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OBITUARY

A EULOGY FOR CARLOS GARCÍA ESQUIVEL (1939-2020)

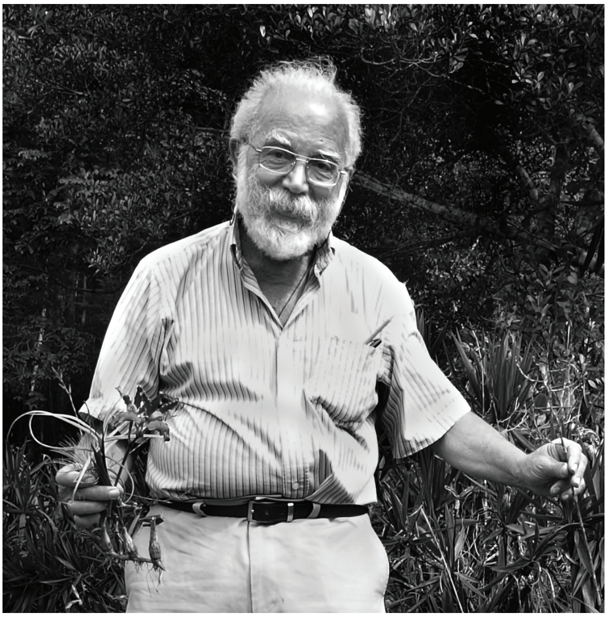

Photo by A. Zaslawski, Brazil, 2009.

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Carlos Simón García Esquivel (CGE), commonly known by his orchid friends as "García Esquivel" or simply, "Esquivel" or "Dr. Esquivel", was born in El Hatillo, Estado Miranda (at the outskirts of Caracas), Venezuela, in November 28, 1939. After his high school studies, in 1956, he became an associate of the La Salle Natural Sciences Foundation, where he begun collecting insects, orchids, and other plants. He entered medical school in 1956, at the Universidad de los Andes, Mérida, Venezuela, eventually obtaining his medical degree in 1960 at the Universidad Central de Venezuela in Caracas (he specialized in psychiatry and gastroenterology). He collaborated with G.C.K. Dunsterville when the second volume of *Venezuelan Orchids Illustrated* was being prepared, where four of his orchid collections were treated, one of them named by Garay and Dunsterville after CGE for his efforts: *Epidendrum garcianum* Garay & Dunsterv. [now *Prosthechea garciana* (Garay & Dunsterv.) W. Higgins]. Eventually, 22 additional collections by CGE appeared in the following four volumes of this monumental work and, in the acknowledgments of the sixth and last volume, he was cited by the authors among a small group of "friends and colleagues" for their "...active participation and continuous interest".

He also collaborated with Ernesto Foldats in the 1960´s when the five volumes of Orchidaceae for the *Flora of Venezuela* were being prepared and eventually published in 1969 (the first volume) and 1970 (the four additional ones). CGE also collaborated with all subsequent Venezuelan orchid specialists (G. Carnevali, I. M. Ramírez-Morillo, G. A. Romero-González, and

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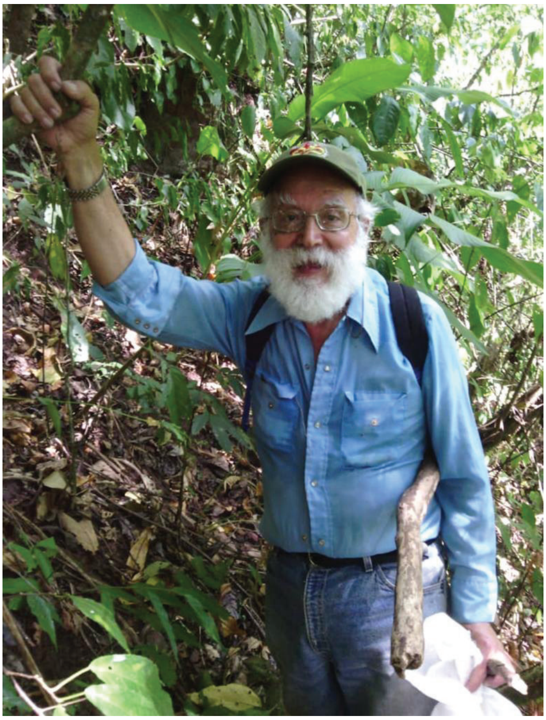
Carlos García Esquivel with María Belén Arreaza, 2019. Photo by R. Gorrochotegui.

Prosthechea garciana, one of the orchid species named after Carlos García Esquivel. Photo by F. Pupulin.

M. Speckmaier), all working in other countries today due to the diaspora of Venezuelan scientists, or with international experts interested in Venezuelan orchids (e.g., Eric Hágsater and collaborators at AMO for *Epidendrum*; Günter Gerlach for Stanhopeinae, and Carl Luer for the Pleurothallidinae), to whom he sent material and photographs as well as accounts of the habitats of the plants. Besides *Epidendrum garcianum*, he had four other species named after him: *Masdevallia garciae* Luer (syn: *Alaticaulia garciae* (Luer) Luer), *Pleurothallis garciae* Luer (= *Acianthera garciae* (Luer) Pridgeon & M.W.Chase), *Encyclia garcia-esquivelii* Carnevali & I.M.Ramírez, *Epidendrum garcia-esquivelii* Hágsater & L.Sánchez, and *Kefersteinia carolorum* Carnevali & Cetzal (named both after him and his orchid friend Carlos Bello, of San Cristobal, Táchira). He was the co-discoverer of *Miltoniopsis santanaei* Garay & Dunsterv., the only Guayanan member of an otherwise west Andean genus; the plant was eventually named after Gustavo Santana, a fellow physician and orchid collector. We have two additional orchid species new to science that we planned to name after him, a species of *Rudolfiella*, and an *Anathallis*. He discovered many other species that were new to science at the time he collected them and further contributed documenting the distribution of many other orchid taxa.

His interests grew from Venezuelan orchids to the family worldwide, eventually acquiring and encyclopedic knowledge that was tapped by orchid scientists and *aficionados* alike. His orchid expertise spanned the taxonomy, distribution, and horticultural information for many orchids from all over the world. Also, of people, places, and the history of Venezuelan orchid science and horticulture. He amassed a large and diverse orchid collection of his own, with emphasis on species and primary hybrids. During part of his retirement, in the late 2000's, he held a job at the largest orchid nursery in Venezuela (Plantío La Orquídea, property of Enrique Graf), where he was in charge of the breeding program, and focused on selected orchid species and primary hybrids.

CGE was an activist in orchid societies, being a life-long member of several of them in Venezuela, and of international associations such as the American Orchid Society. He was frequently invited as a guest speaker and judge in orchid conferences, shows, and society meetings where he thrived sharing his knowledge with friends and fellow orchid lovers. In orchid shows, he would often be called upon to participate in the judgement of orchid species, both Venezuelan and from other countries. Many of us will remember him as a teacher and as a friend. He



Carlos García Esquivel in the field in Venezuela, *ca.* 2009. Photo by C. Fernández.

Carlos García Esquivel in his house in Caracas with a plant of *Orleanesia pleurosthachys*, *ca.* 2012. Photo by G. Carnevali.

took genuine care of his friends (orchid and others) drawing from his experience as a psychiatrist and often provided needed council and support for them. He also was the ultimate collector: he wanted to possess the superlatives and diminutives of the genera he liked, or aimed at having all of the members of a particular group (provided they could be grown under his conditions at his house in Caracas). He was particularly interested in *Coryanthes* and the mystery of its successful cultivation. CGE was also interested in *Encyclia*, *Schomburgkia*, and the Pleurothallidinae. If he was interested in an orchid species, we would become obsessed until obtaining it, often after years or decades of waiting. He kept his interest in insects (particularly Dynastid beetles) throughout his life, frequently asking friends and orchid aficionados to collect the beetles for him in their travels. Later in life, he developed an interest in parrots, doves, and cracid birds, and built a large collection of them. This ornithological collection (along with his orchid collection) demanded of him much time and effort, particularly during his last years, when he depended solely on his retirement money and the Venezuelan economy was less than buoyant.

As a physician, CGE would willingly share his knowledge and expertise, offering advice, and taking care of friends and relatives. He helped curing many seriously ill patients on a *pro bono* basis, just because they were his friends, a friend's friend or relative, or an orchid lover. As an individual, and even in the face of economic, family, and health hardship, CGE was always an engaging, jovial, cheerful, selfless person. He would attentively listen, closely staring at the eyes of his interlocutor, and showing genuine interest in him. You could always tell he was interested in the conversation by the way he would arch his browns while he listened. A conversation with CGE was always an adventure. He was a great storyteller, spinning engaging arguments, drawing from his almost photographic memory of people, places, and plants. His stories ranged from how he discovered and collected a particular plant, how he conceived a particular orchid cross (and what resulted from it) to the tales of how even his entire family would collaborate in the incubation and hatching of the eggs of his favorite birds. He would remember the names of his myriads of friends, even those that he had only met by mail.

He was the ultimate, old-fashioned, postal mail correspondent, even in the age of computers, e-mails, and the social media, which he would only reluctantly (and clumsily) use. His long letters were always meticulously handwritten with impeccable orthography and flawless calligraphy, and as a rule included a long list of the orchids he would like to obtain and what he would like to trade for them. For those of us who were lucky enough to receive these letters, they will be treasured and cherished as fond memories of a true friend that will be forever missed.

It is difficult to understand the history of Venezuelan orchidology during the last half century without CGE. He collaborated both with the orchid scientists and the orchid *aficionados* and was greatly cherished by both. He left a legacy of orchid knowledge as well as many friends and admirers who will miss his company and love for orchids.

CGE died Thursday, April 10, 2020, after the attack of thousands of Africanized honeybees in his house while taking care of his orchid collection in Caracas.

Acknowledgements. The authors would like to thank many individuals for their comments and suggestions to this homage, all friends of CGE. Among them, we would particularly like to acknowledge (in alphabetical order): María Belén Arreaza, Armando Betancourt, Gerardo Castiglione, César Fernández, Rafael Gorrochotegui, Ramón López, Armando Mantellini, Santos Michelena, Manfred Speckmaier, and Alek Zaslawski. The authors want to thank the staff of *Lankesteriana*, particularly Noelia Belfort, Adam Karremans, and Franco Pupulin for their interest and willingness that allowed us to put together this obituary in a matter of two days.

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THE ELUSIVE *SOBRALIA AMABILIS* (ORCHIDACEAE): A RANGE EXTENSION OF ITS DISTRIBUTION TO ALTA VERAPAZ, GUATEMALA, WITH NOTES ON ITS HABITAT

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Abstract. We present a range extension for *Sobralia amabilis*, previously recorded from the southeastern border of Guatemala to Panama and Ecuador. Here, we detail its taxonomic history, description, and geographical distribution, along with photographs of the plants *in vivo*, habitat notes and recommendations for collecting and preparing herbarium specimens.

Resumen. Presentamos una extensión en el rango de distribución de *Sobralia amabilis*, la cual solo ha sido registrada desde la frontera suroriental de Guatemala hasta Panamá y Ecuador. Aquí se detalla su historia taxonómica, descripción y distribución geográfica, junto con fotografías de las plantas *in vivo*, notas sobre su hábitat y recomendaciones para colectar y preparar especímenes de herbario.

Key words / Palabras clave: América Central septentrional, bosque de neblina, cloud forest, Northern Central America, occurrence, ocurrencia, orchid, orquídea, Sobralieae

Introduction. The genus *Sobralia* Ruiz & Pav. includes about 200 species native to the Neotropics (Baranow, Dudek & Szlachetko 2017, Dressler, Acuña Zamora & Pupulin 2016). These are known for their bamboo-like stem, with showy and ephemeral flowers that have taxonomic importance for discriminating sections and species (Brieger 1983). Dressler (2009) recognized the difficulty to classify sobralias by enumerating a few species complexes. The whole genus is noted for its lack of clarity in taxonomy and distribution, due mainly to intraspecific variation, and the difficulty of observing flowering plants in the field, because they are short-lived and have floral synchrony. Additionally, taxonomically informative herbarium specimens are hard to obtain, since the flowers deteriorate during the collection procedures (Baranow & Szlachetko 2016, Dressler & Bogarín 2007). In Guatemala, about 14 *Sobralia* species have been generally accepted (CONAP 2019, Dix & Dix 2000, WCSP 2019). Among these species, *Sobralia amabilis* (Rchb.f.) L.O.Williams has been classified under four synonyms in two genera since its discovery and description in 1852 (Reichenbach 1852, Reichenbach 1866, Schlechter 1923, WCSP 2019, Williams 1946, Williams 1956). Even in recent years, the need to improve the diagnostic characteristics among closely related species, *S. amabilis* (Rchb.f.) L.O.Williams, *S. lepida* Rchb.f. and *S. wercklei* (Schltr.) L.O.Williams has been brought up (Dressler 2003), and the lack of unified criteria in conspecific areas is obvious (Bogarín *et al*. 2014).

These species were first reported in northern Central America within a 44-year gap; *Sobralia wercklei* was first recorded in Honduras (Williams 1956), then as

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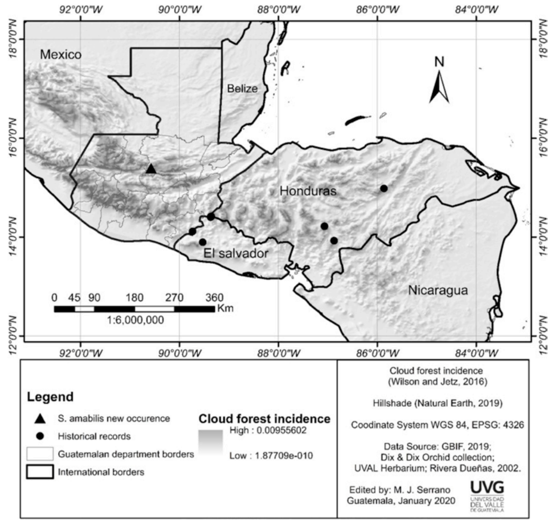

Figure 1. *Sobralia amabilis* reported localities in Guatemala, El Salvador and Honduras.

