilson.

dome-shaped callus on the hypochile (lip base) below the column (Fig. 8). One obvious question presents itself – does this callus contribute in some manner to selecting fly species of the correct size for pollination? Further, since *P. trimeroglossa* seems to be variable in color across its geographic range and to display concomitant differences in height of the callus (Wilson *et al.*, 2017), could this be a mechanism for creating barriers to gene flow through selecting pollinators of different sizes, suggesting that what we call *P. trimeroglossa* is comprised of more than one species?

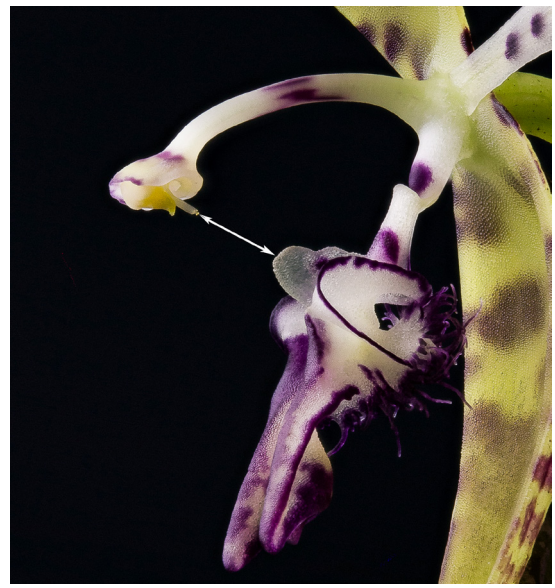

FIGURE 8. Flower of *Pleurothallis trimeroglossa* (lateral view) illustrating the distance between the dome-shaped callus on the hypochile and the viscidium of the pollinarium (double-headed arrow), a potential determinant of pollinator size. Photographs by G. Verhellen. Composition by M. Wilson.

To date, as far as the authors are aware, the only recorded *in situ* observation of an insect visitor and possible pollinator to a member of this species complex, in this case *P. talpinaria* itself, was a wood gnat (*Sylvicola* sp., Anisopodidae, Diptera) (Fig. 9). In this instance, the labellum can be observed to remain in the 'horizontal' or non-depressed position and the fly is positioned with its thorax directly below the clinandrium, appropriately positioned for contact with the pollinarium. Interestingly, when apparently in the correct orientation to interact with the pollinarium, the fly is in a head-up position, suggesting that it is not in the process of attempting to feed on nectar and has been deceived into visiting the flower. Of course, without direct observation of pollinarium acquisition and subsequent deposition under *in situ* conditions, on multiple occasions, we cannot conclude this species is the pollinator. However, the wood gnat does appear to interact with the flower of *P. talpinaria* in a manner indicative that it *could* be a pollinator of this species.

Several questions present themselves regarding pollination in the *P. talpinaria* species complex. Are Dipterans of family Anisopodidae pollinators of these

Figure 9. Flowers of *Pleurothallis talpinaria in situ* being visited by Diptera. **A–B**. A wood gnat (*Sylvicola* sp., *Anisopodidae*, Diptera), a potential pollinator of the species. **C**. Small flies visiting the lip and synsepal. Photographs by Nicolas Baresch Uribe (A, B) and Mateo Hernandez Schmidt (C). Composition by M. A. Sierra-Ariza.

flowers? If demonstrated, this would be the first example of a member of Anisopodidae pollinating *Pleurothallis* (Table 1 in Karremans & Díaz-Morales, 2019). Are these species exhibiting food deceit pollination? What flowers are being mimicked in this deceit pollination? What chemical or visual cues are the pollinators responding to?

Morphology.— In our description of *P. vallejoi*, we characterized the labellum morphology as a trilobed structure, which, in our opinion, provides a clearer understanding of its composition. In contrast to other publications on species of the *P. talpinaria* complex (Wilson, 2018; Wilson *et al.*, 2017), where it was described as a compound lip, we are aware that the label-

lar morphology of these species is difficult to interpret. Therefore, we recommend to consultation of Wilson (2018; Fig. 8), where each part of the lip is clearly labeled, with the following variations: instead of "hypochile" we use "labellum base", instead of "auriculate basal lobe" we use "basal auricles and lateral lobes", instead of "mesochile" and "epichile" we use "tridentate median lobe".

Acknowledgements. We would like to express our sincere gratitude to Eugenio Restrepo for allowing us to use his LCDP of *P. gracilicolumna*, and to Antonio Goicochea and Gerrit Verhellen for sharing their photographs of *P. trimeroglossa*. We also thank Kehan Zhao for providing the scanning electron micrographs, and Nicolás Baresch and Mateo Hernández Schmidt for their valuable photographs of the flower of *P. talpinaria* and its floral visitors. Additionally, we are grateful to Laura Ximena Castillo Balaguera, Jorge Arturo Romero Barrera, and Nicolle Milena Mendoza Beltrán for their participation and support during field outings and monitoring of this species in its natural habitat. Wilson thanks Colorado College for provision of greenhouse space, laboratory space and access to the scanning electron microscope. Finally, we thank the Universidad del Tolima for supporting this research through the collection permit granted under Resolution No. 000009 of the ANLA, and the TOLI Herbarium for accepting the deposit of the type specimen in their collection.

Funding. This work was carried out with personal funds.

Author contributions. MSA: Writing – Original draft, review & editing, Abstract, Keywords, Introduction, Materials and methods, Diagnosis, Description, Discussion, Conservation status, Morphological note**,** Acknowledgments, and Figures 1, 2, 3, 4, 6, and 9, as well as Table 1. JAD: Eponymy, Distribution, Habitat and Ecology, and Phenology. FT: Distribution, Habitat and Ecology. MW: Ecological discussion and Figures 7 and 8.

Conflict of interest. The authors confirm that they have no financial conflicts of interest or personal affiliations that could have influenced the findings presented in this paper.

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NEW EVIDENCE CORROBORATES THE PRESENCE OF *SOLENIDIUM LUNATUM* (ONCIDIINAE) IN COLOMBIA

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Abstract. *Solenidium* is a South American genus of three species characterized by free, similar sepals and petals, a trilobed lip with a grooved claw, and a winged column bearing two ceraceous pollinia. Here we confirm the presence of *Solenidium lunatum* in Colombia, based on a well-documented collection from Sierra de la Macarena National Natural Park. Previous records of *S. lunatum* in the country were doubtful, relying solely on catalog citations derived from a fragmented specimen at K (K–SPC–61118.000) lacking precise locality data. The new collection provides the first unequivocal evidence of the species in Colombia and confirms its known range, which extends from northern South America (Colombia, Venezuela) to the Brazilian Cerrado, mainly in areas with granitic outcrops.

Resumen. *Solenidium* es un género sudamericano de tres especies caracterizadas por sépalos y pétalos libres y similares, un labelo trilobulado con una uña acanalada y una columna alada que porta dos polinias ceráceas. Aquí confirmamos la presencia de *Solenidium lunatum* en Colombia, con base en una colecta bien documentada del Parque Nacional Natural Sierra de la Macarena. Los registros previos de *S. lunatum* en el país eran dudosos, pues se sustentaban únicamente en citas de catálogos derivadas de un espécimen fragmentado en K (K–SPC–61118.000) sin datos de localidad precisos. La nueva colecta constituye la primera evidencia inequívoca de la especie en Colombia y confirma su rango conocido, que se extiende desde el norte de Sudamérica (Colombia, Venezuela) hasta el Cerrado brasileño, principalmente en áreas con afloramientos graníticos.

Keywords/Palabras clave: Colombian Orinoco, Orinoquía Colombiana, orquídeas, orchids, plantas, plants, taxonomía, taxonomy

Introduction. The genus *Solenidium* Lindl. was established in 1846 by John Lindley, based on a specimen collected by Jean Linden in 1842 in forests of Pamplona, Colombia. Its type, *Solenidium racemosum* Lindl., is characterized by the free, similar sepals and petals, a labellum with a grooved basal claw and bidentate apex, a winged column with a trilobed apex, and two ceraceous pollinia inclined over a small subrounded gland (Dalström & Whitten, 2016; Lindley, 1846; Sweet, 1973). These distinctive features differentiate *Solenidium* from related genera such as *Oncidium* Sw. and *Brassia* R.Br. Recent DNA studies, however, indicate a closer relationship with *Capanemia* Barb. Rodr., and *Sanderella* Kuntze, emphasizing the need for a further systematic revision within the Oncidiinae (Buzatto *et al.*, 2020; Chase *et al.*, 2005; Dalström & Whitten, 2016; Neubig *et al.*, 2012).

The second species of the genus, *Solenidium lunatum* (Lindl.) Schltr., was originally described as *Oncidium lunatum* Lindl. in 1838, based on a specimen imported from Demerara (Guyana) and subsequently transferred to *Solenidium* by Schlechter (1914[1915]).

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Received 28 July 2025; accepted for publication 13 October 2025. First published online: 20 November 2025.

Solenidium lunatum is widely distributed across South America, from Colombia to Bolivia and Brazil. In Brazil, it inhabits several threatened ecoregions, including the Amazon, the Cerrado, the seasonal forests of Goiás and Mato Grosso, the savannah of Roraima, and the wetland mosaics of Maranhão and Pará (Barros *et al.*, 2015; Dos Santos Cardoso *et al.*, 2019). In these ecoregions, the expansion of agribusiness and the replacement of native vegetation by monocultures and grasslands have promoted habitat fragmentation and biodiversity loss (Laurance & Vasconcelos, 2009; Queiroz, 2009; Dos Santos Cardoso *et al.*, 2019).

A third species, *Solenidium portillae* Dalström & Whitten (Dalström & Whitten, 2003), was described from Ecuador and has since been recorded in Colombia. In Colombia it occurs in the cloud forests of Bolívar municipality in Valle del Cauca, where it has been reported growing epiphytically on lianas of the genus *Paullinia* L. (Sapindaceae) (Cárdenas-Contreras, 2014). This record not only expands the chorological knowledge of *Solenidium* in the country, but also underlines the importance of these Andean ecosystems for the conservation of rare and little-known species. Intertropical areas, such as the Andes and the Amazon, concentrate most of the biodiversity hotspots, regions that host at least 1,500 endemic species of vascular plants and have lost more than 70% of their original cover (Myers *et al.*, 2000, CONABIO, 2009). In this context, orchids stand out as one of the most threatened plant groups due to their high ecological specialization and pressures such as overexploitation, illegal trade, habitat loss, and climate change, making them flagship taxa in global conservation efforts (Cribb *et al.*, 2003; Dressler, 1993; Swarts & Dixon, 2009; Tejeda-Sartorius *et al.*, 2017).

For many years, the inclusion of *Solenidium lunatum* in Colombian floristic catalogues has remained questionable, primarily due to the absence of corroborating herbarium collections or the discovery of living specimens. This uncertainty is resolved here. In 2025, we confirmed the native presence of the species in Colombia upon discovering four vouchered populations of *S. lunatum* from the Amazon–Orinoquía (Meta, Colombia), supported by field photographs, detailed floral dissections, precise georeferences, and comparative diagnoses against allied genera.

Materials and methods. The specimens examined were collected during botanical explorations conducted from February to May 2025 in the municipalities of Mesetas, San Juan de Arama, Vista Hermosa, and Puerto Rico in the Meta department of Colombia. The comparative morphological study was based on examinations of living plants, dried specimens, illustrations and spirit material preserved in 75% ethanol, stored at Kew Gardens (Royal Botanic Gardens, Kew). Photographs were taken with a Nikon D5300 equipped with an AF-S Nikkor 50mm f/1.8G lens, and images were post-processed for clarity using Adobe® Photoshop CS6. The terminology used for the morphological descriptions follows Beentje (2016). Identification was achieved by comparing the morphometric characteristics of fresh specimens with the original descriptions (Lindley, 1829). To characterize the species' distribution and area of occurrence, we examined digital and physical collections at Colombian, North American, and European herbaria (AMES, COAH, COL, G, MO, NY, U, and US; acronyms follow Thiers, 2024). We also reviewed the designated holotypes of names currently treated as synonyms of *S. lunatum*. Finally, we combined these data with records from GBIF (2025) and iNaturalist (2025) to construct the geographical distribution map using QGIS version 3.40 (2024).

Taxonomic treatment

Solenidium lunatum (Lindl.) Schltr., Orchideen Beschreib. Kult. Zücht.: 525. 1914[1915]. *Oncidium lunatum* Lindl., Edwards's Bot. Reg. 23: t. 1929. 1837. *Rodriguezia lindmanii* Kraenzl., Kungl. Svenska Vetenskapsakad. Handl. n.f, 46(10): 75, t. 12. 1911. *Leochilus mattogrossensis* Cogn., Relat. Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas Annexo 5, Bot. III. 13. 1912; Fedde, Repert. xiii. 492. *Solenidium mattogrossense* Schltr., Repert. Spec. Nov. Regni Veg. 15: 215. 1918. *Solenidium lunatum* Kraenzl., Pflanzenr. (Engler) Orchid. Monandr. Oncid. 316. 1922. Isonym (Fig. 1)

TYPE: GUYANA. Demerara: This very pretty species of *Oncidium* was imported from Demerara by Messrs. Loddiges, with whom it flowered in their stove of epiphytes in June last. s.coll. [s.n.]. (holotype: K–000718291!).

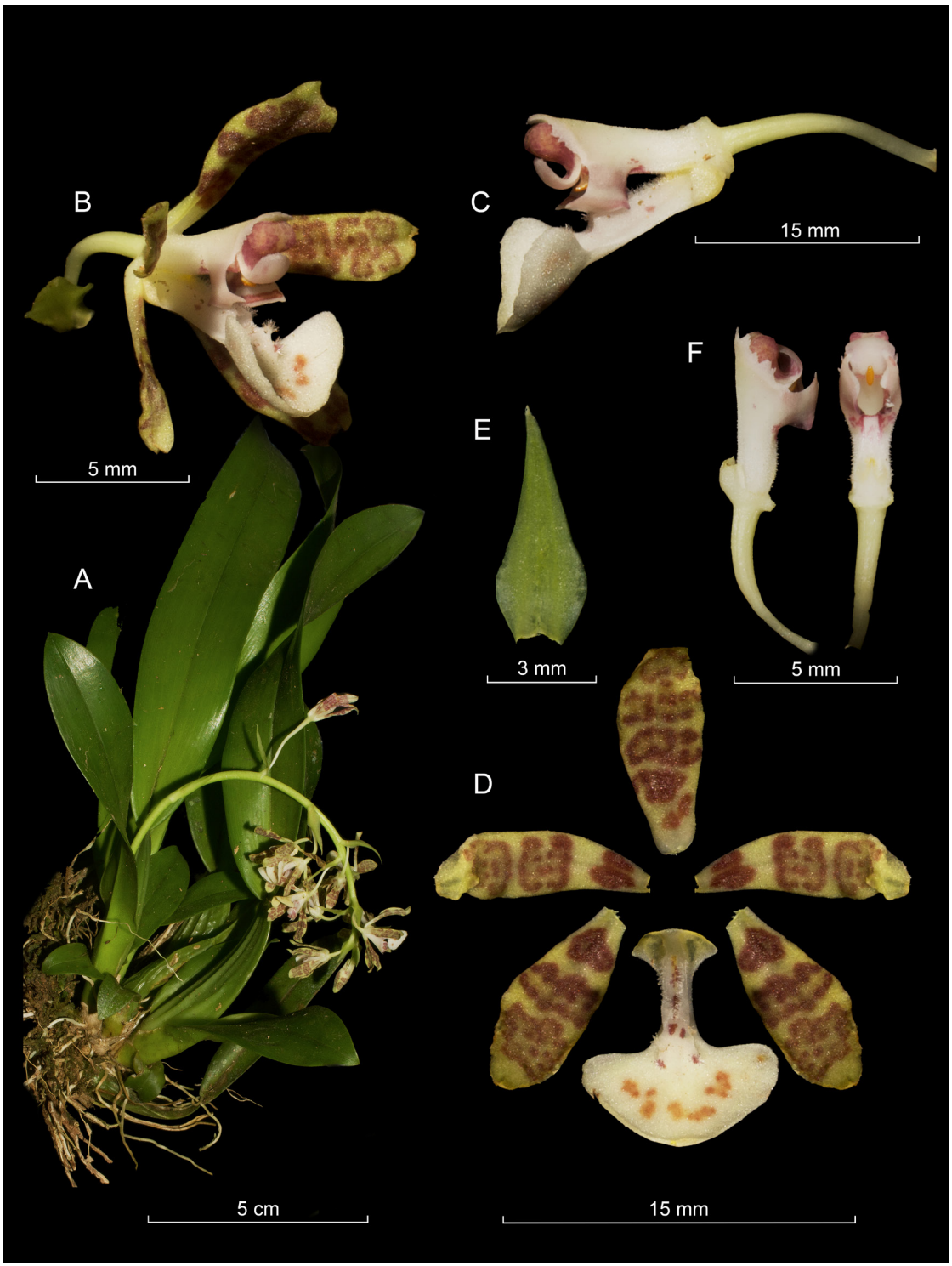

Figure 1. Lankester Composite Digital Plate of *Solenidium lunatum*. **A**. Habit. **B**. Flower. **C**. Lip and column in lateral view. **D**. Dissected perianth. **E**. Flower bract. **F**. Column. Photographs by D. Lozano-Cifuentes.

Epiphytic *herb*. *Pseudobulbs* 3.5–4.5 × 2.0–2.5 cm, caespitose, blunt elliptic, ancipitous, more or less sulcate, unifoliate, green, subtended basally by 3 to 4 distichous sheaths, the uppermost foliaceous, lustrous green. *Leaves* (8.0–)10–12(–14) × 1.8–2.2 cm, conduplicate, elliptic, acute, apiculate. *Inflorescence* 10–15 cm, axillary, from the base of the uppermost sheaths, arching, up to 14 flowered; flower bracts 10–12 × 3–4 mm, adpressed, lanceolate, light green. *Flowers* 25–30 mm long, slightly campanulate; pedicel 10–12 mm long × 1.5–1.7 mm diameter, cylindrical, glabrous, whitish green; ovary 3–4 mm long, slightly ribbed, whitish green. *Sepals* 8–11 × 4.0–4.5 mm, unguiculate, oblong, apex obtuse, greenish yellow with red-brown spots. *Petals* 8–11 × 2.5–3.0 mm, unguiculate, oblong, apex obtuse, greenish yellow with red-brown spots. *Lip* 10–12 mm long, rigidly attached to the base of the column and through a short, central, longitudinal, fleshy keel, trilobate, with barely developed lateral lobes 1.0 × 1.4–1.8 mm, visible only as small auricles; intermediate lobe 10 × 8 mm, formed by a long, linear keel, which expands into a deeply cordate, anteriorly rounded, slightly crenulate, almost crescentic in outline, markedly dilated lamina; lamina 6 × 9–10 mm, whitish, covered with minute trichomes and scattered spots red-brown; keel provided with two fleshy, very thin, depressed lamellae, bidentate on both margins, extending over a densely pubescent central disc. *Column* 6–7 mm long, shortly clavate, pubescent basally and ventrally, canaliculated ventrally, with a pair of two distinct, falcate lobes, revolute and a well-developed, entire anther cap 2.0 × 1.6 mm, bilobulate, with an intermediate lobule, elongated, apex obtuse, revolute; clinandrium with laciniate edge; pollinarium 1.3–1.5 × 0.6–0.8 mm, of two, cleft, pyriform pollinia on a narrowly elongate, obovate, stipe on a flat viscidium.

Material examined: BOLIVIA: [without data collected], *R. Vasquez 81*. (LPB!). BRAZIL. **Mato Grosso**: Valle del Paraguay cerca de Tapirapuã, 09 Nov. 1909, *Hoehne s. n.* (BR– 0000006590161!); Rio dos Bugres, ad arbores silvae ripariae quam inundavit fluvius, 13 Mar. 1894, *C. A. M. Lindman* A-2847. (S–R–5497!). COLOMBIA. **Meta**: Puerto Rico, vereda Santa Lucía, sendero hacia las lagunas, 230 m, 2.78106 N, 73.42918 O, 18 Feb. 2025, *D. Lozano-Cifuentes, Amilcar Santos & Fausto Riaño 2505*. (LLANOS!). ECUADOR. **Napo**: [without data collected], 200 m, 08 May. 1980, *J. S. Brandbyge & E. Asanza 30598*. (MO–656180!). PERU. **Huánuco**: Provincia de Pachitea, Distrito de Honoria, carretera Miel de Abeja, 13 Feb. 1967, *J. Schunke 1600* (F–1686843!). **Loreto**: Alto Amazonas. Andoas, río Pastaza near Ecuador border. Mature and disturbed forest, 210 m, 14 Aug. 1980, *A. L. Gentry et al. 29634*. (MO–679796!). **Madre de Dios**: Tambopata. Las Piedras. Cusco Amazónico. Plantas colectadas en la claro de alberque, 200 m, 08 Dec. 1991, *M. E. Timaná 3678*. (MO–670138!). GUYANA. **Upper Takutu-Upper Essequibo**: Swamp riverine forest and bank vegetation, 250 m, 15 Mar. 1994, *T. Henkel et al. 5221*. (US!).

Other Records: COLOMBIA. Two flowers in 70% ethanol. *Hermans 1833* (K–61118.000!).

Habitat and Ecology: Four populations of up to 13 individuals of *Solenidium lunatum* have been found in the Monserrate Alto rural settlement of the Municipality of San Juan de Arama (1 population) and the Santa Lucía rural settlement of the Municipality of Puerto Rico (3 populations). These populations are located in three biomes within the Sierra de la Macarena National Natural Park: the Amazonian-Orinoquía Tropical Humid Zonobiome, the Amazonian-Orinoquía Helobiome, and the Macarena Orobiome. The plants grow as epiphytic herbs in lowland tropical rainforests on tree species of *Protium rhoifolium* (Benth.) Byng & Christenh. (Burseraceae), *Heisteria acuminata* (Bonpl.) Engl. (Olacaceae), and *Matayba purgans* (Poepp.) Radlk. (Sapindaceae). Additionally, it forms epiphytic orchid communities that include *Acianthera discophylla* (Luer & Carnevali) Luer, *Gongora arcuata* G.Gerlach & Toulem., *Rudolfiella aurantiaca* (Lindl.) Hoehne, and *Scaphyglottis bidentata* (Lindl.) Dressler. It has been found flowering from January to March.

Notes: The first record cited to support the presence of *Solenidium lunatum* in Colombia was a fragmented collection (K–SPC–61118.000) housed at the Royal Botanic Gardens, Kew. It consists only of two flowers originating from Hermans nursery, without specific collection data (Fig. 2). The absence

Figure 2. Hermans collection #1833, K–SPC–61118.000. Photo provided by the Board of Trustees of the Royal Botanic Gardens, Kew.

of vegetative material and verifiable provenance limits its value as concrete evidence, even though the species has been reported as widely distributed from northern South America (Colombia and Venezuela) southwards to the Brazilian Cerrado (Fig. 3). Associated with granitic formations, such as tepuis and vertical walls, this rare species extends across Guyana, Suriname, Colombia, Venezuela, and Brazil. For a long time, the lack of a reliable reference specimen led to its presence in Colombia being regarded as doubtful in the literature (Bernal *et al.*, 2015, 2016; Ministerio de Ambiente y Desarrollo

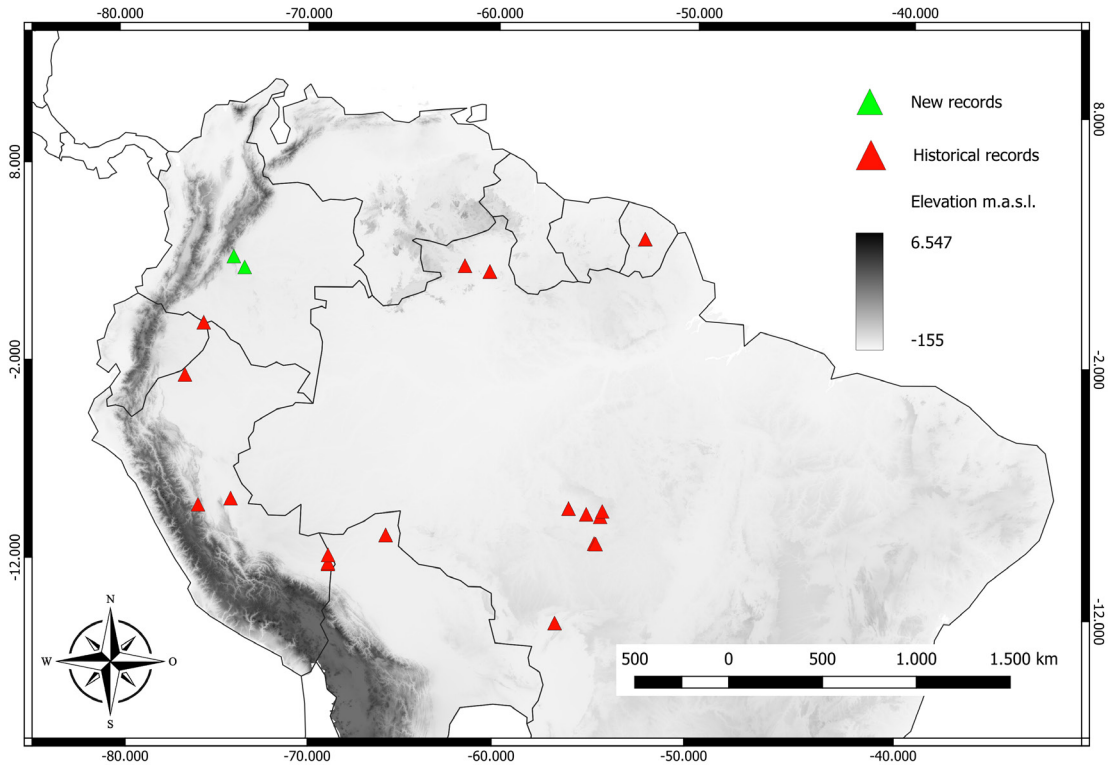

FIGURE 3. Updated geographical distribution of *Solenidium lunatum*. Elaborated by Amilcar Santos.

Sostenible y Universidad Nacional de Colombia, 2015). The recent discovery of this species during floristic surveys in Sierra de la Macarena National Natural Park now provides unequivocal evidence of its native occurrence in Colombia and argues for its prioritization in conservation and propagation programmes, alongside related Colombian species such as *S. racemosum* and *S. portillae*. In contrast, the presence of *S. portillae* in Ecuador remains questionable, as the species was described by Dalström & Whitten (2003) solely from cultivated material (Ecuagenera) and has not yet been documented from wild populations there, whereas several populations have been recorded in Valle del Cauca, Colombia (Fig. 4; Alomia, 2025).

The definitive confirmation of *Solenidium lunatum* in Colombia not only expands the known distribution of *Solenidium* but also underscores the critical role of the Andean and Amazonian intertropical regions in global biodiversity conservation. These areas are recognized biodiversity hotspots (CONABIO, 2009; Myers *et al.*, 2000), where orchids are particularly threatened due to their ecological specialization and the combined effects of habitat loss and illegal trade (Cribb *et al.*, 2003; Dressler, 1993; Swarts & Dixon, 2009; Tejeda-Sartorius *et al.*, 2017). This finding highlights the urgent need for focused conservation measures to protect these flagship plant species.

ACKNOWLEDGEMENTS. We sincerely thank the communities of the Sierra de la Macarena National Natural Park for their dedication to ecosystem conservation and to the park's technical team for their support and assistance during field trips. We are also appreciate Juan Sebastián Moreno and Milton Rincón González for their valuable collaboration and feedback on this discovery. Additionally, our gratitude extends to Gustavo A. Romero (General Curator of the Oakes Ames Orchid Herbarium), André Schuiteman (Curator of Orchidaceae at the Kew Gardens Herbarium), Yasmin Alomia (Serraniagua Corporation), and Stig Dalström for their studies on Oncidiinae.

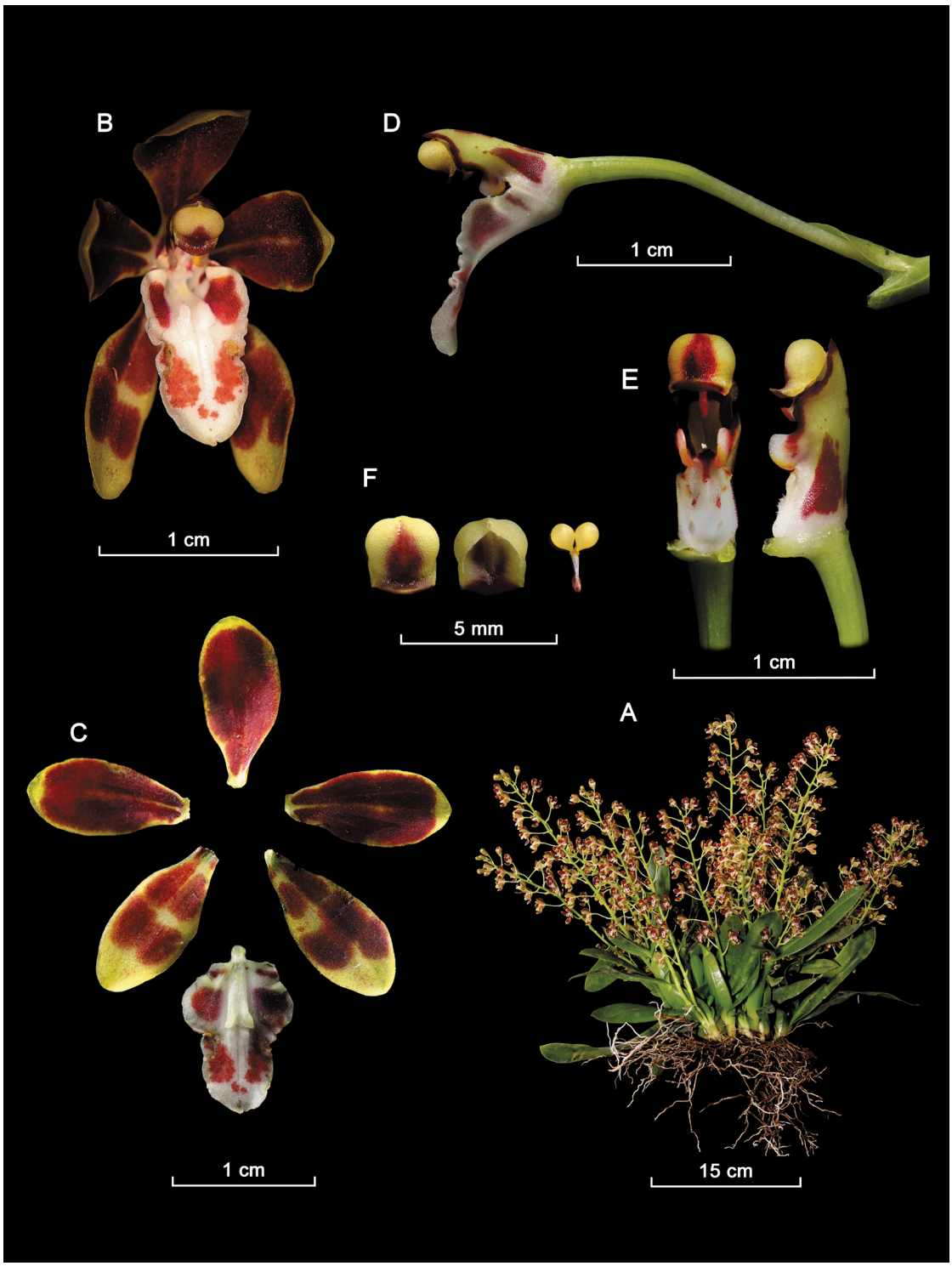

Figure 4. Lankester Composite Digital Plate of *Solenidium portillae* **A**. Habit. **B**. Flower. **C**. Dissected perianth. **D**. Lip and column in lateral view. **E**. Column. **F**. Anther cap and pollinarium. Photographs by Y. Alomia.

Author contributions: DLC, ASM; Data curation: DLC, JLP, and ASM; Investigation: DLC, ASM, JLP and FR; Writing - original draft: DLC and ASM; review & editing: DLC, ASM, JLP and FR.

Funding: The authors did not receive support from any organization for the submitted work.

Conflict of interest: The authors declare no conflicts of interest.

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RECRUITMENT BOTTLENECK IN APHYLLOUS *VANILLA* SEEDLINGS FACING DROUGHT CONDITIONS

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Abstract. Seedling survival is strongly dependent on forest environmental conditions, which in Madagascar have been heavily degraded. Rising temperatures and declining rainfall further exacerbate the vulnerability of these ecosystems. We investigated seedling recruitment across ecologically diverse sites to identify the key factors influencing germination and seedling survival in aphyllous *Vanilla* species. *In situ* seedling monitoring and *in vitro* seed germination trials were conducted to assess tolerance to water stress at various developmental stages. Among the 13 study sites, only three showed positive recruitment rates, with two sites exhibiting rates exceeding 50%. Recruitment was influenced by factors such as fruiting success, soil acidity, high silt content, and clay-rich soil composition. After 12 months of monitoring, approximately 85% of seedlings were lost following severe drought conditions. *In vitro* asymbiotic germination assays revealed two distinct peaks: rapid germination of immature white seeds after four months, followed by delayed germination of mature black seeds eight months later. The assessment of drought tolerance across protocorm developmental stages exposed to a high-concentration gelling agent revealed that advanced-stage protocorms had increased resistance to water stress. However, no developmental stage was capable of surviving a prolonged six-month drought. Due to the absence of seedling recruitment in several locations, aphyllous vanilla recruitment may benefit from assisted propagation through seed cultures and the subsequent reintroduction of young plantlets into natural habitats. Moreover, conservation and restoration programs should incorporate research on developing soil conditions that facilitate vanilla seedling recruitment.

Keywords: aridity, aridez, deforestación, deforestation, establecimiento de plántulas, mortalidad de plántulas, orchids, orquídeas, protocorm, protocormo, seedling establishment, seedling mortality

Introduction. Understanding species recruitment mechanisms and limitations is crucial for the conservation and restoration of orchid populations (Phillips *et al*., 2020), particularly regarding the factors that control seed germination and seedling establishment (McCormick & Jacquemyn, 2014; McCormick *et al.*, 2012; Rasmussen *et al*., 2015). Seed and microsite limitations are the two primary mechanisms that restrict species recruitment (Dalling *et al*., 2002; Eriksson & Ehrlén, 1992; McCormick & Jacquemyn, 2014). Microsite limitations occur when recruitment is hindered by the quantity and quality of suitable sites for establishing new plants. Conversely, seed limitation arises when seeds fail to reach appropriate sites or when seed production is insufficient to occupy potential recruitment sites, even if dispersal is possible (Nathan *et al*., 2000). The recruitment rate of orchids in their natural habitats generally remains low (Bell, 2021; Hens *et al*., 2017; McCormick *et al.*, 2012; Rasmussen *et al*., 2015). This is primarily due to the difficulty of seed germination, which is often constrained by various types of dormancy. Three main types of dormancy have been identified: (1) morphological dormancy, caused by underdeveloped embryos (Arditti & Ghani, 2000; Bewley & Black, 2013; Prutsch *et al*., 2000; Yeung, 2017); (2) physical dormancy, due to a thick and impermeable seed coat (Cameron & Chase, 1998; Nishimura & Tamura, 1993; Yeh *et al*., 2021);

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Received 2 April 2025; accepted for publication 10 November 2025. First published online: 4 December 2025.

and (3) physiological dormancy, resulting from the accumulation of inhibitors in mature seeds (Lee *et al*., 2015; Xu *et al*., 2020).

Given that orchid seeds typically lack an endosperm (Arditti & Ghani, 2000; Yeung, 2017), their reliance on fungal partners during germination is widely recognized (Gao *et al*., 2019; Li *et al*., 2021; Rasmussen, 1992; Sousa *et al*., 2019; Yoder *et al*., 2000). Symbiotic fungi provide most of the minerals, nutrients, vitamins, and water necessary for seed germination and seedling development (Herrera *et al*., 2019; Li *et al*., 2021; McCormick *et al*., 2018). Increased mycorrhizal inoculum significantly enhances the germination and recruitment of young seedlings (McCormick *et al*., 2016; Těšitelová *et al*., 2022).

The availability of symbiotic fungi alone does not guarantee successful orchid seed germination; other environmental factors are often overlooked (Fay *et al*., 2015; Izuddin *et al*., 2019; McCormick & Jacquemyn, 2014; Rasmussen & Whigham, 1998; Stuckey, 1967; Yang *et al*., 2017). Abiotic factors, such as temperature and humidity, also play important roles in germination (Izuddin *et al*., 2019, Rasmussen *et al*., 2015). Also, light is crucial for orchid recruitment and influences various stages of the life cycle. It affects reproductive success (Horth, 2019; Kirillova & Kirillov, 2020) and influences seed germination (Kartzinel *et al*., 2013; Kirillova & Kirillov, 2019; Mahdavi *et al*., 2023; Sorgato *et al*., 2020) and the survival of adult plants and seedlings (Fritsche *et al*., 2022; Scade *et al*., 2006). Soil structure, which determines the ability of seeds to reach an optimal depth, may affect germination (Kinderen, 1995; McCormick *et al*., 2013; Wright *et al*., 2007). Several studies have also indicated that germination success can be associated with the presence of litter (Higaki *et al*., 2017; Li *et al*., 2022), lower pH (Batty *et al*., 2001; Higaki *et al*., 2017), and increased soil moisture and organic matter content (Batty *et al*., 2001; Diez, 2007; Rasmussen *et al*., 2015).

Host trees may influence seedling establishment due to the roughness of certain bark structures that can provide better support and moisture retention, thereby enhancing germination rates (González-Orellana *et al*., 2024; Timsina *et al*., 2016; Zarate-García *et al*., 2020). It is estimated that 69% of orchid species are epiphytes (Zotz, 2013; Zotz *et al*., 2021a), and their life cycle is intricately connected to that of phorophytes. In addition to survival, the distribution of orchids and the organisms that interact with them is also influenced by climatic factors such as temperature, precipitation, and humidity (Swarts & Dixon, 2009). These factors can affect orchid distribution, particularly regarding climate change (Fay, 2015) or specific changes following forest degradation, such as alterations in light levels and quality (Abeli *et al*., 2013; Falara *et al.*, 2013; Kindlmann *et al*., 2014) and soil characteristics (Hempel *et al*., 2013).

The aphyllous vanillas of Madagascar are orchids derived from an African ancestor that have diversified on the island, with seven known morpho-species. They are sold as medicinal plants (Rakotoarivelo *et al*., 2019; Randriamiharisoa *et al*., 2015). Like many climbers, they may also serve as connections between trees for primates and arboreal animals (Dunn *et al*., 2012; Montgomery & Sunquist, 1978; Rendigs *et al*., 2003; Yanoviak, 2015). As crop wild relatives, they may provide a potential gene pool for breeding new varieties to tackle climate uncertainties (Flanagan *et al*., 2018; Maxted & Kell, 2009; Pimentel *et al*., 1997). Understanding their recruitment dynamics under increasing drought conditions is critical for developing effective conservation and restoration strategies. Aphyllous vanillas are located in the humid forests of the north and east, as well as the dry forests of the west and south of the island (Andriamihaja *et al*., 2022). They are nomadic vines because their seeds germinate in the soil before the plant climbs and develops on a host tree (Zotz *et al*., 2021b). Their reproduction is both sexual and vegetative (Botomanga *et al*., 2024a; Gigant *et al*., 2014, Petersson, 2015).

Spontaneous seedling establishment has rarely been reported in aphyllous vanillas, and the conditions favorable for *in situ* germination and seedling development remain unknown. However, habitat degradation and severe drought episodes have been noted to cause recruitment bottlenecks in several species (Gale *et al*., 2018; Garnier *et al*., 2021; Muñoz-Rojas *et al*., 2016; Phillips *et al*., 2020). Drought is expected to further challenge the survival and recruitment of new orchid seedlings (Ackerman, 2021; Evans *et al*., 2020; Janissen *et al*., 2022). Rampant deforestation (Rafanoharana *et al*., 2024; Suzzi-Simmons, 2023), coupled with increasing climate risks in Madagascar (Hending *et al*., 2022; Tadross *et al*., 2008; Weiskopf *et al*., 2021),

suggests that aphyllous *Vanilla* Mill. species may have low *in situ* recruitment rates.

The primary objective of this study was to quantify natural recruitment in aphyllous *Vanilla* species by recording seedling abundance *in situ* across multiple forest sites. We examined how indicators of forest degradation such as forest structure, soil physicochemical properties, and litter quantity, influence seed germination, and we identified microsite characteristics associated with successful recruitment. Seedling survival was monitored throughout the dry season to evaluate sensitivity to drought prior to the onset of rains. Because protocorms are extremely small and difficult to assess under natural conditions, we complemented field observations with *in vitro* assays, exposing different protocorm developmental stages to media with reduced water availability to evaluate their drought responses under controlled conditions.

Materials and methods. *Study sites*.— In November 2019, fieldwork was conducted at 13 sites across different regions of Madagascar (Fig. 1, Table 1). The country's landform features an elevated central plateau that gently slopes toward coastal plains in the west, south, and north, separated from the eastern littoral region by steep escarpments. This topographical diversity, combined with winds and maritime currents, has created different regional climates (Table 1), as shown by Donque (1972) and Cornet (1974). The eastern regions experience a rainy climate, without a marked dry season due to the "*Alizé*" winds that bring high humidity from the Indian Ocean (Cornet, 1974). Manompana (MAN), a study site in the east, has an average annual precipitation of 3042 mm (Table 1). In contrast, the western regions experience moderate precipitation, with about eight dry months annually (Cornet, 1974) and a mean temperature of 26 °C (Table 1). The central plateau generally has cooler temperatures. The Andringitra (AND) mountains in the highlands have an annual mean temperature of 20 °C (Table 1). Rainfall is irregular, with the dry season mitigated by frequent fog and light rain (Cornet, 1974). In the south and southwest, the climate is semi-arid to arid, characterized by low precipitation and a long dry period. The mean annual temperature is 25 °C (Table 1). This considerable climatic variation has resulted

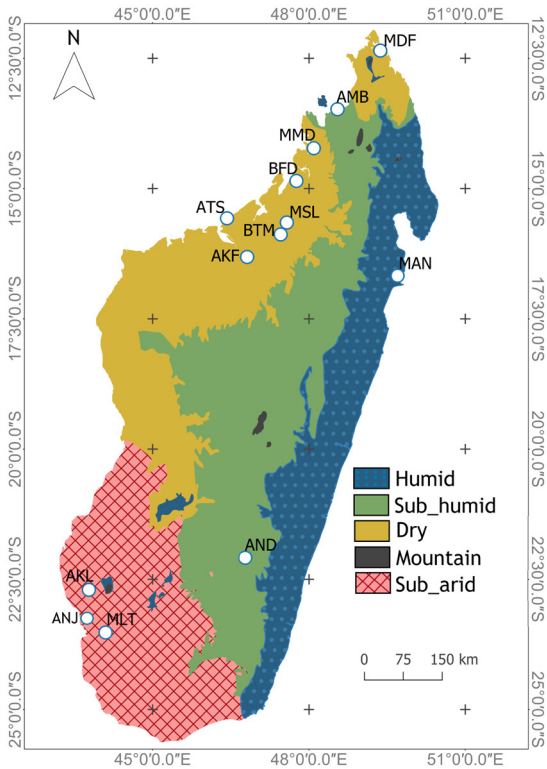

Figure 1. Map showing the five bioclimatic zones of Madagascar after Cornet (Madagascar Catalogue, 2023). Circles indicate the geographic positions of the 13 study sites.

in diverse vegetation types, ranging from evergreen rainforests with closed canopies in the east, to deciduous forests in the west, and spiny thickets with gallery forests in the south (Moat & Smith, 2007).

Study species.— Seven aphyllous *Vanilla* species have been documented in Madagascar: *V. bosseri* L.Allorge and *V. decaryana* H.Perrier, *V. humblotii* Rchb.f., *V. madagascariensis* Rolfe, *V. perrieri* Schltr, *V. allorgeae* C.F.Andriam. & Pailler, and *V. atsinananensis* C.F.Andriam. & Pailler (Allorge-Boiteau, 2005, 2013; Andriamihaja *et al*., 2022; Cribb & Hermans, 2009; Portères, 1954). The aphyllous habit (Fig. 2A), has a polyphyletic origin and is thought to have evolved independently at least three times across Africa, Asia, and the Americas as a convergent adaptation to drought conditions following coastal establishment and island colonization (Bouetard *et al*., 2010). Flowers are produced once a year (Fig. 2B), with colors ranging from

Table 1. Soil parameters and climatic information of 13 study sites in Madagascar. Climatic data were obtained from https://www.worldclim.org (data for 1970 – 2000) and soil data were obtained from SoilGrids™ (https://soilgrids.org)

	AMB	AND	ANJ	AKF	AKL	ATS	BFD	BTM	MLT	MAN	MMD	MSL	MDF
Bioclimat	Sub humid	Sub humid	Sub arid	Dry	Sub arid	Dry	Dry	Dry	Sub arid	Humid	Dry	Dry	Dry
Litter (cm)	1.3	6.5	2	6.5	1.8	1	1.5	3	4	7.8	1.2	1.3	1.5
pH	5.4	6	6.5	6.2	6.3	5.9	6.2	6.2	6.6	5.9	5.8	6.2	6.1
Sand (g/kg)	391	542	518	531	555	424	571	557	540	511	498	571	505
Clay (g/kg)	327	277	291	253	257	293	218	252	257	270	281	243	262
Silt (g/kg)	281	181	191	216	188	283	211	190	202	210	221	186	233
OC (g/kg)	40.6	24.6	28.1	64.3	34.3	75.7	29.2	33.9	26.1	38.8	42.1	36.7	33.6
BIO1 (mm)	2089	963	575	1442	671	1683	1552	1474	614	3042	1797	1496	1139
BIO2 (°C)	26	20	25	26	24	26	26	26	25	24	26	25	25
BIO3 (°C)	21	14	18	20	17	21	20	19	17	20	21	19	20
BIO4 (°C)	31	26	32	32	30	32	32	33	32	28	31	32	29
BIO5 (kPa)	2.2	1.69	2.2	1.9	2.4	2.3	2.2	2.5	2.1	2.3	2.4	2.2	2.3
BIO6 (%)	15.6	42.1	11.6	51.3	28.5	17.8	26	21	39	61.8	16.3	22	25.5

Study sites: AMB: Ambanja, AND: Andringitra, ANJ: Anja, AKF: Ankarafantsika, AKL: Ankililoaka, ATS: Antsianitia, BFD: Befandrama, BTM: Betaramahamay, MLT: Mahaleotse, MAN: Manompana, MMD: Maromandia, MSL: Marosely, MDF: Montagne des Français

Environmental variables: Litter: Litter thickness, pH: Soil pH in H_20 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

white to yellow, and their sizes vary depending on the species (Andriamihaja *et al*., 2022). Flowering times differ accross species and regions (Andriamihaja *et al*., 2020). Based on taxonomic studies by Andriamihaja *et al*. (2021, 2022) and considering the geographical origin of the plant materials used in this study, the *Vanilla* species found at the 13 study sites are as follows: *V. madagascariensis* at both Ambanja (AMB) and Maromandia (MMD), *V. humblotii* at Montagne des Français (MDF), *V. allorgeae* at Andringitra (AND) and Anja (ANJ), *V. atsinananensis* at MAN, *V. decaryana* at Mahaleotse (MLT), *V. perrieri* at Befandrama (BFD) and Betaramahamay (BTM), *V. bosseri* at Antsianitia (ATS) and Marosely (MSL), and hybrids of *V. bosseri* and *V. perrieri* at Ankarafantsika (AKF) and Ankililoaka (AKL).

Characterization of vanilla plants and seedlings.— A 50 × 20 m plot was established in an area with homogeneous vegetation where vanilla vines were present according to the Braun-Blanquet method (Braun-Blanquet, 1932). Three plots were created at each site. The number of vanilla plants in each plot was recorded, along with the number of flowers and fruits for each individual. The seeds of aphyllous vanilla plants are so small that observing the protocorms is extremely challenging. Therefore, we counted seedlings at advanced developmental stages by carefully inspecting surfaces conducive to seed germination, including the soil, tree trunks, and rocks. The physical characteristics of the seedlings, such as size (length and diameter) and color, were also recorded. Additionally, the distances between the nearest neighboring seedlings and between each seedling and the nearest adult individual were measured to understand the mode of dispersal. The fruiting rate of vanilla plants was calculated as the percentage of fruit-bearing individuals relative to the total number of individuals per plot. Similarly, the recruitment rate of aphyllous vanilla plants was calculated as the percentage of young vanilla seedlings relative to the total number of individuals per plot.

Forest vertical structure.— Gautier's method (Gautier *et al*., 1994) was used to investigate the vertical structure of the vegetation. Briefly, a transect line was

Figure 2. Recruitment of aphyllous *Vanilla*: (A). Aphyllous vanilla individual; (B). Flower visited by an insect; (C). Mature fruit blackening at the capsule tip; (D). Spontaneous seedlings under litter; (E). Seed baits placed in the soil; (F). Seedling characterized by the presence of tubers (arrow) in the underground part. Scale bar = 5 cm. Photographs by Botomanga A.

drawn through the center of the plot along its length. We divided the transect line into 50 sampling points spaced at regular intervals of 1 m and erected a vertical graduated pole at each point. Thereafter, we measured the height of all plants that came into contact with the pole, as well as those present within a one-meter width on both sides of the transect line. The data collected from the height contact points of each vegetation facies enabled us to characterize the vertical structure of each plot and analyze the cover by stratum, which corresponded to the average percentage of height classes (Fig. S1). The scale for forest cover, which corresponds to the stratum openness established by Godron *et al*. (1983), was used to interpret the results (Fig. S1).

In situ germination.— To identify microsites favorable for the germination and establishment of aphyllous vanilla seedlings, seed baits were prepared using 6 cm diameter cotton cleansing disks. Seeds were extracted from fully mature *V. madagascariensis* pods (Fig. 2C), collected in December 2019 at AMB and MMD sites. One hundred seeds were placed between two thin layers of cotton disks and wrapped in mesh fabric with a pore size of 100 µm to protect the seeds while allowing air and moisture exchange (Fig. 2E). Five seed baits were placed in each of the tested microsites, including tree trunks, rocks, dead wood debris, under leaf litter, and 5 cm deep topsoil. Each bait location was tagged to facilitate monitoring. Baits were inspected with a magnifying glass every two months over a one-year period to observe germination. The germination rate was calculated by dividing the number of newly formed protocorms by the total number of seeds per disk and multiplying by 100.

Seedling development monitoring.— We monitored the development of *V. madagascariensis* seedlings at the AMB and MMD sites. Sixty seedlings, with an initial average size of approximately 5 cm (Fig. 2F), were observed. Every two months, over the course of 18 months, we collected data on stem diameter, length, color, and mortality rate of the seedlings to assess their growth and survival dynamics. Mortality rate was calculated as the percentage of dead seedlings relative to the total number of seedlings.

Soil analysis.— Soil profiles up to a depth of 50 cm were established to evaluate the characteristics of each horizon and the thickness of the leaf litter, which is important for understanding the environmental condi-

tions that support vanilla plant development. Five topsoil variables, namely pH, sand content, clay content, silt, and organic carbon (Table 1), were extracted from raster data at a 250-meter resolution provided by SoilGrids 2.0 (Poggio *et al.*, 2021).

Seedling in vitro germination.— Five green pods of *V. madagascariensis*, likely resulting from flowers that were naturally pollinated earlier in the year, measured approximately 15 cm in length and 4 cm in diameter. They were harvested along with their flower stalks in December 2019 in the MMD forest. After harvesting, the pods were transported and stored at room temperature for four days. First, the pods were cleaned with a detergent and then thoroughly rinsed with tap water. Next, they were immersed for one hour in a fungicide solution (Mancolaxyl 720 WP containing 6% mancolaxyl (64% mancozeb + 8% metalaxyl)) combined with detergent, and then rinsed five times with distilled water. Surface sterilization continued with a 2.6% sodium hypochlorite solution for 30 minutes, followed by five washes with distilled water. The pods were then immersed in 70° ethanol for ten seconds and briefly flamed with a Bunsen burner. Afterward, the pods were opened under sterile conditions, and the seeds were carefully extracted and placed on sterile filter paper.

The germination medium, hereafter referred to as MS medium, consisted of half-strength Murashige and Skoog (1962) medium, supplemented with 30 g/L sucrose. The pH was adjusted to 5.7 before adding 8 g/L agar (Type E bacteriological agar, Biokar Diagnostics, France). Culture media were sterilized by autoclaving at 120°C and 1.5 bar for 20 minutes, then dispensed into 9 cm diameter Petri dishes. The green pods varied in maturation age, resulting in the extraction of both immature white and mature black seeds (Fig. S2A). During the culture period, immature seeds were separated from mature seeds (Fig. S2B, C). For each pod, approximately 100 seeds were placed on the medium in five replicates, resulting in a total of 25 Petri dishes containing around 2500 seeds. The cultures were incubated for 18 months without any subculturing in a controlled growth room maintained at 25°C, under direct exposure to a light intensity of 3000 lx and a photoperiod of 16 hours of light and 8 hours of darkness. Culture monitoring was conducted every two weeks using a binocular microscope with digital image capture. MESURIM software (Madre, 2011) was utilized to count the germinated seeds, while the dimensions (length and width) of seeds and the various developmental stages of seedlings were measured and analyzed using ImageJ software (Schneider *et al.*, 2012). The germination percentage was determined by calculating the ratio of seeds with a ruptured seed coat to the total number of cultured seeds, which was then multiplied by 100.

Effects of low water availability medium on seed germination and protocorm survival.— To mimic drought conditions in the natural environment of *V. madagascariensis*, we observed protocorm responses to limited water availability by transferring protocorms at different developmental stages onto a medium with a high concentration of a gelling agent. The excess agar does not draw water quickly from plant tissue but increases the matric potential of the medium to reduce free water availability (Klimaszewska & Smith, 1997; Owens & Wozniak, 1991), and thus mimicking the desiccating conditions encountered by seeds and protocorms during drought periods. We extended the water restriction experiment to 24 weeks to simulate the dry season in northwestern Madagascar (April – October) while monitoring the responses and survival of the protocorms. The drying medium (M4) is similar to the MS medium but is supplemented with 16 g/L agar. Protocorms derived from previous germination experiments were used in this study. The staging of protocorm development was adapted from Fang *et al.* (2021) with slight modifications (Fig. S3). These include Stage 1 (P1: white protocorm), Stage 2 (P2: chlorophyllous protocorm), Stage 3 (P3: protocorm with a single leaf primordium), and Stage 4 (P4: protocorm with two leaf primordia). Thirty mature seeds or 20 protocorms were placed in each Petri dish. The experiment was conducted using 10 Petri dishes, repeated five times for mature seeds and for each protocorm developmental stage, resulting in a total of 50 Petri dishes per developmental stage. Subsequently, five seeds or protocorms from different boxes were transferred to the MS medium every two weeks for six months, totaling 50 seeds or protocorms per treatment. Seed and protocorm development were monitored on both culture media for an additional six months following transfer. Monitoring included counting germinated seeds and assessing protocorm size and color.

The size of the protocorms was evaluated by calculating the sum of their lengths and widths, dividing by two. A protocorm was considered dead when it exhibited complete necrosis and was unable to resume development after being transferred to MS medium. The survival rate of the protocorms was determined by calculating the ratio of protocorms that continued their growth to the total number of transferred protocorms and then multiplying by 100.

Analysis of climate data in Maromandia and Ambanja.— Using historical monthly weather data from the WorldClim 2 database (Fick & Hijmans, 2017), we analyzed variations in average monthly precipitation (Fig. S4) and maximum temperatures (Fig. S5) by comparing two distinct periods: before 2000 (1960–1999) and after 2000 (2000–2019). This analysis was conducted at two study sites, where we monitored the development of young vanilla seedlings to better understand potential changes in rainfall and temperature patterns and their impact on the growth and recruitment of these species.

Statistical analysis.— The data were analyzed after confirming normal distribution using the Shapiro-Wilk test. All analyses were performed with R version 4.3.1 (R Core Team, 2023). The germination percentages of immature and mature seeds were analyzed separately. For each two-week period, the average germination rate was determined using a one-way ANOVA. Multiple comparisons of means were conducted using Tukey's post hoc test with the "glht" function from the R package "multcomp" (Hothorn *et al*., 2015) at a significance level of $p < 0.05$. A similar analysis assessed the variation in protocorm size across MC and M4 culture media. The half-life (T50) required for protocorm necrosis was evaluated separately for each developmental stage. Similarly, the survival rate was assessed independently for each developmental stage. To assess the distance between the seedlings and aphyllous adult vanilla plants, we combined data from the two sites. Principal component analysis (PCA) was performed using the FactoMineR package (Lê *et al*., 2008) to explore the relationship between aphyllous vanilla recruitment and the environmental factors at the study sites. The factoextra package (Kassambara & Mundt, 2017) was used to enhance the visualization of the PCA results. A Pearson correlation test was conducted to evaluate the relationship between environmental factors and the recruitment of aphyllous vanilla plants.

Results. *Description of recruitment capacities in different ecoregions*.— Across the 13 study sites, the density of adult aphyllous vanilla plants varied significantly ($p < 0.001$), ranging from 33 individuals per hectare in MAN to 2036 individuals per hectare in BTM. Almost all sites exhibited fruiting vanilla plants, except for MAN and AKF (Table 2). Fruiting rates ranged from 1.3% to 35.6% for MDF and MMD, respectively (Table 2). *Vanilla* seedlings were recorded at only three sites in northwestern Madagascar, with moderate rainfall. MMD and AMB, featuring *V. madagascariensis* populations, exhibited the highest recruitment rates of 53.5% and 51.7%, followed by ATS, where *V. bosseri* is present, at 13.2% (Table 2). Interestingly, AMB was the only site in the study where *V. planifolia* was grown, and positive recruitment of aphyllous vanilla was also observed. The seedlings displayed a green hue and were found growing within the soil, with heights ranging from 0.5 to 6.7 cm (Fig. 2D, F). The underground portions of the seedlings consisted of white tubers (Fig. 2F). The average distance between an adult individual and a young vanilla seedling was 0.95 ± 0.5 m, with a maximum distance of 2.42 m. Seedlings were distributed at an average distance of 0.18 ± 0.12 m apart, with maximum spacing of 0.52 m.

Assessment of environmental factors contributing to aphyllous vanilla recruitment.— Principal Component Analysis (PCA) of the environmental variables classified the 13 sites into four distinct groups (Fig. 3). Group 1 (G1) comprised the single-site AND, characterized by low monthly temperatures (20.1 ± 0.7 °C), soil with low organic carbon content (24.6 g/kg), and a thick litter layer (6.5 cm) (Table 3). Group 2 (G2) included AKF and MAN, exhibiting a semi-open canopy, zero fruiting rate, very high monthly precipitation (186.8 ± 30 mm), thick litter layer (7.1 ± 0.6 cm), and soils rich in organic carbon (51.5 ± 12 g/kg) (Table 3). Group 3 (G3) consisted of AKL, ANJ, BFD, BTM, MLT, MDF and MSL, characterized by low fruiting rates, low

TABLE 2. Population size, fruiting rate, and recruitment rate of aphyllous *Vanilla* species across 13 study sites in Madagascar.

Sites	Code	Species	Vanilla number	Fruiting (%)	Recruitment (%)
Ambanja	AMB	*V. madagascariensis*	137	30.2	51.7
Andringitra	AND	*V. allorgeae*	127	4.5	0
Anja	ANJ	*V. decaryana*	513	3.7	0
Ankarafantsika	AKF	*V. bosseri-perrieri*	42	0	0
Ankililoaka	AKL	*V. bosseri-perrieri*	423	5.4	0
Antsianitia	ATS	*V. bosseri*	241	7.4	13.2
Befandrama	BFD	*V. perrieri*	529	3.7	0
Betaramahamay	BTM	*V. perrieri*	611	2.1	0
Maleotsy	MLT	*V. decaryana*	89	1.6	0
Manompana	MAN	*V. atsinananensis*	10	0	0
Maromandia	MMD	*V. madagascariensis*	173	35.6	53.5
Marosely	MSL	*V. bosseri*	236	2.2	0
Montagne des Français	MDF	*V. humblotii*	159	1.3	0

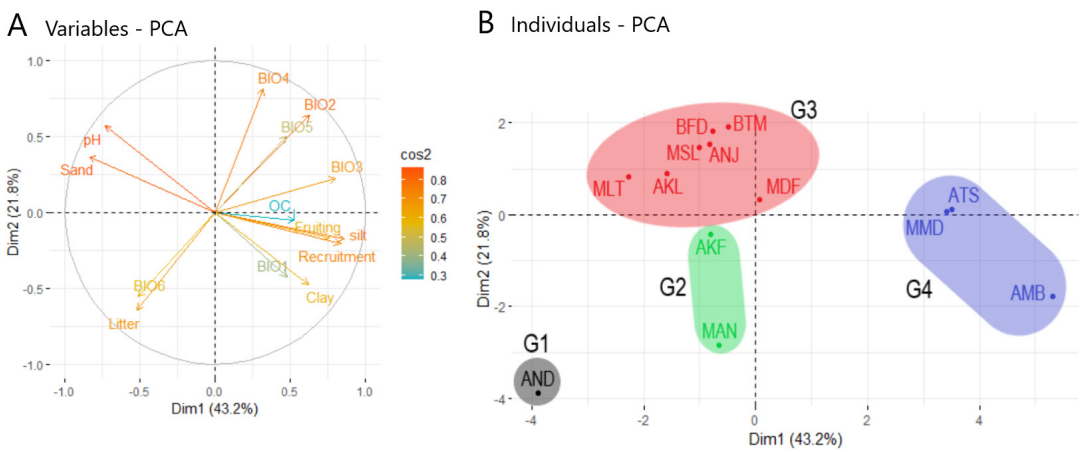

FIGURE 3. Factorial plans of Principal Component Analysis. (**A**) Variables consisting of abiotic and vegetation characteristics of the study sites. pH – Soil pH in H_20 at 5 cm depth, Sand – Quantity of sand in g/kg, at 5 cm depth, Clay – Quantity of clay in g/kg at 5 cm depth, Silt – Quantity of silt in g/kg at 5 cm depth, OC – Quantity of organic carbon in g/kg at 5 cm depth, BIO1 – Annual precipitation, BIO2 – Average annual temperature, BIO3 – Minimum annual temperature, BIO4 – Maximum annual temperature, BIO5 – Water vapor pressure, BIO6 – Canopy cover rate. (**B**) Factor map showing the distribution of localities. Ambanja – AMB, Anja – ANJ, Andringitra – AND, Ankarafantsika – AKF, Ankililoaka – AKL, Antsianitia – ATS, Befandrama – BFD, Betaramamay – BTM, Maleotsy – MLT, Manompana – MAN, Maromandia – MMD, Marosely – MSL, Montagne des Français – MDF. The variables are colored according to their Cos2, and color gradients are used to highlight their degree of correlation: variables with low, mid, and high Cos2 values are colored in blue, nanking yellow, and red, respectively.

monthly precipitation (89.5 ± 13.2 mm), and sandy soils (545.2 ± 9.6 g/kg) (Table 3). Group 4 (G4) included the AMB, ATS, and MMD sites, featuring a very open canopy, high fruiting rates (24.4 ± 8.6%), a thin litter layer (1.1 ± 0.8 cm), average monthly precipitation (154.6 ± 25.7 mm), and clayey-silty soils (Table 3). Recruitment showed strong positive correlations with fruiting rate ($r = 0.9$, $p < 0.001$), soil silt content ($r = 0.59$, $p < 0.05$), and soil clay content ($r = 0.64$, $p < 0.05$) (Fig. S6). In contrast, it

Table 3. Comparison of ten environmental parameters (mean ± SE) among the four PCA groups. Means followed by the same superscript within a column are not significantly different ($p > 0.05$), according to Tukey's post-hoc test.

Group	Canopy closure (%)	Fruiting (%)	Litter (cm)	Precipitation (mm)	Temperature (C°)	pH	OC (g/kg)	Clay (g/kg)	Silt (g/kg)	Sand (g/kg)
1	42.1^{ab}	4.5^{b}	6.5^{a}	80.2 ± 25.6^{ab}	20.1 ± 0.7^{c}	6.0^{ab}	24.6^{a}	277^{ab}	181^{b}	542^{ab}
2	56.5 ± 5.2^{a}	0 ± 0^{b}	7.1 ± 0.6^{a}	186.8 ± 30.0^{a}	25.0 ± 0.4^{ab}	6.1 ± 0.1^{ab}	51.5 ± 12^{a}	261.5 ± 8.5^{ab}	213 ± 3^{ab}	521 ± 10^{ab}
3	24.8 ± 3.1^{b}	2.8 ± 0.5^{b}	2.1 ± 0.3^{b}	89.5 ± 13.2^{b}	25.1 ± 0.2^{b}	6.3 ± 0.1^{a}	31.7 ± 1^{a}	254.2 ± 8.2^{b}	200.1 ± 6^{b}	545.2 ± 9.6^{a}
4	16.5 ± 0.6^{b}	24.4 ± 8^{a}	1.1 ± 0.8^{b}	154.6 ± 25.7^{ab}	26.2 ± 0.2^{a}	5.7 ± 0.3^{b}	52.8 ± 11^{a}	300.3 ± 13.7^{a}	261.6 ± 20^{a}	437.7 ± 31^{b}

Group 1: AND; Group 2: AKF and MAN; Group 3: AKL, ANJ, BFD, BTM, MDF, MLT and MSL; Group 4: AMB, ATS, and MMD.

Environmental variables: pH: Soil pH in H20 at 5 cm depth, OC: Quantity of organic carbon in g/kg at 5 cm depth, Clay: Quantity of clay in g/kg at 5 cm depth, Silt: Quantity of silt in g/kg at 5 cm depth.

was negatively correlated with soil pH ($r = -0.75$, $p < 0.01$) and sand content ($r = -0.68$, $p < 0.05$) (Fig S6). When the MAN and AKF sites, where no fruiting was recorded, were excluded from the analysis, precipitation showed a significant positive correlation with recruitment ($r = 0.69$, $p < 0.05$) (Fig. S7). Notably, no recruitment was observed at the southern Madagascar sites, which are characterized by low annual precipitation, despite significant fruit production (Table 2).

Responses of seed baiting.— After 12 months of monitoring, the seed baits placed on different substrates, including those buried in soil, showed no signs of germination.

Seasonal growth and mortality of young seedlings.— Throughout one year, the increments in length and diameter of young vanilla seedlings exhibited significant variations correlated with precipitation (Fig. 4A–C). From January to June, seedling length increased by 3.6 ± 0.9 cm at AMB and by 2.4 ± 0.3 cm at MMD, while diameter increased by 0.67 ± 0.14 mm at AMB and 0.57 ± 0.16 mm at MMD (Fig. 4A–C). During the dry season (June – October), the length increment was limited to 1.2 ± 0.3 cm at AMB and 0.9 ± 0.1 cm at MMD, while seedling diameter decreased to 0.63 ± 0.25 mm at AMB and 0.57 ± 0.22 mm at MMD (Fig. 4A–C). During this period, the seedlings turned yellow (Fig. 5C, D, E). From November through February, during the heavy rainy season, the surviving seedlings showed a marked increase in length (5.8 ± 1.8 cm at AMB and 5.4 ± 2.8 cm at MMD) and diameter (1.1 ± 0.4 mm at AMB and 0.7 ± 0.4 mm at MMD) (Fig. 4A, C). Over the year, young aphyllous vanilla plants demonstrated a total length growth of 10.5 ± 2.7 cm (Fig. 5A, B). However, a high mortality rate was observed, with 24/30 (80%) of the seedlings at AMB and 27/30 (90%) at MMD not surviving through December; the majority of losses occurred between October and December (Fig. S8). Between 2000 and 2019, there was a significant decrease in annual precipitation ($p < 0.05$), with a reduction of 151 mm at AMB and 109 mm at MMD compared to the 1970–1999 period. Simultaneously, maximum temperatures increased by 0.5 °C at AMB and 0.6 °C at MMD.

In vitro asymbiotic germination.— The highest cumulative germination percentage occurred in immature seeds, reaching 98% after 210 days of cultivation (Fig. 6A), while mature seeds displayed a cumulative germination rate of 25% after 390 days (Fig. 6B). Germination peaked at 105 days after cultivation (DAC) for immature seeds (Fig. 6A) and at 270 DAC for mature seeds (Fig. 6B). Asymbiotic germination of aphyllous vanilla is initiated by seed imbibition and subsequent rupture of the seed coat (Fig. 7A, B), followed by the release of a whitish embryo (Fig. 7C). Non-chlorophyllous protocorms emerged from immature seeds at 75 DAC on MS medium (Fig. 6A), whereas germination was noted only

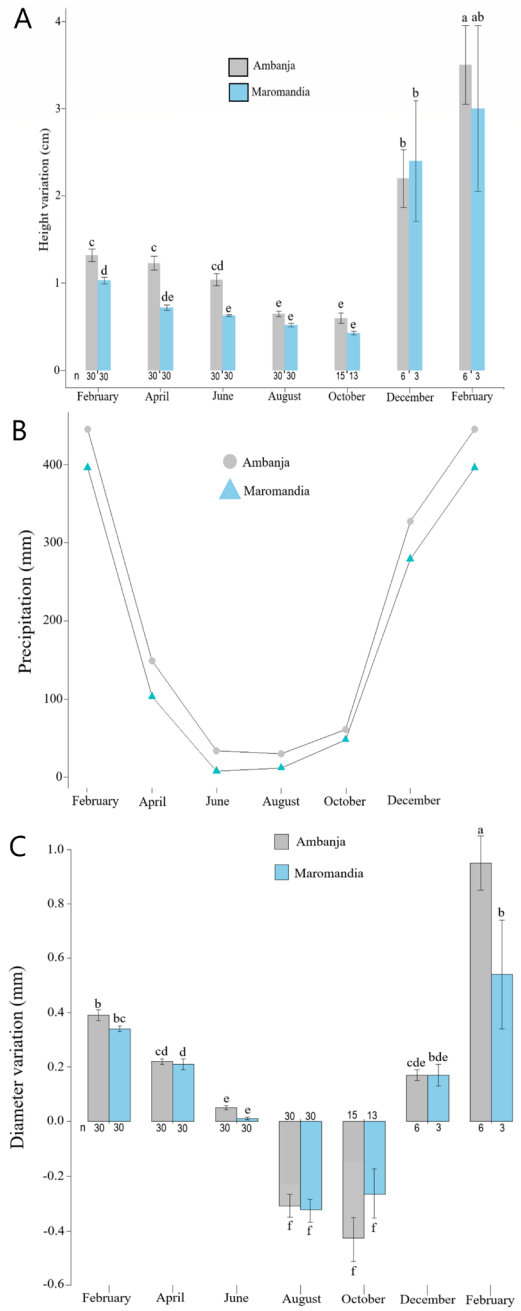

FIGURE 4. Annual variations in precipitation, seedling height, and seedling diameter at the Maromandia and Ambanja sites. (**A**) Variation in seedling height (cm); (**B**) average monthly precipitation (https://www.worldclim.org); (**C**) variation in seedling diameter (mm). (n = Sample sizes); bars with different letters are significantly different after Tukey post hoc test with $P < 0.05$.

at 195 DAC in mature seeds (Fig. 6B). Initially, white protocorms enlarged and gradually developed green pigmentation (Fig. 7D) after an average period of 3 ± 1.2 weeks post seed coat rupture (WPSCR). However, a few protocorms (1.2%) exhibited atypical development, remaining white before turning brown, and subsequently dark brown at 21 ± 2.2 WPSCR. Chlorophyllous protocorms continued to grow, forming a cotyledon (Fig. 7E) at 8 ± 1.6 WPSCR, and developing the first leaf (Fig. 7F) at 15 ± 2.3 WPSCR. The protocorm developed into a young seedling with roots (Fig. 7G) at 34 ± 3.2 WPSCR. At 390 DAC, the germination of some mature seeds was still observed (Fig. 6B).