S. lepida in El Salvador (Hamer 1981)*,* as *S. amabilis* and *S. wercklei* in El Salvador (Siegerist 1984), and *S. amabilis* in Guatemala (Dix & Dix 2000). The Guatemalan specimen was registered near the summit of Volcán Chingo, above coffee plantations in broad-leaf oak forest, close to the border with El Salvador. We know of no other citation for a locality in Guatemala. However, during an Ecological Assessment course field trip from Universidad del Valle de Guatemala, in June 2018, we collected *S. amabilis* (Fig. 1) on a private farm in western Alta Verapaz, Guatemala. This specimen represents a new location for the distribution of *S. amabilis*. Below, we detail its taxonomic history, description *in vivo* and specimen photographs, habitat notes, and recommendations for collecting *Sobralia* specimens.

Taxonomic History

Heinrich G. Reichenbach first described this orchid as *Fregea amabilis* Rchb.f. (1852: 712–713), from a specimen collected in Cordillera de Chiriquí, Panama, by Józef Warszewicz. Fourteen years later, Reichenbach described a similar orchid from Paso del Desengaño in Costa Rica and named it *Sobralia lepida* Rchb.f. (1866: 68). Later, Rudolf Schlechter described a new species, *F. wercklei* Schltr. (1923: 9), collected in San Jerónimo, Costa Rica, and distinguished it from *F. amabilis* for its greater height, broader leaves, and larger labellum. Then, Louis O. Williams (1946), while working on the Orchidaceae section of the Flora of Panama, determined that there are no differences between the genus *Fregea* Rchb.f. and *Sobralia*, so he included *Fregea* as a synonym of *Sobralia*, and he transferred *F. amabilis* to *S. amabilis*. Williams (1956), during the enumeration of the orchids of Central America, included *S. amabilis*, *S. lepida* and synonymized *F. wercklei* to *S. wercklei*, each one as a separate species. Right now, *S. amabilis*, *S. lepida*, and *S. wercklei* are not included in any of the Sections accepted for *Sobralia* (Dressler 2002).

Taxonomic treatment

Sobralia amabilis (Rchb.f.) L.O.Williams, *Ann. Missouri Bot. Gard*. 33: 30. 1946.

Synonyms (WCSP 2019):

Sobralia lepida Rchb.f., *Beitr. Orchid. -K. C. Amer.*: 68 (1866).

Fregea wercklei Schltr., *Repert. Spec. Nov. Regni Veg. Beih*. 19: 9 (1923).

Sobralia wercklei (Schltr.) L.O.Williams, *Ceiba* 5: 25 (1956).

Bas.: *Fregea amabilis* Rchb.f., *Bot. Zeitung (Berlin)* 10: 712–713. 1852. TYPE: Panama. Cordilleren von Chiriqui, *J. Warszewicz s.n.* (holotype, W).

Epiphytic *herbs*. *Stem* erect, cylindric, 10–30 cm long, 0.4 cm in diameter, covered with brownish sheaths, verrucose at the apex. *Leaves* up to 4, plicate, 4.33–11.10 cm long × 1.4–3.2 cm wide, lanceolate to long acuminate, strongly corrugated. *Inflorescence* brown or green bracts 3.4–5.0 cm × 0.5–0.9 cm, ellipsoid, verrucose, clustered, 1–2 flowers. *Flowers* 5-6 cm wide, color magenta, with a paler throat that has darker spots. *Sepals* elliptic to oblong or ovate, acute to apiculate, base connate; *dorsal sepal* 3.19 × 1.24 cm; *lateral sepals* 1.33 × 2.95 cm. *Petals* 2.97 × 1.24 cm, elliptic or obovate, obtuse. *Labellum* 4.18 × 3.64 cm, forming a basal tube 1.40 × 0.49 cm, covering the column, and extending abruptly into a flabellate–obovate, emarginated lamella, with wavy edges. *Column* 1.21–1.25 × 0.5-0.6 cm, white, slightly curved, apex dilated. *Fruits* and *seeds* not seen.

The characters measured are in the range of the data reported for *S. amabilis* (Archila *et al.* n.d., Dressler

FIGURE 2. *Sobralia amabilis*, *in situ* A. Leaf and Flowers, close up. B. Whole plant. C. Habit. Photographs A & B by Isabella Rosito; C by Rafael Grajeda–Estrada.

2003, Rivera–Dueñas 2002), except for the basal tube of the labellum (2 mm shorter), the sepals (10–20 mm wider), and column (2 mm longer). We also noticed that the dorsal sepal is slenderer than the lateral sepals, and we suggest keeping a separate record of these measurements.

EXAMINED MATERIAL. Guatemala. Alta Verapaz: San Cristóbal Verapaz, 2080 m elev., 3 June 2018, in bloom, collection project number *R. Grajeda–Estrada et al. EER18c2* (Herbarium number *UVAL–19920*, dried collection) Figs. 1–2, 4

DISTRIBUTION: *Sobralia amabilis* had been located from Ecuador to almost every country of Central America, except from Guatemala and Belize (Dressler 2003, GBIF.org 2019). Nevertheless, in 1991 Margaret and Michael Dix collected the species near the summit of Volcán Chingo, close to the border with El Salvador (Dix & Dix 2000); however, the specimen is not available. Besides, there were no specimens in the herbarium of the Universidad del Valle de Guatemala (UVAL) and the Universidad de San Carlos de Guatemala herbaria: Herbarium of the Escuela de Biología (BIGU), Herbarium of the Centro de Estudios Conservacionistas (USCG) and Herbarium of the Facultad de Agronomía (AGUAT) (pers. obs.). Neither are records of *S. amabilis* or the related species, *S. lepida* and *S. wercklei*, from Guatemala at the free access database of the Global Biodiversity Information Facility (GBIF.org 2019).

HABITAT AND ECOLOGY. The specimen comes from a flowering plant found in Northern Guatemala and corresponds to the northernmost occurrence of this species in Central America, about 168.57 km from the nearest locality previously registered at Volcán Chingo, Guatemala or 169.48 km from Trifinio, El Salvador (Fig. 1). Several epiphytic individuals of *S. amabilis* were found on a *Hedyosmum mexicanum* C.Cordem. (Chloranthaceae) tree, about 6 meters above ground (Fig. 2C) in a broadleaf, evergreen, tropical lower

FIGURE 3. Habitat view at collection site, San Cristóbal Verapaz, Alta Verapaz, Guatemala. Photograph by Marcelo Serrano.

FIGURE 4. Collected specimen A. Before drying and pressing (*R. Grajeda–Estrada et al. EER18c2*). B. Herbarium specimen (*UVAL–19920*). Photographs by Rafael Grajeda–Estrada.

montane wet forest on a private farm in San Cristóbal Verapaz, Alta Verapaz. The altitude range goes from 1700 to 2200 m elev., the average temperature is 16°C; annual precipitation is 1946 mm (IARNA 2018). The evergreen forest includes trees like *Alfaroa guatemalensis* (Standl.) L.O.Williams & Ant.Molina (Juglandaceae), *Cojoba arborea* (L.) Britton & Rose (Leguminosae), *Dendropanax arboreus* (L.) Decne. & Planch. (Araliaceae), *Parathesis leptopa* Lundell (Primulaceae), oaks (Fagaceae) like *Quercus lancifolia* Schltdl. & Cham., and *Q. conspersa* Benth., arborescent ferns (Cyatheaceae) like *Cyathea divergens* Kunze and *Alsophila tryoniana* (G.J.Gastony) D.S.Conant. The understory includes shrubs like *Miconia petiolaris* (Schltdl. & Cham.) Michelang. (Melastomaceae), small trees like *Amphitecna montana* L.O.Williams (Bignoniaceae) and several palms (*Chamaedorea* spp.). Also, there are various lianas (*Ficus* sp. (Moraceae) and *Clusia* sp. (Clusiaceae), epiphytic mosses, ferns, bromeliads and orchids (Fig. 3). So far, we have found 46 orchid species at the site.

Across its distributional range, *Sobralia amabilis* occurs in montane forests at elevations between 900 m to 3100 m like the Cordillera of Chiquirí, Panama and the Cordillera de Talamanca, Costa Rica. In Costa Rica, the orchid can be found on various volcanoes and natural reserves with lower montane rain forests (Bogarín *et al*. 2014, GBIF.org 2019, Monro *et al*. 2017, Pupulin 2010). *Sobralia amabilis* was reported at Volcán Mombacho, Granada, Nicaragua (GBIF.org 2019). There are reports of *S. amabilis* in the Reserva Biológica de Yuscarán in central Honduras, characterized by mountains and high annual precipitation (Rivera–Dueñas 2002). Also, in El Salvador, a plant of *S. amabilis* has been registered at the Parque Nacional Montecristo, part of Reserva de la Biosfera Trifinio, a tri-national reserve of the two previous countries and Guatemala established to preserve the Cerro Montecristo cloud forest (GBIF.org 2019, Hamer 1981). We describe the habitat of our recent find as a broadleaf cloud forest, with high epiphytes diversity, annual precipitation between 1600–4000 mm or more, from 20% to 60% of cloud coverage daily (Dix & Dix 2018). Schuster *et al*. (2000), listed as cloud forests: the Sierras de las Minas, Santa Cruz, and Chamá; La Unión in Zacapa, and other localities in the mountains and volcanoes in southeastern Guatemala; these are found between the new site of occurrence and the one from Volcán Chingo, therefore there may be other populations of *S. amabilis* in these localities.

Recommendations. Collecting *Sobralia* specimens for dried preservation is difficult because the flowers of many species are fragile and tend to deteriorate quickly (Fig. 4), probably as a result of their ephemeral flowers and anatomy; even in spirit preservation, some material decays depending on the curation process (Dressler & Bogarín 2007). Dressler (2002) suggests studying *Sobralia* using photographs and drawings based on living material from collections or field studies to achieve better documentation and understanding of the species, considering that the genus has been studied mainly on dried material (Baranow *et al*. 2017). Coinciding with Dressler (2002), we recommend collecting material *in situ*, immediately taking quality photographs with DSLR camera and an 18-55 mm f/3.5–5.6 lens. Also, the flowers should be preserved in a spirit collection with a solution of 75% ethanol, 5% glycerin and 20% distilled water to prevent deterioration; if there are more flowers available, at least one should be dissected and dry pressed to mount each floral part separately.

Acknowledgments. Dedicated to the memory of Robert Louis Dressler, who worked tirelessly with neotropical orchids and contributed greatly to *Sobralia* research. We thank the Serrano family and especially Ruth Prem for their efforts to preserve their forest and for letting us explore the place; Juan Pablo Pinto, for his preliminary orchid identification *in situ*, and Isabella Rosito for her photographs. To the Universidad del Valle de Guatemala and Daniel Ariano for organizing the field trip during which this specimen was found; Diego Incer for facilitating the climatic data; CONAP for collection permit (A–003267). To the editors and both reviewers for their recommendations and improvement of this paper. Finally, to Michael W. Dix for sharing his love and knowledge about Guatemalan epiphytes.

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EPIDENDRUM CURIMARCENSE (ORCHIDACEAE), A NEW SPECIES FROM CENTRAL PERU

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Abstract. A new species of *Epidendrum* from Peru, *Epidendrum curimarcense*, is described, illustrated and compared with the similar species *Epidendrum ampelospathum.* The new finding represents an important record for the flora associated with *Polylepis* forests of central Peru. The species has stems produced from a middle internode of the previous cane-like stem, a spathe, an elongate peduncle with several similar spaced bracts, flowers opening in succession, petals narrower than the sepals, a 3-lobed lip which is bicallose with rounded sub-equal lobes, and 4 obovoid, laterally compressed pollinia.

Resumen. Una nueva especie de *Epidendrum* del Perú, *Epidendrum curimarcense*, es descrita, ilustrada y comparada con la especie similar *Epidendrum ampelospathum.* El nuevo hallazgo representa un importante registro para la flora asociada a bosques de *Polylepis* en el centro del Perú. La especie tiene tallos tipo caña producidos de un internodo medio del tallo anterior, una espata en la base de la inflorescencia, con varias brácteas semejantes espaciadas a lo largo del pedúnculo alargado, las flores abren en sucesión, los pétalos son más angostos que los sépalos, labelo 3-lobado, lóbulos redondeadas semejantes, bicalloso; polinios 4, obovoides, lateralmente comprimidos.

Key Words / Palabras clave: Andean orchids, central Peru, *Epidendrum,* Junín, orquídeas andinas, *Polylepis*

Introduction. The *Epidendrum* genus was assigned by Linnaeus in 1763, for all orchids that grew on trees. None of the early species designated by Linnaeus remain today in the genus, as orchids are predominantly epiphytic, so many genera were later described, and all the original species placed in them. In the early botanical expeditions to Peru and Chile, Ruiz and Pavón (1798) published 19 species of *Epidendrum* based on their collections and their illustrations which are deposited in Real Jardín Botánico de Madrid Herbarium (MA).

Epidendrum is one of the most diverse genera within the Orchidaceae family, distributed from sea level to 4,200 meters altitude (Hágsater & Soto 2005). *Epidendrum* are epiphytic, lithophytic, or rarely terrestrial herbs, caespitose, sympodial or rarely monopodial, and then usually branching above, erect, creeping or hanging. The stems are usual1y cane-like, simple or branching, sometimes thickened and then fusiform, rarely pseudobulbous. The inflorescence is apical, lateral, or rarely basal, single-flowered, subcorymbose, racemose to paniculate, producing flowers only once, or producing new racemes from an old inflorescence over several years, thus producing in time a pluri-racemose structure from what was a racemose inflorescence at first flowering, sessile to long-pedunculate, when apical often produced from within one or two spathaceous bracts that develop long before the inflorescence. The labellum is, in most species, united to the column lateral margins along the entire length of the column, however some groups of *Epidendrum* species have labella that are partially to totally free. The column-labellum structure forms a nectar tube that usually penetrates the ovary. The labellum is usually ornate with two basal, globose calli and a pair of laminar calli ending in a narrow keel or a complex, tuberculate callus; however, some species lack any callus and instead have one or several parallel

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to radiating keels, sometimes ornate with trichomes or papillae. The labella range from entire to deeply 3- or 4-lobed, mostly distinct from the sepals and petals. The pollinarium usually has 4 pollinia, rarely 2 or 8. The rostellum is parallel to the longitudinal column axis, arching downwards at the apex, producing a semi-liquid viscidium attached to the caudicles of the pollinarium as it is removed from the column during pollination, leaving an elliptical slit.

Agricultural expansion, cattle ranching, logging, burning, among other human activities are the main causes of habitat loss of *Epidendrum* and other types of plants that are dependent on trees (García & Galeano 2006).

Peru was credited with around 2034 species of orchids (Brako & Zarucchi 1993, Ulloa, Zarucchi & Léon 2004), among them 775 endemics, 98 of the endemics correspond to the genus *Epidendrum* with only 14 species registered for the Junín region (Roque & León 2006). Ulloa *et al.* (2017) published a new number of 2,120 species for Peru, including 316 species of *Epidendrum*. Several additions have been made since 2017 (Damian & Hágsater 2016, Hágsater & Santiago 2018a, 2018b, Acuña-Tarazona *et al*. 2019, Gutierrez *et al*. 2019). The most recent publication of Hágsater and Santiago (2019) includes 18 new species from Peru; several, including some old species, cited for Junín (*Epidendrum choccei* M.E.Acuña, Hágsater & E.Santiago, *E. trullatum* Hágsater, L.Valenz. & E.Santiago, *E. holochilum* (Schltr.) Mansf. ex Hágsater and *E. megagastrium* Lindl.). These, including the new species here described, would increase the orchids numbers to 2142 for Peru, and 338 for Junín. Hágsater, in the AMODATA digital information bank (2019), has registered 439 species of *Epidendrum* for Peru (Santiago & Hágsater 2019, Goicochea *et al*. 2019). A count of orchid species prepared by Ulloa (2019) from the Vascular Plants of the Americas – Tropicos sets the number of orchids at 2219 of which 355 are *Epidendrum* species. That list corrected by Hágsater in late November 2019 (unpubl. data), deleting incorrect citations, synonyms and adding recently published species not yet included in Tropicos, sets the number of *Epidendrum* species for Peru at 446. Current work by a team of Peruvian botanists will probably greatly increase that number of species. This work, along with work in herbaria and in the field, is proving that species thought to be endemic to either Colombia, Ecuador, Peru or Bolivia, are in reality more widespread, and thus the number of species is increasing but endemism may be less frequent (Hágsater unpubl. data).

High altitude Andean forests (3000–5200 m, Kessler 2006) harbor diversity and very high rates of endemic species of flora and fauna (Fjeldså & Kessler 2004). In the last decade, several new records have been made for the *Epidendrum* genus throughout the Andes as more areas that were not previously well-explored are being examined, thus demonstrating that the *Epidendrum* genus possesses much greater diversity than previously estimated. This article describes and illustrates a new species for the Department of Junín of Peru.

Materials and methods. The plant material was collected in Curimarca, Molinos District, Jauja Province, Junín Department (Fig. 1) in fieldwork in which the flora associated with the high Andean forests of *Polylepis* was evaluated. Photographs were taken in the field and laboratory for preparing and diagramming a Lankester Composite Digital Plate (LCDP). Searches were made in AMODATA, where 691 records were retrieved for *Epidendrum* from Junín, out of a total of 10,165 for Peru. Other herbaria recorded include AMES, AMO, B (photographs lodged at F), CAS, COL, F, G, GH, HB, HBG, HOXA, K, LE, LL, M, MA, MO, MOL, NY, OXF, P, PR, RENZ, S, SEL, TNS, UC, US, USM and WIS. These records are based on images taken at these herbaria through the years and do not necessarily include recent collections. Records of specimens and illustrations from David E. Bennet Jr. (at MOL and AMO) were also searched. The records for Junín were studied for possible matches. Comparisons were made with *E. ampelospathum* Hágsater & Dodson (2004) and *E. ampelomelanoxeros* Hágsater, E.Santiago & E.Parra (2013) (images of type and live material at AMO, as well as descriptions), the species most similar vegetatively to *E. curimarcense*. In addition, due to the floral and inflorescence details, the species was also compared to *Epidendrum totoroense* J.S.Moreno, Hágsater, E.Santiago & Erazo (2016). The pressed material was deposited at HOXA and HUT herbaria.

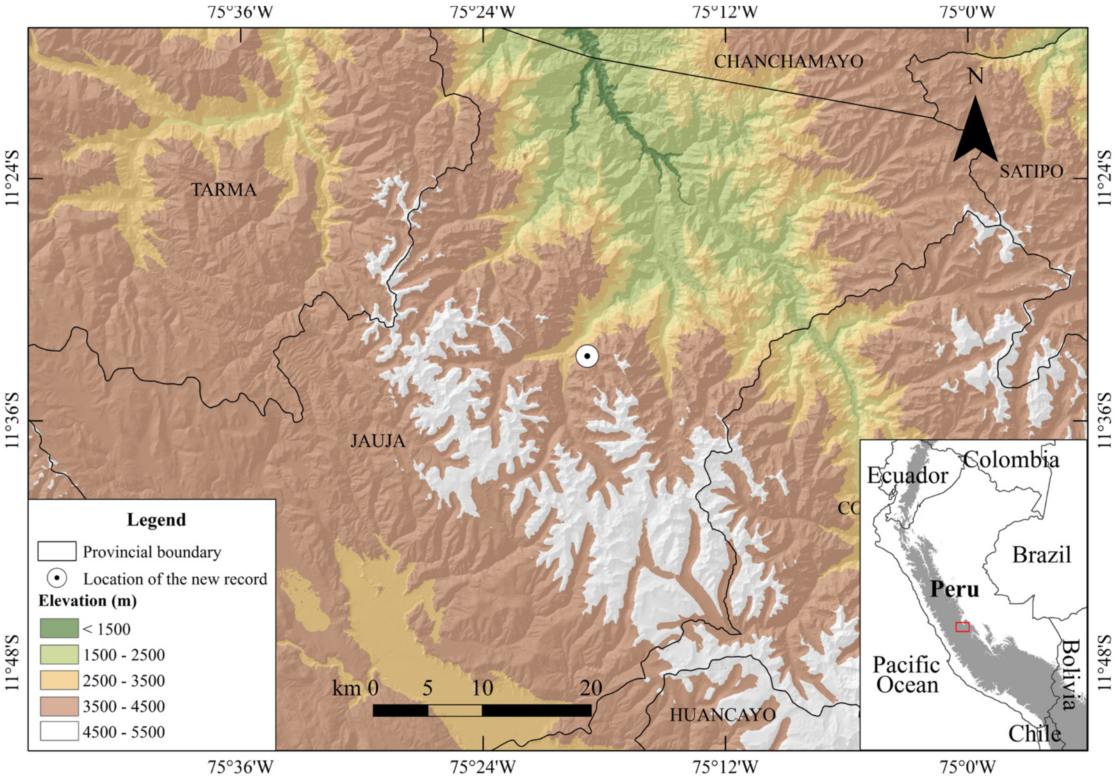


Figure 1. Distribution map of *Epidendrum curimarcense* (white circle) in Peru.

Taxonomic treatment

Epidendrum curimarcense Hágsater, W.C.Navarro & H.R.Quispe, ***sp. nov.*** (Fig. 2–3)

TYPE: Peru. Junín: Jauja Province, Molinos District; Curimarca River, 3450 m, humid montane forest, epiphyte on *Polylepis canoi* W.Mend., *Oreopanax oroyanus* Harms, and *Miconia sp.*, collected 17 March 2018, pressed 20 March 2018, *W. C. Navarro & H. R. Quispe 050*. Holotype HOXA! (LCDP & photo voucher). Isotype HUT!

Diagnosis: *Epidendrum curimarcense* is similar to *E. ampelospathum* Hágsater & Dodson, but plants are much smaller, to 30 cm tall, elliptic leaves, 2.5–7.3 × 1.0–2.5 cm, inflorescence 17.5 cm long with a spathe 2.3 × 0.5 cm, peduncle elongate, ancipitose, provided with 2–3 bracts similar to the spathe but progressively shorter, flowers greenish buff, column and lip green, sepals 16 × 4–5 mm, petals oblanceolate, 12.0 × 2.5 mm, and lip base slightly cordate, lateral lobes transversely ovoid; mid-lobe sub-quadrate.

Epiphytic, sympodial, scandent, erect to arching *herb*, *ca*. 30 cm long, of successive lateral growths. *Stems* 10–14 × 0.3–0.4 cm, produced from a middle internode of the previous stem (2nd to 4th internode, below the first leaf), cane-like, terete. *Leaves* 5–6 distributed throughout the upper half of the stem, spreading, sub-coriaceous; sheath 1.0 × 0.4 cm, tubular; blade 2.5–7.3 × 1.0–2.5 cm, elliptic, acute, smooth, margin entire, spreading. *Spathe* 2.3 × 0.5 cm, single, conduplicate, tubular below, open above, narrowly elliptic, acuminate, margin entire, light brown. *Inflorescence* 17.5 cm long, apical, racemose, arching, nutant, flowering only once; peduncle 5–8 cm long, elongate, ancipitose, provided with 2–3 bracts, up to 23 × 5 mm, similar to the spathe but progressively smaller and developed as the inflorescence grows, conduplicate, tubular below, open above, narrowly elliptic, acuminate, margin entire, light brown.

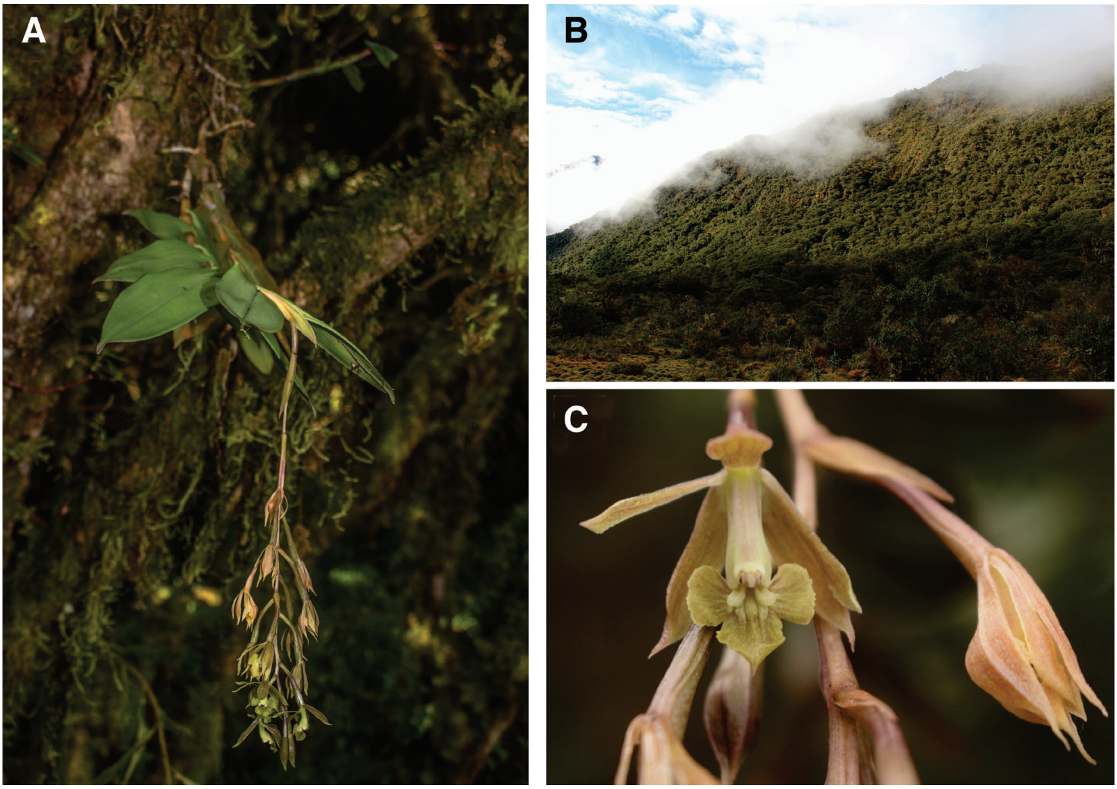
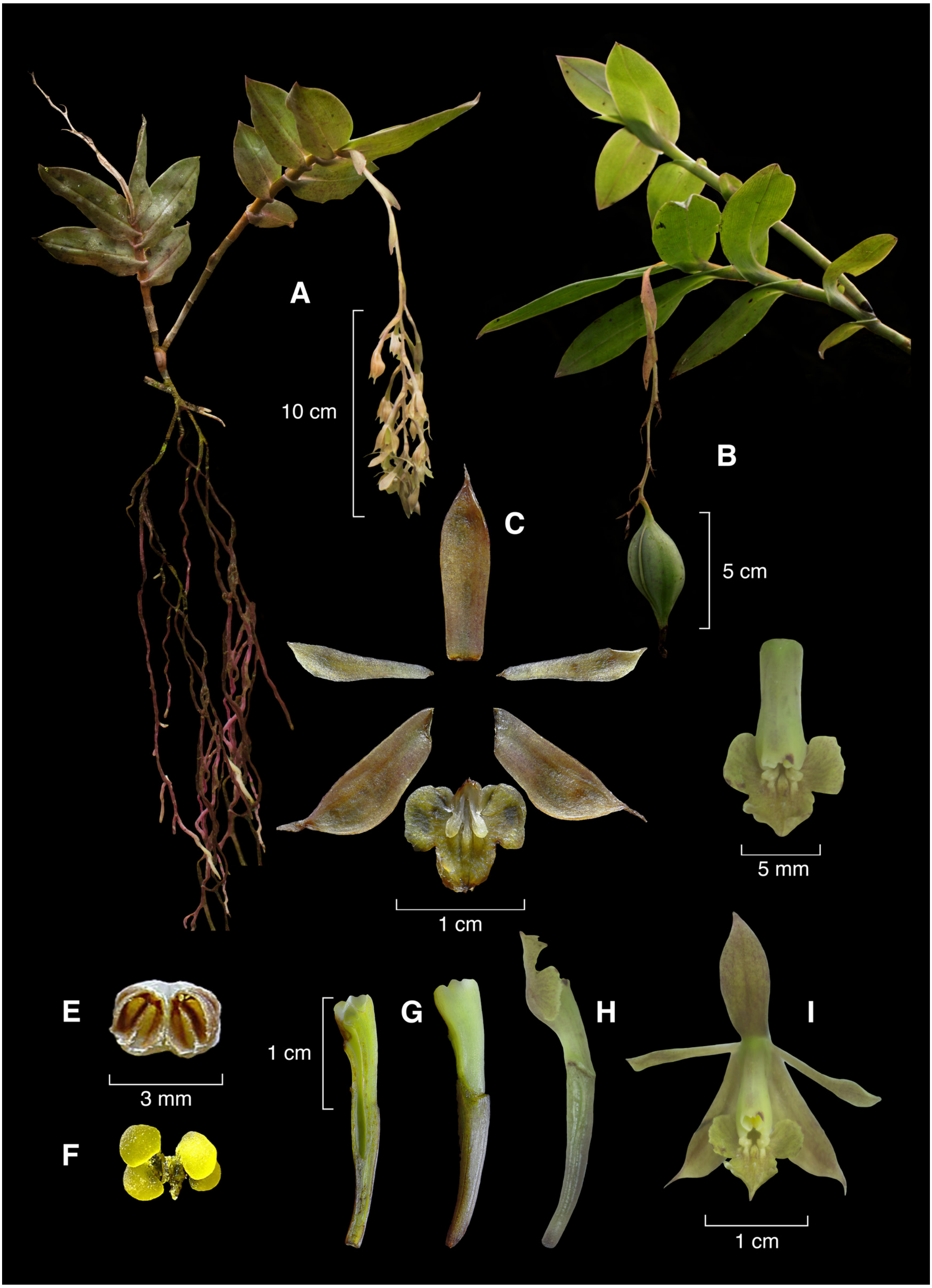