Effect of the M4 drying medium on seed germination and protocorm development.— After 24 weeks of culture in M4 medium, there was no germination observed in the mature seeds. In contrast, seeds transferred to the MS medium had a cumulative germination rate of 6% over the same period. Regarding growth, the size of the protocorms in the M4 medium remained unchanged after 24 weeks of cultivation (Table 4). Conversely, all protocorms cultured in MS medium showed a significant increase in size compared to the beginning of the cultivation period (Table 5). Over several weeks in M4 medium, the protocorms gradually turned brown (Fig. S2D), although the time required for this color change varied depending on the stage of the protocorms. P1, initially white, turned brown after 6 ± 1.5 weeks in the M4 medium, and then dark brown after 10 ± 1.7 weeks (Fig. S9). P2, P3, and P4, which were initially green, turned dark brown after 14 ± 1.5, 17 ± 2, and 21 ± 2 weeks, respectively, in the M4 medium (Fig. S9). The transfer of protocorms from M4 to MC revealed differing capacities to resume development after water stress, which is influenced by both the duration of exposure to the M4 medium and the developmental stage of the protocorms. For the P1, P2, P3, and P4 protocorms, the times required to reach 50% mortality (T50) in M4 medium were 10, 12, 14, and 16 weeks, respectively (Fig 8). Complete mortality, with no protocorms surviving after transfer to the MS medium, was observed after 14, 16, 18, and 22 weeks (Fig 8).

Discussion. *Fruiting rate and soil properties are important factors controlling seedlings recruitment*.— Across 13 sites, aphyllous vanilla seedlings were observed at

Figure 5. Seedling evolution. (**A–B**) Healthy seedlings observed at the beginning of the year (**A**) and after one year of monitoring (**B**). (**C–D**) Dehydrated seedlings observed in August (**C**) and an advanced stage of dehydration leading to necrosis of the upper parts (**D**). (**E**) Close-up of a necrotic seedling stem caused by severe dehydration. Scale bar: (A, C) = 1 cm; (B, D) = 2 cm. Photographs by Botomanga A.

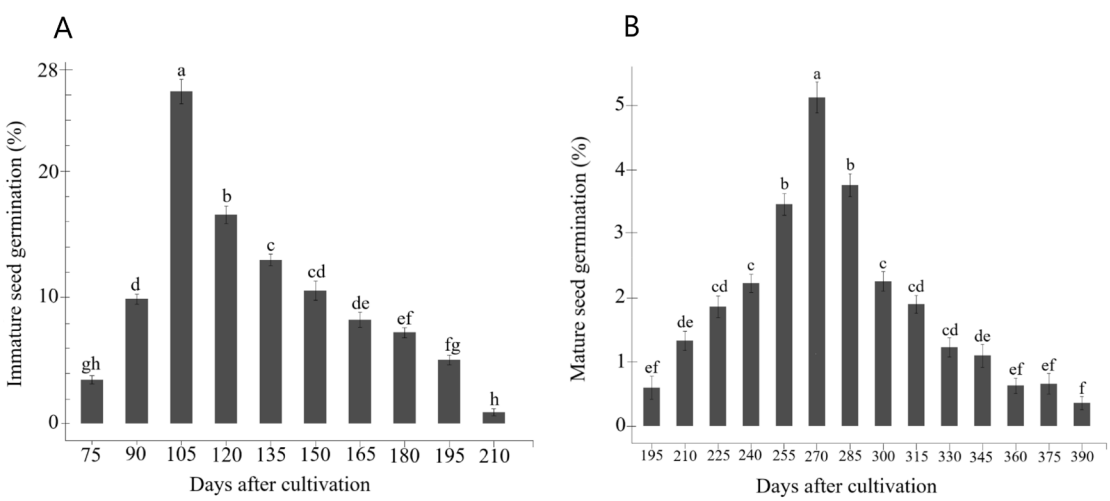

Figure 6. Percentage of seed germination of aphyllous vanilla in MC culture medium over 390 days. (**A**) Germination of immature seeds; (**B**) Germination of mature seeds. Bars correspond to the standard error (SE) of means. Sample sizes were as follows: immature seeds (n = 1000), mature seeds (n = 1500). Bars with different letters are significantly different after Tukey post hoc test with $P < 0.05$.

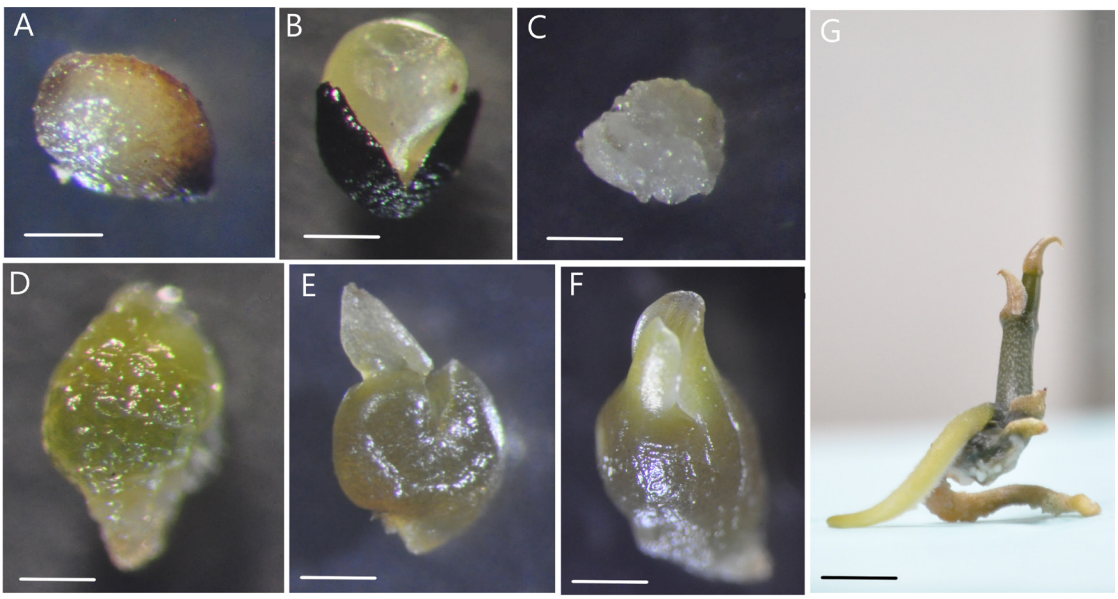

FIGURE 7. Development of protocorms and growth of aphyllous vanilla seedlings: (**A**). Imbibed seed; (**B**). Protocorm emerging from the seed coat; (**C**). White protocorm after seed coat rupture; (**D**). Green protocorm (4 weeks after seed coat rupture); (**E**). Protocorm with cotyledon (8 weeks after seed coat rupture); (**F**). Protocorm with the first leaf (14 weeks after seed coat rupture); (**G**). Seedlings with healthy roots (34 weeks after seed coat rupture). Scale bars: (A, B, D) = 0.1 mm; (C) = 0.2 mm; (E) = 0.25 mm; (F) = 1 mm; (G) = 1 cm. Photographs by Botomanga A.

only three northwestern sites (AMB, ATS, and MMD), highlighting the stringent recruitment requirements for orchid species under natural conditions (Batty *et al*., 2001; Gill, 1996; Hens *et al*., 2017). These three sites likely provide environmental conditions conducive to both germination and seedling growth, including a high fruiting rate, high silt content, high clay content, and acidic soil. In contrast, at the other 10 sites, particularly those in southern Madagascar, we assume that recruitment in the aphyllous vanilla occurs exclusively through vegetative reproduction via stem branching.

Fruit production, which is essential for seed availability, is crucial for orchid recruitment (Ackerman *et al*., 1996; Hens *et al*., 2017; Nathan & Muller-Landau, 2000). Our results suggest that higher fruiting rates correlate with a greater likelihood of encountering wild seedlings. In populations influenced by density-dependent factors such as suitable microsite availability, individuals producing more seeds have a better chance of locating an appropriate microsite than those producing fewer seeds (Hens *et al*., 2017; Shefferson *et al*., 2020).

Seedling occurrence was also associated with lower pH. Soil acidity can directly or indirectly influence orchid seed germination (Diez, 2007). Due to their reliance on mycorrhizae for germination, factors affecting fungal distribution indirectly influence germination success, and soil pH significantly affects the distribution of mycorrhizal fungi (Janowski & Leski, 2022). Soil fungi tend to be more diverse in low-pH environments (Blagodatskaya & Anderson, 1998; Rousk *et al*., 2010). Furthermore, HCl solutions are commonly used to mimic animal digestive acids and to break down the impermeable seed coats of endozoochorous plants (Jaganathan *et al*., 2019; Kleyheeg *et al*., 2018; Šoch *et al*., 2023), suggesting that soil acidity may directly impact seed germination by acting similarly to that of orchid seeds.

The silt and clay contents of soils play a critical role in geological and environmental processes because of the fine particle size and porous structure of these components (Huntley, 2023; Li *et al*., 2018). Soil pore space governs the movement of air and water through its porosity and permeability, which in turn determine the availability of these resources to plant roots (Matus, 2021). These physical properties endow soils with high water retention capacity and substantial potential for carbon and nitrogen stor-

Table 4. Variation in protocorm size (mean ± SE) on drying M4 medium after 24 weeks of culture. Means followed by the same superscript in a column were not significantly different ($p > 0.05$) according to Tukey post-hoc test.

Temps (T)	P1 (mm)	P2 (mm)	P3 (mm)	P4 (mm)
T0	0.37 ± 0.03^{e}	0.81 ± 0.02^{d}	1.14 ± 0.08^{c}	2.98 ± 0.26^{b}
T1	0.41 ± 0.01^{e}	0.80 ± 0.08^{d}	1.12 ± 0.11^{c}	3.04 ± 0.13^{b}
T2	0.32 ± 0.03^{e}	0.71 ± 0.06^{d}	1.10 ± 0.12^{c}	3.12 ± 0.71^{ab}
T3	0.30 ± 0.05^{e}	0.63 ± 0.04^{d}	1.08 ± 0.11^{c}	3.08 ± 1.1^{b}

Table 5. Variation in protocorm size (mean ± SE) on germination media after 24 weeks of culture. Means followed by the same superscript in a column were not significantly different ($p > 0.05$) according to Tukey post-hoc test.

Temps (T)	P1 (mm)	P2 (mm)	P3 (mm)	P4 (mm)
T0	0.35 ± 0.02^{i}	0.83 ± 0.07^{i}	1.15 ± 0.30^{h}	2.94 ± 0.37^{e}
T1	0.77 ± 0.02^{i}	1.52 ± 0.09^{g}	2.39 ± 0.21^{f}	4.7 ± 0.38^{c}
T2	1.36 ± 0.04^{g}	2.47 ± 0.11^{f}	3.11 ± 0.41^{d}	7.2 ± 1.3^{b}
T3	2.85 ± 0.04^{ef}	3.2 ± 0.14^{de}	4.2 ± 0.35^{c}	13.7 ± 2.4^{a}

P1: stage 1 protocorm, P2: stage 2 protocorm, P3: stage 3 protocorm, P4: stage 4 protocorm, T0: culture initiation, T1: 8 weeks after initiation, T2: 16 weeks after initiation and T3: 24 weeks after initiation.

age (Li *et al*., 2018; Matus, 2021; Moreno-Maroto & Alonso-Azcárate, 2018; Rabot *et al*., 2018). Although we did not observe a direct correlation between precipitation and aphyllous vanilla plant recruitment, G4 sites received an average of 154 mm of rainfall per month, ensuring consistent moisture. The clayey soils at these sites may be instrumental in maintaining moisture, facilitating germination by preventing seed desiccation, and promoting water imbibition. The negative charge of clay particles enables them to attract and retain essential cations such as calcium, potassium, and magnesium (Kumari & Mohan, 2021; Schoonheydt *et al*., 2018). These nutrients, which are vital for plant growth, are gradually released by clay and serve as reservoirs for seedling root development. Loamy soils, characterized by intermediate particle size and low compaction, provide favorable conditions for the development of fungal networks (Pauwels *et al*., 2023). The genus *Vanilla* is frequently associated with various fungal partners, such as *Rhizoctonia solani* J.G.Kühn, *Tulasnella bifrons* Bourdot & Galzin, *Tulasnella deliquescens* Juel (Juel), and *Ceratobasidium cornigerum* (Bourdot) D.P.Rogers (Porras-Alfaro & Bayman, 2007; Sathiyadash *et al*., 2020). These fungi display a variety of ecological strategies ranging from saprotrophic growth to symbiotic associations with plant roots. They colonize root tissues and extend their hyphae beyond the rhizosphere, facilitating the uptake and transfer of water and nutrients to host plants (Bahadur *et al*., 2019; Cheng *et al*., 2021).

An additional biotic factor in orchid recruitment is the positive influence of seedling recruitment near adult individuals. We found an average distance of less than 1 m between seedlings and adults, corroborating results that indicate germination and successful recruitment often occur preferentially near parental or conspecific plants (Diez, 2007; González-Orellana *et al*., 2024; McCormick *et al*., 2018). This spatial proximity may be attributed to the enhanced availability of mycorrhizal fungi and optimal microsite conditions in the vicinity of adult specimens (Diez, 2007; Fernández *et al*., 2023; Petrolli *et al*., 2022). Read *et al*. (2024) confirmed that adult plants transfer carbon to seedlings via mycorrhizal fungal networks that connect the roots. They suggested that this mechanism may play a crucial role in orchid establishment and development in natural habitats.

Delayed germination of mature seeds to align with favorable environmental conditions.– The immature seeds of the aphyllous *Vanilla* species exhibited nearly complete germination, while only 25% of the mature seeds germinated in the culture medium. This indicates that seed coat impermeability and the deposition of inhibitory substances occur late in seed development, as observed in many orchid seeds (Šoch *et al*., 2023; Yamazaki & Miyoshi, 2006; Yeung *et al*., 2018; Zhang *et al*., 2013). The results indicated that the germination of mature seeds peaked at approximately nine months and persisted for just over one year. In their natural habitat, the pods of *V. madagascariensis* reach ma-

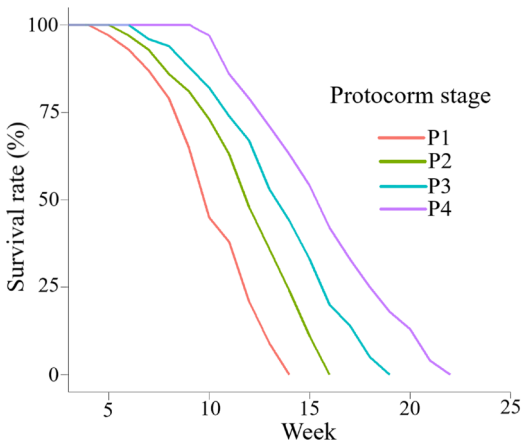

FIGURE 8. Survival rates of various protocorm stages after several weeks of culture on M4 medium. For each protocorm developmental stage, n = 600.

turity between June and August. They likely dehisce between September and November in response to the high temperatures and dryness typical of that period, thereby releasing seeds at the onset of the rainy season, between November and January. In line with the observed results, germination began around mid-August with a peak recorded in early October, which historically marked the beginning of the rainy season (Fig. S4). Due to a prolonged interval between the onset of germination and seed coat rupture, germination likely occurs during dry periods, and protocorm development coincides with the following rainy season, which is favorable for their growth. However, under current climate change conditions in Madagascar, the first rains tend to occur later, often in December. This could affect the survival rate of developing protocorms. The peak germination rate of the mature seeds was 5%, which is low. However, considering the number of seeds in an aphyllous vanilla pod, this still represents a significant number of seedlings. Consequently, it is not surprising that our seed-baiting experiment failed to yield positive results, as the experiment was terminated prematurely. In certain orchid species, as reported by Rasmussen & Whigham (1998), seed germination was not observed within 20 months following bait installation but only in the subsequent year.

High seedling mortality occurs as the dry season intensifies.– Our findings revealed that 85% of the young seedlings perished during the 18-month monitoring period in their natural habitats. This high mortality rate was primarily attributed to excessive dehydration, characterized by a progressive reduction in seedling diameter, yellowing of the stem, and ultimately, collapse of the inner tissues. The elevated mortality observed at the onset of the rainy season suggests that the adverse conditions during the dry season inflicted irreversible damage on the seedlings. An analysis of climate data over the past six decades has indicated a decline in annual rainfall and a rise in temperature in AMB and MMD. These results corroborate a recent analysis that incorporated controlled and homogenized data from 28 meteorological stations in Madagascar between 1950 and 2018 (Randriamarolaza *et al*., 2022). The rising temperatures likely led to increased evaporation and surface drying, thereby intensifying drought conditions caused by the lack of rainfall (Gebrechorkos *et al*., 2025). Such dual effects of water limitation and extreme heat were found to cause seedling mortality among different pine species, with rising temperatures exacerbating moisture stress (Hankin *et al*., 2025). Furthermore, recruitment is strongly affected by climate change even among species that have adaptive traits to withstand drought (Félix-Burruel *et al*., 2025; Milton *et al*., 2024).

Responses of protocorms to low water availability are dependent on their developmental stages.– In the drying medium, all stages of protocorms ceased development, as indicated by the lack of significant size variation over the 24-week culture period. Water restriction limits the growth and development of germinating embryos (Bazalar, 2020; Klimaszewska *et al*., 2000). Prolonged water restriction results in plant death (Huang & Song, 2013; Ntuli, 2012; Wojtyla *et al*., 2020), as shown in this study. However, we observed that drought tolerance varied depending on the developmental stage of protocorms. P1-stage protocorms are more sensitive to drought than P4-stage protocorms, supporting the hypothesis that body size is a key life-history trait influencing drought survival in plants (Midgley & Van Der Heyden, 1999; Milton *et al*., 2024). The P4 protocorms, which were significantly larger than P3, P2, and P1, exhibited greater drought resilience, with a notable delay in mortality. This increased resistance is likely due to the higher water storage capacity of the well-developed tissues. This mechanism enhances survival

probability in water-limited environments (Midgley & Van Der Heyden, 1999; Ripley *et al*., 2013). In the present study, no new seedling recruitment was observed at any of the sites located within ecoregions characterized by low precipitation. This absence of recruitment is concerning as it highlights the vulnerability of *Vanilla* species in increasingly arid environments. These findings underscore the urgency of prioritizing conservation actions for vanilla populations inhabiting drought-prone regions, where natural recruitment appears to be critically limited.

Conclusion and implications to conservation. The recruitment of young plants and successful sexual reproduction play crucial roles in maintaining high genetic diversity and promoting effective gene flow within populations (Berry *et al*., 2019; Paulo *et al*., 2019; Salgotra & Chauhan, 2023). The absence or low recruitment rate observed in this study, combined with the high clonality found in certain aphyllous vanilla populations (Botomanga *et al*., 2024a), may further constrain seedling recruitment. Indeed, reduced genetic diversity and increased inbreeding are associated with lower germination rates and decreased seedling fitness at early developmental stages (Booy *et al*., 2000; Capblancq *et al*., 2021). This highlights the need for corrective action, such as translocation strategies, including reintroduction and assisted colonization, to support species recovery (Phillips *et al*., 2020; Shao *et al*., 2017; Zhao *et al*., 2021). The results presented here lay the groundwork for developing habitats conducive to the recruitment of aphyllous *Vanilla* within a restoration program. Such approaches have proven successful for some orchid species (Wright *et al*., 2007). To preserve and enhance the genetic diversity of aphyllous *Vanilla* orchids, seed germination success may be enhanced using tissue culture techniques (Jolman *et al*., 2022). The resulting seedlings can then be reintroduced into their natural habitat once they attain sufficient robustness to withstand drought.

The aphyllous vanilla species found in Madagascar have demonstrated a remarkable capacity to adapt to heterogeneous environments, including humid and dry forests. Interspecific comparisons of root anatomical traits revealed an increased number of aerenchyma and vascular bundles in low-rainfall regions (Botomanga *et al*., 2024b). Such attributes could be used to select drought-tolerant *V. planifolia* genotypes to help farmers facing climate change. Interestingly, two aphyllous species, *V. perrieri* and *V. bosseri*, displayed a wide distribution, spanning different bioclimatic regions. In contrast, they are candidates for genome–environment associations (GEA) studies at the intraspecific level to identify genomic signatures of drought adaptation that are potentially useful in future breeding programs (Ravelonanosy *et al*., 2025).

Acknowledgements. Our work received research permit no. 004/17/MEEF/54/DGF/DSAP/SCB.Re. from the Ministry of Environment and Sustainable Development of Madagascar. We express our gratitude to Nivohanintsoa Ravoniarison for granting access to the Tissue Culture Laboratory. The vanilla conservation program was supported by Chanel Parfums Beauté. We extend our heartfelt thanks to Hanitriniaina Razaiarimanana for her valuable technical expertise and support.

Author contributions. Writing review & editing: AB, M.T.G.D, JGA, HNN, VHJ, NF, AVR. Writing original draft: AB, VHJ, AVR. Methodology: AB, M.T.G.D, JGA, HNN, VHJ, AVR. Validation: AB, M.T.G.D, JGA, HNN, VHJ, AVR. Formal Analysis: MTGD., AB. Investigation: AB, MTGD, JGA, HNN, AVR. Data curation: JGA, HNN, AB. Supervision: VHJ, AVR. Conceptualization: AB, VHJ, NF, AVR. Funding acquisition: NF, AVR. Visualization: AVR. Resources, Project administration: AVR.

Funding. This research received financial support from Chanel Parfums Beauté.

Conflict of interest. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supporting Information: Additional material related to this article is available in the online Supporting Information section.

Figure S1. Stratum closure rates for the thirteen study sites.

Figure S2. Aseptic culture of aphyllous vanilla seeds. (**A**). Immature (white) and mature (black) seeds of aphyllous vanilla. (**B**) Germination of immature seeds after 38 weeks of culture. (**C**) Germination of mature seeds after 38 weeks of culture. (**D**) Necrotic protocorms after 18 weeks of culture on M4 medium. Bar (A) = 1 mm; Bar (B, D) = 5 mm; Bar (C) = 2 mm. Photographs by Botomanga A.

Figure S3. Description of the developmental stages of *V. madagascariensis* protocorms cultivated in vitro. (**A**). Stage 1 : protocorm barely emerged from the seed coat, white in color, with an average size of 0.35 ± 0.02 mm. (**B**). Stage 2 : protocorm having acquired a green coloration, with an average size of 0.83 ± 0.07 mm. (**C**). Stage 3 : protocorm with a cotyledon (arrow) and an average size of 1.15 ± 0.30 mm. (**D**) Stage 4 : protocorm with a cotyledon and one leaf primordial (arrows) and an average size of 2.94 ± 0.37 mm. Bar (A) = 0.2 mm; Bar (B) = 0.5 mm; Bar (C) = 1 mm; Bar (D) = 1 cm. Photographs by Botomanga A.

Figure S4. Variations in precipitation between the 1960–1999 period (40-year average) and the 2000–2019 period (20-year average) at Maromandia and Ambanja.

Figure S5. Differences in maximum temperatures between the 1960–1999 period (40-year average) and the 2000–2019 period (20-year average) at Maromandia and Ambanja.

Figure S6. Pearson correlation among the environmental factors studied. Environmental variables: Litter: Litter thickness, pH: Soil pH in H20 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

Figure S7. Pearson correlation Analysis of environmental variables excluding MAN and AKF. Environmental variables: Litter: Litter thickness, pH: Soil pH in H20 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

Figure S8. Seedling mortality rate after 12 months of development monitoring at the Maromandia and Ambanja sites. Sample sizes were as follows: Ambanja (n = 30), Maromandia (n = 30).

Figure S9. Change in coloration of protocorms to black (necrosis) over time on the M4 culture medium. Histogram colors accurately represent the browning of protocorms. For each developmental stage, n = 200.

IN VITRO SEED PROPAGATION CONSTRAINTS OF MEXICAN LADY'S SLIPPER ORCHID, PICHOUAXTLE, *CYPRIPEDIUM IRAPEANUM*

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Abstract. *Cypripedium irapeanum* known in Mexico as "*Pichohuaxtle"* in Nahuatl, is the most emblematic Mexican lady's slipper orchid and one of the most studied cypripedioid species in Mesoamerica. Despite its protected status in Mexico, habitat loss driven by land-use change has severely reduced its populations. *Ex situ* conservation approaches are urgently needed to safeguard its genetic diversity, yet seed germination in the genus remains challenging due to innate dormancy and specific nutritional requirements. We collected seeds from two Mexican ecosystems where this species is distributed —*Quercus* and Tropical Dry Forests— and isolated mycorrhizal fungi from adult plants to examine both symbiotic and asymbiotic cultures. We found limitations in seed production due to flower ripening, ineffective pollination, or parasitism. Furthermore, germination was achieved after a cold treatment, and the best medium to obtain seedlings *in vitro* was Norstog medium. Two mycorrhizal fungi, isolated from adult plants belonging to the family Tulasnellaceae, formed a clade with previous isolates from American slipper orchids. One of these isolates promoted germination but later produced fungal incompatibility *in vitro*. Our study contributes to clarify previously undescribed processes in the biology of this highly appreciated and vulnerable species, supporting future efforts to propagate it from seed and obtain successful cultures.

Resumen. *Cypripedium irapeanum*, conocida en México como "Pichohuaxtle" en náhuatl, es la orquídea zapatilla de dama más emblemática del país y una de las especies cipripedioideas más estudiadas en Mesoamérica. A pesar de su estatus de protección en México, la pérdida de hábitat asociada al cambio de uso del suelo ha reducido severamente sus poblaciones. Las estrategias de conservación *ex situ* son urgentemente necesarias para salvaguardar su diversidad genética; sin embargo, la germinación de semillas en el género sigue siendo un reto debido a su dormancia innata y a sus requerimientos nutricionales específicos. Recolectamos semillas en dos ecosistemas mexicanos donde se distribuye la especie —bosques de *Quercus* y selva tropical seca— y aislamos hongos micorrízicos de plantas adultas para evaluar cultivos simbióticos y asimbióticos. Detectamos limitaciones en la producción de semillas debido a la maduración floral, la polinización ineficaz o el parasitismo. Asimismo, la germinación se logró únicamente tras un tratamiento de frío, y el medio más eficaz para obtener plántulas *in vitro* fue el medio Norstog. Dos hongos micorrízicos aislados de plantas adultas, pertenecientes a la familia Tulasnellaceae, formaron un clado con aislados previamente reportados en orquídeas zapatilla americanas. Uno de ellos promovió la germinación, aunque posteriormente produjo incompatibilidad fúngica en condiciones *in vitro*. Nuestro estudio contribuye a esclarecer procesos previamente no descritos en la biología de esta especie apreciada y vulnerable, y proporciona bases para futuros esfuerzos de propagación a partir de semillas orientados a obtener cultivos exitosos.

Keywords / Palabras clave: Ceratobasidiaceae, *Epulorhiza*, germinación simbiótica, incompatibilidad micorrícica, micorriza orquideoide, mycorrhizal incompatibility, orchid mycorrhiza, symbiotic germination, Tulasnellaceae

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Received 5 August 2025; accepted for publication 10 November 2025. First published online: 5 December 2025.

Introduction. Many cypripedioid orchids are endangered due to anthropogenic activities and climate change. These factors affect ecological distribution and their associations, reducing seed production by decreasing pollinator populations or increasing parasites that prey on seed capsules (Faleiro *et al*., 2018; Liu *et al.,* 2021; Mcgough *et al.,* 2006; Rankou & Salazar, 2014). Mesoamerican *Cypripedium* L. is a group of interest because it is a sister clade to the rest of the *Cypripedium* (Guo *et al.*, 2012; Li *et al.*, 2011): a particular group formed by *C. irapeanum* Lex., *C. molle* Lindl. and *C. dickinsonianum* Hágsater, of which *C. irapeanum* is the most widely distributed in several habitats (Soto-Arenas & Solano-Gómez, 2007). All species have experienced a decline of almost 50% in population size as a result of habitat transformation, and propagation for reintroduction remains challenging due to persistent difficulties related to *in vitro* culture and mycorrhizal fungi associations (Moreno-Camarena & Ortega-Larrocea, 2022).

While habitat conservation has become a priority, understanding the life history of this elusive group is important as it may contribute to establishing protocols for propagation. In nature, protocorm and seedling recruitment (addition of new individuals to a population) in orchids are considered negligible contributors to population models because of the rarity of *in situ* germination observations (Nicolè *et al*., 2005). Therefore, a deeper understanding of the germination process and its relationships with orchid mycorrhizal fungi (OMF) is imperative to prevent further loss of genetic variability. Few seed-based propagation protocols have been developed, and most have been successfully applied only to European or North American species (Seaton & Ramsay, 2005; Shefferson *et al*., 2007, 2019; Shimura *et al*., 2009). In this study, we developed a set of methods for symbiotic and asymbiotic *in vitro* propagation based on previous protocols to test their effectiveness in *C. irapeanum*, the most valued of the Mexican lady's slippers.

Material and methods. *Collection of biological material.*— We sampled five populations of *Cypripedium irapeanum* (Fig. 1A), three in *Quercus* L. spp. forests (State of Mexico, Morelos and Puebla), and two in Tropical Deciduous Forests (TDF) (Veracruz). We also tried to isolate mycorrhizal fungi from *C. molle* in *Quercus* Forest in Oaxaca state (Fig. 1B**)**. Due to the endangered status of the genus, sampling authorization was granted by local institutions (SEMARNAT, Secretaría de Medio Ambiente y Recursos Naturales, SGPA/DGVS/07200/14 and SGPA/DGVS/5126/19).

Fungi: In each population, roots were collected from plantlets and adult non-flowering plants separated by at least one meter. One root per plant, including secondary roots, was collected, cutting it from the base of the rhizome (Fig. 1C–F). Roots were wrapped in aluminum foil with a small amount of soil and kept at 10 °C until isolation.

Seeds: Mature capsules were collected during the rainy season (August–September) and kept in paper bags then stored in a desiccator with silica gel at 24 °C and a relative humidity of 24%; once ripened, seeds from capsules belonging to the same plant were evaluated for their quality (amount of seeds without embryos, presence of fungi or other pests in dehiscent capsules) before being mixed. Semi-permanent slides were made (Fig. 2). Capsules collected from the State of Mexico and Veracruz were aborted due to wasp infestation.

Isolation, morphological and molecular characterization of orchid mycorrhizal fungi.— Roots were superficially washed to remove soil and organic matter. Colonization and digestion of hyphal coils was evaluated through semi-permanent slides of transverse root sections (*ca.* 0.5–1.0 cm) stained with acid fuchsin (0.1% in lactic acid and glycerol) and mounted in polyvinyllactoglycerol alcohol (PVLG) (Fig 1I–J). The velamen was removed from colonized segments, which were then rinsed in sterile distilled water and disinfected in 10% NaOCl (Brand Puro Sol®, 5% active Cl) for 1–2 min, followed by three additional rinses in sterile distilled water.

Under sterile conditions and using a microscope, root segments were cut in a Petri dish with a drop of distilled water; hyphal coils were extracted with a pipette and incubated onto three culture media: GPA (supernatant of green pea; Shimura *et al*.,2009), FIM (Fungal Isolation Medium; Clements *et al*., 1986) and AWA (Acidic Water Agar; Stewart, 2004). Media pH was adjusted to 5.8 (HCl 0.1 N and NaOH 0.1 N). All cultures were kept in the dark at 25 ± 1 °C.

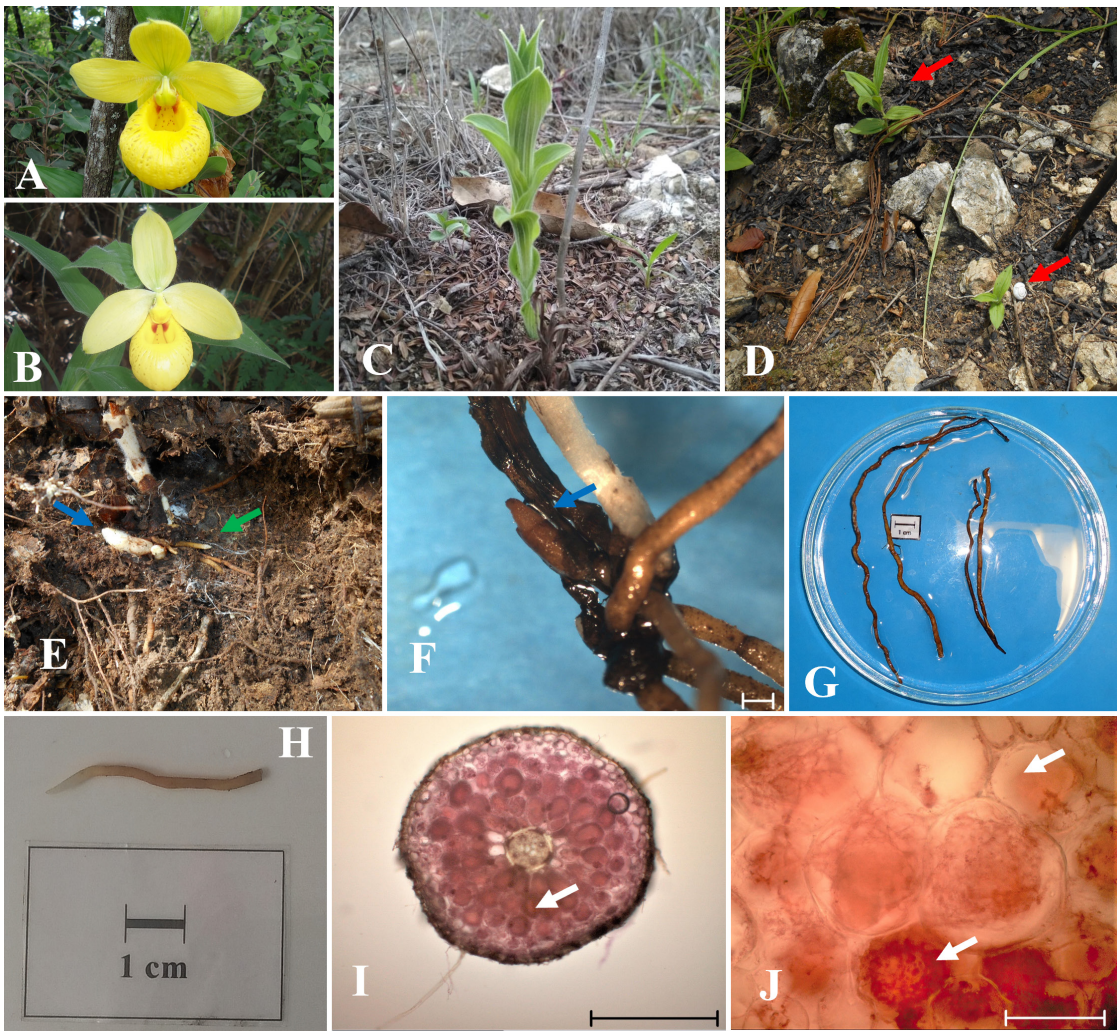

Figure 1. Flowers, collection, and root colonization diagnosis. **A, C**. *Cypripedium irapeanum* from *Quercus* forest at Puebla state, Mexico. **B, D**. *Cypripedium molle* from *Quercus* forest in Oaxaca state, Mexico. **E**. Underground shoot and root of *C. irapeanum* in Tropical Dry Forest. **F**. Lateral shoot in a 5-year plantlet. **G**. Aspect of long reddish adult roots. **H**. Aspect of lateral root. **I**. Mycorrhizal colonized cortex of transverse segment of lateral root. **J**. Hyphal coils in different degradation stages. Photographs by M. Moreno-Camarena.