Figure 2. *Epidendrum curimarcense*. **A**. Habit and complete plant. **B**. Habitat. **C**. Flowers. Photograph by Harold R. Quispe-Melgar.

Floral bracts 5–8 × 1 mm, shorter than the ovary, triangular, acute. *Flowers* 6–15, opening in succession, eventually most open together, greenish buff (turning reddish brown as they wilt), column and lip green; without fragrance. *Ovary* 12.5–16.0 mm long, terete, progressively thickened towards the apex. *Sepals* partly spreading, 3-veined, long apiculate, margins entire; dorsal sepal 16 × 4 mm, free, oblong, widened slightly near the apex, acuminate; *lateral sepals* 16 × 5 mm, obliquely united to the base of the column, obliquely oblong-obovate, acuminate. *Petals* 12.0 × 2.5 mm, partly spreading, free, oblanceolate, acute, somewhat oblique, the upper margin straight, 1-veined, margin entire. *Lip* 9 × 10 mm, united to the column, 3-lobed, the base slightly cordate, margin entire to erose, bicallose, the calli divergent, prominent, digitiform, a low fleshy mid-rib runs down the middle of the mid-lobe to the apex with a pair of lower, shorter ribs on the side; lateral lobes 3 × 5 mm, transversely ovoid, rounded; mid-lobe 4 × 5 mm, sub-quadrate, apex rounded. *Column* 10 mm long, thick, straight, apex truncate; *clinandrium* hood short, margin entire. *Anther* reniform, 4-celled. *Pollinia* obovoid, laterally compressed; *caudicles* granulose, short; *viscidium* semi-liquid. *Rostellum* apical, slit. Lateral lobes of the *stigma* short, the viscidium itself also short, about the same size as the clinandrium. *Nectary* penetrating half the ovary, smooth, not inflated, unornamented. *Capsule* 46 × 19 mm, ellipsoid, pedicel 7 mm long, short thin, body 36 × 19 mm, and neck *ca*. 3 mm long.

Distribution and ecology: Known presently only from the type, from central Peru, Department of Junín, upper basin of the Río Curimarca, an affluent of the Río Uchubamba, on the upper eastern slopes of the Cordillera Oriental de Los Andes. In montane

→ Right, Figure 3. Plate of *Epidendrum curimarcense*. **A**. Habit and inflorescence. **B**. Plant in fruit. **C**. Dissected perianth. **D**. Column and lip. **E**. Anther. **F**. Pollinarium. **G**. Column, dissected and lateral view. **H**. Column and lip, lateral view. **I**. Flower, front view. Photographs by Jonathan Chancasana (C, E, F & G) and Harold R. Quispe-Melgar (A, B, D, H & I).


A
10 cm
B
5 cm
C
5 mm
1 cm
E
3 mm
F
1 cm
G
H
I
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humid forest, epiphytic on *Polylepis canoi*, *Oreopanax oroyanus*, and *Miconia sp.*, at 3450 m in elevation. Flowering in March.

Etymology: In reference to the locality where the species was collected, in the basin of the Comunidad Campesina de Curimarca/Jauja.

Epidendrum curimarcense belongs to the Ampelospathum group which is recognized by the scandent habit, with new stems produced from the middle of the previous stems, roots produced at the base of the main stem, and occasionally from the base of the successive stems, an apical, arching, racemose inflorescence subtended by 1–2, large, acute spathes, and flowers with a 3-lobed lip with 2 small, basal calli. The new species has short plants, *ca*. 30 cm tall, elliptic leaves 2.5–7.3 × 1.0–2.5 cm, a nutant inflorescence, an elongate, ancipitose peduncle with a spathe, 2.3 × 0.5 cm, that is conduplicate and acuminate with similar but progressively smaller bracts spaced along the peduncle, flowers greenish buff, column and lip green, sepals 16 × 4–5 mm, and a lip 3-lobed, the base slightly cordate, lateral lobes transversely ovoid, rounded, mid-lobe sub-quadrate and the apex rounded. *Epidendrum ampelospathum* has plants up to 2.5 m tall, the stems with only 5–7 narrowly lanceolate leaves, 11.2–18.5 × 1.3–1.6 cm, and yellow to yellowish green flowers with very wide, trullate petals, and a 3-lobed lip, the base deeply cordate, lateral lobes reniform, obliquely orbicular, rounded, the mid-lobe triangular, apex truncate, rounded. *Epidendrum ampelomelanoxeros* Hágsater, E.Santiago & E.Parra has plants to 70 cm tall, a raceme with 17–26 flowers that are simultaneous, fleshy, greenish yellow tinged reddish, sepals 16–18 mm long, petals obliquely oblanceolate, acute, and dolabriform lateral lobes of the lip with the apical margin erose. *Epidendrum totoroense* has short, erect stems produced from the base of the previous stem, to 7.5 cm tall, with 2–4 elliptic leaves, 2.5–5.0 × 1.3–2.0 cm, an erect inflorescence 13.5 cm long, flowers yellowish brown with the apex of the column and disc tinged purple, sepals 19 mm long, and the lateral lobes of the lip smaller than the mid-lobe; the flowers are very similar to those of the new species in color and proportions, but the plant habit is clearly different.

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A NEW GOLDEN YELLOW SMALL-FLOWERED *CYRTOCHILUM* SPECIES (ORCHIDACEAE: ONCIDIINAE) FROM PERU

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Abstract. A new golden yellow small-flowered species of *Cyrtochilum* is described and illustrated with a line drawing and photographs. It is compared with superficially similar species in the *C. aureum* complex, in which the new species differs by the lack of lacerate column wings typical for that group. It is distinguished from all other known small-flowered *Cyrtochilum* species by the golden yellow color in combination with the distinctly pandurate lip lamina where the front lobe is wider than the basal lobes combined.

Key Words: Ancash, *Cyrtochilum*, new species, Oncidiinae, Peru

Introduction. While exploring the western slopes of the Peruvian Andes the authors have observed that the climate there is markedly less humid than on the eastern slopes. This appears to be the effect from the Humboldt Current that brings cold water from the Antarctic region up along the Peruvian coast, which in turn produces less evaporation of water. This climatologic and hydrologic fact simply means less rain over the coastal area. Being more densely populated than the eastern slopes, it also means a heavier toll on existing fresh water sources and on other natural resources such as original forests. Slash and burn techniques to clear land for pastures, and mining operations are other human activities that disturb the natural orders. This has created a landscape commonly dominated by shrubs and grasslands. The orchid diversity in these plant communities therefore seem to be relatively poor, according to the authors' observations. Species of the Oncidiinae appear to be scarce in this region in contrast to the eastern slopes of the Peruvian Andes where this subtribe is highly diverse. At elevations above 3000 m only two Oncidiinae species have been currently recorded from the central-northern highlands of Peru (Departments of Ancash and La Libertad): *Trichoceros tupaipi* Rchb.f. and *Cyrtochilum cochleatum* (Lindl.) Dalström (Fig. 1) (Trujillo 2012). This latter species has been misidentified by several authors and reported as "*Odontoglossum mystacinum* (Lindl.) Lindl." (Weberbauer 1945), "*O. rigidum* Lindl.", (Kolff & Kolff 1997; Smith 1988) and *C. aureum* (Lindl.) Senghas (Trujillo 2012, 2013). The *C. aureum* complex consists of four hitherto described species: *C. aureum*, *C. cochleatum*, *C. mystacinum, C. rigidum* (Lindl,) Dalström (Dalström 2013), and one undescribed species from the Department of Huanuco (in cultivation by the Perúflora nursery). During a recent revision of the *Cyrtochilum* specimens of the David Bennett Collection kept at the herbarium of the National Agrarian University La Molina in Lima (MOL), a new species with golden yellow flowers from the highlands of Ancash was found that superficially resembles the members of the *C. aureum* complex but differs in not having distinct lacerate column wings and by having a distinctly pandurate lip lamina, and is described here.

Taxonomic treatment

Cyrtochilum panduratum Dalström & D.Trujillo, ***sp. nov.*** (Fig. 2–4).

TYPE: Peru. Ancash: Yungay; Huascarán National Park, Lago Llanganuco area on rocky slopes, 3800 m, 20 Nov. 1998, *M. León* & *B. Collantes 2825* (Herbarium Bennettianum 7848) (holotype: MOL).

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FIGURE 1. *Cyrtochilum cochleatum* from Peru, Dept. La Libertad. Photo by D. Trujillo.

DIAGNOSIS: *Cyrtochilum panduratum* is distinguished from the species in the superficially similar *C. aureum* complex by the combination of the wingless column and the distinctly pandurate lip lamina.

Terrestrial or lithophytic *herb*. *Roots* thick, *ca*. 3–4 mm in diameter. *Pseudobulbs* caespitose, ovoid elongate, *ca*. 9 × 3 cm, bifoliate or trifoliate, surrounded basally by 5 to 6 distichous sheaths, the uppermost foliaceous. *Leaves* subpetiolate, conduplicate, elliptic to obovate-lanceolate, obtuse apiculate,19–28 × 1.5–2.0 cm. *Inflorescence* erect, an almost straight to indistinctly flexuous panicle with many widely spaced, indistinctly flexuous 2–10 cm long side-branches, total length 118 cm long [according to the original collector's notes]. *Inflorescence bracts* and *floral bracts* similar, scale-like, appressed, acute, 2–15 mm long. *Pedicel* with *ovary* 8–15 mm long. *Flowers* golden yellow; *dorsal sepal* unguiculate, obovate, indistinctly canaliculate, apiculate with 5 veins, *ca*. 8 × 5 mm; *lateral sepals* fused for *ca*. 1/3 of their length then spreading, unguiculate, elliptic to indistinctly ovate and slightly oblique, indistinctly canaliculate, apiculate, with 5 veins, *ca*. 10 × 4 mm; *petals* shortly unguiculate, obliquely ovate, *ca.* 7 × 4.5–5.0 mm, with 5 veins; *lip* rigidly attached to the base of the column, unguiculate, broadly cuneate, pandurate, retuse, *ca*. 10–11 × 6 mm; *callus* with a fleshy elevated central ridge emerging at the base of the lip, extending to the constricted part of the lip lamina, consisting of a pair of parallel basal ridges, diverging into spreading rounded angles, with an intermediate pair of blunt and fleshy angles apically, and with a smaller, blunt

Figure 2. Holotype of *Cyrtochilum panduratum* Dalström & D.Trujillo, *M. León M.* & *B. Collantes M. 2825* (MOL). Photo by D. Trujillo.

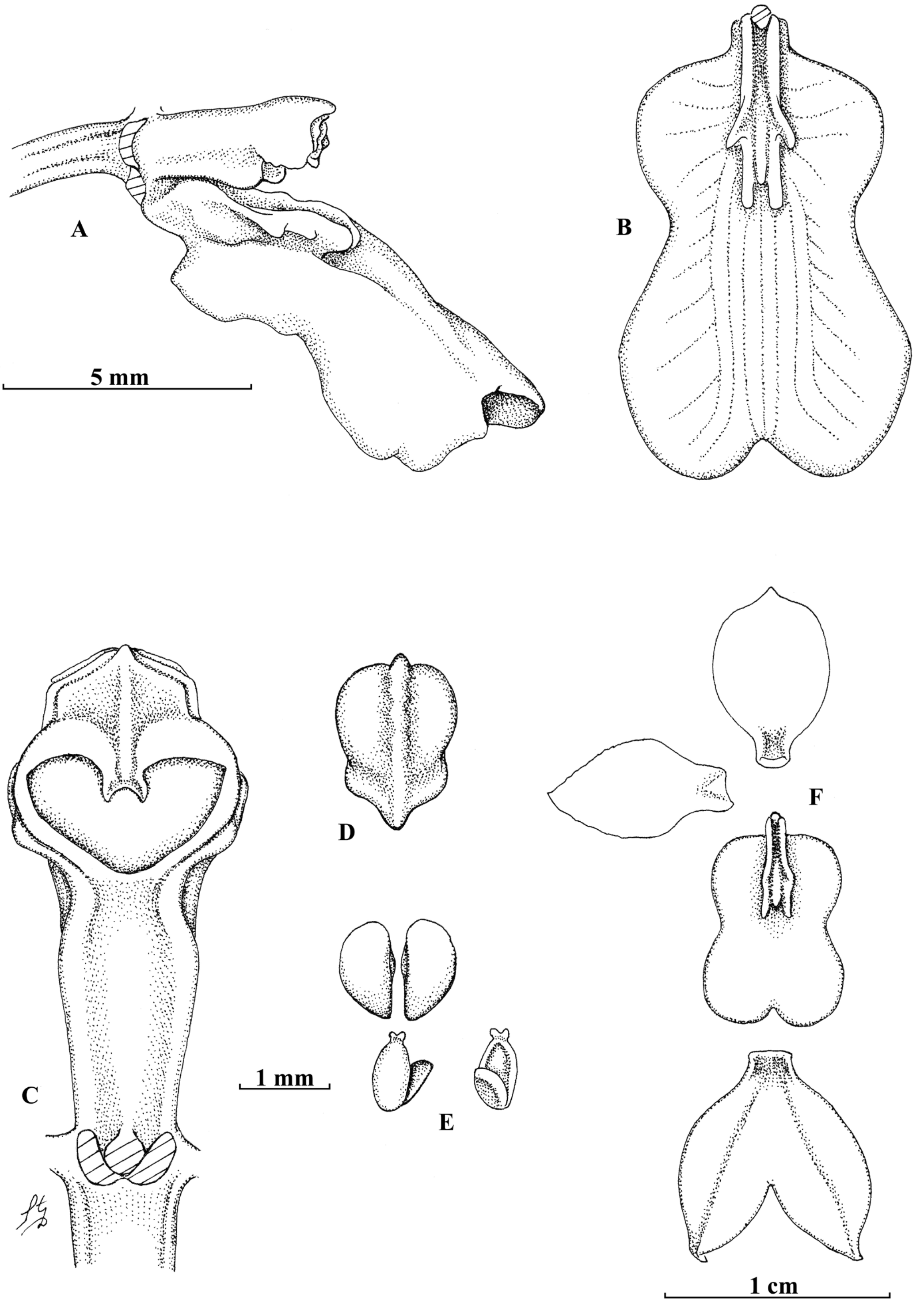

FIGURE 3. *Cyrtochilum panduratum*. A. Column and lip lateral view. B. Lip frontal view. C. Column ventral view. D. Anther cap. E. Pollinarium. F. Dissected flower. Drawn from the holotype by Stig Dalström.

angle in between, *ca*. 4 mm long. *Column* clavate, straight, dorsally ridged, ventrally canaliculated with distinct apical angles below the stigmatic surface, and with a pair of apical more or less rounded wings, *ca*. 4 mm long; *anther cap* campanulate with a dorsal ridge, shortly rostrate; *pollinarium* of two obovoid, cleft/folded pollinia on a *ca.* 1 mm long ovate and strap-like stipe, longitudinally indistinctly revolute along the edges, on a pulvinate viscidium.

Paratype: No additional material seen.

Distribution: *Cyrtochilum panduratum* is only known from the type location, at high elevation rocky slopes in the area surrounding the Llanganuco lakes located in the Huascarán National Park, Department of Ancash, Peru.

Etymology: Named in reference to the pandurate lip lamina.

From a floral morphology aspect *Cyrtochilum panduratum* does not seem to have any close relatives that it can be confused with. The combination of the distinctly pandurate lip lamina with a front lobe wider than the rounded basal lobes combined, together with the partially fused lateral sepals easily distinguish it from other yellow small-flowered species such as C. *longipes* Rchb.f. and *C. soennemarkii* Dalström, which have basal lobes wider than the front lobe. In overall morphological features *Cyrtochilum panduratum* is superficially similar to the yellow-colored *C. cochleatum*, *C. mystacinum* and *C. rigidum* but differs in not having any kind of apical column wings, versus large, lacerate and downward directed wings for the latter species.

Figure 4. Close-up of a flower of *C. panduratum*, preserved in alcohol at MOL. Photo by D. Trujillo.

Acknowledgments. We thank the staff and curators of MOL for allowing access to their collections and permitting using photographs thereof for this article. Author Trujillo sincerely thanks Diana Bennett and Richard Bennett for access to David Bennett's notes and records. We also thank Wesley Higgins for viewing and commenting on the manuscript.

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NEW COMBINATIONS IN *CYRTOCHILUM* (ORCHIDACEAE: ONCIDIINAE)

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Abstract. Two species, originally described as *Odontoglossum amphiglottis* and *Odontoglossum sodiroi* are here transferred to *Cyrtochilum* (Orchidaceae: Oncidiinae), based on molecular data and morphological features, accompanied by brief discussions about their taxonomic background and natural history, and illustrated with line drawings and photographs. Both species were previously considered to be synonyms of *C. ramosissimum* by the author of this paper and others, due to limited access of type material. But recent field discoveries reveal that a much stouter growth habit and distinctly corymbose inflorescences distinguish *O. amphiglottis* from *C. ramosissimum*. In the case of *O. sodiroi* the smoothly rounded pair of basal lip calli, which are marginally covered externally by the ventral flanks of the column, distinguish this species from *C. ramosissimum*, which has more or less denticulate basal callus keels on the lip, and where the ventral columnar flanks are marginally placed between these keels.

Key Words: *Cyrtochilum*, new combinations, Oncidiinae, Orchidaceae

Introduction. The genus *Cyrtochilum* Kunth never stops presenting surprises. Striking, as well as insignificant new species appear in a seemingly never-ending flow. Some new names turn out to be synonyms of older ones, and some older synonyms turn out to represent distinct species as we go back and re-study the type specimens, decipher obscure descriptions and compare them with freshly made field observations.

John Lindley (1852) described *Odontoglossum ramosissimum* Lindl. (Fig. 1–3), from a plant collected in Venezuela by Jean Linden (*J. Linden 661*, K-L, W). Lindley placed the plant in *Odontoglossum* Kunth, based primarily on the flower morphology. The shape of the column and the lip were early on considered important taxonomic features, and in *Odontoglossum* the lip and column were supposed to be “parallel” (in a very broad sense). But then it was discovered that many not closely related plants also produce flowers with a similar shape. In addition, not all true *Odontoglossum* species share the same lip-column structure and angle. Based on a broader morphological concept, including vegetative features in combination with molecular data (Williams *et al*. 2001), *O. ramosissimum* together with many closely related species were transferred to *Cyrtochilum* in a synopsis of the genus (Dalström 2001). In this synopsis *O. amphiglottis* Rchb.f. (originally described as “*amphiglottii*”, which is believed to be a misspelling) and *Odontoglossum sodiroi* Schltr., were included as synonyms of *C. ramosissimum*.

In the case of *Odontoglossum sodiroi*, Rudolf Schlechter based his description on two different Ecuadorean collections made by the Jesuit priest Luis Sodiro (1836–1909), which unfortunately represent two different species; *L. Sodiro 112* (BR) = *Cyrtochilum angustatum* (Lindl.) Dalström (Fig.4), and *L. Sodiro 23b* (Fig. 5–7) represents what has long been known as *Odontoglossum*, and later *Cyrtochilum ramosissimum* in horticulture and various literature (Bockemühl 1989, Dalström 2001, 2010). After more detailed analysis of type specimens and other collections it has become clear that *L. Sodiro 23b*, which is distributed from Colombia to central Peru, in fact represents a distinct species that is different from the typical *C. ramosissimum*, which is only reported from Venezuela. The main difference being that the basal and ventral flanks of the column lay on the outside of the basal lip callus in *O. sodiroi*, versus being held between the basal callus keels in *C. ramosissimum*.

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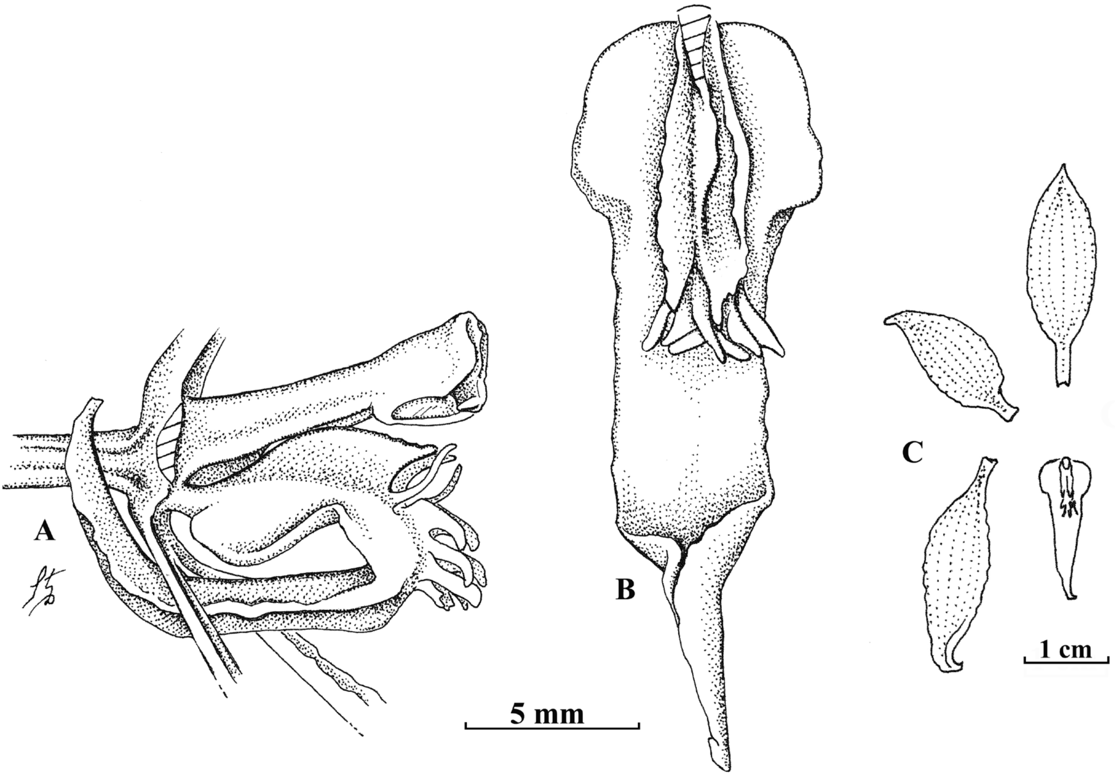

FIGURE 1. *Cyrtochilum ramosissimum* (Lindl.) Dalström. A. Lip column lateral view. B. Lip dorsal view. C. Flower dissected. Drawn from *N. Funck & L. J. Schlim 1041* (W) by Stig Dalström.

FIGURE 2. *Cyrtochilum ramosissimum*. Plant from Venezuela. Photo by Carlos Jerez.

The basal and dominating callus keels in *O. sodiroi* emerge abruptly near the base of the lip and are perfectly smooth and rounded (Fig. 6–7), as opposed to emerging more gradually at a short distance from the base of the lip and being more or less denticulate near the apex in *C. ramosissimum*. But because *L. Sodiro 112* is listed first in Schlechter's description it was originally considered by Dalström (2001, 2010) that it would represent the type of *O. sodiroi*, which therefore constitutes a synonym of *C. angustatum*. The *L. Sodiro 23b* specimen would then become a new species. But Schlechter mentions in the type description that *O. sodiroi* is similar to *O. ramosissimum*, and may be the same thing, but that it differs in the base of the lip not being cordate (heart-shaped), and the callus

→ Right, Figure 3. *Cyrtochilum ramosissimum*. Plant from Venezuela. Photo by Leonore Bockemühl, provided by Karlheinz Senghas.