After one or two days, hyphal tips growing from coils were excised and incubated on PDA medium (potato dextrose agar) or concentrated GPA (mashed peas without filtering it) and cultivated under the same conditions to obtain pure strains. From these colonies, hyphae were taken and stained with acid fuchsin and mounted on semi-permanent slides with PVLG to characterize micromorphology. *Rhizoctonia*-like fungi were selected considering branching of hyphae (*ca.* 90°), presence of basal septum and monilioid cells as well as color, appearance, and growth rate of colonies.

PCR amplification, sequencing, and phylogenetic analysis.— DNA was extracted from colonies with characteristics of *Rhizoctonia*. First, they were cultured in liquid PDB medium (potato dextrose broth) or GPE (green pea extract, boiled, non-crushed, and filtered pea) (pH 5.8) and incubated under agitation at 25 ± 1 °C. Once a colony had formed, the hyphae

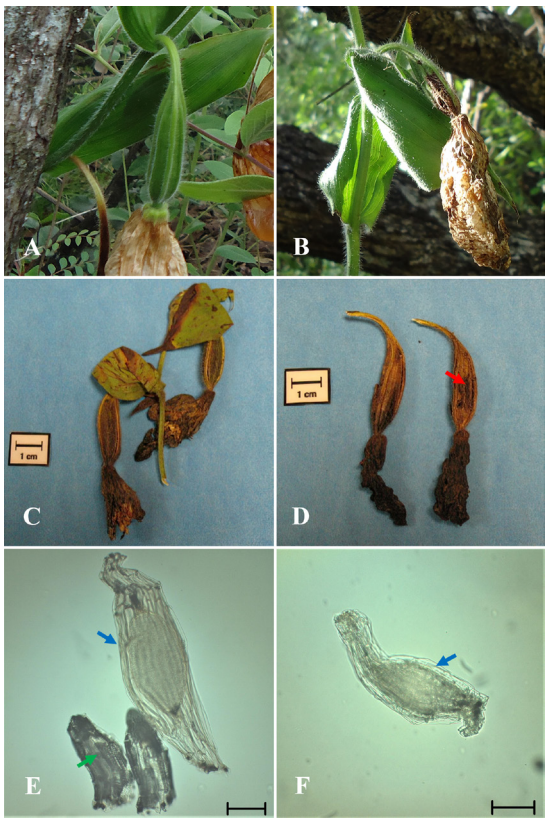

FIGURE 2. Aspect of capsules and seeds from *Cypripedium irapeanum*: **A**. from plants growing in Puebla state. **B**. Veracruz state aborted capsule. **C**. Dried capsules of *C. irapeanum*. **D**. Capsule of *C. irapeanum* damaged by parasites. **E**. Viable (blue arrow) and without embryo seeds (green arrow) from a capsule of *C. irapeanum* from Puebla state. **F**. Aspect of a single unviable seed due to the non-turgid appearance of the embryo cells that have adhered to those of the testa. Bar = 100 µm. Photographs by M. Moreno-Camarena.

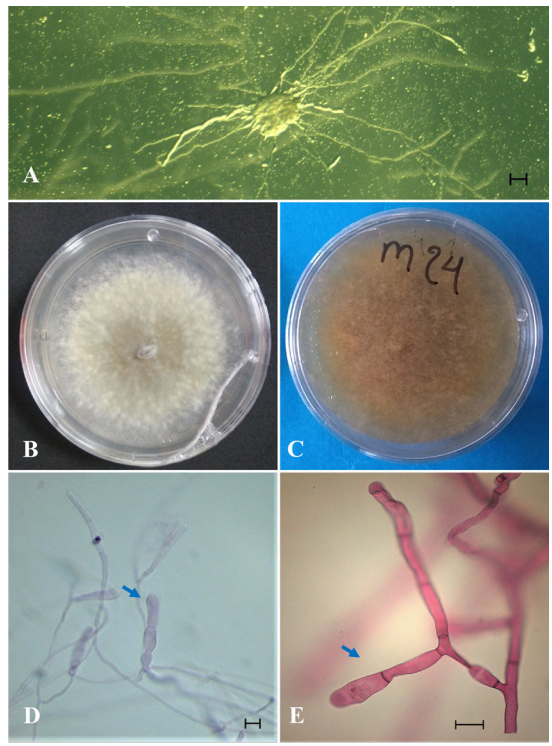

FIGURE 3. Mycorrhizal fungal isolates from *Cypripedium irapeanum*. **A**. Hyphal coil regrowing on green pea agar (GPA) medium (Bar = 100 µm). **B**. Aspect of a *Epulorhiza* colony on GPA. **C**. Aspect of a colony of *Ceratorhiza* isolated from *C. molle*. **D**. Monilioid cells (blue arrows) from *Epulorhiza* **E**. Monilioid cells from *Ceratorhiza* (Bar = 10 µm). Photographs by M. Moreno-Camarena.

were washed three times in sterile distilled water using a vacuum system to remove any residual medium. Extraction was performed with commercial kit RED Extract-N-Amp Plant PCR-(SIGMA®), following the manufacturer's recommendations.

PCR was performed in a thermocycler T100™ Thermal Cycler (BIORAD) with the primer combination ITS 1/ITS 4 and ITS1-ITS4 Tul (Jacquemyn *et al*., 2012; Shefferson *et al*., 2005; Shimura *et al*., 2009; Valdés *et al*., 2011). PCR mix contained 5.0 µL of Taq polymerase (Taq-&GO™ mastermix, MP Biomedicals), 0.25 µL of each primer, 3.0 µL of DNA (1:10 v:v), and 16.5 µL of deionized water for a final volume of 25.0 µL. PCR conditions consisted of a pre denaturation at 95 °C for 120 s, 35 cycles of denaturation at 95 °C for 60 s, annealing at 55 °C for 60 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 600 s (Gardes & Bruns, 1993; Shefferson *et al*., 2005; 2007). PCR products were verified in an electrophoresis gel at 2% stained with Midori Green. Successfully amplified samples were sequenced in LANABIO (Laboratorio Nacional de Biodiversidad, Biology Institute, UNAM).

Sequences were assembled and edited in Geneious Prime and deposited in GenBank under accession numbers (ON620090- ON620092). BLAST search (Basic Local Alignment Search Tool, blast, Max E-Value:10, Word size: 11, Gap cost: 5 2) (Altschul *et al*., 1997), MAFFT alignment (Katoh *et al*., 2002; Ka-

toh & Standley, 2013) and phylogenetic reconstruction (maximum likelihood) were performed with PHYML (substitution model Tamura-Nei TN93, 1000 bootstrap for node support) (Guindon *et al*., 2010), using plugins included on Geneious. *Multiclavula corynoides* (Peck) R.H.Petersen and *Scleroderma* Pers. (Accession numbers MCU66440 and HM196776, respectively) were used as outgroups (Yuan *et al*., 2010). An additional analysis was performed using representative sequences of OMF from different species of *Cypripedium* including *C. irapeanum* from previous reports (Valdés *et al*., 2011) and *C. molle* (Moreno-Camarena & Ortega-Larrocea, 2022). The same parameters for phylogenetic reconstruction were applied, with MCU66440 used as the outgroup.

Asymbiotic and symbiotic germination.— For *in vitro* germination assays, 0.5 mg of mature seeds were stored in envelopes of filter paper Whatman No. 1 (Seaton & Ramsay, 2005) in darkness for four months at 4 °C. After this time, seeds were disinfected by agitation for 30 min in a solution of $NaOCl:C_2H_6O:H_2O$ (15:5:80 v:v:v) plus 2 drops of Tween 20; envelopes were washed three times in sterile distilled water and incubated on Phytamax medium (modified) (SIGMA 1990), MS (Murashige & Skoog, 1962) and Norstog (Norstog, 1970). Symbiotic germination was conducted using two OMF isolates from adult plants of *C. irapeanum* population growing in a TDF and *Quercus* forest; fungi and seeds were sown simultaneously on Oatmeal Agar (OMA) (Clements *et al.,* 1986). Fungi were inoculated by placing a circle of 0.5 cm^3 of each inoculum; a negative control of OMA without fungi was also prepared. All Petri dishes were incubated in a light/darkness photoperiod (16/8) at 25 ± 1 °C and light intensity of 50 μmol m^{-2} s^{-1}.

Photographic records were taken every 12 days after sowing (das) at the following time points: T1:12, T2:24, T3:36, T4:48, T5:60, T6:72, T7:84, T8:96 and T9:108 das. A final account was done at 256 das to evaluate development. Morphogenesis was recorded according to developmental stages proposed by Stewart and Zettler (2002), with modifications due germination behavior of *Cypripedium* (Curtis, 1943). The stages were defined as follows: Stage 1—mature seeds; Stage 2—imbibed seeds; Stage 3—protocorm polarization; Stage 4—protocorm development with one leaf primordium; Stage 5—development of radicular meristem (pre-seedling); and Stage 6—seedling with developed roots and leaves (Fig. 4Q). ANOVA was applied to evaluate differences in germination between treatments. For statistical analysis, Stage 1 was not considered. A Least Significance Difference (LSD) was calculated using Statistica software (Ver. 7.0) (Stat Soft, 2004). Developed plantlets were cultivated in flasks containing the same germination medium to stimulate growth.

Results. *Seed collection.*— Collection of good quality seeds of this species was difficult to obtain because of the asynchronous formation of mature capsules, which were successfuly collected from one site in Puebla. In the State of Mexico, flowering was reduced due to harvesting pressure by local villagers, while populations in Veracruz failed to produce capsules with viable seeds. In all cases, several indehiscent capsules were infested with parasites (Fig. 2D).

Root colonization.— Primary roots were longer than 50 cm, and mycorrhizal colonization was scarce and heterogeneous along the length; the hyphal coils that were observed were normally digested and surrounded by numerous starch granules. Secondary roots were short (*ca.* 2–3 cm), thin (*ca.* 1 mm), and many had high levels of mycorrhizal colonization along the length. Hyphal coils were mostly undigested. Plantlets had numerous roots, and their colonization pattern was similar to that of secondary roots (Fig. 1F, I).

Fungal isolation and identity.— We isolated 33 strains from *C. irapeanum* from *Quercus* forest and TDF (Table 1). All fungi recovered from *C. irapeanum* belonged to the anamorphic genus *Epulorhiza* R.T.Moore (hyphae less than 4 μm, pearly monilioid cells, creamy submerged colonies on PDA, slow growing rate *ca.* 0.2 mm per day) (Fig. 3A–C). In contrast, those recovered from *C. molle* roots belonged to the anamorph genus *Ceratorhiza* R.T.Moore (hyphae of more than 4 μm, barrel-shaped monilioid cells, brownish colonies on PDA, aerial mycelium, growing rate of 0.5 mm per day). In all cases, monilioid cells were long and irregular (Fig. 3D, E). The best media for isolation and conservation of these fungi were GPA and AWA. Colonies maintained on PDA, or OMA exhibited a scarce growth rate and failed to survive over the long term.

Table 1. Description of orchid mycorrhizal isolates obtained from *Cypripedium irapeanum* (Mexican lady's slipper) and incubated on PDA medium (Potato Dextrose Agar). Values are presented as mean ± standard error (SE).

Habitat	Growth by day (cm)	Basal septa of hyphae (μm)	Diameter of hyphae (μm)	Monilioid cells (μm)	
				width	length
TDF	1.14 ± 0.22 a	1.79 ± 1.28 a	3.3 ± 1.1 a	7.7 ± 2.0 a	16.1 ± 4.5 a
QF	0.44 ± 0.25 b	1.44 ± 0.38 a	2.7 ± 0.4 a	7.8 ± 1.2 a	18.5 ± 4.2 a

Habitat: TDF-Tropical deciduous forest, QF-*Quercus forest.* Different letters represent statistical significance within a column.

Phylogenetic analysis (Fig. 4, green clade) revealed that isolates from *C. irapeanum* grouped into a clade comprising two sequences that belong to a clade of Tulasnellaceae associated with sequences recovered from *Vanilla planifolia* Andrews from Veracruz, Mexico (PQ423669) (Alejo-Viderique *et al*., 2025); *V. poitaei* Rchb.f. from Puerto Rico (DQ834391) (Porras-Alfaro & Bayman, 2007) and *Spathoglottis plicata* Ridl. from Singapore (AJ313456) (Ma *et al*., 2003). All reference sequences were obtained from roots growing in soil. The closest related fungi matched with uncultured endophytes from *Cypripedium parviflorum* Salisb. (DQ925544) (Shefferson *et al*., 2007) and a sister OMF clade from *Paphiopedilum armeniacum* S.C.Chen & F.Y.Liu (Yuan *et al*. 2010) followed by more distant clades in Tulasnellaceae. An analysis incorporating OMF from other *Cypripedium* species (Fig. S1) revealed that isolates of this study belong to one of two distinct clades, associated with *C. candidum* Muhl. ex Willd. and *C. parviflorum* from Illinois, USA (DQ925539 and DQ925543). One OMF isolate from *C. irapeanum* in Puebla and State of Mexico (JF313324) (Valdés *et al*. 2011) is distantly related to the rest of OMF *Cypripedium* from Asiatic and North American species.

In the case of isolates from *C. molle* (Fig. 4, red clade), the sequences belonged to Ceratobasidiaceae, a group in which clades are less well supported. The closest related sequences where those from *Cephalanthera rubra* Rich. (Bell *et al*., 2020), *Thanatephorus* Donk associated with *V. aphylla* Blume from soil mycelium in Cuba (Porras-Alfaro & Bayman, 2007) and one isolate from *Cremastra variabilis* from Japan, all terrestrial species. When OMF from other slipper orchids were analyzed (Fig. S2), two well-supported clades were recovered: one formed by OMF associated with *Cypripedium tibeticum* King ex Rolfe, and *C. flavum* P.F.Hunt & Summerh and another within a clade including Asian species (*C. guttatum* Sw., *C. subtropicum* S.C.Chen & K.Y.Lang, *C. plectrochilum* Franch) to which the *C. molle* isolate is related. The isolate recovered from *C. irapeanum* in Puebla by Valdés *et al.* (2011) also grouped separately.

Asymbiotic and symbiotic germination.— Successful germination occurred only on asymbiotic Norstog medium (Fig. 5) and was very low on MS and Commercial Phytamax™, reaching only protocorm polarization with no further development and eventual tissue oxidation (Fig. 5D). Conversely, polarization of protocorm and morphogenesis were observed only on Norstog medium and symbiotic-assisted germination. In general, seeds germinated 12–14 das, starting with embryo swelling and testa rupture, followed by protocorm formation and differentiation on a promeristematic zone (Fig. 5J). Morphogenesis continued until root and foliar meristems developed, with no differences in timing between symbiotic or asymbiotic treatments. Protocorms developed rhizoids only in symbiotic assays (Fig. 6D). Color varied from faintly green in symbiotic treatments to intense green on asymbiotic Norstog medium; additionally, protocorms on Norstog developed long roots and several foliar meristems (Fig. 5N–O). In symbiotic germination, although both isolates belong to the same molecular clade (Fig. 4), the isolate from oak-forest promoted 12% of germination, while the isolate from TDF reached only 0.6 % but promoted better development (Fig. 6C–E, Fig. 7). Unfortunately, neither promoted development beyond Stage 4 with a leaf primordium due to long-term incompatibility, expressed as invasion of the meristematic tissues of the protocorms (Fig. 5F–G). On asymbiotic Norstog medium, development continued until seedlings with multiple lateral shoots and long root development were obtained. Further culture in flasks with the same me-

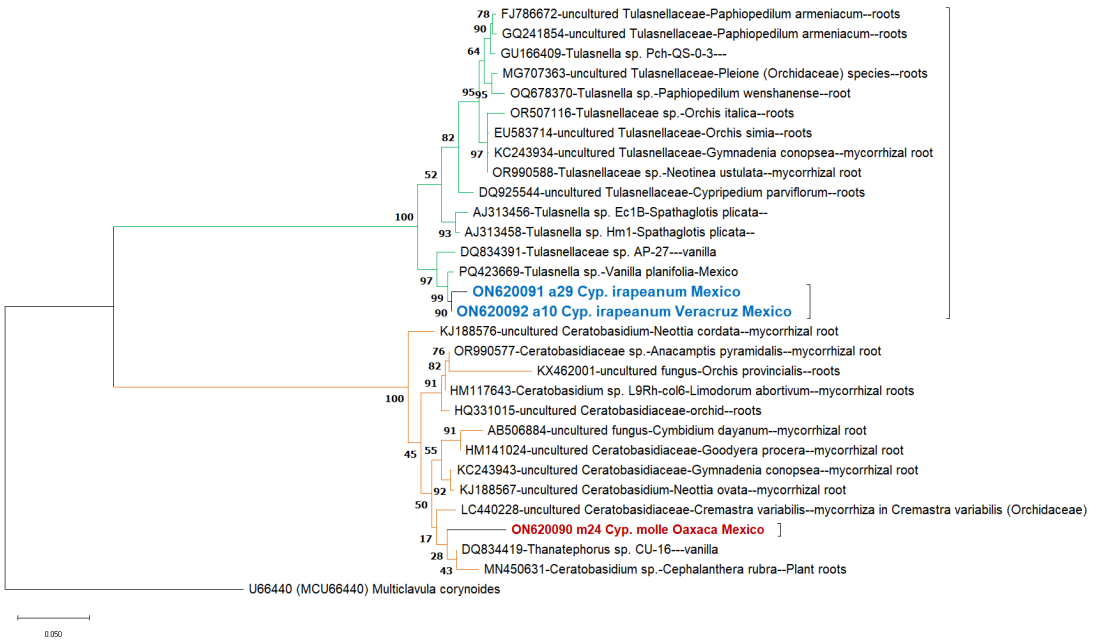

Figure 4. Consensus tree of phylogenetic relationships of *C. irapeanum* and *C. molle* (bold, italic, red labels) recovered by BLAST algorithm (Altschul *et al*. 1997). The green clade includes the isolates recovered in this study from *Quercus* forest and Tropical Dry Forest. Tree inferred using the Maximum Likelihood method and the Tamura-Nei 93 model. Branch lengths labels represent bootstrap support. Analyses and editions were performed in Geneious.

dium resulted in plantlets with several well-developed stems and leaves; however, plantlets died before *ex vitro* acclimatization (Fig. 5P–Q).

Discussion. Mycorrhizal colonization in *C. irapeanum* shows the same patterns previously described (Colin Rivera & Ortega-Larrocea, 2012; Moreno-Camarena & Ortega-Larrocea, 2022) and observed in other species of the genus, characterized as scarce, erratic, and sometimes rich in starch granules (Shefferson *et al*., 2007; 2019). Secondary roots—particularly in plantlets—are densely colonized by mostly undigested hyphal coils, likely indicating a strong mycorrhizal dependency at these stages (Rasmussen *et al*., 2015; Rasmussen & Pedersen, 2012).

However, the presence of mycorrhizal-compatible fungi in soil does not guarantee the successful recruitment of new individuals into a population, even though seed production is not low by orchid standards (*ca.* 100,000 seeds). *In situ* germination remains extremely low—estimated at around 0.001% (Hernández-Apolinar *et al*., 2012)— and is also remarkably slow. In a previous study, we reported a germination event after ten years of sowing seed baits (Moreno-Camarena & Ortega Larrocea, 2022). Low recruitment can be due to several seed-related factors, such as the presence of polyphenols and lignin in the coat—which make it highly hydrophobic —and the presence of abscisic acid— which inhibits seed development, increases as seeds reach maturity (Barsberg *et al*., 2013; Lee *et al*., 2005, Zeng *et al*., 2014). Additionally, compatibility issues with symbiotic partners also contribute to low recruitment, resulting in less than 1% of *in vitro* germination, as also observed by Shimura & Koda (2005).

This loss of symbiotic compatibility appears as browning and necrosis in protocorms and seedlings of *C. macranthos* var. *rebunense* (Kudô) Miyabe & Kudô (Shimura & Koda, 2005). In the case of *C. irapeanum,* incompatibility may arise because mycorrhizal fungi were isolated from adult plants that may not be responsible for germination, as the range of fungal associates changes throughout orchid's life cycle (Bidartondo & Read, 2008; Meng *et al*., 2019, Rasmussen *et al*., 2015). Shimura & Koda (2005) reported that protocorms develop numerous rhizoids, indicating that mycorrhizal colonization

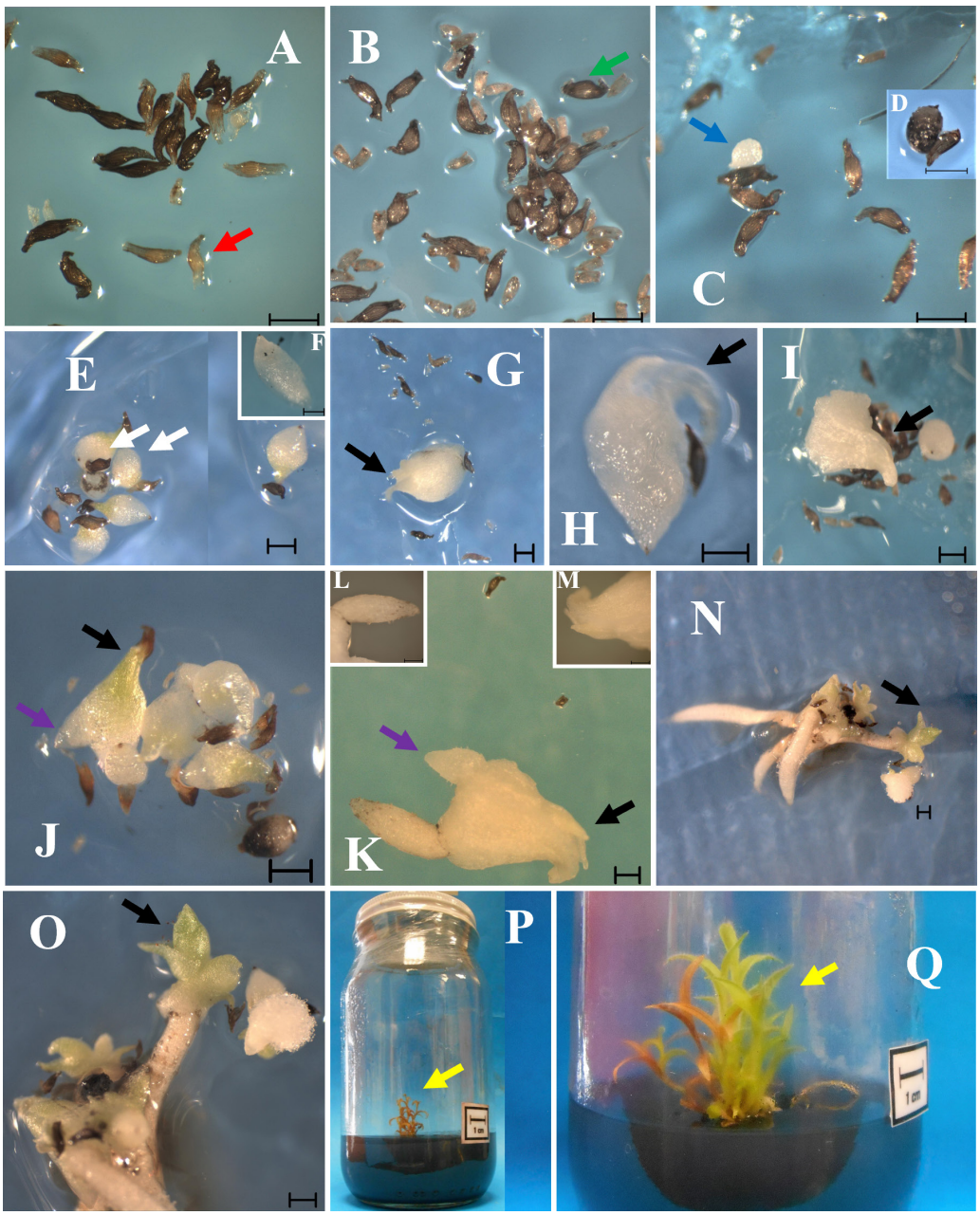

FIGURE 5. Asymbiotic germination on *C. irapeanum* in Norstog medium. **A**. Seeds at Stage 1, red arrow signals immature seeds, 0 days after sowing (das). **B**. Seed at stage 2 (imbibed) and **C** protocorms at stage 3 (polarization), 12 das. **D**. Protocorm oxidation at stage 3 in MS medium. **E**. Protocorms at stage 4 with leaf primordium at 54 das. **F**. Protocorm at stage 3 in Phytamax media 54 das with no photosynthetic activity. **G-I**. Protocorms at stage 4 at 96 das with different degree of development of leaf primordium (black arrow). **J**. Protocorm at stage 5 with leaf primordium and promeristematic zone (purple arrow) at 96 das. **K**. Seedling with developed leaf primordium (m, black arrow) and developed true roots (l, purple arrow), notice root hairs in **L**, and apical leaf in **M,** 122 das. **N-O**. Seedling with different degrees of development, 300 das. **P**. Seedlings after subculture on Norstog medium with added activated charcoal after 280 das. Bar from a-o represent 500 µm. **Q**. Same seedlings with first sprouting of leaves after 300 das. Photographs by M. Moreno-Camarena.

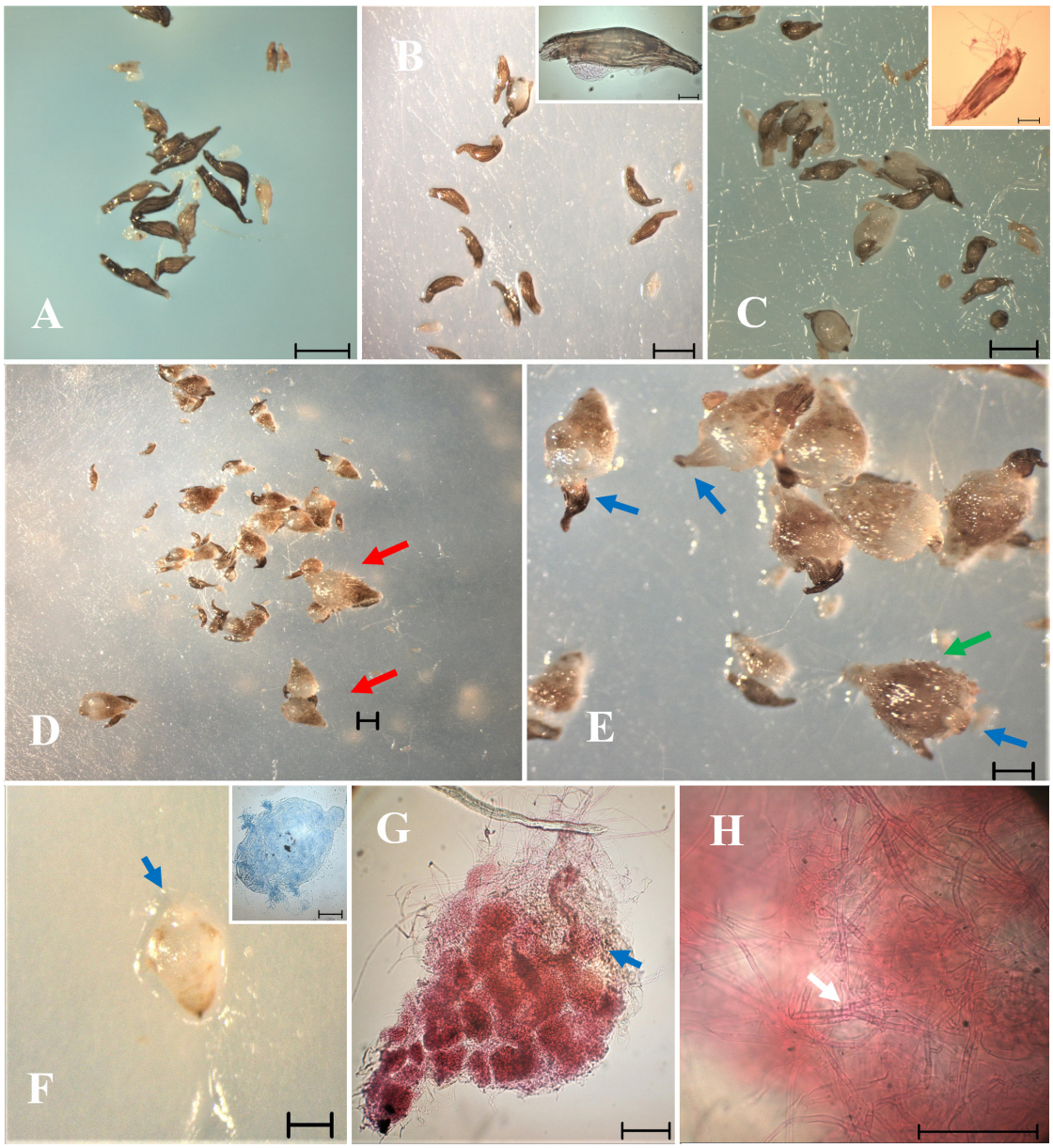

Figure 6. Symbiotic germination on *Cypripedium irapeanum*. **A**. Seeds at Stage 1, 0 days after sowing (das). **B**. Seed at stage 2 (imbibed) and 3, 12 das, detail (Bar = 100 µm). **C**. Protocorms at stage 3 (polarization), 22 das. **D-E**. Protocorms at stage 4 (red arrow) with leaf primordium (blue arrow) and colonized area (green arrow), 54 das. **F**. Protocorm at stage 4 at 109 das, detail to observe hyphal coils in base of protocorm. **G**. Protocorm at stage 4 at 187 das with leaf meristem (blue arrow) and hyphal coils colonizing it. Bar in A-G represents 500 µm. **H**. Detail of basal segment of protocorm, note hyphae branched *ca.* 90° (white arrow). Bar = 50 µm. Photographs by M. Moreno-Camarena.

begins via rhizoids; however, field observations and *in vitro* tests on *C. irapeanum* and *C. calceolus* L. (Rasmussen & Pedersen 2012, Moreno-Camarena & Ortega Larrocea, 2022) suggest that colonization starts from micropylar end cells before rhizoid formation. In fact, our findings illustrate that rhizoid development is a consequence of symbiotic colonization, occurring only after the association has been

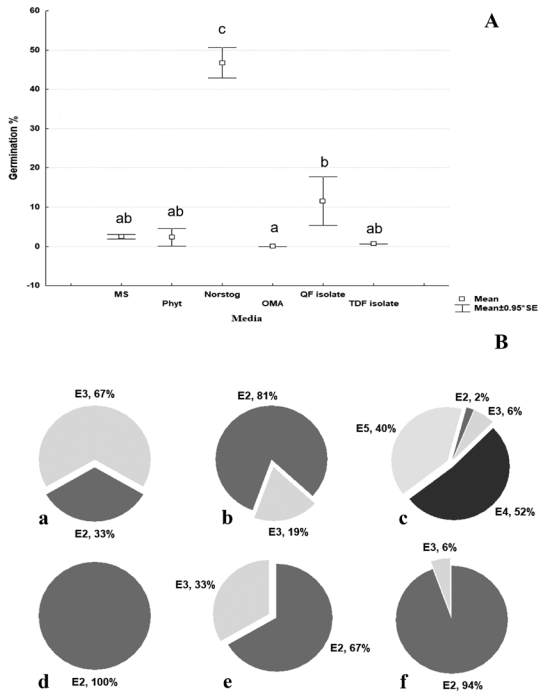

FIGURE 7. **A**. Germination percentage of *Cypripedium irapeanum* on different media. Letter indicates significative differences (LSD at 0.05). **B**. Developmental stages reached in different asymbiotic (a-d) and symbiotic (e-f) media: a) Murashige & Skoog; b) Phytamax; c) Norstog; d) Oat Meal Agar; e) *Quercus* Forest isolate; f) Tropical Dry Forest isolate.

established. Plantlets may possess the ability to control fungal growth within their tissues by phytoalexins in response to changing environmental conditions, such as nutrient and moisture availability, or other physical factors. For example, when *C. macranthos* var. *rebunense* was cultured on a medium with fungicide (Benomyl), it continued to grow and produced healthy plantlets (Shimura & Koda 2005). In further studies, two antifungal compounds—lusianthrin and chrysin— were found in the cytoplasm of orchid cells, both capable of inhibiting fungal growth (Shimura *et al*., 2007). Orchid mycorrhizal fungi appear to be a life-and-death struggle regulated by numerous factors, like seed composition and the fungi's degradation capacity (Barsberg *et al*. 2013; Zeng *et al.*, 2014), environmental conditions such as humidity and temperature (De Pauw & Remphrey, 1993, Shimura & Koda, 2005), the light/darkness regime, and nutrient availability. Loss of compatibility observed in our study may be due to the absence of specific stimuli needed to trigger plantlets to produce natural antifungal compounds, or to inherent *in vitro* conditions, as we did not evaluate complex fungal media or substrates that could help regulate fungal growth. *Epulorhiza* grow slowly and do not exhibit invasive growth over protocorms; instead, incompatibility occurring within the tissues. Further research is needed using different substrates and media, as well as with mycorrhizal isolates obtained from naturally occurring seedlings—even when recruitment is rare in the wild.

Undeniably, *in vitro* germination is consistently more successful than *in situ* germination, because many factors are stable. *In vitro* conditions provide seeds with continuous and regulated humidity for imbibition, readily available nutrients, and the possibility of scarifying or treat seeds to break dormancy. Dormancy-breaking in this genus has been linked to cold treatment when seeds come from temperate habitats (Rasmussen & Pedersen, 2012; Shimura & Koda, 2004; 2005), allowing for successful colonization by OMF. Methods to break dormancy can be physical, chemical or biological (*e.g.* sonication, solution of NaOCl or with mycorrhizal fungi, respectively), as long as they diminish the effect of inhibitors of germination (*e.g.* ABA, hydrophobic testa).

In situ germination requires several successive cold treatments, with germination rates increasing to 30% after the second period (Pedersen *et al*., 2012). This suggests that germination in this genus is not only extremely low but also likely spans several years in order to progress through each life stage (Curtis, 1943; Moreno-Camarena & Ortega-Larrocea, 2022, Pedersen *et al*., 2012; Rasmussen & Pedersen, 2012, Shefferson *et al*., Hutchings 2017). Optimum germination rates vary amongst species; and depend on the relationship between embryo development and barriers of the seed (formation of testa, chemical inhibitors, etc.); the days after pollination (40–105 days), and the environmental conditions of the species (with subtropical species requiring the longest periods) and may follow a possible phylogenetic gradient in germination requirements (Perner *et al*., 2022). Light or dark conditions requirements for incubation seem not constant in the genus, also suggesting an evolutionary tendency (Kaur, 2023;

Park *et al.,* 2023). Mesoamerican *C. irapeanum* follows this pattern: *in vitro* germination is accelerated by cold treatment (*ca.* 12 das), even though it grows in temperate natural habitats where prolonged cold dormancy periods in the soil are not assumed (Moreno-Camarena & Ortega Larrocea, 2022). On the other hand, nutrient medium preferences for this genus were described long ago by Harvais and Norstog (Oliva & Arditti, 1981) and confirmed recently (Kaur, 2023). Media with high phosphate and sugar contents such as MS and Phytamax (Murashige & Skoog, 1962; Seaton *et al*., 2005, Sigma-Aldrich, 1990), traditionally used for orchid germination, do not favor *C. irapeanum* germination nor *C. macranthos* Sw. (Huh *et al.,* 2016a, b). It remains unclear whether this effect may be dependent on seed origin, storage conditions, or seed maturity, since germination on MS medium has previously reached about 25 % (Hernández-Apolinar *et al*., 2012). However, in *C. acaule* Aiton, *C. formosanum* Hayata, and *C. macranthos* germination on media such as MS, Thomale, and VWD (Van Waes and Debergh) decreases as seed maturity increases, becoming null at full maturity (Zeng *et al*., 2012; 2014, Zhang *et al*., 2013). Conversely, germination of mature *C. debile* Rchb.f. seeds reaches up to 80 % in liquid MS medium, as the liquid environment may physically reduce resistance of the seed coat. Several studies use immature seeds (40-90 days after pollination-dap) to avoid dormancy and prevent seed coat impermeability, achieving considerably high germination rates on MS and Harvais— 56 and 25 % respectively— (Taniguchi *et al*., 2008; Zhang *et al.,* 2013). Root system formation may predate shoot leaves, varying amongst species but appears to be the predominant pattern in the genus (Kaur, 2023). The effects are often not predictable among species, population and even the year of experimentation (Hsu & Lee, 2012; Jiang *et al*., 2017).

Although these protocols do not reflect natural conditions, they may serve as a strategy to obtain seedlings that are more susceptible to inoculation by OMF, since adult stages likely have a greater ability to regulate the orchid-fungi relationship effectively (Fay *et al.,* 2018; Shimura *et al.,* 2007; Shimura & Koda, 2004; Yuan *et al*., 2010). Additionally, conventional media do not support the maintenance and growth of fungal strains well. Preferences for OMF media seem quite specific; for example, pea starch appears to be a more suitable energy source. Nevertheless, further studies are needed to determine whether this represents a limitation for isolating and culturing Tulasnellaceae fungi (Shimura *et al.,* 2009). Although OMF from European, Asian, and North American *Cypripedium* have been extensively studied (Shefferson *et al*., 2005; 2007; Yuan *et al*., 2010), attempts at isolation have only been reported for *C. macranthos* var. *rebunense* (Shimura & Koda 2005) and *C. irapeanum* (Valdés *et al*. 2011). In this study, we obtained several strains from *C. irapeanum* belonging to *Epulorhiza* species, which supports Shefferson *et al*.'s (2019) proposition of dominance by *Tulasnella cystidiophora* Höhn. & Litsch. associates within the genus. We found that OMF from *C. irapeanum* (Tulasnellaceae) and *C. molle* (Ceratobasidiaceae) are not closely related to clades recovered from other lady's slipper orchids, with the exception of a clade from *C. parviflorum.* Moreover, previously reported isolates of *C. irapeanum* from another locality in the state of Puebla belong to a different Tulasnellaceae clade than those found in our study. Ceratobasidiaceae has also been reported in association with *C. californicum* A.Gray and *C. fasciculatum* Kellogg ex S.Watson (Shefferson *et al*., 2005; 2019; Whitridge, 2004; Whitridge & Southworth, 2005); but symbiotic germination tests have not yet been performed. Such tests could provide valuable insights, especially since isolates obtained from plantlets of *C. molle* clearly differ from those found in adult plants (Moreno-Camarena & Ortega Larrocea, 2022). Isolates obtained from both OMF families are also related to fungi associated with *Vanilla* Mill. species, whose symbiotic germination has also been difficult as their seeds show very similar properties in the waterproofing of the testa by pigments, both families are phylogenetically related as ancestral clades of orchids (Givnish *et al*., 2015).

Final remarks. Mesoamerican *Cypripedium irapeanum* may associate with other clades of Tulasnellaceae mycorrhizal fungi, differring from those previously documented for other species of the genus. As in many *Cypripedium* taxa, in vitro germination proved difficult, likely reflecting nutritional and physiological requirements that differ between subtropical and temperate species. Germination success is shaped by multiple interacting factors, including seed dormancy, culture

medium composition, and fungal compatibility. The challenges in implementing effective propagation and conservation strategies may stem from several constraints: the use of asymbiotic media formulated for other recalcitrant taxa (e.g., cycads), and the specific requirements of fungal growth media, particularly the quality and type of starch used as a carbon source. Our results contribute to the limited body of information on the germination and early development of a Mesoamerican lady's slipper orchids. Despite the loss of compatibility during the process, adult fungal isolates remained capable of colonizing and initiating seed germination. Future work should focus on optimizing the balance between orchid and mycorrhizal fungi to produce robust seedlings suitable for greenhouse cultivation or reintroduction into natural habitats. Increasing the number of isolates and sequenced strains will be essential to determine whether the apparent preference of the Irapeana clade for Tulasnellaceae represents an evolutionary pattern or reflects sampling limitations.

Acknowledgments. We acknowledge M. J. Escalona for English proofreading.

Author contributions. MMC: contribution research, methodology, data curation, formal analysis, Writing (Original Draft). MPOL: conceptualization, writing (revision and editing), supervision, fundraising, project management, and resources.

Funding. This research was funded by the project SEP-CONACYT-ANUIES-ECOS-NORD-FRANCIA 299021, DGAPA-PAPIIT IT-201422, PAPIME PE115024 and grant for Conahcyt-Posgrado en Ciencias Biológicas from the Universidad Nacional Autónoma de México.

Conflict of interest. The authors declare no conflict of interest.

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Supporting Information: Additional material related to this article is available in the online Supporting Information section.

Figure S1. Consensus tree of phylogenetic relationships of *Cypripedium irapeanum* recovered by BLAST algorithm and orchid mycorrhizal fungi (OMF) from other *Cypripedium* species in Tulasnellaceae family. Tree inferred by using the Maximum Likelihood method and the Tamura-Nei 93 model. Branch lengths labels represent bootstrap support. Analyses and editing were done using Geneious.

Figure S2. Consensus tree of phylogenetic relationships of *Cypripedium molle* recovered by BLAST algorithm and Orchid Mycorrhizal Fungi (OMF) from other *Cypripedium* species in Ceratobasidiaceae family. The tree was inferred by using the Maximum Likelihood method and the Tamura-Nei 93 model. Branch labels' length represents bootstrap support. Analyses and editing were performed using Geneious.

 doi: http://dx.doi.org/10.15517/0efjpg42

DIVERSITY AND CONSERVATION OF ORCHIDS IN SAN ANTONIO DEL TEQUENDAMA, CUNDINAMARCA, COLOMBIA

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Abstract. The municipality of San Antonio del Tequendama, Cundinamarca, Colombia, harbours remnants of cloud forests that support a rich diversity of orchids, primarily threatened by habitat loss. This study aims to guide conservation efforts through fieldwork and herbarium records, focused on orchid spatial distribution. The study area covers 82 km², situated at elevations between 1090 and 2700 meters. We conducted 31 field trips between 2017 and 2019. A total of 151 species from 54 genera were documented, offering the most comprehensive list of orchid species recorded in the region, accounting for 3.51% of Colombia's orchid species. The most diverse genera were *Epidendrum* (23 spp.), *Maxillaria* (13 spp.), *Stelis* (10 spp.), and *Cyrtochilum* (10 spp.). Thirty genera (19.86%) were represented by only one species. Six threatened species were identified: *Cattleya trianae* and *Masdevallia caudata* (Endangered), *Cyrtochilum ioplocon*, *Dracula psittacina*, *Oncidium gloriosum* , and *Pleurothallis mundula* (Vulnerable). Although the conservation status of some endemic species (26 spp., 17.21%) remains unknown or unassessed, many observed during field trips are rare and vulnerable to illegal collection for commercial purposes. The remaining forests of San Antonio del Tequendama, especially those within protected natural reserves, are crucial for in situ conservation actions at the local level. Our findings on species distribution provide a baseline for assessing the conservation status of orchid populations in cloud forests.

Resumen. El municipio de San Antonio del Tequendama, Cundinamarca, Colombia, alberga relictos de bosque de niebla, ecosistemas clave para una gran diversidad de orquídeas, cuya principal amenaza es la pérdida de hábitat. Este estudio presenta la diversidad de orquídeas de San Antonio del Tequendama, su estado de conservación y algunas proyecciones para su manejo, con el fin de promover su gestión y uso sostenible. El área de estudio abarcó 82 km², en elevaciones entre los 1090 y 2700 metros. Entre 2017 y 2019, se realizaron 31 salidas de campo en distintas localidades, empleando transectos lineales. En total, se registraron 151 especies distribuidas en 54 géneros, lo que constituye el listado más completo de la región, representando el 3.51 % de las orquídeas del país. Los géneros más diversos fueron *Epidendrum* (23 spp.), *Maxillaria* (13 spp.), *Stelis* (10 spp.) y *Cyrtochilum* (10 spp.). Sin embargo, un 19.86 % de los géneros (30 spp.) estuvieron representados por una sola especie. Se identificaron cinco especies amenazadas a nivel nacional: *Cattleya trianae* y *Masdevallia caudata* están catalogadas como especies En Peligro, mientras que *Cyrtochilum ioplocon*, *Dracula psittacina* y *Oncidium gloriosum* se clasifican como Vulnerables. Aunque la mayoría de las especies endémicas nacionales (26 spp., 17.21 %) no han sido evaluadas, se consideran raras en la zona y enfrentan la amenaza de extracción ilegal con fines comerciales. Los bosques remanentes de San Antonio del Tequendama, especialmente en áreas protegidas, son fundamentales para la conservación in situ de estas orquídeas a nivel local. Estudiar la

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Received 3 June 2025; accepted for publication 17 November 2025. First published online: 12 December 2025.

demografía de estas especies podría proporcionar las bases necesarias para evaluar su estado de conservación en los bosques de niebla.

Keywords/Palabras clave: Andean forest, bosque Andino, bosque nuboso, cloud forest, diversidad, diversity, endemic, endémico, *Epidendrum*, epífitas, epiphytes

Introduction. Colombia is one of the most orchid-rich countries in the world, with over 4300 documented species (Betancur *et al.*, 2015; POWO, 2025). However, this remarkable diversity is concentrated in ecosystems such as Colombia's cloud forests, where between 60% and 73% of the species reported for the country are found (Orejuela-Gartner, 2010; Ospina, 1996). These ecosystems, though vital for biodiversity conservation, face threats from habitat fragmentation, loss, and climate change, factors that directly threaten both overall biodiversity and the health of orchid communities (Karmalkar *et al*., 2008; Parra-Sánchez *et al.*, 2016; Saunders *et al*., 1991).

The municipality of San Antonio del Tequendama, located in the department of Cundinamarca, harbours remnants of cloud forests. These ecosystems are characterized by high species richness, but their coverage has been severely reduced 50% of their original coverage has been lost in the country, (Armenteras *et al.*, 2007).

Known for its richness in orchids, the Tequendama region has been a focal point of botanical exploration since the 19th century. One of the most influential works documenting the local flora was *"Florae Columbiae"* by Hermann Karsten, who described numerous orchid species, significantly advancing botanical knowledge of the region (Tryon, 1963). Similarly, in the *"Geografía pintoresca de Colombia",* Karsten highlighted the diversity and beauty of Tequendama's orchids (Saffray, 1971). These explorations not only cataloged species but also emphasized the importance of conserving these valuable ecosystems (Saffray, 1971). However, currently, there is no comprehensive list of orchid species, especially for key areas like the Cuchilla de Peñas Blancas, an Integrated Management District, and the Chicaque Reserve (Castellanos-Castro & Torres-Morales, 2018), representing a gap in our knowledge of orchid diversity and conservation (Calderón González *et al.*, 2017).

Our research addresses the biogeographical gap regarding regional orchid distribution by providing a comprehensive and updated inventory of species richness, geographic distribution, and conservation status of orchids in San Antonio del Tequendama. This work establishes the baseline for developing *in situ, ex situ*, and *circa situm* conservation strategies, which are essential for preserving orchid species both in their natural habitats and under controlled conditions.

Material and methods. *Study Area.*— San Antonio del Tequendama is situated in the eastern mountain range of Colombia, within the department of Cundinamarca (4°36′58″N 74°21′08″W, Fig. 1). The local temperature ranges from 12 °C to 18 °C (IDEAM, 2024), with annual rainfall between 900 and 1700 mm, following a bimodal rainfall pattern. Peak rainfall occurs in April and November (around 200 mm), while July is typically the driest month with about 50 mm of rain. The study area spans elevations from 850 to 2700 meters and covers 82 km². Notably, above 1800 meters, the area is heavily influenced by fog originating from the Magdalena River valley for much of the year. According to Holdridge's classification (1996), San Antonio del Tequendama falls within the very humid low montane forest life zone, characterized by high humidity and dense vegetation typical of mountainous regions between 1000 and 2500 meters above sea level.

Collection and identification.— Between January 2017 and November 2019, a comprehensive inventory of the orchids in the region was compiled. Eighteen localities were visited. The inventory relied on direct observation of the species, and binoculars were used when needed to examine treetops. Phenological and ecological data, including hosts, elevation, and geographic coordinates, were recorded, along with photographic documentation of the species. A total of 186 hours of field observation was conducted. Fertile botanical samples were collected under the umbrella of the framework collection permit granted by ANLA to the Jardín Botánico José Celestino Mutis in Bogotá for the period 2017–2019, which included the lead author, including vouchers of miniature species stored in ethanol, especially when multiple individuals were found in a location. Some species lack information because they are species for which there are no herbarium records and for which only a single indi-

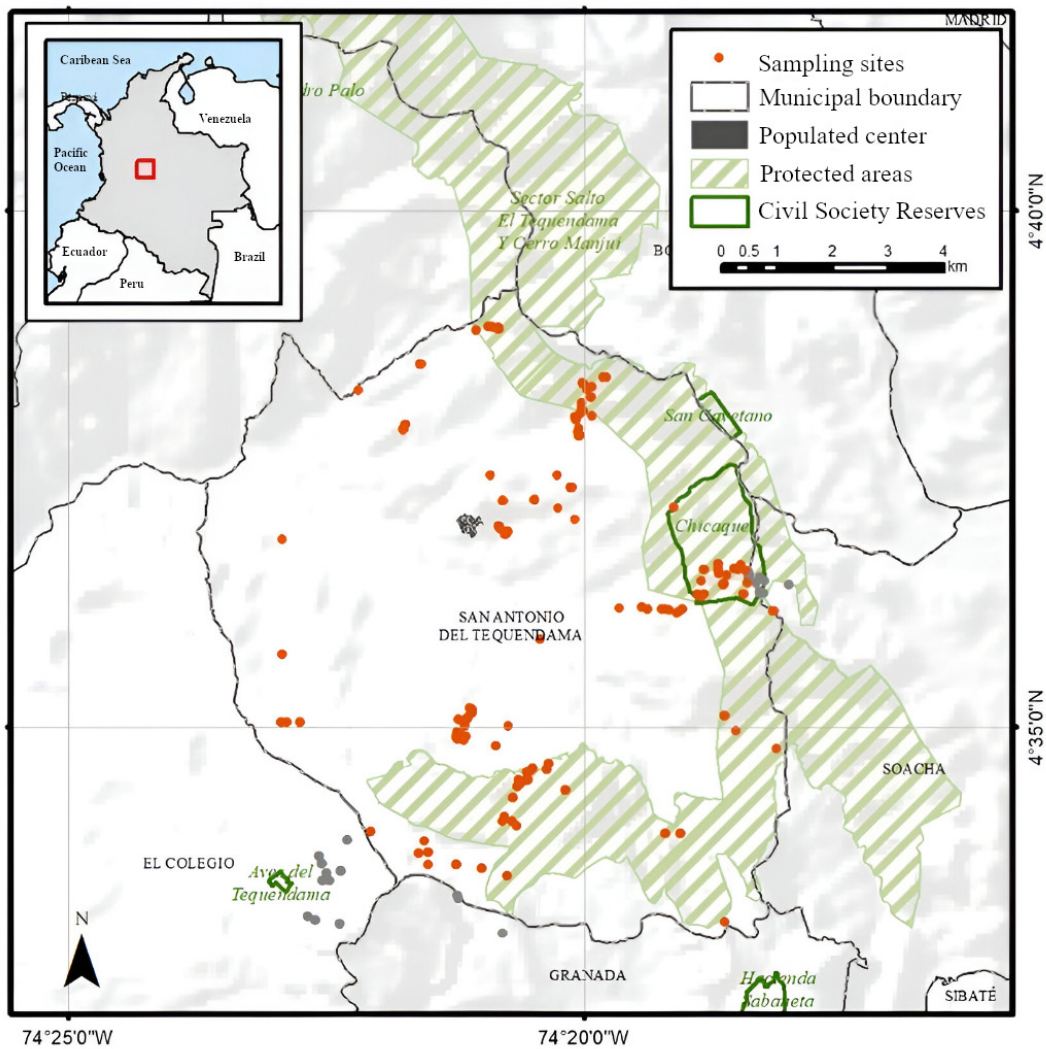

Figure 1. General map of the study area. The localities of the recorded specimens are detailed. The orange colour shows the collection points within the municipality and the grey colour shows populations located on the borders of neighbouring municipalities.

vidual was found in the field, so they were not collected. Samples were processed following standard herbarium techniques and deposited in the herbarium JBB. Additionally, live individuals were collected for the JBB living collections to enable monitoring and future preservation as herbarium specimens.

For the compilation of the species list, sources such as the work of Castellanos-Castro and Torres-Morales (2018) were used and further complemented by examination of specimens from the herbaria ANDES, BOG, COL, FAUC, UDBC, HPUJ, HUQ and JBB. Species identification was performed using taxonomic keys and specialized literature (Chiron, 2005; Christenson, 2009; Dorr *et al.*, 2000; Dueñas & Fernández, 2009; Dunsterville & Garay, 1979; Escobar *et al.*, 1991; Farfán *et al.*, 2003; Hágsater & Sánchez-Saldaña, 2001, 2004; Karremans & Rincón-González, 2015; Luer, 1986, 1994; Nowak *et al.*, 2015; Romero

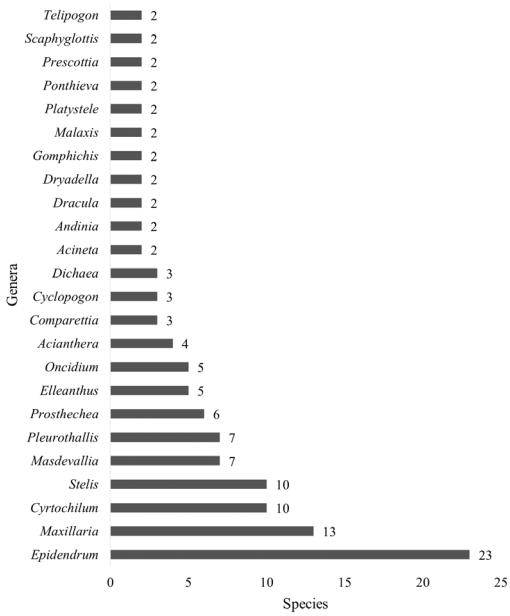

Figure 2. Most diverse orchid genera in San Antonio del Tequendama (Cundinamarca, Colombia).

& Carnevali, 2000; Uribe, 2015). We also consulted specialists to assist with species identification. We collaborated with AMO Herbarium of Mexico for the genus *Epidendrum* L. and Stig Dalström for Oncidiinae.

Accepted species names were verified using online databases such as Govaerts *et al.* (2019), Missouri Botanical Garden (2024) and POWO (2025), Conservation status was determined by referencing Resolution 0126 of 2024 from the Ministry of Environment and Sustainable Development, the International Union for Conservation of Nature (IUCN) Red List online database (IUCN, 2025), the "Libro Rojo de Plantas de Colombia" (Calderón-Sáenz, 2007), and the "Lista Roja de Plantas Vasculares Endémicas de la Alta Montaña de Colombia" (Baca Gamboa *et al.*, 2021). Taxonomic, biogeographic, ecological, and conservation data were also reviewed in the "Catálogo de plantas y líquenes de Colombia" and the "Plan para el estudio y la conservación de las orquídeas en Colombia" (Bernal *et al.*, 2019; Betancur *et al.*, 2015).

A Bray-Curtis dissimilarity analysis was performed in R studio (Nenadic & Greenacre, 2007) to evaluate significant differences (where 0 = identical, 1 = completely different) between the orchid species composition in this study and the records by Castellanos-Castro and Torres-Morales (2018).

Results. *Diversity.*— The 31 field trips conducted between 2017 and 2019 allowed us to identify 151 orchid species across 54 genera (Annex 1, Fig. 5–16). The most diverse genera were *Epidendrum* (23 species), *Maxillaria* Ruiz & Pav. (13 species), *Stelis* Sw. (10 species), and *Cyrtochilum* Kunth (10 species) (Fig. 2). Conversely, 30 of the genera (19.86%) included only one species, and 58 species (38.41%) were found in just one location (Table 1). To supplement the information on regional species, specimens from local herbaria such as ANDES, BOG, COL, FAUC, UDBC, HPUJ, HUQ and JBB were examined, revealing 16 species collected between 1949 and 1986 that were not identified in this study (Fig. 3).

There are significant differences between the composition of orchid species in our field study and previous records (BC = 0.799), both from herbariums and those reported by Castellanos-Castro & Torres-Morales (2018). Our findings differ greatly from other sources, indicating possible ecological changes or potential sampling biases (Fig. 3).

Life form.— Most of the species inventoried are epiphytes, which comprise 69 species (45.69%) of the total recorded. Geophytes comprise 21 species, equivalent to 13.90%, while lithophytes comprise 17 species, corresponding to 11.25% (Annex 1). Likewise, species with two life forms were identified: epiphytes-lithophytes, with 22 species representing 14.56%; epiphytes-geophytes, with 11 species equivalent to 7.28%; and lithophytes-geophytes, with 7 species constituting 4.63%. In addition, five facultative species (3.31%) were recorded: *Cyrtochilum exasperatum* (Linden & Rchb.f.) Kraenzl., *C. orgyale* (Rchb.f. & Warsz.) Kraenzl., *Epidendrum arachnoglossum* Rchb.f. ex André, *Maxillaria aurea* (Poepp. & Endl.) L.O.Williams, and *M. quelchii* Rolfe (Fig. 4).

Geographic distribution.— We identified 26 species (17.2%) as Colombian endemics and 21 species (13.9%) as binational endemics, occurring in Colombia and one neighbouring country. Sixty-seven species (44.4%) have a broader South American distribution, and 36 species (23.8%) are found across the Neotropical region. Additionally, we recorded one naturalized species, *Eulophia maculata* (Lindl.) Rchb.f., native to tropical Africa and now widespread in the Neotropics, where it

Table 1. Colombian endemic orchids recorded in San Antonio del Tequendama (Cundinamarca, Colombia). Elevation range and distribution in Colombia by departments are listed, and species that constitute a new locality for Colombia are indicated in asterisk and in bold (Betancur *et al.*, 2015). Antioquia (Ant), Boyacá (Boy), Caldas (Cal), Casanare (Cas), Cauca (Cau), Chocó (Cho), Cundinamarca (Cun), Huila (Hui), Magdalena (Mag), Meta (Met), Nariño (Nar), Norte De Santander (Nsa), Quindio (Qui), Putumayo (Put), Risaralda (Ris), Santander (San), Tolima (Tol), Valle Del Cauca (Val).

Species	Elevation above sea level		Distribution in Colombia
	Midlands (1000–1500 m)	Highlands (>1500 m)	
Acianthera adeodata* P.Ortiz, O.Pérez & E.Parra		×	Val, Cun
Acianthera serratifolia* Rinc.-González & Karremans		×	Tol, Cun
Andinia pendens (Garay) Karremans & S.V.Uribe		×	Boy, Cau, Cun, Cas
Bulbophyllum antioquiense Kraenzl.	×		Ant, Cau, Cun, Met, San, Tol
Cattleya trianae Linden & Rchb.f.	×		Cun, Hui, Tol
Comparettia macroplectron Rchb.f. & Triana	×	×	Boy, Cas, Cun, Mag, Met
Cyrtochilum baldeviamae (Rchb.f.) Kraenzl.		×	Cun
Cyrtochilum ioplocon (Rchb.f.) Dalström		×	Ant, Cal, Cun
Dracula houtteana (Rchb.f.) Luer	×	×	Ant, Cau, Cho, Cun, Ris, Tol
Dracula psittacina (Rchb.f.) Luer & R.Escobar		×	Ant, Cun
Epidendrum cleistocoleum Hágsater & E.Santiago		×	Ant, Cun, Ris, Val
Epidendrum fusagasugaënse E.Parra, Hágsater & L.Sánchez		×	Cun
Epidendrum mamapachae* Hágsater, F.O.Espinosa & E.Santiago		×	Boy, Cun
Epidendrum ortizii Hagsater & Santiago		×	Cun, San
Epidendrum scytocladium Schltr.		×	Ant, Boy, Cal, Cho, Cun, Hui, Qui, Ris, San, Tol
Epidendrum tequendamae F.Lehm. & Kraenzl.		×	Cun
Maxillaria carrilloi Christenson		×	Cun
Maxillaria tenuibulba Christenson		×	Ant, Cun, Val
Oncidium luteopurpureum (Lindl.) Beer		×	Ant, Boy, Cal, Cun, Hui, Put, Ris, San, Tol, Val
Platystele schneideri P.Ortiz		×	Ant, Boy, Cau, Cun, Val
Pleurothallis mundula Luer & R.Escobar		×	Cun, Tol
Rodriguezia granadensis (Lindl.) Rchb.f.	×	×	Ant, Boy, Cun, Mag, Nar, Nsa, San, Tol, Val
Scaphyglottis bicornis (Lindl.) Garay		×	Ant, Cau, Cun, Hui, Nsa, Ris, San
Sobralia mutisii P.Ortiz		×	Ces, Cun, Hui
Stelis alba Kunth		×	Ant, Boy, Cal, Cau, Cun, Ris
Telipogon albertii Rchb.f.		×	Cun

behaves invasively (Cohen & Ackerman, 2009; Baptiste *et al*., 2010; see Annex 1, Fig. 10B). We also provide new distribution records in Colombia for three species: *Acianthera adeodata* P.Ortiz, O.Pérez & E.Parra (Fig. 5A), *Acianthera serratifolia* Rinc.-González & Karremans (Fig. 5C), and *Epidendrum mamapachae* Hágsater, F.O.Espinosa & E.Santiago (Fig. 9B).

Altitudinal distribution.— Altitudes above 1500 m showed the greatest species richness (139 species), and a total of 48 species were recorded between 1000 and 1500 m. Finally, although no sampling was carried out in areas below 1000 m, 17 species are found in lowland areas (below 1000 m) and six species are from temperate lands (between 1000 and 1500 m).

Conservation status.— Of the 151 orchid species documented in San Antonio del Tequendama, only 11.9% (18 species) have been officially evaluated and classified as nationally threatened under IUCN-based national assessments. Among these, three species are categorized as Endangered (EN): *Cattleya trianae* Linden & Rchb.f., *Masdevallia caudata* Lindl., and *Pleurothallis mundula* Luer & R.Escobar. Another three species are listed as Vulnerable (VU): *Cyrtochilum ioplocon* (Rchb.f.) Dalström, *Dracula psittacina* (Rchb.f.) Luer & R.Escobar, and *Oncidium gloriosum* (Linden & Rchb.f.) M.W.Chase & N.H.Williams (Annex 2). The remaining 133 species (88.1%) have not yet been assessed, highlighting a gap in conservation evaluation.

Discussion. Endemism in San Antonio del Tequendama represents 1.65% of Colombia's national orchid flora, increasing to 2.98% when binational endemics are considered. This study confirms the presence of ten species endemic to Cundinamarca and twenty-six species (17.21%) endemic to Colombia. These results corroborate the high endemism levels reported for the Andean region, where at least 944 endemic orchid species have been documented (Betancur *et al*., 2018) and are consistent with the remarkable orchid richness of Cundinamarca, which ranks third nationally with 273 endemic species, including 87 restricted to the department.

This high species richness is closely linked to the presence of cloud forest ecosystems, which are affected by moisture-laden currents from the Magdalena River Valley (Ospina-Arguello & Silva-Tabio, 2015). This pattern is also seen in other tropical regions (Haber, 2000). In the Andes, orchid diversity especially of epiphytes peaks between 1000 and 2000 m in elevation, where plants take advantage of available niches, access to sunlight, and steady moisture from runoff and canopy drip (Gentry & Dodson, 1987; Granados-Sánchez *et al.*, 2003; Molina-García *et al.*, 2024; Parra-Sánchez *et al.*, 2016). In our study, 139 species (92.05%) were found above 1500 m, where diversity is highest and conservation concerns are greatest (Dressler, 1981; Willis, 2017). The reliance of epiphytes on host trees makes them vulnerable to deforestation and microclimatic changes (Benzing, 1990; Valencia, 2014).

Despite this ecological richness, threats to orchid populations are evident. Secondary forests, especially in protected areas like Cuchilla de Peñas Blancas and the Subia Integrated Management District are essential for orchid growth conservation.

Yet, these Andean remnants face increasing pressures from deforestation, selective harvesting, and climate change (Christmann *et al.*, 2023; Fay, 2018; Ospina, 1996; Parra-Sánchez *et al.*, 2016). Species such as *Dracula psittacina*, *Kefersteinia tolimensis* Schltr., *Masdevallia caudata* Lindl., and *Oncidium luteopurpureum* (Lindl.) Beer, continue to be collected for commercial purposes (Hinsley *et al.*, 2018a; Hinsley & Roberts, 2018b; Parra-Sanchez & Baquero, 2023). The rarity of species like *Cattleya trianae* and *Acianthera adeodata* likely reflects decades of extraction, severely threatening their survival (Calderón-Sáenz, 2007).

The remarkable orchid diversity in this region is characterized by the dominance of the epiphytic habit. Geophytes make up 21 species (13.90%), typically found in moist, well-preserved habitats that support stable populations (Zhang *et al.*, 2018). Lithophytes, with 17 species (11.25%), thrive in niches within humid rock formations, benefiting from the abundant mosses, liverworts, and lichens typical of cloud forests (Dressler, 1981; Viancha-Plazas & Córdoba-Cárdenas, 2019). The coexistence of diverse life forms indicates the presence of unique microhabitats in the region complexity.

Our results differ from the 163 species listed by Castellanos-Castro and Torres-Morales (2018). The difference probably comes from including cultivated specimens in the earlier study voucher specimens from the HPUJ herbarium that were not collected in their natural habitat. For example, *Maxillaria lawrenceana* (Rolfe)

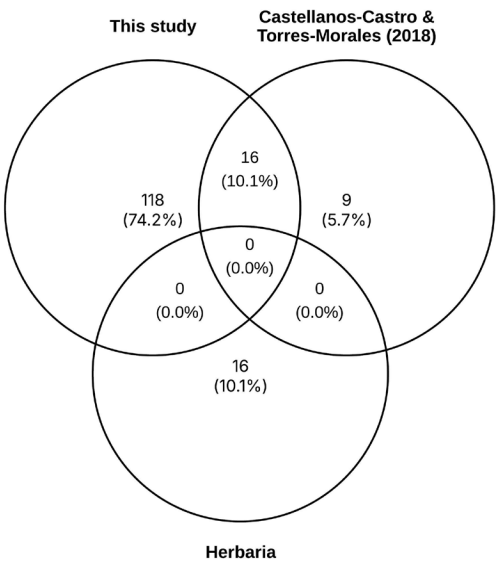

Figure 3. Venn diagram showing the number of shared and exclusive records between the current study, the study by Castellanos-Castro and Torres-Morales (2018), and local herbaria.

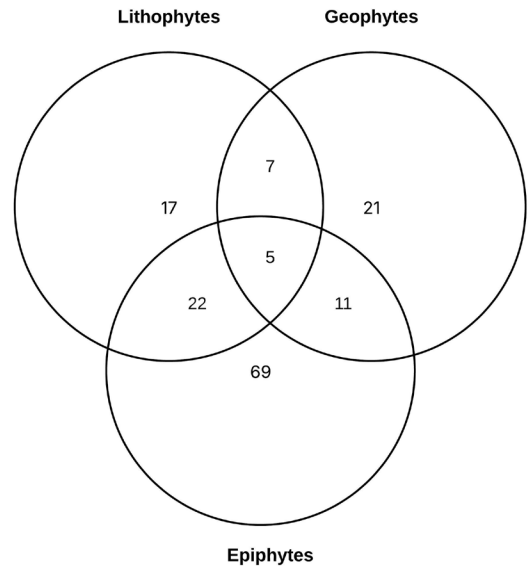

Figure 4. Distribution of orchid species according to life form in the municipality of San Antonio del Tequendama, Cundinamarca, Colombia.

Garay & Dunst., and *Masdevallia coccinea* Linden ex Lindl. are not known to occur naturally in the area. In our research, we included 16 species last collected between 1949 and 1986 that have not been reported since, indicating potential local extinction. However, our findings are consistent with other studies in Andean forests where Pleurothallidinae, Laeliinae, and Oncidiinae are predominant (Cascante-Marín & Hernández, 2019; Dressler, 1981; Orejuela-Gartner, 2012).

Finally, this study highlights the urgent need for targeted conservation efforts. Many species in the region are endemic, rare, or have not been observed in decades, as those marked in bold and with an asterisk in Annex 1, actors that increase their risk of extinction. However, knowledge of their ecology and population status remains limited. Geographic distribution data, like that presented in this study, are crucial for predictive modelling of extinction risk (Darrah *et al.*, 2017). Effective conservation in San Antonio del Tequendama should include: (1) *in situ* conservation through habitat protection and species reintroduction, (2) *ex situ* strategies including germplasm banks and nursery propagation, and (3) *circa situm* approaches involving local communities in sustainable propagation and ecotourism (Flanagan & Mosquera-Espinosa, 2016; Swarts & Dixon, 2009).

Acknowledgements. We thank the José Celestino Mutis Botanical Garden, especially the Living Collections Section, and the community of San Antonio del Tequendama, particularly Nelsy Quintero, Pedro Neiva, Carlos Zea, Carlos Arias, Rocío Camargo, Lina Pedraza, and Néstor Benavides. We also thank Carlos López for his support with GIS, and Diego Moreno, Eder Vanegas, Manuela Báez, Julián Ordóñez, and Nicolás Santos for their collaboration. We appreciate the contributions of specialists C. Castro, E. Santiago, L. Baquero, M. Kolanowska, S. Dalström, and S. Nowak. We are grateful to Ana B. Hurtado, José Luis Alanís, Fabio Ávila, and Susana Rudas for their contributions to the manuscript, and to Viateur Boutot for his work as editor and proofreader. We declare that the collection and storage of herbarium samples was covered by the ANLA collection and transportation permits issued by the Bogotá Botanical Garden for the years 2017–2019.