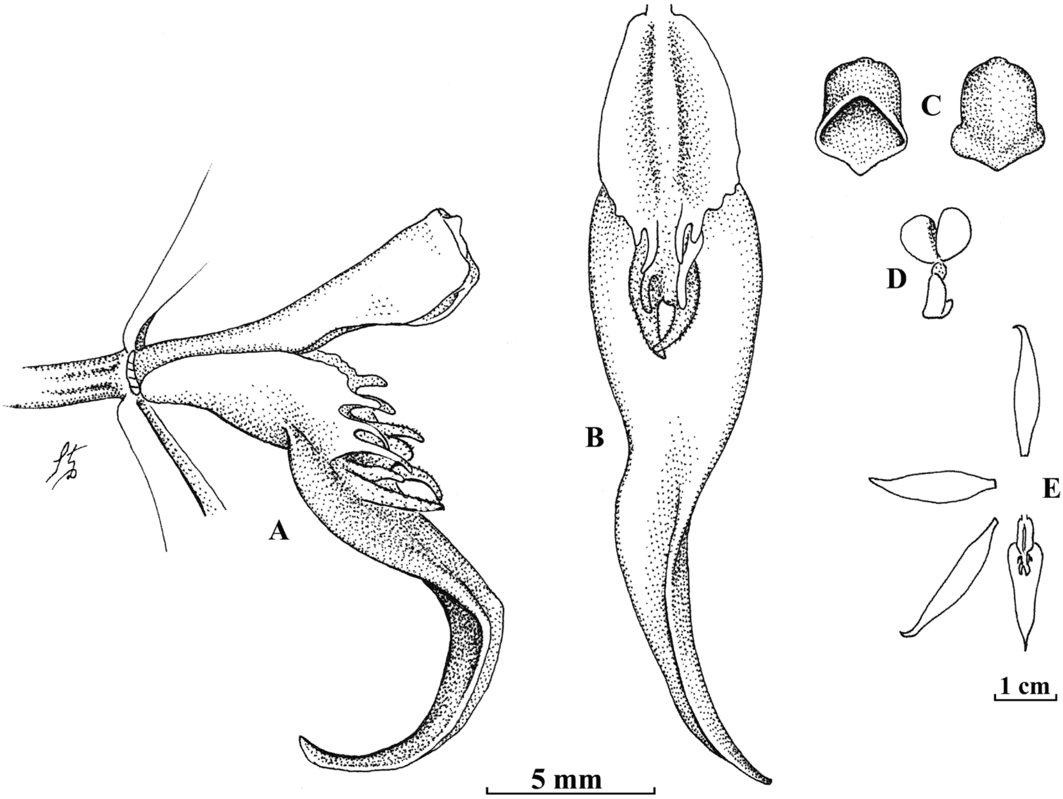

Figure 4. *Cyrtochilum angustatum* (Lindl) Dalström. A. Lip column lateral view. B. Lip dorsal view. C. Anther cap, front and back views. D. Pollinarium. E. Flower dissected. Drawn from *L. Sodiro 112* (BR) by Stig Dalström.

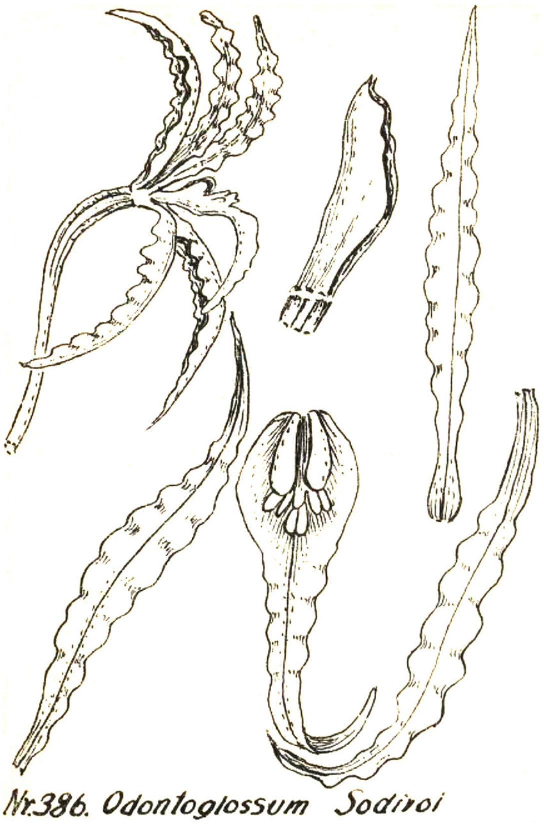

Figure 5. *Cyrtochilum* (as "*Odontoglossum*") *sodiroi* (Schltr.) Dalström. Based on *L. Sodiro 23b*, published in Repert. Spec. Nov. Regni Veg., Beih. 57, plate 99, no. 386 (1929).

being glabrous and shaped differently. This is a rather confusing statement because the spreading basal lobes of the lip of the true *C. ramosissimum* from Venezuela are not cordate, but hastate to indistinctly cuneate. The highly variable lip base of what *L. Sodiro 23b* represents in the wild, on the other hand, has spreading side-lobes that can be cordate as well as hastate to cuneate, and is often virtually identical with the illustration of *O. sodiroi* in Schlechter ex Mansfeld (1929) (Fig. 5). The basal lobes of the lip of *C. angustatum* (*L. Sodiro 112*), (Figs. 4, 9), on the other hand, are erect and fused to the lip callus and structurally very different from both *L. Sodiro 23b* and *C. ramosissimum*. The lip callus is also pubescent as opposed to glabrous for the other two. It is therefore unclear why Schlechter mentions that the callus of *O. sodiroi* differs from *C. ramosissimum* by not having a cordate lip base (which *L. Sodiro 23b* in fact sometimes has, but not so for *C. ramosissimum*). Schlechter also points out that the lip callus for his "*O. sodiroi*" is glabrous thus suggesting that the lip callus of *C. ramosissimum* is not, when in fact they both have glabrous lip calli. The only member of this triad that has a pubescent lip callus is *C. angustatum* (hence *L. Sodiro 112*). In order to straighten out this confusion it seems therefore reasonable and pragmatic to accept *L. Sodiro 23b* as what Schlechter envisioned as being *O. sodiroi* and treat it as a distinct species that needs to be transferred to *Cyrtochilum*. The name "*sodiroi*" has been used in publications for this taxon (Dodson & Dodson 1984), and is currently becoming more and more used by people, so it is proposed here to attach this name permanently for this species. The transfer to *Cyrtochilum* is made below.

In the synopsis of the genus *Cyrtochilum* (Dalström 2001) it is mentioned that *Odontoglossum amphiglottis* (Fig. 8, 10) differs from *C. ramosissimum* by a smaller habit with fewer flowers on a shorter inflorescence, differences which do not justify specific status. This statement was based on studies of the poor and insignificant type specimen of *O. amphiglottis* in the Museum of Natural History in Vienna (*B. Rözl s.n.* W). However, this statement could not be farther from the truth. Recent observations of living plants in the field by Fernando Ramírez, Esteban Domínguez, Sebastián Moreno, Sebastián Vieira and others show a very large and sturdy-looking plant that displays the distinguishing floristic features of *O. amphiglottis*, and is also coming from the same general area as the type collection (Fig. 10). The straight and erect corymbose inflorescence of *O. amphiglottis* differs from the generally laxly arching and loosely branched inflorescence of the sympatric *O. sodiroi* (Fig. 11). The flowers of the former appear to be consistently rosy purple, while the flowers of the latter vary considerably from white to pale yellow with purple to brown spots and stripes (Fig. 7, 11–12). It is therefore concluded here that these species represent distinct taxa, and that they both need to be authoritatively transferred to *Cyrtochilum*, which is made below.

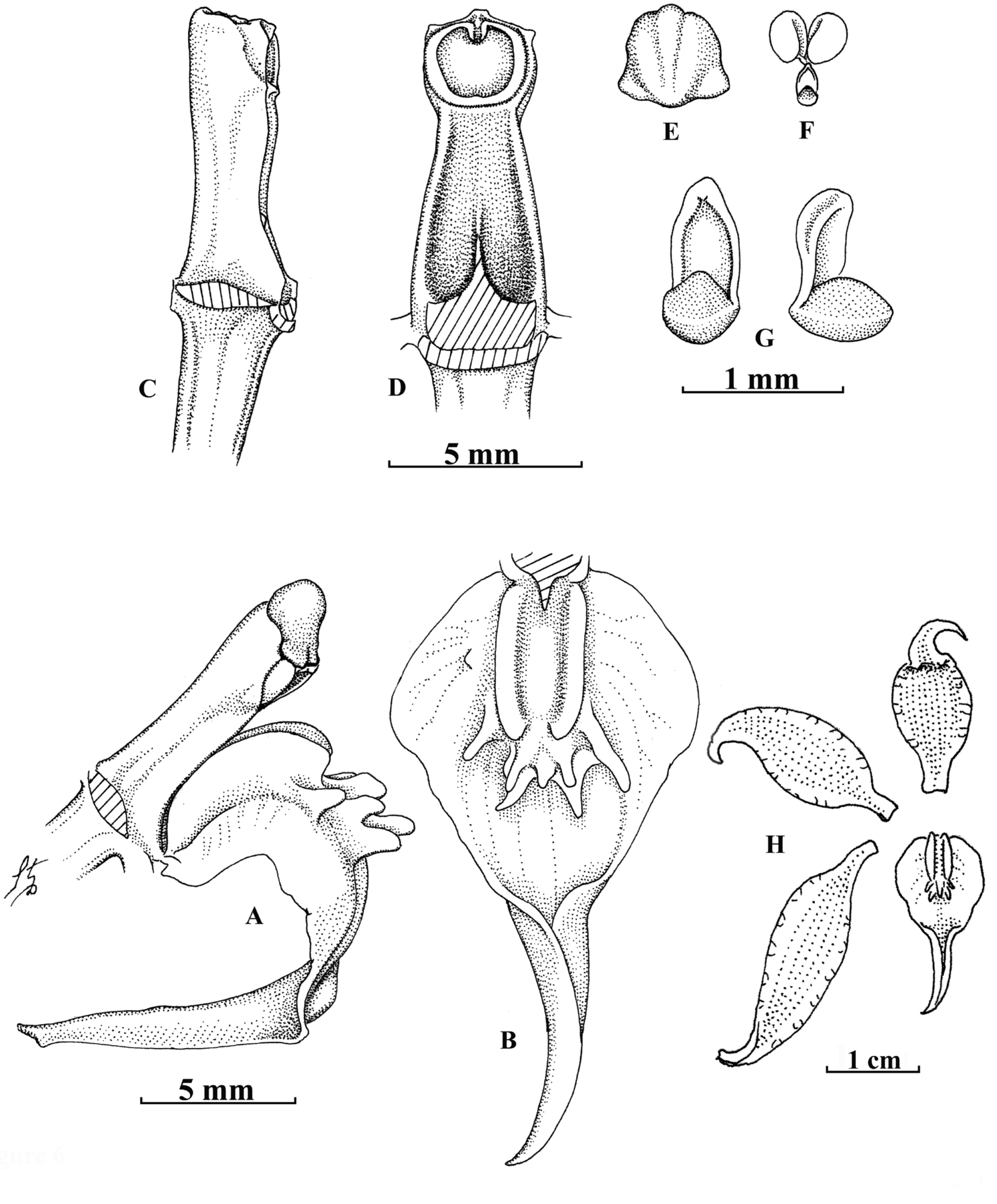

Figure 6. *Cyrtochilum sodiroi* (Schltr.) Dalström. A. Lip column lateral view. B. Lip dorsal view. C. Column lateral view. D. Column ventral view. E. Anther cap front view. F. Pollinarium back view. G. Stipe and viscidium front and back views. H. Flower dissected Drawn from *S. Dalström 606* (SEL) by Stig Dalström.

Figure 7. *Cyrtochilum sodiroi* (Schltr.) Dalström. Plant cultivated and photographed by Guido Deburghgraeve (*G. Deburghgraeve 410*).

Taxonomic treatment

Cyrtochilum amphiglottis (Rchb.f.) Dalström, ***comb. nov.*** *Odontoglossum amphiglottis* Rchb.f., Linnaea 41. 1877. TYPE: Colombia, Medellín, *B. Rözl s.n.* (holotype: W). Fig. 8, 10.

Cyrtochilum sodiroi (Schltr.) Dalström, ***comb. nov.*** *Odontoglossum sodiroi* Schltr., Repert. Spec. Nov. Regni. Veg. Beih. 8: 101. 1921. SYNTYPES: Ecuador. Pichincha: in silvis subandinis montis Corazon-Miligally, *L. Sodiro 112* (BR); Ecuador. [Napo:] in silvis subandinis Andium Orientalium supra Oyacachi, *L. Sodiro 23 b* (holotype: B, probably destroyed; illustration of the type, **selected here as the lectotype**: *Odontoglossum sodiroi.* Repertorium Specierum Novarum Regni Vegetabilis, Beihefte 57**,** plate 99, no. 386. Fig. 5–8, 11–12.

The Biblioteca Ecuatoriana Aurelio Espinosa Pólit (QPLS) in Quito, Ecuador, hosts a number of Sodiro's specimens, which have not been seen by the author. If an isotype of *Sodiro 23 b* would possibly be found there, this would supersede the present lectotypification.

Acknowledgments. I thank the curators of the following herbaria for allowing access to important specimens: AMES, BR, BM, COL, F, HEID, JAUM, K, K-L, M, MO, MOL, NY, QCA, S, SEL, US, USM and W. I also thank Guido Deburghgraeve for his help with the introduction to the herbarium in Meise, and Wesley Higgins for viewing the manuscript.