Author contributions. Conceptualization: J.C.O.B. and G.M. Methodology: J.C.O.B., G.M., and C.C. Formal Analysis: J.C.O.B. and E.P.S. Writing – Original Draft Preparation: J.C.O.B. Writing – Review & Editing: J.C.O.B., E.P.S., and J.S.G.

Funding. Several stages of the project were supported through service contracts from the José Celestino Mutis Botanical Garden of Bogotá.

Conflict of interest. The author declares no commercial, financial, academic, or personal conflicts of interest related to this study.

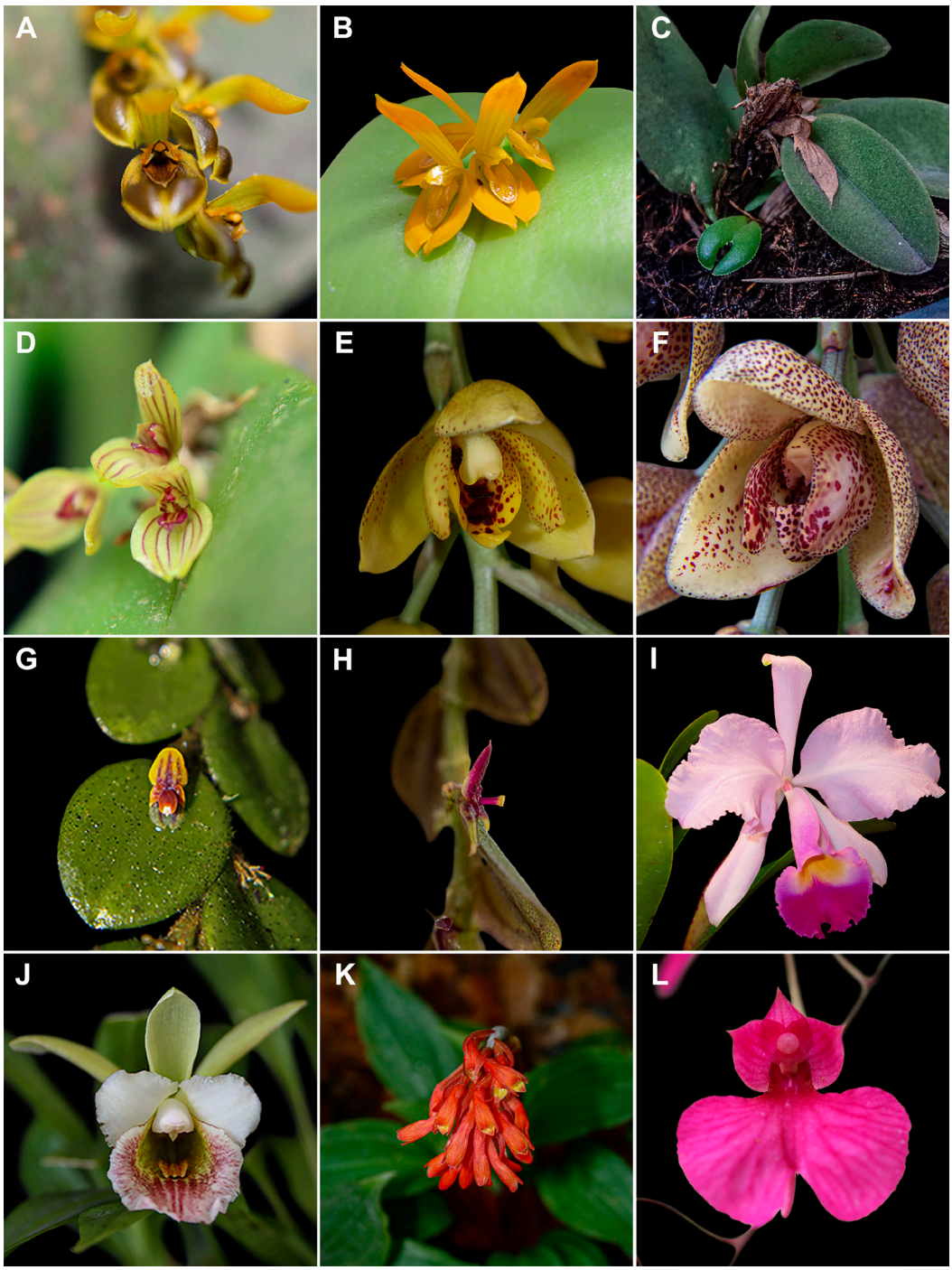

FIGURE 5. **A**. *Acianthera adeodata* P.Ortiz, O.Pérez & E.Parra. **B**. *Acianthera polystachya* (Ruiz & Pav.) Pupulin. **C**. *Acianthera serratifolia* Rinc.-González & Karremans. **D**. *Acianthera sicaria* (Lindl.) Pridgeon & M.W.Chase. **E**. *Acineta cryptodonta* Rchb.f. **F**. *Acineta superba* (Kunth) Rchb.f. **G**. *Andinia nummularia* (Rchb.f.) Karremans & S.V.Uribe. **H**. *Andinia pendens* (Garay) Karremans & S.V.Uribe. **I**. *Cattleya trianae* Linden & Rchb.f. **J**. *Chondrorhyncha hirtzii* Dodson. **K**. *Coccineorchis cernua* (Lindl.) Garay. **L**. *Comparettia falcata* Poepp. & Endl. Photos by J. C. Ordóñez-Blanco.

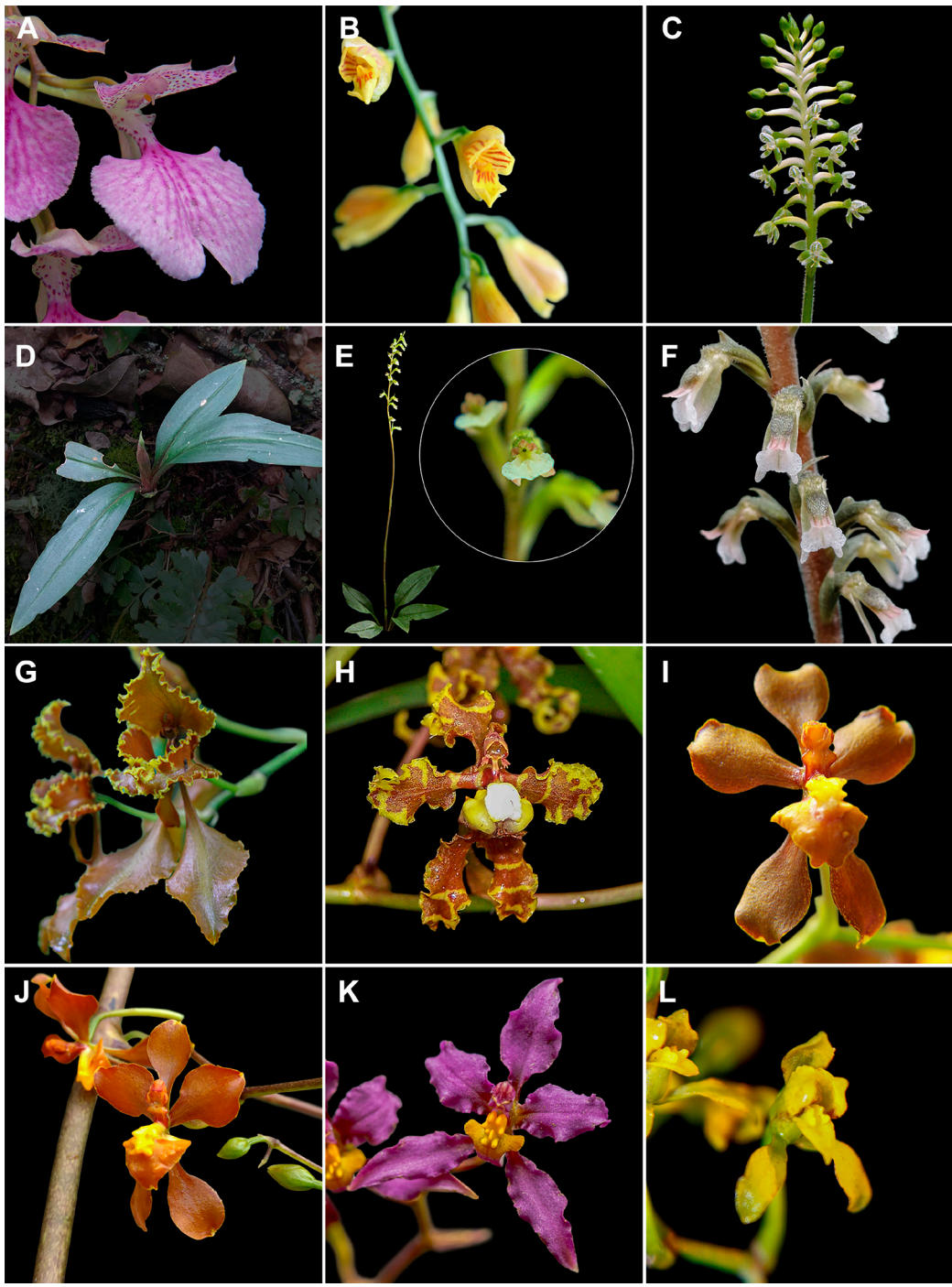

Figure 6. **A**. *Comparettia macroplectron* Rchb.f. & Triana. **B**. *Comparettia ottonis* (Klotzsch) M.W.Chase & N.H.Williams. **C**. *Cranichis antioquiensis* Schltr. **D**. *Cyclopogon* cf. *peruvianus* (C.Presl) Schltr. **E**. *Cyclopogon elatus* (Sw.) Schltr. **F**. *Cyclopogon lindleyanus* (Link, Klotzsch & Otto) Schltr. **G**. *Cyrtochilum baldeviamae* (Rchb.f.) Kraenzl. **H**. *Cyrtochilum divaricatum* (Lindl.) Dalström. **I**. *Cyrtochilum exasperatum* (Linden & Rchb.f.) Kraenzl. **J**. *Cyrtochilum flexuosum* Kunth. **K**. *Cyrtochilum ioplocon* (Rchb.f.) Dalström. **L**. *Cyrtochilum megalophium* (Lindl.) Kraenzl. Photos by J. C. Ordóñez-Blanco.

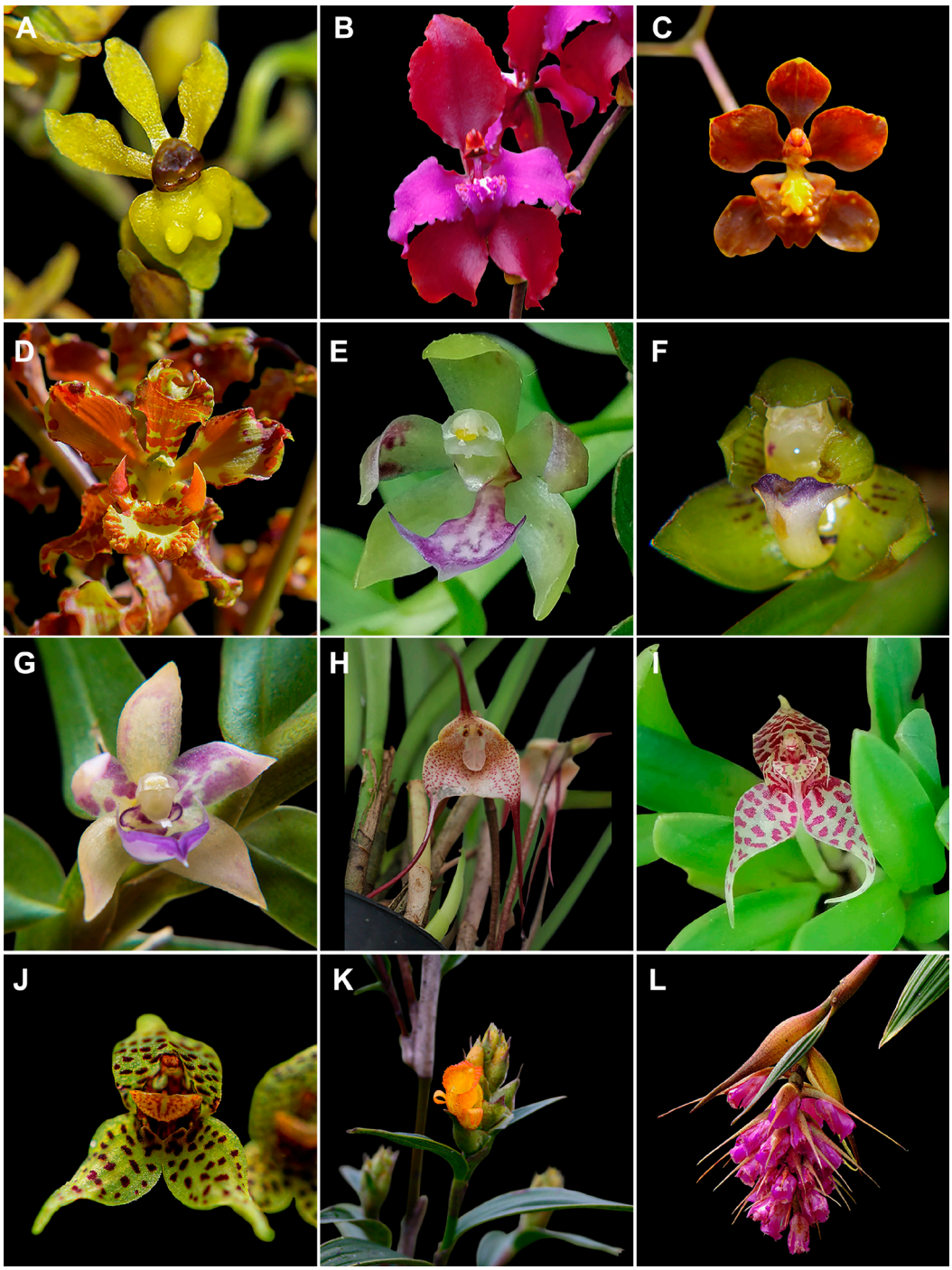

FIGURE 7. **A**. *Cyrtochilum murinum* (Rchb.f.) Kraenzl. **B**. *Cyrtochilum orgyale* (Rchb.f. & Warsz.) Kraenzl. **C**. *Cyrtochilum porrigens* (Rchb.f.) Kraenzl. **D**. *Cyrtopodium punctatum* (L.) Lindl. **E**. *Dichaea longa* Schltr. **F**. *Dichaea morrisii* Fawc. & Rendle. **G**. *Dichaea pendula* (Aubl.) Cogn. **H**. *Dracula psittacina* (Rchb.f.) Luer & R.Escobar. **I**. *Dryadella minuscula* Luer & R.Escobar. **J**. *Dryadella simula* (Rchb.f.) Luer. **K**. *Elleanthus aurantiacus* (Lindl.) Rchb.f. **L**. *Elleanthus ensatus* (Lindl.) Rchb.f. Photos by J. C. Ordóñez-Blanco.

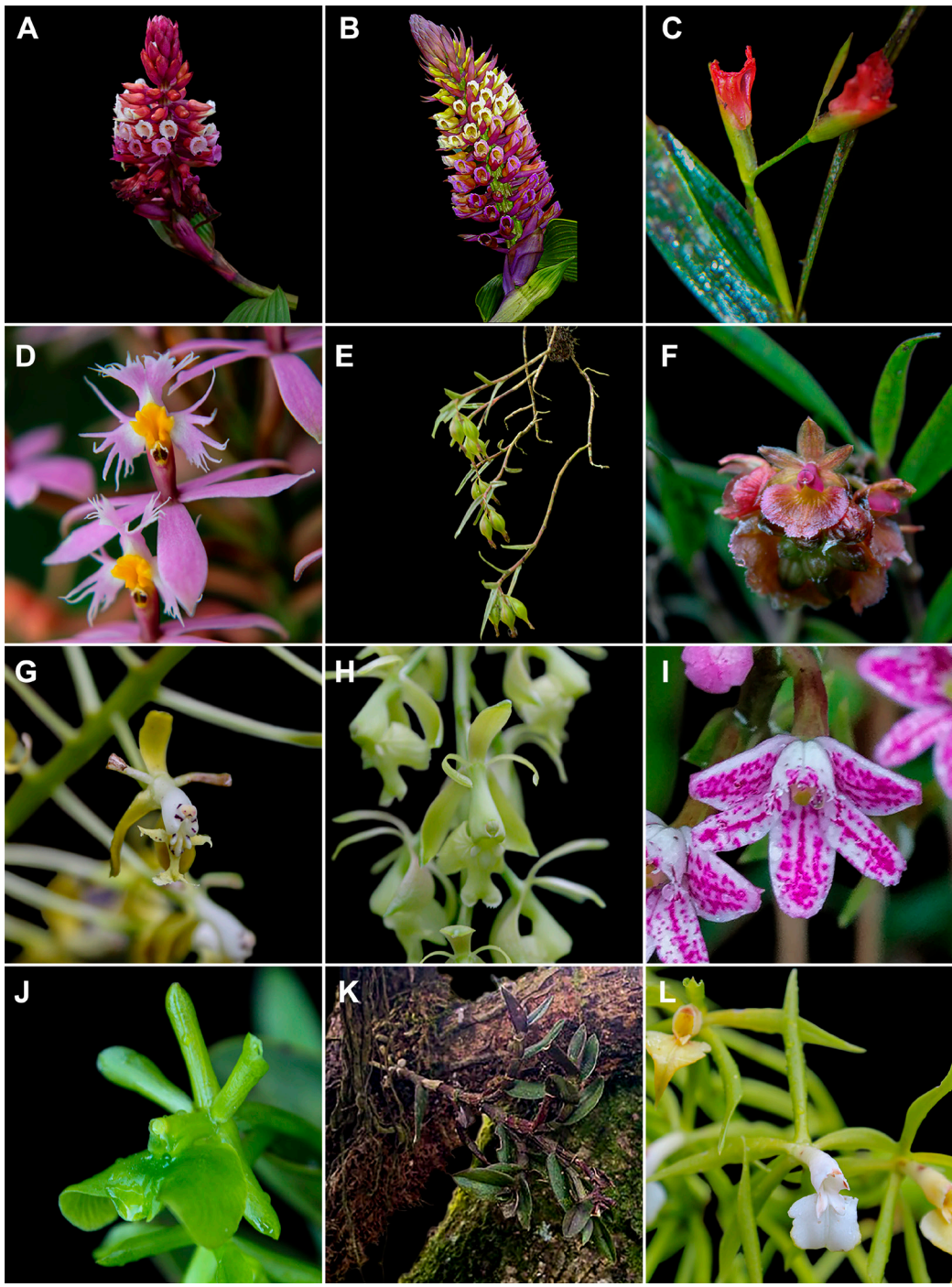

Figure 8. **A**. *Elleanthus purpureus* (Rchb.f.) Rchb.f. **B**. *Elleanthus smithii* Schltr. **C**. *Elleanthus virgatus* (Rchb.f.) C.Schweinf. **D**. *Epidendrum arachnoglossum* Rchb.f. ex André. **E**. *Epidendrum cleistocoleum* Hágsater & E.Santiago. **F**. *Epidendrum cottoniiflorum* (Rchb.f.) Hágsater. **G**. *Epidendrum cylindraceum* Lindl. **H**. *Epidendrum excisum* Lindl. **I**. *Epidendrum fimbriatum* Kunth. **J**. *Epidendrum fusagasugaënse* E.Parra, Hágsater & L.Sánchez. **K**. *Epidendrum gratissimum* (Rchb.f.) Hágsater & Dodson. **L**. *Epidendrum lacustre* Lindl. Photos by J. C. Ordóñez-Blanco (A–J, L) and C. Arias (K).

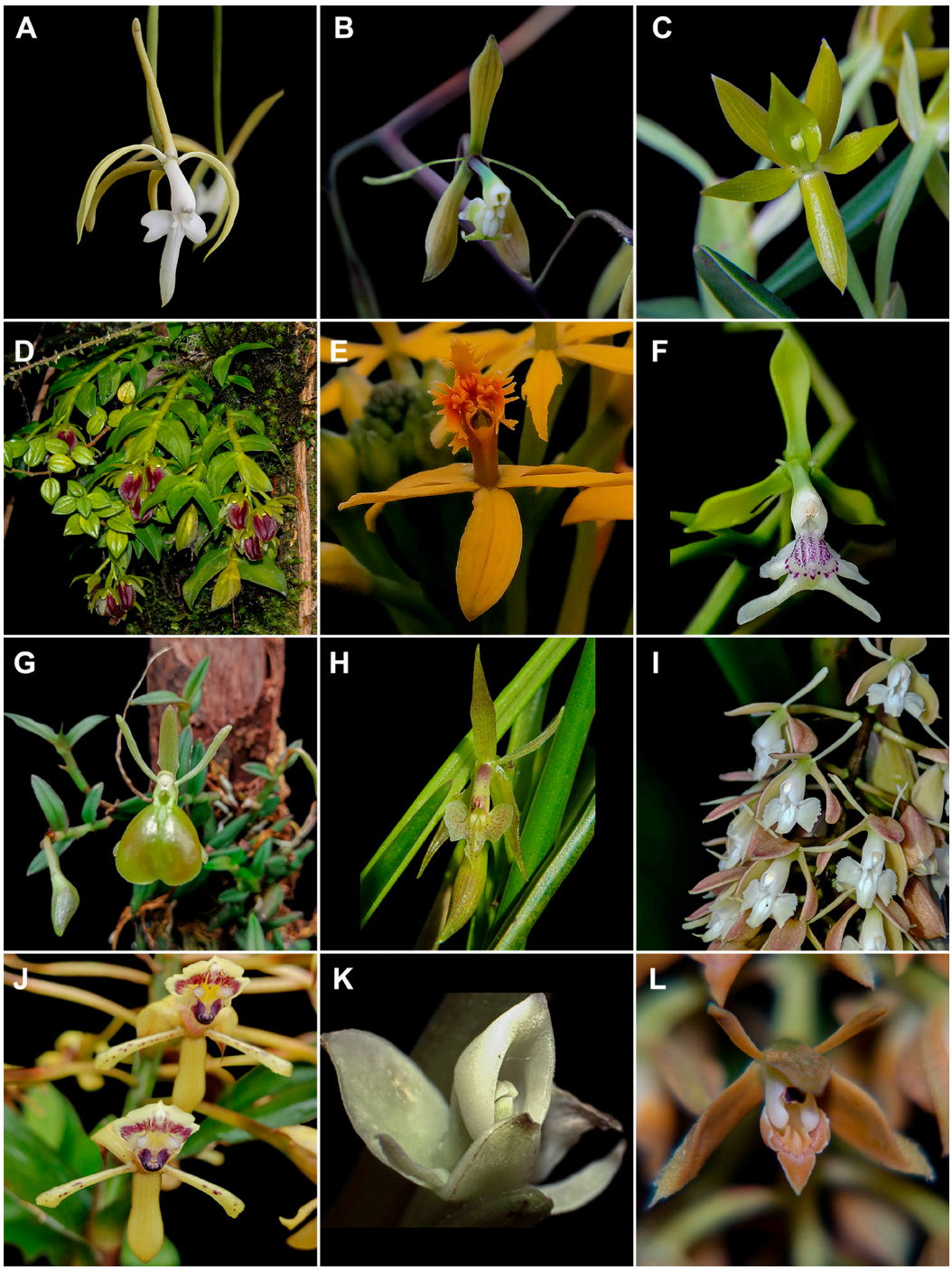

FIGURE 9. **A**. *Epidendrum leucochilum* Link, Klotzsch & Otto. **B**. *Epidendrum mamapachae* Hágsater, F.O.Espinosa & E.Santiago. **C**. *Epidendrum marsupiale* F.Lehm. & Kraenzl. **D**. *Epidendrum megalospathum* Rchb.f. **E**. *Epidendrum melinanthum* Schltr. **F**. *Epidendrum ortizii* Hagsater & Santiago. **G**. *Epidendrum peperomia* Rchb.f. **H**. *Epidendrum porquerense* F.Lehm. & Kraenzl. **I**. *Epidendrum ruizianum* Steud. **J**. *Epidendrum scytocladium* Schltr. **K**. *Epidendrum stenobractistachyum* Hágsater & E.Santiago. **L**. *Epidendrum tequendamae* F.Lehm. & Kraenzl. Photos by J. C. Ordóñez-Blanco.

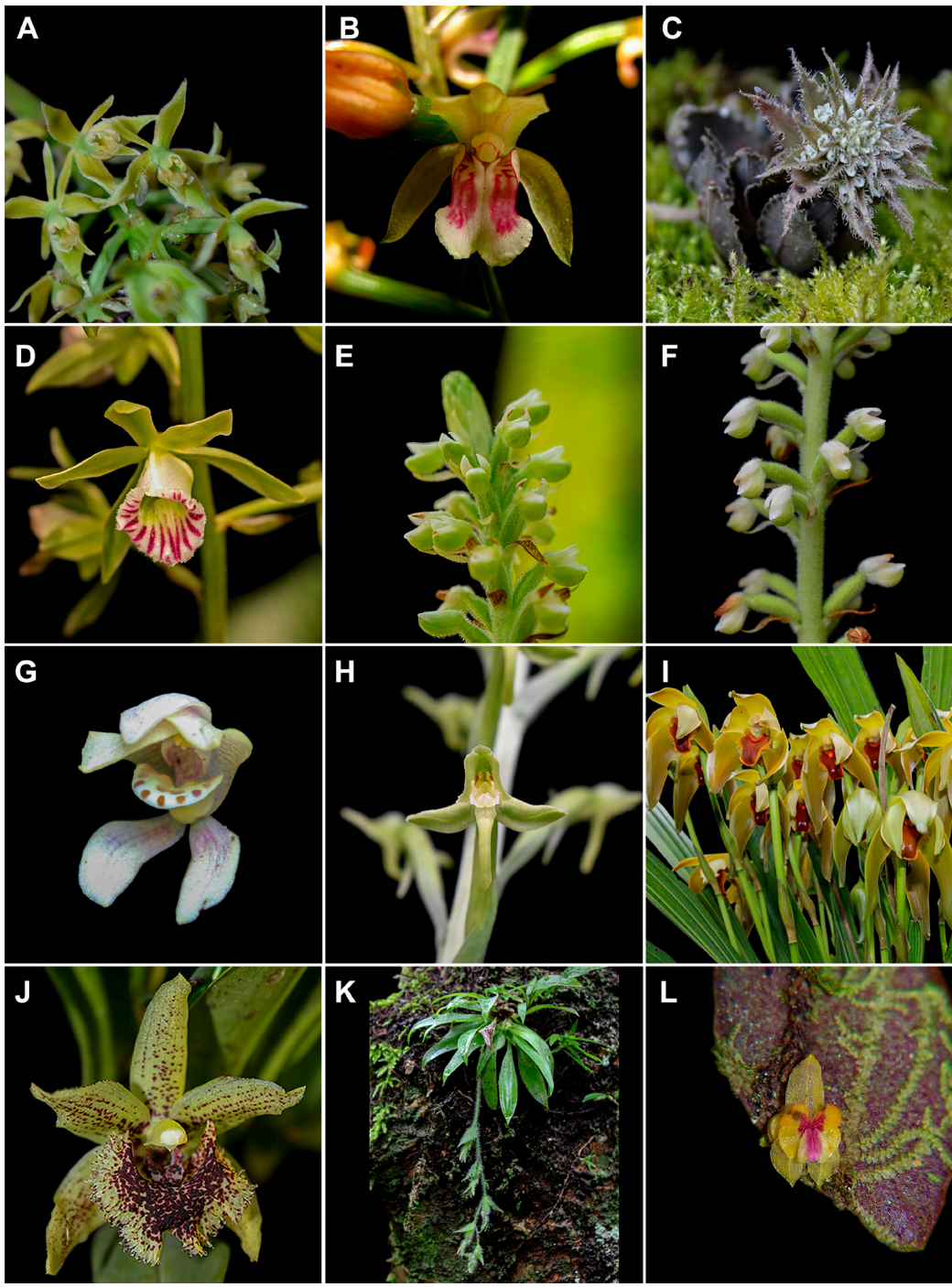

Figure 10. **A**. *Epidendrum viridialpicola* Hágsater & Ordóñez-Blanco. **B**. *Eulophia maculata* (Lindl.) Rchb.f. **C**. *Eurystyles cotyledon* Wawra. **D**. *Galeandra beyrichii* Rchb.f. **E**. *Gomphichis hetaerioides* Schltr. **F**. *Gomphichis longifolia* (Rolfe) Schltr. **G**. *Govenia fasciata* Lindl. **H**. *Habenaria floribunda* Lindl. **I**. *Ida castanea* Oakeley. **J**. *Kefersteinia tolimensis* Schltr. **K**. *Lankesterella orthantha* (Kraenzl.) Garay. **L**. *Lepanthes wageneri* Rchb. Photos by J. C. Ordóñez-Blanco.

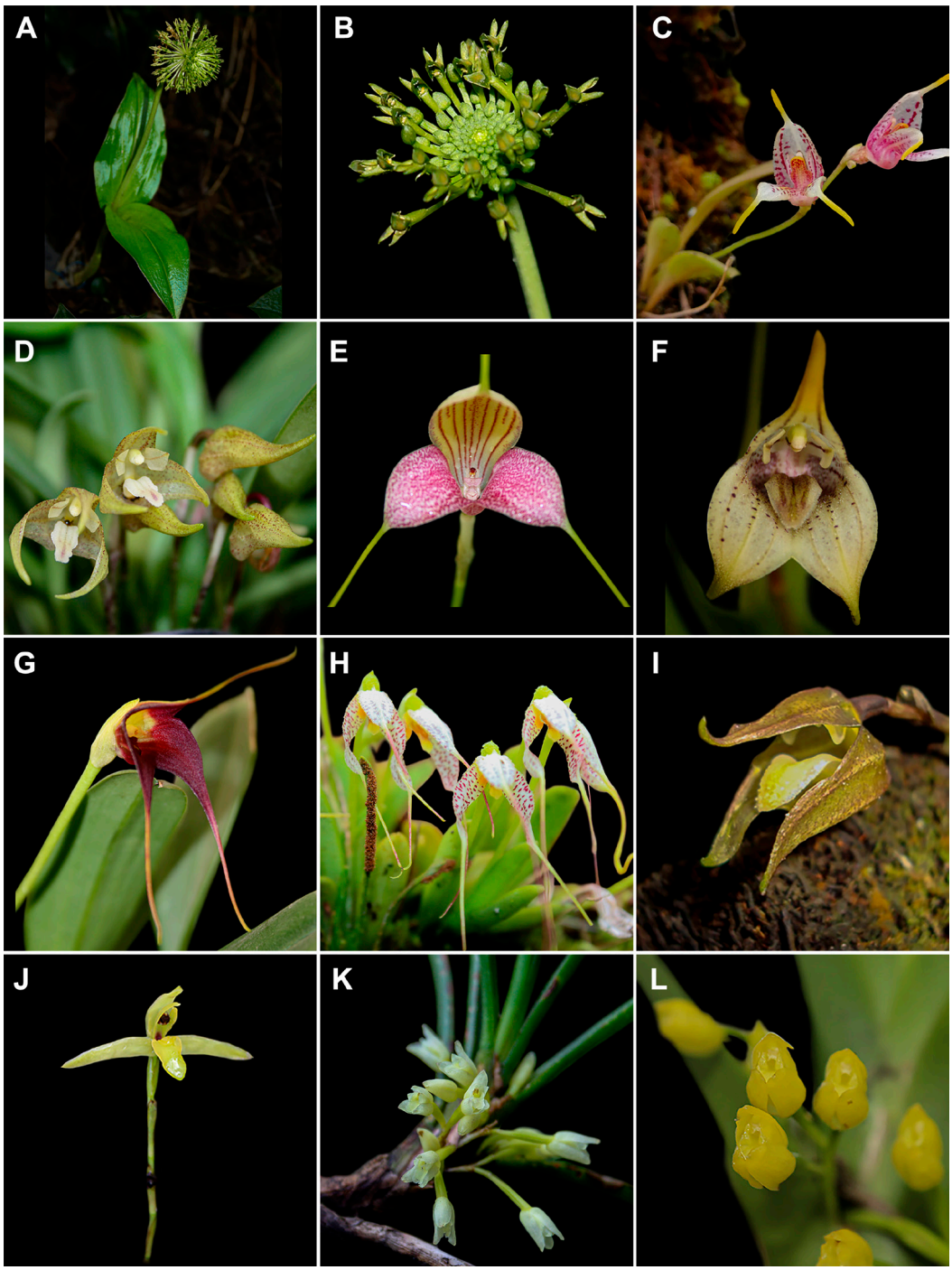

FIGURE 11. **A**. *Malaxis crispifolia* (Rchb.f.) Kuntze. **B**. *Malaxis* cf. *excavata* (Lindl.) Kuntze. **C**. *Masdevallia amanda* Rchb.f. & Warsz. **D**. *Masdevallia campyloglossa* Rchb.f. **E**. *Masdevallia caudata* Lindl. **F**. *Masdevallia coriacea* Lindl. **G**. *Masdevallia cucullata* Rchb.f. **H**. *Masdevallia picturata* Rchb.f. **I**. *Masdevallia platyglossa* Rchb.f. **J**. *Maxillaria acuminata* Lindl. **K**. *Maxillaria aggregata* (Kunth) Lindl. **L**. *Maxillaria aurea* (Poepp. & Endl.) L.O.Williams. Photos by J. C. Ordóñez-Blanco.

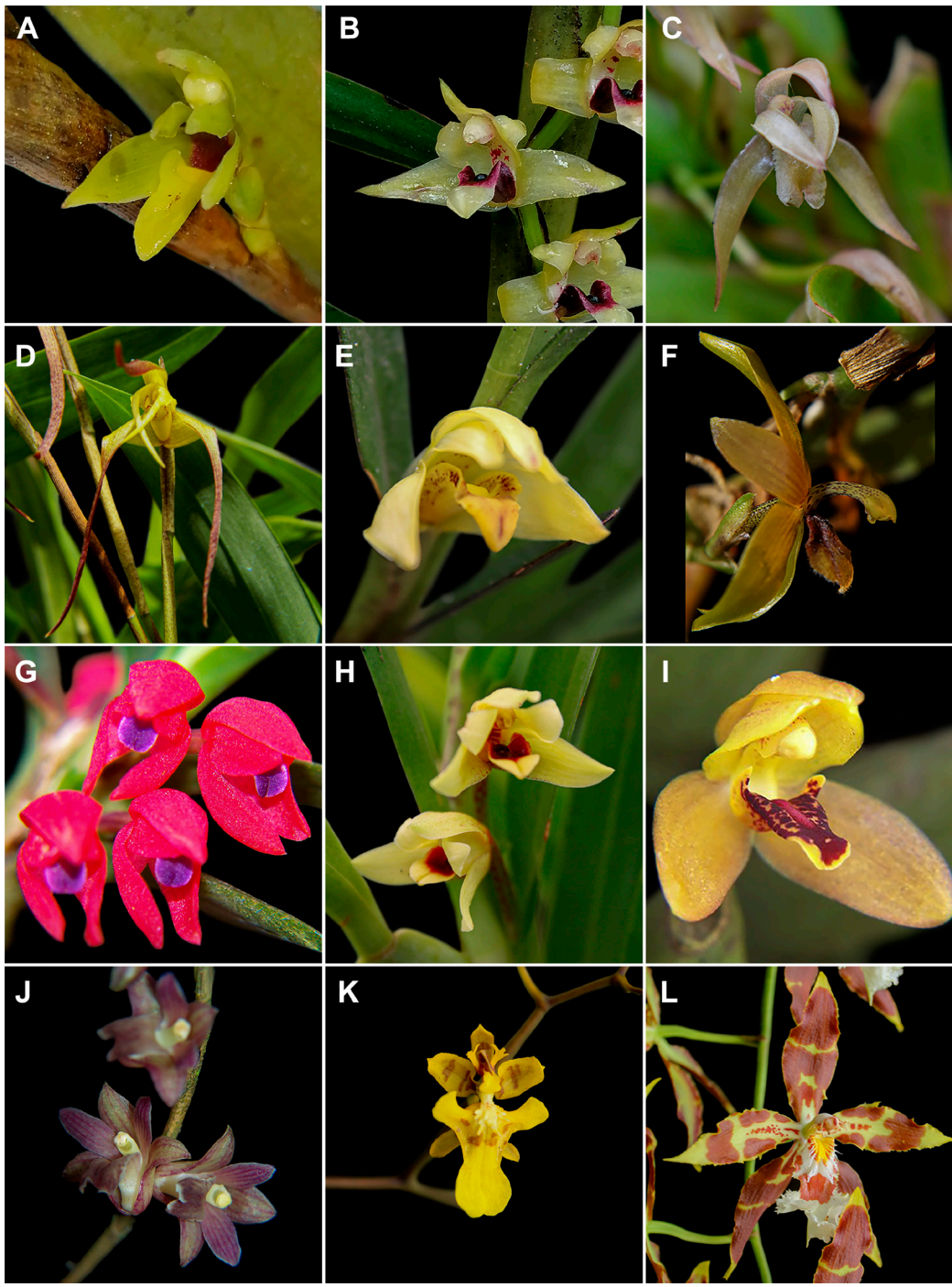

Figure 12. **A**. *Maxillaria carrilloi* Christenson. **B**. *Maxillaria cassapensis* Rchb.f. **C**. *Maxillaria fimbriatiloba* Carnevali & G.A.Romero. **D**. *Maxillaria lepidota* Lindl. **E**. *Maxillaria meridensis* Lindl. **F**. *Maxillaria rhomboglossa* (F.Lehm. & Kraenzl.) Molinari. **G**. *Maxillaria ruberrima* (Lindl.) Garay. **H**. *Maxillaria spilotantha* Rchb.f. **I**. *Maxillaria tenuibulba* Christenson. **J**. *Nemaconia striata* (Lindl.) Van den Berg, Salazar & Soto Arenas. **K**. *Oncidium lancifolium* Lindl. **L**. *Oncidium luteopurpureum* (Lindl.) Beer. Photos by J. C. Ordóñez-Blanco.

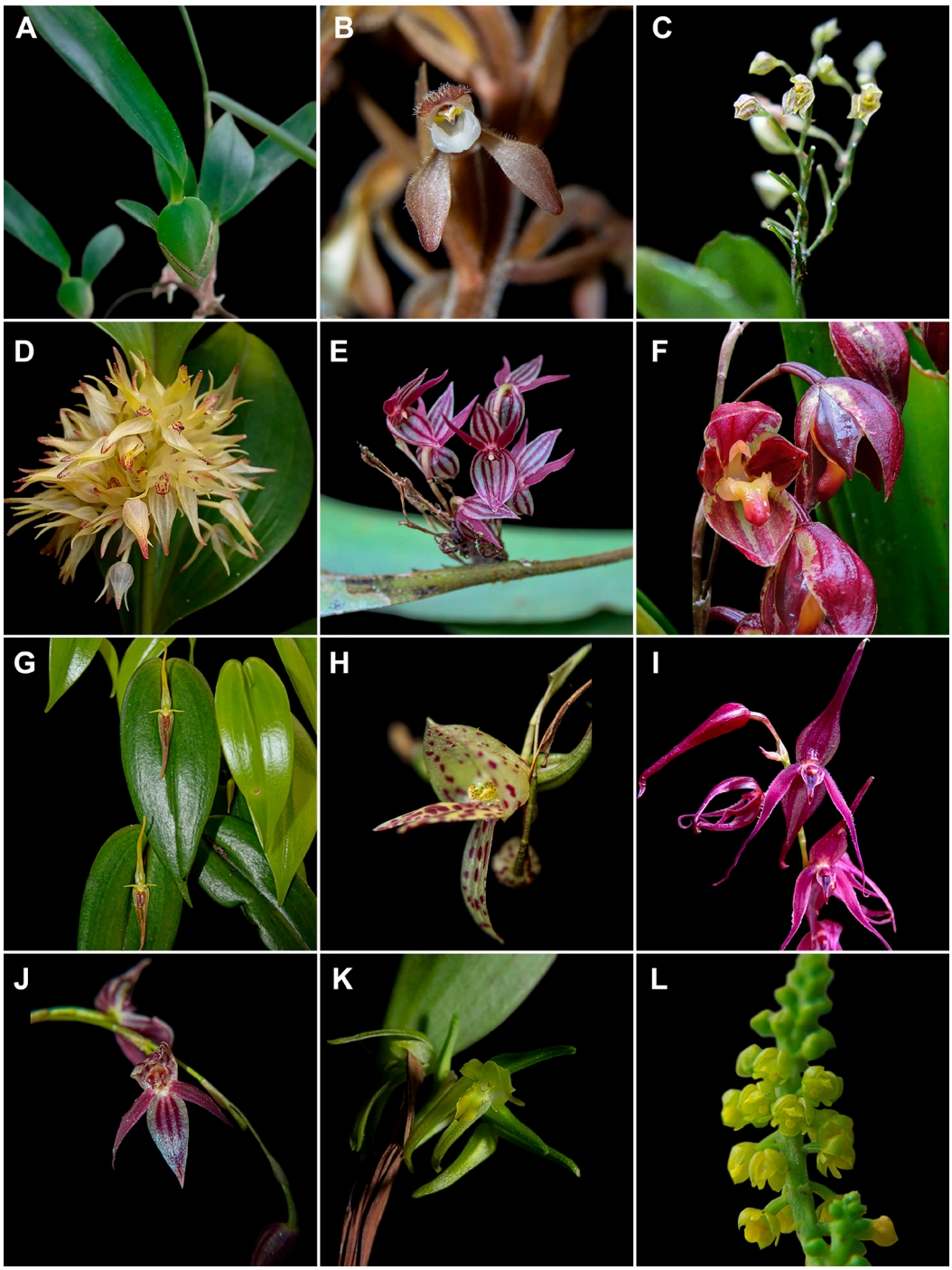

FIGURE 13. **A**. *Otoglossum globuliferum* (Kunth) N.H.Williams & M.W.Chase. **B**. *Pelexia weberbaueri* (Kraenzl.) Schltr. **C**. *Platystele schneideri* P.Ortiz. **D**. *Pleurothallis chloroleuca* Lindl. **E**. *Pleurothallis lindenii* Lindl. **F**. *Pleurothallis macrophylla* Kunth. **G**. *Pleurothallis microcardia* Rchb.f. **H**. *Pleurothallis mundula* Luer & R.Escobar. **I**. *Pleurothallis phalangifera* (C.Presl) Rchb.f. **J**. *Pleurothallis secunda* Poepp. & Endl. **K**. *Pleurothallopsis microptera* (Schltr.) Pridgeon & M.W.Chase. **L**. *Polystachya foliosa* (Hook.) Rchb.f. Photos by C. Arias (A) and J. C. Ordóñez-Blanco (B–L).

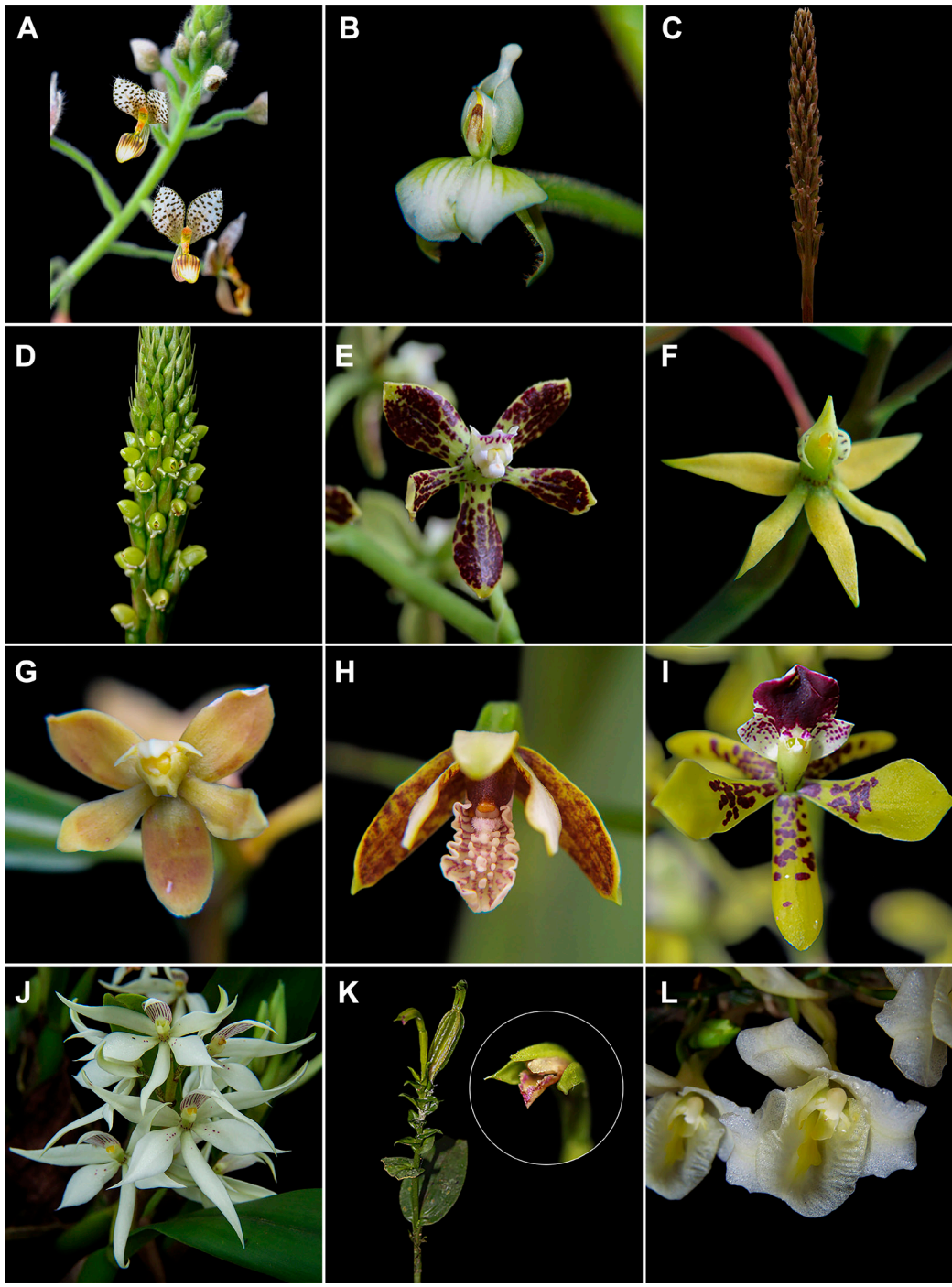

Figure 14. **A**. *Ponthieva maculata* Lindl. **B**. *Ponthieva rostrata* Lindl. **C**. *Prescottia petiolaris* Lindl. **D**. *Prescottia stachyodes* (Sw.) Lindl. **E**. *Prosthechea crassilabia* (Poepp. & Endl.) Carnevali & I.Ramírez. **F**. *Prosthechea grammatoglossa* (Rchb.f.) W.E.Higgins. **G**. *Prosthechea hartwegii* (Lindl.) W.E.Higgins. **H**. *Prosthechea livida* (Lindl.) W.E.Higgins. **I**. *Prosthechea sceptra* (Lindl.) W.E.Higgins. **J**. *Prosthechea sima* (Dressler) W.E.Higgins. **K**. *Psilochilus macrophyllus* (Lindl.) Ames. **L**. *Rodriguezia granadensis* (Lindl.) Rchb.f. Photos by J. C. Ordóñez-Blanco.

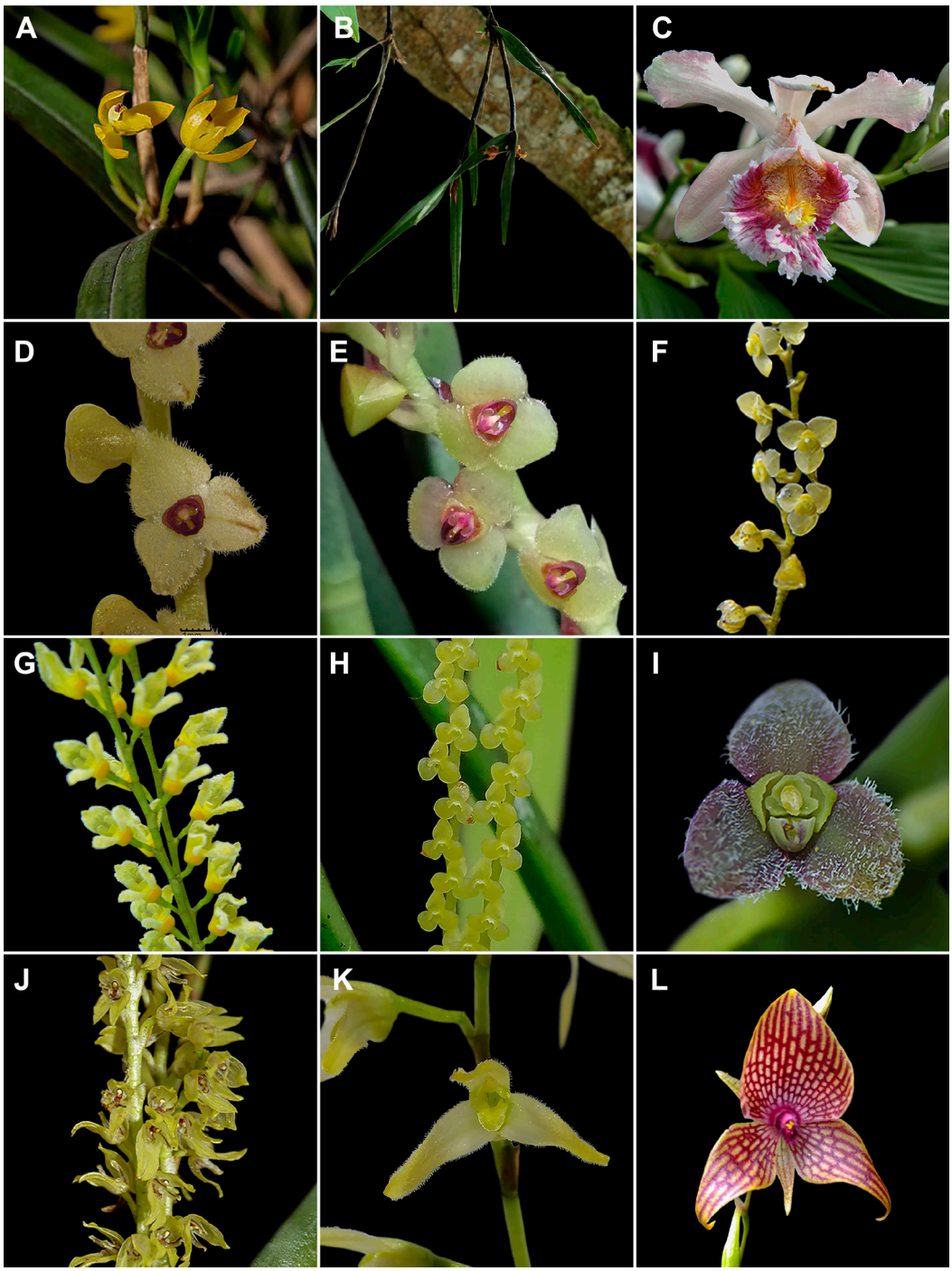

FIGURE 15. **A**. *Scaphyglottis bicornis* (Lindl.) Garay. **B**. *Scaphyglottis* sp. **C**. *Sobralia mutisii* P.Ortiz. **D**. *Stelis alba* Kunth. **E**. *Stelis angustifolia* Kunth. **F**. *Stelis ascendens* Lindl. **G**. *Stelis gelida* (Lindl.) Pridgeon & M.W.Chase. **H**. *Stelis hylophila* Rchb.f. **I**. *Stelis oblonga* (Ruiz & Pav.) Willd. **J**. *Stelis pulchella* Kunth. **K**. *Stelis spathilabia* (Schltr.) Karremans. **L**. *Telipogon albertii* Rchb.f. Photos by J. C. Ordóñez-Blanco (A, C–L) and C. Arias (B).

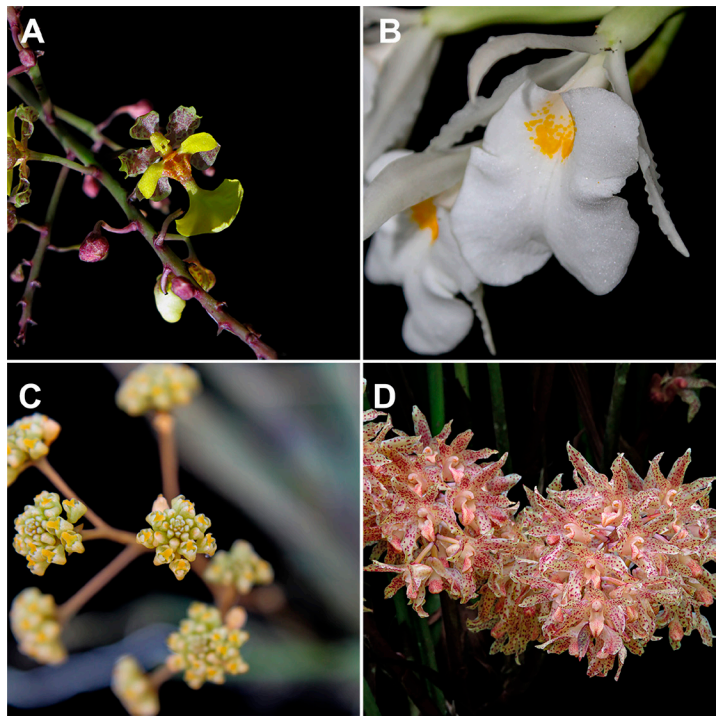

Figure 16. **A**. *Trichocentrum cebolleta* (Jacq.) M.W.Chase & N.H.Williams. **B**. *Trichopilia fragrans* (Lindl.) Rchb.f. **C**. *Trizeuxis falcata* Lindl. **D**. *Xylobium leontoglossum* (Rchb.f.) Benth. ex Rolfe. Photos: **A-D** by J. C. Ordóñez-Blanco.

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Supporting Information: Additional material related to this article is available in the online Supporting Information section.

Annex 1. List of orchids from the municipality of San Antonio del Tequendama (Cundinamarca, Colombia). Distribution (AFRICA: África; ARG: Argentina; BHS: Bahamas; BLZ: Belice; BOL: Bolivia; BRA: Brasil; COL: Colombia; CRI: Costa Rica; CUB: Cuba; CUR: Curazao; CYM: Islas Caimán; DOM: República Dominicana; ECU: Ecuador; GTM: Guatemala; GUY: Guyana; GUF: Guayana Francesa; HND: Honduras; HTI: Haití; JAM: Jamaica; MEX: México; NIC: Nicaragua; PAN: Panamá; PER: Perú; PRY: Paraguay; PRI: Puerto Rico; SLV: El Salvador; SUR: Surinam; TTO: Trinidad y Tobago; URY: Uruguay; US-FL: Florida; VEN: Venezuela), the life form (Epiphyte E, Lithophyte L, Geophyte G), the phenology (month of flowering and fruiting) and we present in the column "Other research" the specimens recorded in other research in the herbaria ANDES, BOG, COL, FAUC, UDBC, HPUJ, HUQ and JBB. In addition, species that were collected and are stored in the living collection of the Botanical Garden of Bogotá are highlighted with an ×. Species not sighted in this research are highlighted in bold.

Annex 2. Conservation status of the orchids of San Antonio del Tequendama (Cundinamarca, Colombia). Conservation status according to: Resolución 1912 de 2017 (Colombia. Ministerio de Ambiente y Desarrollo Sostenible. (2017), IUCN Red List (IUCN, 2025) and Libro Rojo de plantas de Colombia (Cálderon-Sáenz, 2007). Species not sighted in this research are highlighted in bold and with an asterisk.

 doi: http://dx.doi.org/10.15517/xw71n393

OVERLOOKED FOR ALMOST 200 YEARS: CLARIFICATION OF *VANILLA GUIANENSIS* (ORCHIDACEAE), A PRIOR NAME FOR *V. HOSTMANNII*, AND THE REINSTATEMENT OF *V. ACUTA*

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Abstract. The binomial *Vanilla guianensis*, published by Splitgerber in 1841, has long been debated as potentially based on a mixed collection of two taxa belonging to different *Vanilla* subgenera. Most of the literature has placed the name under *Vanilla* subg. *Membranacea*, while it has also been less frequently associated with *Vanilla* subg. *Vanilla*. In this study, we reassessed its typification and demonstrate that the original material and protologue are congruent and not based on a mixed collection of different species. We hypothesize *Vanilla guianensis* is a member of *Vanilla* subg. *Vanilla*, and that the name is conspecific with *V. hostmannii.* We further clarify the nomenclatural and typification status of *V. surinamensis*, a name published by Reichenbach on the basis of Splitgerber material, and identify Pulle as the first author to have effectively neotypified Splitgerber's *V. guianensis*. We reach this conclusion by conducting a comprehensive analysis of Splitgerber's *Vanilla* collections, supported by an examination of his herbarium catalogue and fieldbook, together with an extensive historical review of the post-Splitgerber literature. Finally, we discuss the implications of our proposal for the circumscription of these and other implicated taxa, including *Vanilla acuta.*

Resumen. El binomio *Vanilla guianensis*, publicado por Splitgerber en 1841, ha sido durante mucho tiempo objeto de debate por considerarse potencialmente basado en una colección mixta de dos taxones pertenecientes a diferentes subgéneros de *Vanilla*. La mayor parte de la literatura ha tratado este nombre dentro de *Vanilla* subg. *Membranacea*; con menor frecuencia, también se ha asociado con *Vanilla* subg. *Vanilla*. En este estudio, reevaluamos su tipificación y demostramos que el material original y el protólogo son congruentes y no están basados en una colección mixta de especies distintas. Proponemos que *Vanilla guianensis* es miembro de *Vanilla* subg. *Vanilla* y que el nombre es conespecífico con *V. hostmannii*. Asimismo, aclaramos el estatus nomenclatural y de tipificación de *V. surinamensis*, un nombre publicado por Reichenbach a partir de material de Splitgerber, e identificamos a Pulle como el primer autor que neotipificó de manera efectiva *V. guianensis* de Splitgerber. Llegamos a esta conclusión mediante un análisis exhaustivo de las colecciones de *Vanilla* de Splitgerber, respaldado por el estudio de su catálogo de herbario y cuaderno de campo, junto con una revisión histórica amplia de la literatura posterior a Splitgerber. Finalmente, discutimos las implicaciones de nuestra propuesta para la circunscripción de estos y otros taxones implicados, incluido *Vanilla acuta*.

Keywords / Palabras clave: nomenclatura, nomenclature, Orchidaceae, Reichenbach, Splitgerber, Surinam, Suriname, Vanilloideae

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Received 1 September 2025; accepted for publication 15 December 2025. First published online: 19 December 2025.

Introduction. The pantropical genus *Vanilla* Mill. (Orchidaceae: Vanilloideae) is among the most economically important plant crops worldwide. This importance is primarily due to the cultivation of a single species, *Vanilla planifolia* Andrews, native to Central America. The fruits of *V. planifolia* produce vanillin and other aromatic compounds highly valued across multiple industries, contributing to a global market estimated at USD 1.26 billion (FAO, 2022). Although *V. planifolia* is the main species cultivated for commercial use, the genus comprises *ca*. 128 species distributed throughout tropical regions, with particularly high species richness in the Neotropics (Cameron, 2011; Karremans *et al.*, 2020). From an evolutionary perspective, *Vanilla* is also notable for its suite of traits that are unique among orchids. These include flexible, often long climbing stems; flowers that typically form a floral tube through partial fusion of the labellum and column; and fleshy, often fragrant fruits containing sclerified large seeds (Cameron, 2003; Dressler, 1993; Soto Arenas & Cribb, 2010a).

Based on recent morphological and molecular analyses (Karremans *et al.*, 2025), the genus *Vanilla* has been re-circumscribed into four distinct groups: *Vanilla* subg. *Vanilla*, *Vanilla* subg. *Gondwana* Karremans, Damián & Pupulin, *Vanilla* subg. *Membranacea* Karremans, Pupulin & Damián, and *Vanilla* subg. *Tethyos* Karremans, Damián & Pupulin. Among these, the subgenera *Vanilla* and *Membranacea* encompass the largest number of Neotropical species. The former includes all known fragrant taxa, while the latter is regarded as one of the least studied and most taxonomically overlooked lineages within the genus. This lack of attention is partly attributed to its non-fragrant species and is reflected in the limited literature regarding its biology, ecology, and especially taxonomy (Damián-Parizaca *et al.*, 2022; Soto Arenas & Cribb 2010a). Notably, several species names remain unused or unrecognized in taxonomic treatments, and the group is underrepresented in broader discussions about the genus (Damián-Parizaca *et al.*, 2025b). Members of *Vanilla* subg. *Membranacea*, also referred to as the membranaceous clade, can be distinguished from their Neotropical counterparts by having membranous leaves (*vs*. typically thick and fleshy), greenish-white flowers (*vs*. yellow to orange), the absence of a penicillate callus (*vs*. present), and a column attached to the labellum only at its base (*vs*. deeply connate, forming a distinct floral tube) (Damián-Parizaca *et al.*, in press).

One of the earliest names historically associated with the membranaceous vanillas is *Vanilla guianensis* Splitg., described in 1841 by the Dutch botanist Frederik Louis Splitgerber, based on specimens he collected in Suriname. The name has long been suspected to be based on a mixed collection comprising fragrant fruits and membranaceous-like flowers (Rolfe, 1896). This confusion has persisted to the present, mainly due to the absence of an illustration or reference to a specific specimen in the original protologue. However, during the preparation of the monograph on membranaceous *Vanilla* species (Damián-Parizaca *et al.*, in press), we found no support for the mixed-origin hypothesis of *V. guianensis*. Following a detailed examination of Splitgerber's original material, along with a critical review of his field notes, we argue that he did not intend to describe a membranaceous species but rather a fragrant one. Accordingly, we clarify the taxonomic identity of *V. guianensis* within the fragrant clade (*Vanilla* subg. *Vanilla*) and examine the implications of this re-circumscription for names previously treated as its synonyms, such as *Vanilla acuta*. In addition, we provide live photographs of *V. guianensis* and a diagnostic key to distinguish it from other morphologically similar species.

Materials and methods. Our analyses and conclusions are primarily based on two main sources: herbarium specimen revision and historical literature. For the former, we conducted a comprehensive examination of specimens through direct visual inspection at the following herbaria: AMO, AMES, B, BM, BR, CR, F, FLAS, K, K-L, MA, MEXU, MO, MOL, NY, SEL, UFV, US, USM, W, W-R, and WIS, supplemented by high-resolution images from: BAB, BIGU, FCQ, FTG, G, GH, HA, HB, HJBG, HOXA, JBB, L, LBMBP, LAGU, LL, LY, P, PRG, QCNA, QCNE, R, SCP, SP, SPFR, and UVAL. Particular attention was given to L herbarium, where most of Splitgerber's collections are housed, either directly or accessible through its online database (BioPortal, 2025).

For the literature review, we compiled all relevant documents that mention *Vanilla guianensis* or Frederik Louis Splitgerber, including protologues of all species discussed herein, primarily obtained from BHL (2025).

Figure 1. Illustrations attributed to *Vanilla guianensis* Splitg. in de Vriese (1856a). **A**. Plate V, plant and inflorescence. **B**. Dissection flower and fruits.

Additionally, we studied Splitgerber's field notebooks held at the Leiden University Library, which were digitized for this study upon request. A previous study briefly examined these fieldbooks, bringing their existence to our attention (de Vriese, 2022).

To further clarify the circumscription of *V. guianensis*, we designated an epitype following the International Code of Nomenclature (ICN) guidelines (Turland *et al.*, 2025). We also provide a list of revised species consistent with our species concept of *V. guianensis* for reference. Moreover, we include detailed photographic documentation of the species through a Lankester Composite Dissection Plate (LCDP), and an artificial key for the *V. guianensis* group (*sensu* Soto Arenas & Cribb, 2010b).

Results and Discussion

Search for *Vanilla guianensis* original material. Splitgerber (1841) did not specify the nomenclatural type of *Vanilla guianensis* at the time of publication. Although his protologue was extensive, he did not include any direct or indirect references to an illustration or specimens that could constitute original material. Our current concept of *V. guianensis* is largely based on illustrations by de Vriese's (1856 a, b), which were published a few years after Splitgerber passed away. Subsequent authors have accepted the illustrations as depicting original material under the assumption that they represented the common *Vanilla* found in Suriname (Reichenbach, 1859; Soto Arenas & Cribb, 2010b). Unfortunately, we could find no direct link between de Vriese's illustrations and Splitgerber's original material of *V. guianensis*. To make matters worse, de Vriese's plates represent a mixed collection, depicting flowers of a species belonging to *Vanilla* subg. *Membranacea* alongside thicker, triangular fruits typical of *Vanilla* subg. *Vanilla* (Fig. 1). Rolfe (1896) suggested the possibility of a mixed collections of *V. guianensis*, but instead of referring to de Vriese's illustrations, he claimed that it was Splitgerber himself who had combined the two taxa in his original work. Soto Arenas & Cribb (2010b) agreed with Rolfe's interpretation of two discordant elements, both in the original description by Splitgerber and the illustrations by de Vriese.

FIGURE 2. Splitgerber *Vanilla* collections. **A**. *Splitgerber 409* (W0195938). **B–C**. *Splitgerber 515* (L0778311, W19365). **D**. *Splitgerber sn* [possibly *Splitgerber 529*] (L0778313). **E**. *Splitgerber 925* (W19367). **F**. *Hostmann 1174* (W19375). Photographs by Alexander Damian-Parizaca (C, E, F) and by permission of the Natural History Museum Vienna (A), and The Naturalis Biodiversity Center, Leiden (B, D).

Upon analyzing Splitgerber's field notes, we found that he collected four *Vanilla* specimens during his time in Suriname, collection numbers *Splitgerber 409*, *515*, *529*, and *925* (Fig. 2–3). Additionally, we traced a fifth collection made by Jacob, one of Hostmann's collectors. A specimen was eventually sent to Splitgerber, who assigned it to his number *1174* (Fig. 2F). We were able to locate and unambiguously assign five specimens to these collection numbers: *Splitgerber 409* represented by

Figure 3. Splitgerber fieldbook *Vanilla* collections. **A–B**. *Vanilla guianensis*. **C**. *Vanilla aromatica*. **D**. *Vanilla palmarum*. **E**. Hostmann collection given to Splitgerber. **F–G**. Splitgerber Catalogus Plantarum in Herbario dated 1836 and 1842 respectively. Images by permission of the Collections Archives and Images of The Naturalis Biodiversity Center, Leiden.

the voucher W-R-0195938; *Splitgerber 515*, duplicates represented by L0778311 and W-R-019365; *Splitgerber 925* represented by W-R-019367; and *Splitgerber 1174* represented by W-R-019375) (Fig. 2). It is important to mention that the labels of Splitgerber's specimens in Reichenbach's herbarium in Vienna (W-R) are handwritten by the latter rather than the former, except W-R-019375, which besides Reichenbach's label also carries a slip with the number *Splitgerber 1174* in Splitgerber's handwriting. It is unclear how Reichenbach obtained the information to add to each label, which otherwise lacks any numbering. It is possible that all Splitgerber's specimens in Vienna originally carried the same slips with numbers as *1174*, having been either lost or removed at some point. Furthermore, it is possible

that the specimens came accompanied by correspondence from Leiden that included the information that Reichenbach afterwards transcribed to the labels. Either way, although the labels are in Reichenbach's hand rather than Splitgerber's, the label data were likely supplied to Reichenbach directly because it is not found in either the protologue or the fieldnotes.

The protologue of *V. guianensis* offers little information about which of these specimens he used as original material, but there are clues. Of his *Vanilla* collections, only numbers *409* and *529* are consistent with the protologue. Splitgerber links them directly to his fieldnotes, comparing number *409* to *529* ("*confer N 529*") (Fig. 3A). It is indeed the specimens with the number *Splitgerber 409* (W-R-0195938) and the unnumbered *H.L.B 905,88-84* (L0778313) that best fit the protologue in having broadly elliptic, acuminate leaves, long compact inflorescences, and thick, slightly arcuate fruits (Fig. 2A, D). The protologue indicates that the species blooms in May and June (Majo et Junio in Latin) and this is exactly what can be read on the label under *Splitgerber 409* (W-R-0195938), albeit in Dutch (Mei, Juni). Also noted in the protologue is that the species is "non raro" (common) and "flores albidi" (white flowers), both of which are among the few notes he lists in his fieldbook under number *529*, in Dutch ("zeer gemeen", very common, and, "bloemen witachtig", whitish flowers). This suggests that Splitgerber's concept of *V. guianensis* is in fact based on both his collections, *409* and *529*. One might be tempted to infer, given its morphological similarity with the specimen W-R-0195938 in Vienna, that the specimen *H.L.B 905,88-84* (L0778313) (Fig. 2D) represents Splitgerber's collection number *529*. The latter is the only one of his field numbers referred to a *Vanilla* for which we could not find any voucher specimen, and L0778313 is the only one of his specimens that bears flowers, something Splitgerber described in the protologue. Unfortunately, this specimen lacks the original label, as is the case with several Splitgerber sheets (de Vriese, 2022). Therefore, there is no indication that can directly associate it to the name *V. guianensis* or *Splitgerber 529*, and thus we cannot unambiguously list it as original material.

Although we could not confirm unambiguously that L0778313 corresponds to *Splitgerber 529*, the sheet nonetheless provides several insights pertinent to future investigations of Splitgerber's material. Evidence from Splitgerber catalogues dated 1836 and 1842 (Fig. 3F–G), preserved as part of Herb. Splitgerber when his collection was acquired by the Rijksherbarium (now Naturalis Biodiversity Center, Leiden) (van Steenis-Kruseman, 1979), indicates that three *Vanilla* specimens attributed to Splitgerber were present in the Rijksherbarium when the Splitgerber herbarium was finally incorporated in 1871. Furthermore, *V. palmarum* (*Splitgerber 925*) appears to have been lost from L, a conclusion supported both by its listing in the Splitgerber catalogue and by Pulle (1906), who cited a complete specimen at L that is no longer extant; the only surviving duplicate is now housed at W (Fig. 2E). The unlabeled Splitgerber specimen at L (L0778313) was examined by Cogniaux, as indicated by his handwriting on the sheet (Probst, 2025), yet it was inexplicably omitted from his 1893 treatment, presumably because the absence of an original label rendered it unsuitable for formal inclusion. Additionally, Splitgerber's *Vanilla* material appears to have remained unmounted until the early 20th century. For instance, the catalogue label of L0778313 indicates that the specimen was mounted on 29 March 1905 (the 88th day of 1905), recorded as "Herb. Lugd. Bat. N 905, 88–84," (see Thijsse *et al.*, 2023, for more examples). This timing coincides with the appointment of J.M. Janse as director of the Rijksherbarium and the imminent relocation of the institution to a new building. Before this administrative reorganization, several collections were prone to loss or mixing because numerous specimens remained stored together and unmounted, a situation that changed markedly with the shift toward a more systematic system using designated boxes. This historical context most likely also explains the loss of some Splitgerber material.