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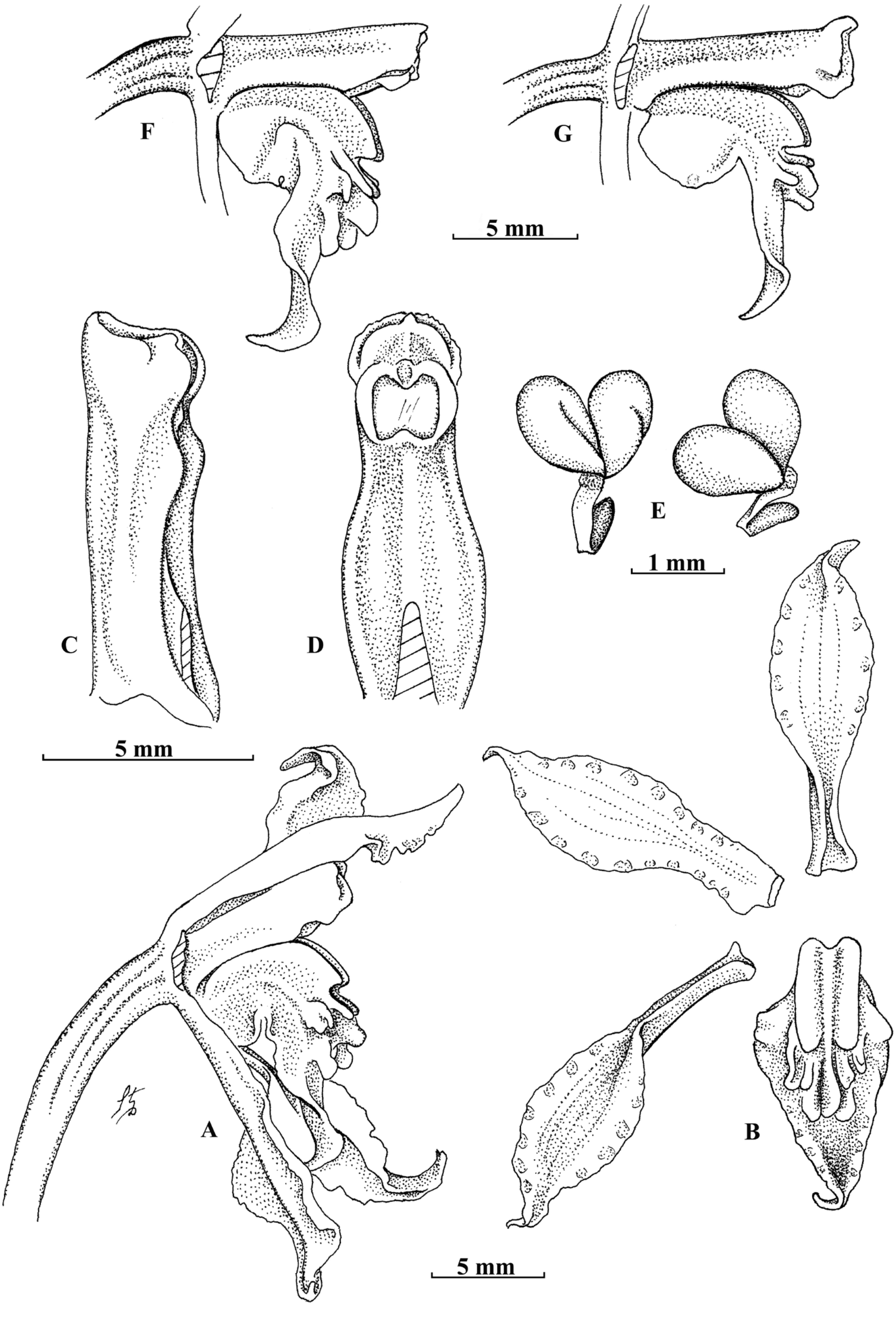

Figure 8. *Cyrtochilum amphiglottis* (Rchb.f.) Dalström. A. Flower with right petal removed, lateral view. B. Flower dissected. C. Column lateral view. D. Column ventral view. E. Pollinarium front-angled and back-angled views. F. Column lip lateral view. Drawn from *Schmidtchen s.n.* (W) by Stig Dalström. G. Lip column lateral view. Drawn from *B. Rözl s.n.* (W) by Stig Dalström.

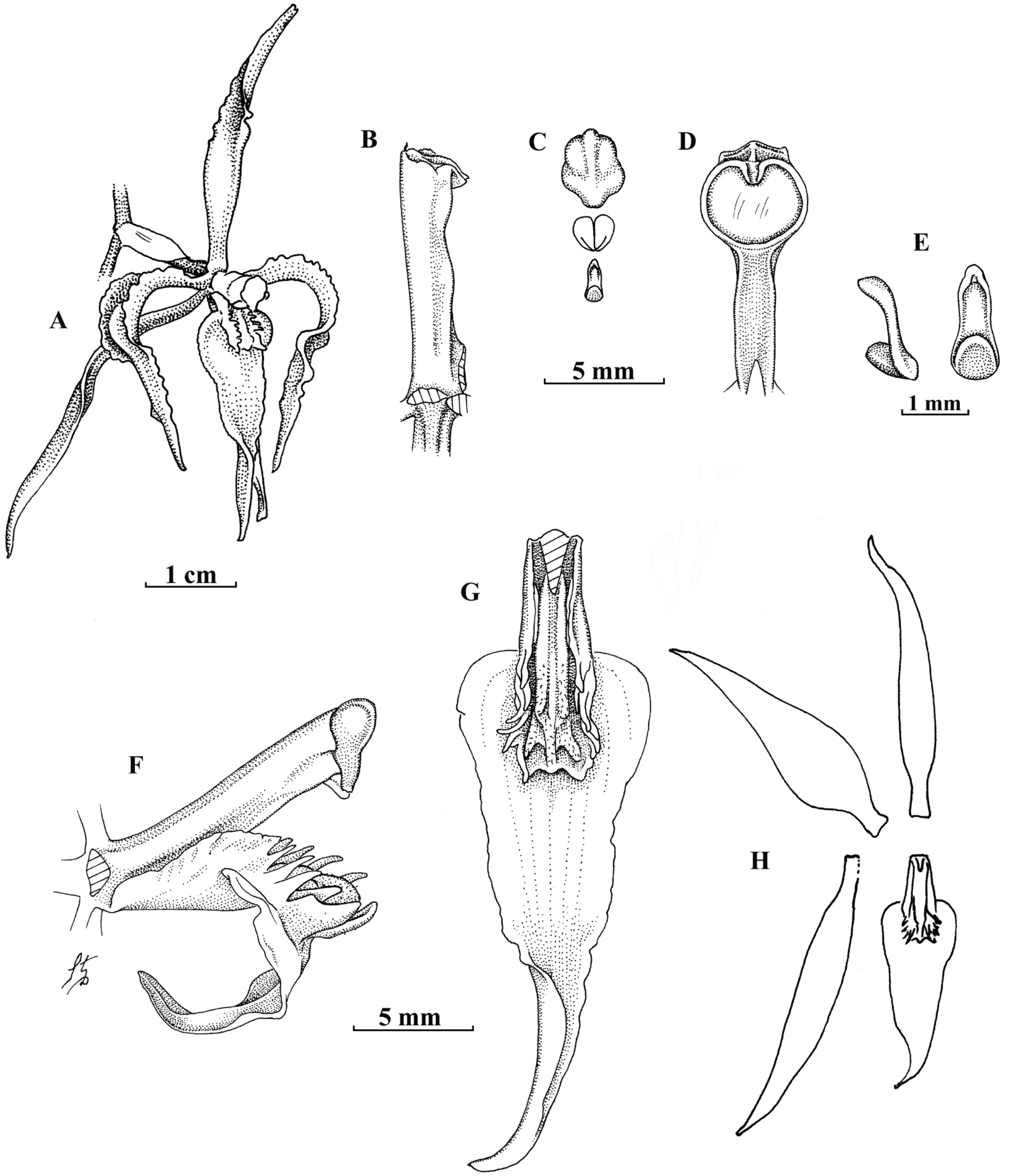

FIGURE 9. *Cyrtochilum angustatum* (Lindl.) Dalström. A. Flower front-angled view. B. Column lateral view. C. Anther cap front view and pollinarium back view. D. Column ventral view. E. Stipe and viscidium lateral and back views. F. Lip and column lateral view. G. Lip dorsal view. H. Flower dissected. Drawn from *S. Dalström 001* (SEL) by Stig Dalström.

Figure 10. Plant of *Cyrtochilum amphiglottis* (Rchb.f.) Dalström, from the natural habitat in the department of Antioquia, Colombia. Photo by Esteban Dominguez.

Figure 11. *Cyrtochilum sodiroi* (Schltr.) Dalström. Plant cultivated and photographed by Jan Sönnemark.

Figure 12. *Cyrtochilum sodiroi* (Schltr.) Dalström. Flower photographed by Gilberto Escobar (*G. Escobar 809*).

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NEW RECORDS OF *BULBOPHYLLUM* IN THE FLORA OF VIETNAM

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ABSTRACT. *Bulbophyllum leysianum* (sect. *Hyalosema*) and *B. ovalifolium* (sect. *Macrocaulia*) are recorded as new for the flora of Vietnam. Both species originate from the Hon Ba Nature Reserve (Khanh Hoa Province, Vietnam). The first was found as an epiphyte on fallen branch in riparian lowland forest, while the second was recorded on small branches in a canopy tree at about 1500 m a.s.l.

KEY WORDS: *Bulbophyllum* sections, Epidendroideae, Orchidaceae, Vietnam

Introduction. The genus *Bulbophyllum* Thouars is one of the largest plant genera, with well over 2000 species in the tropics and subtropics worldwide (Pridgeon *et al*. 2014). So far, about 145 species in 16 sections have been recorded for Vietnam (Averyanov & Averyanova 2003, Nong & Averyanov 2015, Averyanov *et al*. 2016, Truong & Sridith 2016, Averyanov *et al*. 2017, Nguyen & Averyanov 2017, Averyanov *et al*. 2018, Truong *et al*. 2019a, 2019b, 2019c, Averyanov *et al*. 2019a, 2019b).

During the botanical survey in Hon Ba Nature Reserve (Khanh Hoa Province, Vietnam), we found two *Bulbophyllum* species not previously reported from Vietnam, *B. leysianum* Burbidge, of section *Hyalosema* and *B. ovalifolium* Lindl., of section *Macrocaulia*.

Materials and methods. We took photos of the plants with a Canon 600D fitted with an EF–S 60mm f/2.8 Macro USM lens. We preserved material of both species in 70% ethanol; the samples are stored in VNM (Institute of Tropical Biology Herbarium). The descriptions below are based on the plants found in Hon Ba Nature Reserve. Terminology for the morphological description follows Beentje (2012) and Vermeulen *et al*. (2015).

TAXONOMIC TREATMENT

Bulbophyllum leysianum Burb., J. Roy. Hort. Soc. 17.1895; Vermeulen *et al*., Orchid of Borneo: 607, fig. 726, 727, 728. 2015. (Fig. 1).

TYPE: Malaysia. Sabah: Sinaroup Village, *Burbidge s.n.* (not seen).

Long-creeping *epiphyte*. *Rhizome ca*. 3 mm in diameter, sections between pseudobulbs 0.6–1.5 cm long. *Pseudobulbs* 1–leafed, glossy green, obliquely ovoid, 1.1–2.0 cm long, 0.5–1.3 cm wide. *Leaves* green, thick, narrowly elliptic, 6–9 cm long, 2.2–3.0 cm wide, apex obtuse; petiole *ca*. 2 cm long. *Inflorescence* from mature pseudobulbs, 1-flowered, *ca*. 18 cm long, peduncle *ca*. 7.4 cm long, bracts 4, 3–8 mm long, apex acute. *Flowers* slightly to moderately opening, dorsal sepal white, suffused pink, greenish around the midvein and towards the base, lateral sepals white, suffused pink, petals white with green veins and pink appendage, lip white, suffused pink, slightly greenish abaxially, and with patches of pink–red spots; pedicel plus ovary *ca*. 2.7 cm long, floral bract tubular, *ca*. 11 mm long. *Dorsal sepal* concave towards the base, narrowly ovate, *ca*. 5.5 cm long, 1.6 cm wide, apex rounded and mucronate, margins entire, ciliolate, surface almost glabrous, abaxially keeled along the midvein; *ca*. 12–veined. *Lateral sepals* loosely adherent along the lower margins, upper margin curved inwards until it (almost) touches the upper margin of the opposite sepal, flat, obliquely ovate–triangular, clawed, 4.0–4.2 cm long, 7–8 mm wide above the claw, apex acute, margins entire; glabrous, *ca*. 10-veined. *Petals* obliquely elliptic, *ca*. 7 mm long, 2 mm wide, apex with a *ca*. 3 mm long filiform processus ending with a globular, warty apex;