***Vanilla guianensis* typification**. To establish an appropriate typification for *V. guianensis*, it is first necessary to consider any inadvertent lectotypifications that may have occurred prior to 2001. The earliest relevant reference to material attributable to Splitgerber's *V. guianensis* is found in Reichenbach (1859), who examined specimens sent from Leiden and listed three *Vanilla* species, including one he called "*Vanilla surinamensis* Splitg.", a name that has not otherwise been used. Although Reichenbach cited the protologue of *V. guianensis* as "Ann. Sci. Nat. Bot. 15, 1841" with an incorrect page number, he explicitly associated *Split-*

gerber 409 with that publication, and the corresponding sheet in his herbarium bears a label in his own handwriting reading "*V. guianensis* Splitg." with a correct citation to the protologue. Although Reichenbach most probably did not intend to describe a new taxon, the name *V. surinamensis* fulfills all criteria for valid publication under Arts. 32–45 of the Code (Turland, 2025). The differences between *V. surinamensis* and *V. guianensis* extend beyond the scope of typographical or orthographical error as defined by Arts. 60–61, and the name must therefore be treated as validly published. Moreover, it is also legitimate, as none of the provisions for illegitimacy or superfluity under Art. 52 apply. Although one might consider invoking Art. 52.3 because Reichenbach cited Splitgerber's protologue, this interpretation is untenable because he did not reproduce any sequential element or exact wording traceable to Splitgerber's original diagnosis; in fact, his diagnostic statement differs from that of Splitgerber (1841). Consequently, *V. surinamensis* must be regarded as a valid and legitimate name, typified by Reichenbach on the basis of *Splitgerber 409*.

The question therefore remains whether an inadvertent lectotype or neotype for *V. guianensis* might exist. Three later authors cited *V. guianensis*, each introducing potential, but ambiguous, elements relevant to its typification. Cogniaux (1893), in his treatment of Brazilian *Vanilla*, included *Vanilla guianensis* and listed four specimens, including *Splitgerber 409*, yet did not explicitly designate any as a type or type-equivalent. Rolfe (1896) interpreted *V. guianensis* only in part, subsuming it within his broader concept of *V. inodora*, but incorrectly reassigned *Splitgerber 409* to *V. palmarum*, along with three additional, unrelated specimens. Subsequently, Pulle (1906), in his *Enumeration of the Flora of Suriname*, followed Rolfe's taxonomic framework and, under his interpretation of *V. inodora*, listed both *V. surinamensis* (citing *Splitgerber 409*) and *V. guianensis*. For the latter, he cited an unlabeled specimen (L0778313), which he attributed to Splitgerber as "H.L.B. 905, 88–84 named *V. guyanensis* Splitg." We therefore considered whether Pulle's citation of specimen L0778313 might constitute an inadvertent neotype, given that, although atypical for the period, he explicitly provided a specimen, a herbarium, and a name that can be unambiguously correlated.

Our analysis of Pulle's treatment indicates that his use of the term "named" consistently represents his transcription of labels present on herbarium sheets, typically accompanied by complete bibliographic citations, a pattern confirmed by numerous examples throughout his *Enumeration*. He likewise employed "named" when referring to taxa cited within earlier publications, adding "in" in such cases, as illustrated by his treatment of *Adiantum tetraphyllum*, where he listed a specimen as "named: *A. pachysorum* Reichb. in Weigelt, *Plant. Surin.*" Accordingly, his citations should be interpreted as references to material available to him under a given name, rather than as definitive indications of original material or deliberate type selection. Although the current Code explicitly defines the requirements for effective typification and discourages mechanical procedures, criteria that would apply to Pulle's enumerations, such constraints did not exist at the time; indeed, prior to 1990 the Code clarified that citation of herbaria, collections, or institutions was not required for valid typification (Art. 9.22). Consequently, Pulle's (1906) citation, although clearly inadvertent, constitutes a valid typification, and since no original material attributable to *V. guianensis* is extant, *Splitgerber 409* being precluded as it serves as the type of *V. surinamensis*, we regard Pulle's listing as an inadvertent neotype designation.

Correct application of the name *V. guianensis*. The vegetative and floral characteristics inferred from the protologue and type of *Vanilla guianensis* differ markedly from those of the taxon to which this concept has often been applied, which is a member of *Vanilla* subg. *Membranacea* (e.g. Damián-Parizaca, 2019; Householder *et al.*, 2010; Karremans *et al.*, 2020; POWO, 2024; Sambin & Aucourd, 2024; Sambin & Ravet, 2021; Soto Arenas & Cribb, 2010b; Szlachetko & Kolanowska, 2020; Szlachetko *et al.*, 2012, 2017). The protologue provides a detailed diagnosis, defining *V. guianensis* by its fleshy, non-membranous, elliptic-oblong, acuminate leaves, 15–20 × 5–6 cm, internodes 5–10 cm long, axillary inflorescences bearing 5–15 flowers with acute, ovate bracts, 15–20 cm long, a triquetrous and sub-falcate fruit, whitish to yellowish flowers, lanceolate, sub-acuminate sepals with a revolute apex and slightly undulate margins measuring *ca.* 6.0 × 0.6 cm, similar petals, and a labellum that is shorter than both. The base of the labellum is laterally connate to the column, forming a funnel, with a

broadly ovate lamina, an apex that is nearly acute to sub-acuminate, curled edges, and a disc bearing three thick, elevated, longitudinal lamellae that merge at the apex.

These features place *V. guianensis* among a small group of species within *Vanilla* subg. *Vanilla* that includes *V. hostmannii* Rolfe, *V. cribbiana* Soto Arenas, *V. dressleri* Soto Arenas, *V. corinnae* Sambin & Chiron, *V. sekut* Damián, H.Garzón & A.Bentley, *V. rivasii* Molineros, R.T.González, Flanagan, & J.T.Otero and *V. weberbaueriana* Kraenzl., collectively referred to as the *V. hostmannii* group by Soto Arenas & Cribb (2010b) and here renamed the *V. guianensis* group. The group is characterized by a distinctive combination of traits, including distichous floral bracts with a strongly papillose rachis; orange to yellowish flowers with sepals conspicuously papillose on the adaxial surface; and a short, penicillate callus, usually positioned in the lower third of the labellum, with several papillose, congested, and thickened veins toward the apex. Among these taxa, *V. guianensis* is most closely allied to *V. hostmannii*, which was also described from Suriname (Fig. 4 C–E). The two species share densely arranged inflorescences, whitish sepals and petals, and a broadly ovate labellum with an obtuse to sub-acuminate apex, bearing 3 longitudinal warty keels. This contrasts with the flabellate labellum with a broadly rounded apex found in *V. corinnae*, the subrhombic labellum of *V. dressleri*, the presence of more than five papillose keels and lax inflorescences in both *V. cribbiana* and *V. dressleri*, the conspicuously trilobed labellum with triangular midlobe of *V. sekut*, and the rounded lateral labellum lobes and oblong to rounded midlobe found in *V. weberbaueriana* (for a more detailed comparisons of these species refer to Damián-Parizaca *et al.*, 2025a). The similarity between *Vanilla guianensis* and *V. hostmannii* was noted by Hoehne (1945), who in his treatment of Brazil placed the latter under the synonym of *V. guianensis* with a question mark (?) and later stated that he was "...unaware if Rolfe compared *V. hostmannii* with *V. guianensis*, which, based on its details and description, is very similar to the scarce data provided in his description". Our current hypothesis, grounded in a critical examination of the protologues and the corresponding type materials, is that indeed these two taxa refer to the same species, as such, the name *V. guianensis* has priority, and *V. hostmannii* must be placed in synonymy.

By contrast, the membranous-leaved taxon to which the name *V. guianensis* has frequently been misapplied (auct. non Splitg.) remains in need of the correct name. This taxon is characterized by distinctly membranaceous leaves, small floral bracts, and flowers with lanceolate, acute sepals and petals, featuring a hexagonal labellum when extended, with three longitudinal warty keels that converge at the apex. Two names described from Guianan material match that circumscription: *V. acuta* Rolfe, described from Suriname (Rolfe, 1896), and *V. latisegmenta* Ames & C.Schweinf. from Guyana (Ames & Schweinfurth, 1925), the first having priority over the second.

Nomenclatural Summary

Vanilla guianensis Splitg. (1841: 279). NEOTYPE, designated by Pulle (1906): Suriname, without collector data, H.L.B 905, 88-84 (L 0778313!). EPITYPE: Guyana, 1898, *E. F. im Thurn 65* (epitype, designated here: K001551124!).

Vanilla surinamensis Rchb.f. (1859: 321). Type: Suriname, 1838, *Splitgerber 409* (W-R-0195938!).

Vanilla hostmannii Rolfe, J. Linn. Soc. Bot. 32: 462. 1896. TYPE: Suriname, *Hostmann 306* (lectotype, designated by Damián-Parizaca *et al.* 2025a: K000463756!, isolectotype: K-LINDL!)

Vanilla porteresiana Veyret & Szlach. Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér. 4, 16(2–4): 219 (1995). TYPE: French Guiana, crique Mulet mort, Sud de Saul, zone basse, 25 February 1966, *Oldeman 2087* (holotype: P04026364!, isotype: CAY-179135)

Vanilla barrereana Veyret & Szlach. Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér. 4, 16(2–4): 220 (1995). TYPE: French Guiana, Haut Tampoc, Saut Pierourou, sur les berges et dans les petiles iles au miliey du saut, 27 March 1977, *Cremers 4523* (holotype P04026365, isotype: CAY-not seen!)

Distribution: Native to the Guiana Shield in the lowland forests of Brazil, French Guiana, Guyana, Suriname, and Venezuela.

Illustrations: as "*Vanilla cribbiana*" in Koch *et al.* (2013); as "*Vanilla gardneri*" in Silva & Silva (2010); and as "*Vanilla pompona*" in Romero (1998).

Figure 4. *Vanilla guianensis* Splitg. **A**. Epitype designated based on *E.F. im Thurn 65* (K-001551124!). **B**. Lankester Composite Dissection Plate (LCDP) based on *R. Menchaca MEX07* (CITRO!). **C**. Lectotype of *Vanilla hostmannii* Rolfe based on *Hostmann 306* (K000463756!). **D**. Flower close-up of the *V. hostmannii* lectotype. **E**. Flower close-up of the *V. hostmannii* isolectotype (K-LINDL!). **F–G**. Flower close-up of *V. guianensis* epytype. Photographs by Alexander Damián-Parizaca (A, C–G) and Miguel Lozano Rodríguez (B).

Notes: Pulle (1906) neotype coincides well with *V. guianensis* protologue and with the species of *Vanilla* subg. *Vanilla*, because of the leathery (not membranous), elliptic-oblong and subacuminate leaf, the short axillary inflorescence on which some ovate and acute floral bracts are preserved, and the triquetrous, slightly falcate fruit. Although, Pulle material have some flowering material, this is extremely fragmentary. In consideration of the predominantly incorrect interpretation of this species, we therefore believe it appropriate to select a fertile epitype. The selected specimen (*E.F. im Thurn 65*), collected from the Guianas, bears numerous flowers and matches the protologue and neotype in several key traits, including its broadly elliptic, acuminate leaves; flowers with sepals 6–9 cm long; an ovate labellum with a obtuse to subacuminate apex; a disc with three thick, elevated lamellae that converge at the apex; and a subfalcate fruit.

In a previous account (Damián-Parizaca & Mitidieri-Rivera, 2023), we reported some of the specimens cited below as *V. dressleri*, based on a limited comparative assessment. This is corrected herein, as *V. dressleri* is currently known only from Costa Rica, across Panama, and southward through the Chocó biogeographic region along the Pacific coasts of Colombia and Ecuador, with an outlier population in the foothills of the Colombian Central Cordillera (Flanagan *et al.*, 2022).

Additional specimens examined: BRAZIL. Para: Vitoria do Xingu, 10 Nov. 2012, *J. Batista 370* (MBM-405595!); Belem, Utinga, 3 Nov. 1938, *Markgrat 3822* (RB00260219!); Along edge of Rio Cauaburi (in high forest on terra firma) between Rio Maturaca and Rio Ya, Febr. 3, 1966, *N. Silva & U. Brazao 60960* (NY02695607!, US00319636!), Roraima, Canto Galo, Rio mucajai, between Pratinha and Rio Apiau, 22 Jan. 1967, *G.T. Prance et al. 3988* (NY01414978!); Para, Rio Itapacura, BR 163, 24 Nov. 1977, *G.T. Prance et al. 25739* (US00319614, K000940256!, MG60339!); Para, *Wullschlägel 1132* (BR-000032880410!, W-R 35505!, W-R 35454!). FRENCH GUIANA. Layon Eaux Claires: Region de Saul, 250 m, 16 Febr. 1993, *G. Cremers 13016* (NY04170422!, CAY-214558); Counami, Foret primaire degrade, 22 March 200, *Prevost M.-F & Barthelemy D. 3820* (NY04170453!); without exact locality, Jan. 2025, *R. Menchaca MEX07* (CITRO!). GUYANA, Mabuina ck, Yarikita Ck, Amacur River, Northwest District, at sea level, 27 July 1908, *C. Wilgress Anderson 71* (K-001551125!); Northwest District, Wini River, Marabo Shortcut, 3 Febr. 1922, *J.S. de la Cruz 1298* (NY01414948!); Edu Creek, 1898, *E.F. im Thurn 222* (K001551013). SURINAME. Border of Marowijne above base camp. 14 Febr. 1949, L. *Lanjouw et J.C. Lindeman 2074* (K-000395974!). VENEZUELA. Amazonas: Rio Orinoco, Along river just below mouth of Rio Cunucunuma, 20 June 1959, *J.J. Wurdack & L.S. Adderley 43074* (US00319616!, NY01075148!); Monagas. Reserva Forestal de Guarapiche. (Caño Colorado), July 1969, *L. Ariesteguieta et al. 7233* (NY04170463!, US00319635!).

Additional records: FRENCH GUIANA. Cayene: Macouria, 10 Feb. 2022 (Delamarche, 2022); Roura: 4 Feb. 2020 (Cocchi, 2020); Saint-Laurent-du-Maroni: State Saül, 8 March 2019 (Dewynter, 2019). GUYANA, without exact locality, 3 Feb. 2020 (Le Roux, 2020).

Vanilla acuta Rolfe (1896: 453). TYPE: Suriname, Marowyne: Aug 1846, *Kappler 1843* (Lectotype designated by Damián-Parizaca *et al.* 2025b: K-LINDL! without barcode; isolectotypes: W barcode W62925!, P barcode P00612143!). EPITYPE: Guyana (British Guiana), River Berbice, illustration by Robert H. Schomburgk plate 216-267 (epitype designated by Damián-Parizaca *et al.* 2025b: BM, photograph of the epitype at NY, tracings K barcodes K001551121!, W barcodes W19383!, W19384!).

Vanilla latisegmenta Ames & C.Schweinf. (1925: 2). Type. Guyana (British Guiana), Upper Rupununi River, near Dadanawa, 29 May 1922, *J. S. de la Cruz 1404* (holotype: AMES barcode 00090756!; isotypes: GH barcode GH00090757!, US barcode US00093324!, MO barcode MO2602185!).

Notes: *Vanilla acuta* is recognized here as the correct name for the membranaceous taxon to which *Vanilla guianensis* was previously misapplied. *Vanilla acuta* was recently lectotypified by Damián-Parizaca *et al.* (2025b), and a complete description, including ecological and additional taxonomic details, will be provided in the forthcoming monograph on *Vanilla* subg. *Membranacea* (Damián-Parizaca *et al.*, in press.).

Artificial Key to the *Vanilla guianensis* group

1. Inflorescences dense, with up to 60 flowers; typically, 4–10 open simultaneously; sepals adaxially densely verrucose ..... *V. weberbaueriana*
1′. Inflorescences lax, with up to 15 flowers; usually 1–3 open at a time; sepals adaxially sparsely verrucose 2
2. Sepals and petals with acute to subacute apices 3
3. Lateral sepals partially fused at the base, about one-third of their total length; labellum with flabellate margins, midlobe conspicuously bilobed; callus with 7–8 thickened keels ..... *V. rivasii*
3′. Lateral sepals free; labellum with entire margins, midlobe variously triangular; callus with 3–5 main thickened keels 4
4. Floral tube opening ovoid, wider than long; sepals up to 9 cm long; labellum with sinuate apical margins, midlobe broadly triangular; endemic to the Guiana Shield ..... *V. guianensis*
4′. Floral tube opening circular; sepals up to 8 cm long; labellum with flat, non-sinuate apical margins, midlobe shortly triangular; known from the Amazonia ..... *V. sekut*
2′. Sepals and petals with obtuse to rounded apices 5
5. Labellum subtrilobed to rhombic; endemic to the Guianas and Chocó 6
6. Sepals and petals white abaxially; petals rounded; labellum subtrilobed to entire, up to 56 mm long; callus with 5 thickened veins toward the apex ..... *V. corinnae*
6′. Sepals and petals yellowish abaxially; petals obtuse; labellum subrhombic, up to 69 mm long; callus with up to 11 thickened veins toward the apex ..... *V. dressleri*
5′. Labellum conspicuously trilobed; endemic to Central America ..... *V. cribbiana*

Acknowledgements. We extend our sincere gratitude to the directors and curators of all referenced herbaria for their invaluable support during our visits and for facilitating access to photographic materials. Special appreciation goes to Tinde van Andel (Naturalis Biodiversity Center) and the Collection Archives and Images Department for their assistance in scanning Splitgerber's fieldbook. We are particularly grateful to the Department of Botany at the University of Wisconsin–Madison for its financial support, especially to the Hugh H. Iltis Graduate Student Research Award Committee and Mr. Theodore S. Cochrane for their generous contributions. The first author also acknowledges funding from the American Society of Plant Taxonomists (ASPT), the Botanical Society of America (BSA), the Systematics Association, and the American Orchid Society (AOS). Special thanks to CONCYTEC-PROCIENCIA for supporting the first author's research through the program "E041-2023-01 Basic Research Projects" [contract number: PE501082530-2023]. Finally, we thank Miguel Lozano Rodriguez for sharing photographs of *Vanilla guianensis.*

Author contribution. ADP: Conceptualization; Investigation; Writing of the original draft. APK and FP: Data curation; Writing, Reviewing and Editing.

Funding. Department of Botany, University of Wisconsin-Madison; ASPT; BSA; AOS; CONCYTEC-PROCIENCIA.

Conflict of interest. No conflict of interest to declare.

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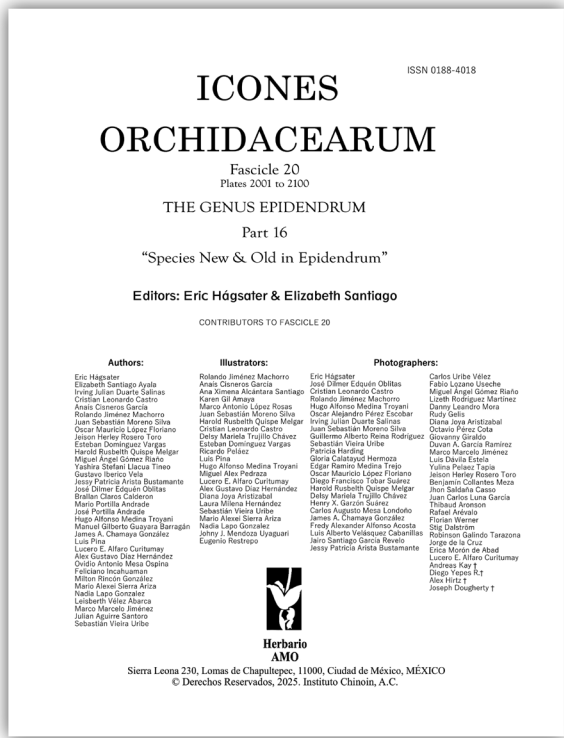

ISSN 0188-4018

ICONES
ORCHIDACEARUM

Fascicle 20
Plates 2001 to 2100

THE GENUS EPIDENDRUM

Part 16

"Species New & Old in Epidendrum"

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For over thirty years, *Icones Orchidacearum* has served as a key reference in the study of the species-rich genus *Epidendrum* L. It was established by Eric Hágsater and Gerardo A. Salazar to share the accumulated knowledge about this genus, particularly in Mexico. However, they recognized that a comprehensive understanding of the taxonomy of this mega genus was unattainable if limited to Mexican species alone. Since then, this publication has advanced the study of the genus for over three decades, featuring hundreds of species, describing new species to science, and clarifying obscure concepts and poorly known species across the Neotropical region.

Under the editorial leadership of Eric Hágsater and Elizabeth Santiago, *Icones Orchidacearum* continues its thorough examination of *Epidendrum* with Fascicle 20 (plates 2001–2100), titled *The Genus Epidendrum* Part 16, "New and Old Species in *Epidendrum*". As in the recent editions, this fascicle highlights an exceptional collaborative effort, involving 34 authors and co-authors, supported by 23 illustrators, 53 photographers, and 19 reviewers. The work represents a broad collaborative initiative across the Neotropics, especially in biodiversity-rich countries such as Colombia, Ecuador, and Peru. Recent volumes of *Icones Orchidacearum* feature texts evaluated by at least two external reviewers, particularly for newly described taxa, adding scholarly rigor to the series.

This issue honors the memory of geologist and renowned orchidologist Alexander C. Hirtz

(1951–2024), who made noteworthy contributions to the understanding and conservation of orchids in Ecuador. Since 1978, his scientific legacy has been documented through extensive herbarium collections and field photographs. Much of his collection formed the basis for describing at least 52 new species within *Epidendrum*, including a species he co-authored, *E. roseobicirrhatum* Hágsater & Hirtz. He passed away in Quito on July 2, 2024, but his influence endures through hundreds of specimens, photographs, and records that remain a valuable resource for the orchid community.

The core of the Fascicle 20 contains 100 texts, of which 87 are already described species. A notable new feature is the republication of 60 species that were previously illustrated only in black and white; these have now been updated with full-color digital images using the Lankester Composite Dissection Plate (LCDP) technique. These plates complement the classic inked illustrations and are particularly helpful when color distinctions are difficult to depict or when photographs can better show differences among species. This issue introduces 13 new species to science, distributed geographically as: one from Peru (*E. caducispathum* Hágsater, E.Santiago & J.Duarte), four from Ecuador (*E. callosum* Hágsater, M.Portilla & E.Santiago; *E. cubicum* Hágsater, H.Medina & J.Portilla; *E. kiat-tanii* Hágsater, E.Santiago, H.Medina & J.Portilla; and *E. parvialbertii* Hágsater & E.Santiago), seven from Colombia (*E. expansilobum* C. Castro & Hágsater; *E. juaicaense* Hágsater, L.Pina & J.Duarte; *E. julieannae* Hágsater & C.Castro; *E. noriadelapaz* Est.Domínguez, O.A.Mesa & Hágsater; *E. quilinsayacoense* Hágsater & E. Santiago; *E. rioalisalense* Hágsater & E.Santiago; and *E. sisavitaense* Hágsater & E.Santiago), and one shared between Colombia and Ecuador (*E. pseudopurum* Hágsater & Sierra-Ariza). Regarding nomenclature, the volume presents the lectotypification of *Epidendrum stramineum* Lindl., considered a synonym of *Epidendrum moritzii* Rchb.f., and the neotypification of *Epidendrum cebolleta* Schltr. (*nom. illeg.*), a synonym of *E. uribei* A. D. Hawkes.

Also, this edition stress the importance of regional herbaria as repositories and essential sources of information for understanding highly diverse genera such as *Epidendrum*. Many of the described species come from herbarium surveys, especially through multiple visits by expert taxonomists to COL, the National Herbarium of Colombia. The editors also stress the need for sustained study in underexplored areas such as northern Peru, particularly the Alto Mayo Protection Forest (BPAM) and the La Pampa del Burro Private Conservation Area, where the flora remains insufficiently documented despite its high diversity. They note that most specimens from these studies are kept at the KUELAP Herbarium (National University Toribio Rodríguez de Mendoza of Amazonas), with appropriate collection permits. The involvement of new generations of orchidologists and taxonomists, especially in Colombia and Peru, is particularly noteworthy. I am confident that, under the guidance of Eric and Elizabeth, these future researchers will continue to advance the understanding of the genus, as many areas of the Neotropics remain unexplored and numerous species await discovery.

The appendix includes corrections to previous volumes, covering amendments to information on 10 species names. Complete holotype data are provided for *E. althaniorum* Hágsater & Collantes, and it is clarified that an additional cited specimen does not exist. Elevation data have been corrected for *E. constricolumna* Hágsater, Chocce & E. Santiago and *E. croceoserpens* Hágsater & Salas Guerr. In *E. franckei* Hágsater, *E. lufinorum* Ocupa & Hágsater, *E. oenochrochilum* Hágsater, Ric.Fernández & E. Santiago, *E. porphyreodiscum* Hágsater, D. Trujillo & E. Santiago, and *E. tetartociclium* Collantes & Hágsater, collection and pressing dates have been updated. For *E. naviculare* Hágsater, M.E. Acuña & E. Santiago, one collection number has been corrected, and in *E. unifoliatum* Schltr., the collection date and elevation have been adjusted. These corrections are important for herbarium curators and researchers to ensure accurate data in their databases and studies, especially concerning taxonomy and species distribution, thereby also preventing nomenclatural issues.

One of the practical advantages of this volume is its availability in both print and free PDF formats via the AMO Herbarium website (the PDF can be downloaded at https://www.herbarioamo.org). Print copies are distributed to libraries and herbaria worldwide. While the PDF version broadens access to the information, the print edition remains the

primary and most reliable means for long-term data preservation. As a suggestion, a general index of the species names treated across the more than 20 published fascicles would be highly beneficial in the future.

Overall, *Icones Orchidacearum* is a critical reference for *Epidendrum* specialists, offering mostly high-quality illustrations along with detailed morphological descriptions, diagnoses, taxonomic and nomenclatural notes, conservation, etymology, phenology, and bibliographic references. As such, the series is also a valuable data resource for research in ecology, biogeography, morphometrics, herbarium and living collection management, and orchid conservation across the Neotropics.

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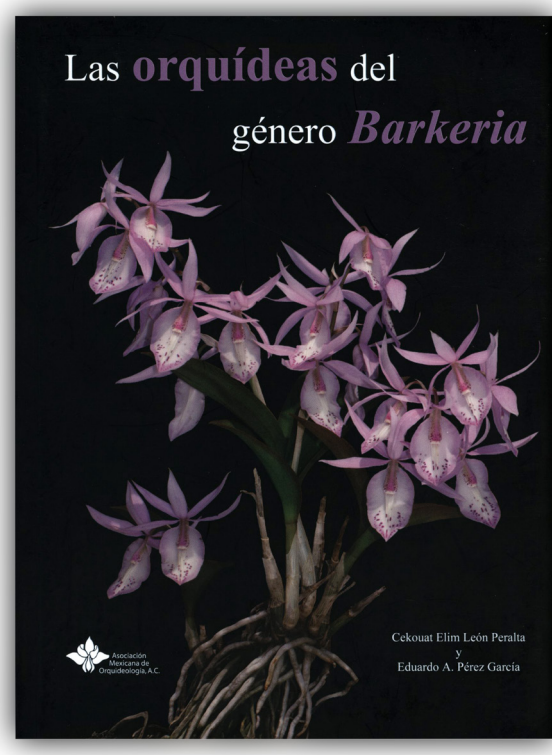
León Peralta, Cekouat, and Eduardo Alberto Pérez García. 2025. **Las orquídeas del género *Barkeria***. 1st ed. Mexico: Asociación Mexicana de Orquideología. 253 pp. 19.5 × 26 cm. Hardcover (tapa dura/cartoné). Price: MXN $500 (USD $28). ISBN 978-970-96997-0-8. Published October 31, 2025. (In Spanish).



Any serious attempt to understand *Barkeria* inevitably begins in Mexico. The genus was described in 1838 by George Beauchamp Knowles and Frederic Westcott in honor of George Barker, the English horticulturist and chemist who introduced *B. elegans* Knowles & Westc. (now regarded as a synonym of *B. uniflora* (Lex.) Dressler & Halb., the type species) to Europe. Although some of the earliest species historically linked to the group were collected in 1825 by Juan José Martínez de Lexarza and published in his *Orchidalium Opusculum* under *Pachyphyllum* Kunth (a genus in Oncidiinae, somewhat distant from Laeliinae), modern classification recognizes 18 species, 17 of which are found in Mexico with 15 endemic, making the country the center of diversity for the genus. The only species not found in Mexico is *Barkeria lindleyana* Bateman ex Lindl., endemic to Costa Rica, where it is popularly known as the "Independence flower" or "September 15th" because its peak flowering coincides with that date.

In this context, "Las orquídeas del género *Barkeria*", by Mexican biologists Cekouat Elim León Peralta and Eduardo A. Pérez García and published by the Asociación Mexicana de Orquideología, offers a modern monograph designed to provide the most up-to-date information on the genus, enable accurate species identification, and sustain the interest of a broad audience. With its monographic purpose and illustrated approach, the work evokes classic titles devoted to other orchid groups in Mexico, such as "El género *Lepanthes* Sw. en México" by Gerardo Salazar and Miguel A. Soto, or "Laelias of Mexico" by Federico Halbinger and Miguel A. Soto; yet it

also offers contributions that strengthen its value as a contemporary synthesis such as phylogenomic information, uptated conservation status and color plates for all the species of the genus. The book begins with brief chapters on general background and taxonomic history, followed by sections on vegetative and floral morphology, discussing the main organs and illustrated by schematic diagrams of the two main diagnostic growth habits present in the genus (caespitose and scandent). A comparative plate of the flattened lip view for all the species is included, and it is useful for comparing size, structure, and coloration of this diagnostic structure among the 18 species.

The book discusses the genus's distribution, which spans from southern Sonora, Mexico, through Central America into western Panama. The ecology chapter covers habitat, forest types, and common phorophytes, and it presents photographs of the habitats where the species occur. It also includes images of floral visitors, such as a *Bombus* carrying the pollinia of *B. spectabilis* Bateman ex Lindl. and a hummingbird visiting *B. whartoniana* (C.Schweinf.) Soto Arenas. Despite these observations, the authors note that pollination in *Barkeria* remains insufficiently understood. For example, one untested hypothesis is that *B. vanneriana* may mimic *Achimenes* (Acanthaceae) species because of similarities in flower color. However, the pollinators of both the orchid and *Achimenes* remain unidentified. However, clarifying species taxonomy is a critical step for advancing ecological research, and in my opinion, the book achieve this objective effectively.

The next chapter provides conservation assessments for all *Barkeria* species based on IUCN categories. Notably, *Barkeria fritz-halbingeriana* Soto Arenas is classified as Critically Endangered (CR) due to the only two known individuals. Additionally, *B. dorotheae* Halb.*, B. melanocaulon* A.Rich. & Galeotti*, B. strophinx* (Rchb.f.) Halb.*, B. uruapani* León-Peralta, Valdez-Partida & Pérez-García*, B. whartoniana* (C.Schweinf.) Soto Arenas*,* and *B. wixarika* León-Peralta & Pérez-García are categorized as Endangered (EN) because of their small, geographically close populations. Currently, the only *Barkeria* evaluated by the IUCN is *B. naevosa*, listed as Least Concern (https://www.iucnredlist.org/species/44392591/44453006); however, the authors advise classifying it as Vulnerable. Overall, these new assessments will significantly aid the development of more effective conservation policies for *Barkeria* species.

In addition, for those interested in cultivating *Barkeria*, the book offers practical guidance in the next chapter on light requirements, substrates, watering, fertilization, pests, and diseases. The scientific content is strengthened by a phylogenetic section that presents a maximum-likelihood tree based on whole plastomes from all 18 species and their close relatives. The results support a division of the genus into three main clades, referred to as the *B. lindleyana*, *B. obovata*, and *B. uniflora* groups, with maximal support. However, given the frequent conflicts between plastid and nuclear signals, the authors should test this hypothesis with multilocus nuclear data to evaluate potential supported incongruences between both datasets.

The core of the book continues with a comprehensive classic taxonomic treatment, including detailed species descriptions, a dichotomous key, etymology, common names, distribution, historical notes, identification, conservation status, and additional examined specimens. This section is richly illustrated with "Lankester Composite Disection Plates" (LCDP), photographs of flowers and plants in their natural habitats, and distribution maps for each species. Although some images are slightly out of focus or could benefit from focus-stacking, most are high-quality and clearly show color patterns and floral variations. The volume concludes with a glossary of botanical terms and a list of references.

Overall, "Las orquídeas del género *Barkeria*" provides the most comprehensive treatment of the genus to date, serving as both an authoritative monograph and a practical guide for researchers, curators, horticulturists, enthusiasts, and anyone interested in the natural history of this iconic, mostly Mexican lineage. Beyond documenting what is known, it also makes clear what remains to be studied, especially the still poorly understood pollination biology of the genus, and calls for urgent conservation of these species and their natural habitats.

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LANKESTERIANA, the International Journal on Orchidology, has been dedicated to publishing articles focused mainly (today exclusively) on orchid science, spanning a wide variety of topics, including anatomy, ecology, evolution, history, physiology, phylogenetics, and systematics. Founded in 2001, LANKESTERIANA is hosted by the University of Costa Rica. The first issue published on the 15th of May, 2001, with the support of Jorge Warner, former Director of Lankester Botanical Garden, and Franco Pupulin, its inaugural Editor in Chief, was funded by Brian Holley from Cleveland Botanical Garden. The journal's early years were marked by enthusiasm and rapid growth despite initial challenges in distinguishing itself from other botanical journals. However, it quickly gained recognition within the scientific community, largely due to contributions from prominent figures in orchid science, including James Ackerman, Germán Carnevali, Phillip Cribb, Stig Dälstrom, Mark Chase, Calaway H. Dodson, Robert L. Dressler, Eric Hágsater, Günter Gerlach, Alec Pridgeon, Gerardo Salazar, and Norris H. Williams.

Initially not exclusively focused on orchids, LANKESTERIANA shifted its scope to solely cover orchid-related research in 2007, filling a gap in orchidology left by the discontinuation of other recognized journals in the field, such as Lindleyana, Orquídea, Orquideología, and Selbyana. With the continuous support of the Vice-Rectorate for Research at the University of Costa Rica and the incorporation of Diego Bogarín, Adam P. Karremans, and Melissa Díaz as Associate and Managing Editors and Noelia Belfort-Oconitrillo as Technical Editor, the journal maintained a steady flow of high-quality publications. Today, LANKESTERIANA is the only scientific journal dedicated exclusively to publishing original research articles on orchid science, along with correspondence, comments, corrigendum, opinions, obituaries, special issue contributions, conference proceedings, checklists, and reviews.

The journal continues to assert its influence within the field of orchidology, evidenced by its high citation in orchid-related literature worldwide and its inclusion in well-recognized indexes such as Scimago and Scopus. LANKESTERIANA is a peer-reviewed, electronic, open-access journal that still distributes printed copies to over 50 institutions worldwide.

Aims and scope: LANKESTERIANA, the International Journal on Orchidology, is an international scientific journal published by the Lankester Botanical Garden Research Center – University of Costa Rica. The journal is globally recognized for its specialization and contributions to the knowledge of the orchid family. In addition to research articles, LANKESTERIANA is dedicated to facilitating scholarly communication and exchange by publishing a comprehensive array of content, including book reviews, checklists, comments, conference proceedings, correspondence, corrigendum, obituaries, opinions, reviews, and special issue contributions.

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Furthermore, LANKESTERIANA values the contributions of authors worldwide, with English being the official language of the journal.

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