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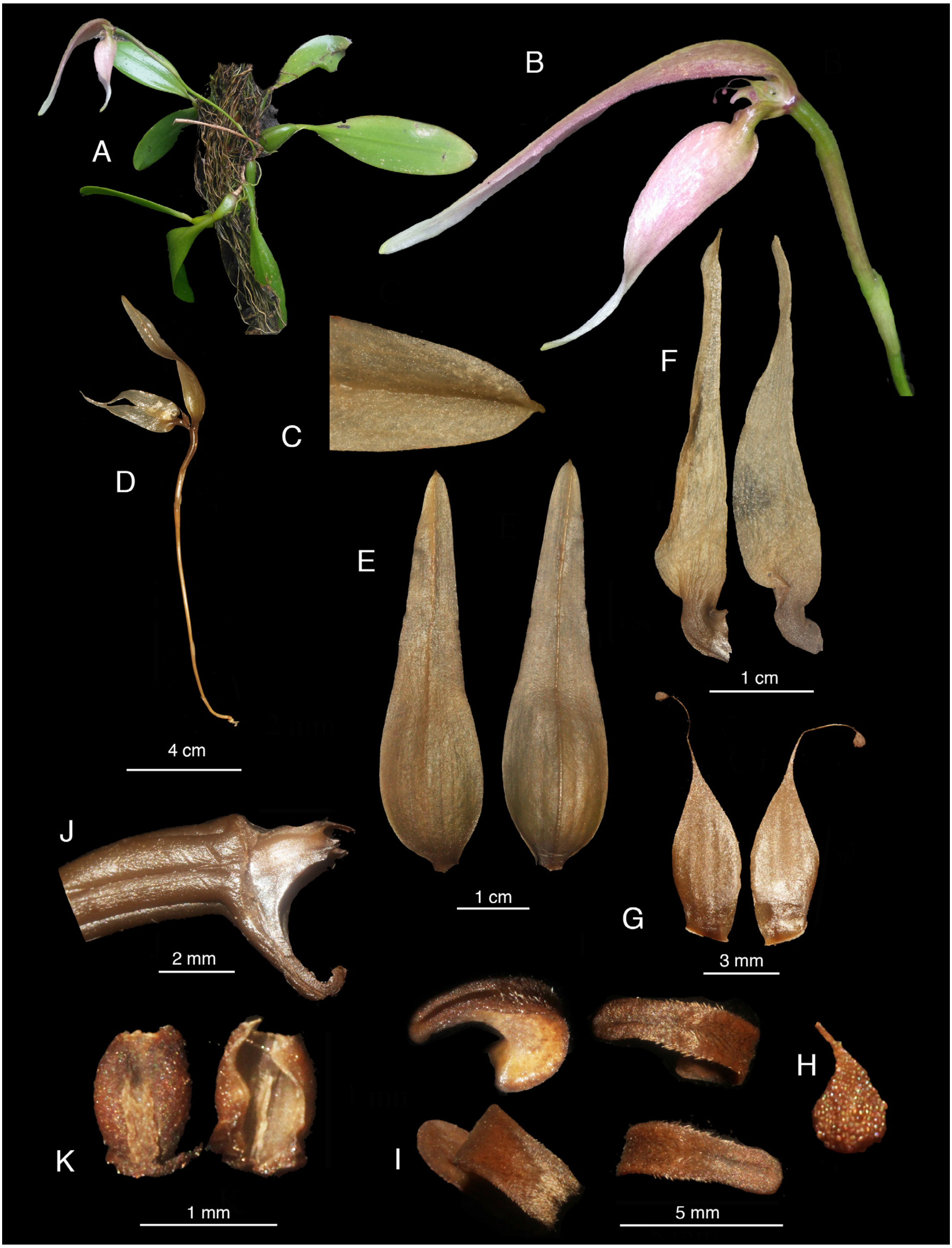

FIGURE 1. *Bulbophyllum leysianum* Burb. **A**. Flowering plant. **B**. Flower, side view. **C**. Leaf apex. **D**. Flower with pedicel and peduncle. **E**. Dorsal sepal. **F**. Lateral sepals. **G**. Petals. **H**. Warty apex of petal. **I**. Labellum, different views. **J**. Column. **K**. Anther cap. (TRUONG B.V., *BV 340*, Hon Ba, Khanh Hoa province, Vietnam). Photos by Truong B. V., 2018.

margins entire; glabrous, 3-veined. *Labellum* fleshy, curved in lateral view, (elliptic-) oblong, *ca*. 5 mm long, 1.8 mm wide, apex rounded, margins ciliate, adaxially slightly concave towards the base, elsewhere with a shallow median furrow which ends just short of the slightly convex apex, surface almost glabrous towards the base, elsewhere hirsute, shorter so towards the apex, abaxially almost glabrous, abaxially with a distinct keel, surface glabrous. *Column ca*. 2.8 mm long; stelidia short, triangular, acute, with a small, antrorse, acute tooth along the upper margin, and deltoid, obtuse tooth along the lower margin. *Anther cap* with the frontal margin drawn out into a widely rounded beak.

Ecology and phenology: Epiphyte on small branches along a stream over granite bedrock, *ca*. 400 m a.s.l. Very rare in Hon Ba reserve. Flowering in November.

Distribution: Peninsular Malaysia, Borneo, Java. This species has been known only from Malesia and hence its occurrence in Vietnam shows a very disjunct distribution range.

Material examined: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 8 November, 2018, *Truong Ba Vuong, Mang Van Lam BV 340* (VNM 00023601); Khanh Hoa Province, Hon Ba Nature Reserve, 19 February, 2019, *Truong Ba Vuong, Mang Van Lam BV 350* (VNM 000023603)

This species may be confused with *B. antenniferum* (Lindl.) Rchb.f., which has been reported from Peninsular Malaysia and peninsular Thailand (Vermeulen *et al.* 2015). *Bulbophyllum leysianum* differs by the nearly oblong, adaxially coarsely hirsute labellum of less than 2 mm wide (*vs*. labellum ovate-triangular, adaxially finely hirsute only in the centre and otherwise glabrous, and more than 2 mm wide in *B. antenniferum*).

Bulbophyllum ovalifolium (Blume) Lindl., Gen. Sp. Orchid. Pl.: 49. 1830; Seidenf. & Wood, Orch. Malay. Sing.: 513, fig. 234, a-j. 1992; Vermeulen *et al*., Orchid of Borneo: 552, fig. 669, 670, 671, 672, 673. = *Diphyes ovalifoli*a Blume, Bijdr. Fl. Ned. Ind.: 318. 1825. = *Phyllorkis ovalifolia* (Blume) Kuntze, Revis. Gen. Pl. 2: 677. 1891. (Fig. 2)

TYPE. Indonesia: Java, Mount Gedeh, *Blume 509, HLB 902, 322-463* (L! L0058263)

Small, creeping, *epiphyte* on branches orchid. *Rhizomes ca*. 0.5 mm in diameter. *Pseudobulbs* 1-leafed, ovoid, narrowly elliptic, *ca*. 6 mm long. *Leaves* 1-leafed, elliptic *ca*. 9.5 mm long, 5 mm wide, obtuse, petiole *ca*. 1.5 mm long. *Inflorescence* orange color, from base of mature pseudobulbs, 1-flowered, *ca*. 1.5 cm long, usually from pseudobulbs without leaves, floral bract, tubular, *ca*. 1 mm long. *Flower* open widely, tepals orange, labellum red, column red. *Dorsal sepals* elliptic, *ca*. 3 mm long, 2 mm wide, subacute and minutely apiculate, margins entire, glabrous, 5-veined. *Lateral sepals* elliptic, *ca*. 4.5 cm long, 2.5 cm, rounded, margins entire, glabrous, 5-veined. *Petals* elliptic, *ca*. 1.5 mm long, 0.5–0.6 mm wide, obtuse, margins entire, glabrous, 1-veined. *Labellum* simple, attached to the column foot by white ligament, recurved proximally, elliptic, *ca*. 2.1 mm long, 1.2 mm wide, margin distally folded downward, abaxial surface concave and glabrous proximally, convex and coarsely verruculose distally. *Column ca*. 0.3 cm long, column foot *ca*. 1 mm long, stigma elliptic; stelidia ca. 0.8 mm long, triangular subulate, slightly downwards curved, acute. *Pollina* 4. *Anther cap* not seen.

Ecology and phenology: Epiphyte on small branches in a canopy tree, *ca*. 1500 m a.s.l. Very rare in Hon Ba Nature Reserve. Flowering in June.

Distribution: China (Yunnan), Thailand, Peninsular Malaysia, Borneo (Sabah, Sarawak), Sumatra, Java, Flores, Sulawesi.

Material examined: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 21 June, 2019, *Truong Ba Vuong, Tu Bao Ngan & Mang Van Lam, BV 378* (VNM 00023604)

Very variable in flower size and color, and lip shape. The adaxial surface of the lip varies from smooth to coarsely verruculose. Specimens from Vietnam have a verruculose lip; these differ from *Bulbophyllum catenarium* Ridl. (Fig. 3) by the general outline (without spreading) of the lip: elliptic to ovate in *B. ovalifolium*, *versus* triangular proximally and globose distally in *B. catenarium*.

Bulbophyllum ovalifolium is similar to *Bulbophyllum moniliforme* C.S.P.Parish & Rchb.f. but the latter has spherical (slightly inflated) bulbs

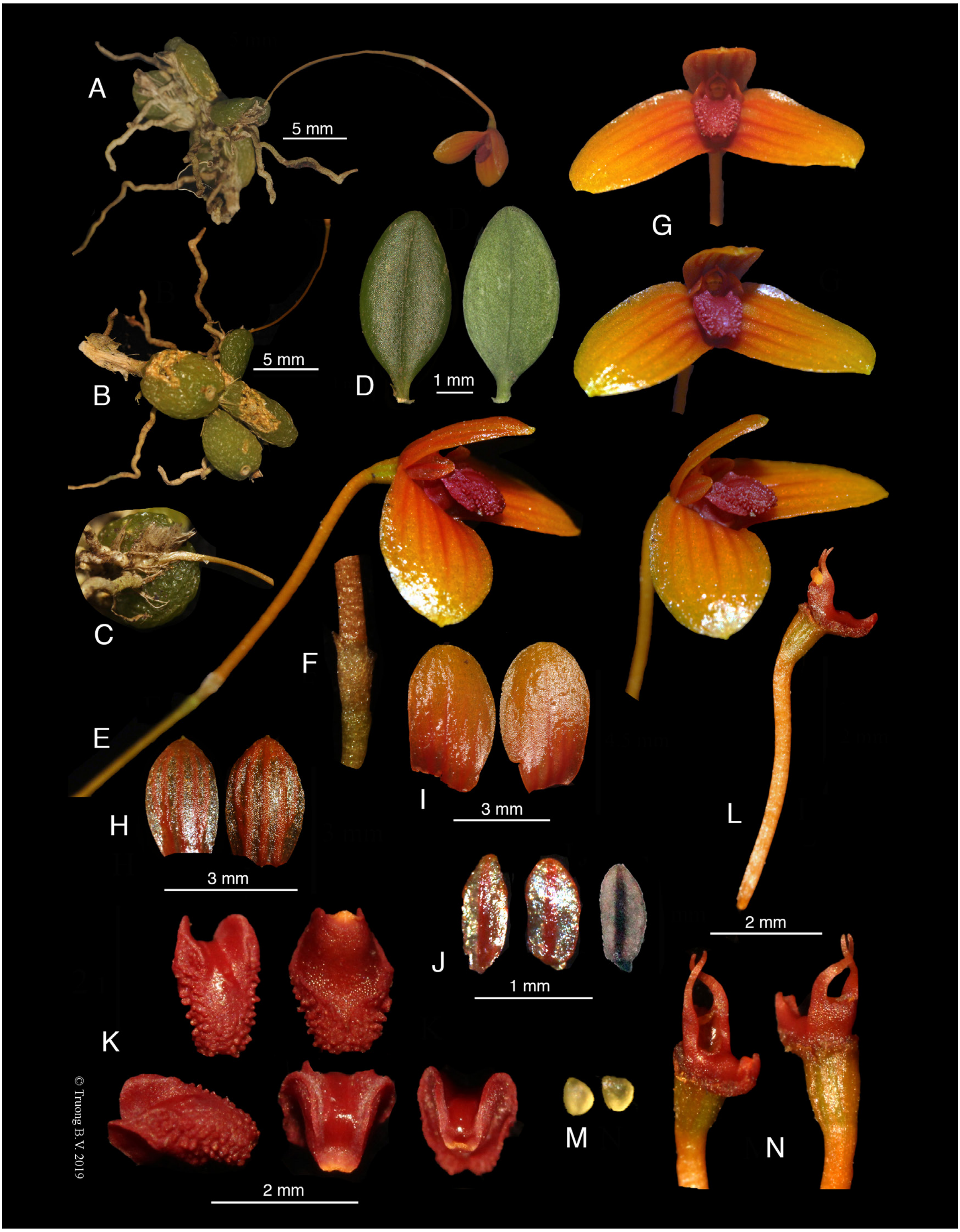

FIGURE 2. *Bulbophyllum ovalifolium* (Blume) Lindl. **A**. Flowering plant. **B**. Pseudobulbs. **C**. Base of inflorescence arising from pseudobulb. **D**. Leaves. **E**. Flower with pedicel and peduncle. **F**. Floral bract. **G**. Flower, different views. **H**. Dorsal sepal. **I**. Lateral sepals. **J**. Petals. **K**. Labellum, different views. **L**. Column with pedicel. **M**. Pollinia. **N**. Column and stelidia. (*Truong Ba Vuong, Tu Bao Ngan & Mang Van Lam, BV 378*, Hon Ba, Khanh Hoa Province, Vietnam). Photos by Truong B.V., 2019.

Figure 3. *Bulbophyllum catenarium* Ridl. (*Vermeulen 4329*, Malaysia, Sabah, Crocker Range, G. Alab, 2000 m a.s.l.). Photo by Jaap J. Vermeulen.

which do not usually bear leaves in flowering season. However, *B. ovalifolium* has slightly inclined ovate bulbs usually bearing leaves while flowering. Labellum in *B. moniliforme* has smooth surface bearing very inconspicuous side lobes, however it is rough or warted on the upper surface in *B. ovalifolium* with very distinct side lobes.

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ENDOPHYTIC FUNGI ASSOCIATED WITH ROOTS OF EPIPHYTIC ORCHIDS IN TWO ANDEAN FORESTS IN SOUTHERN ECUADOR AND THEIR ROLE IN GERMINATION

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Abstract. Orchids are known to establish complex relationships with endophytic fungi throughout their life cycle, and particularly during germination of their reserves-deprived seeds. Characterizing generalist or specialist interactions between orchids and associated fungi is key to supporting orchid conservation efforts. Here, endophytic fungi associated with roots of epiphytic orchids were studied in two montane Andean forests in Southern Ecuador. Orchid root samples were collected from ten ~500 m^2 plots distributed between two neighboring forests. Endophytic fungi associated with these roots were then isolated, cultured, and identified by sequencing of rDNA markers. In total, 52 pure isolates were recovered from the roots of 10 orchid species. These isolates were classified into nine taxonomic groups except for one isolate that remained unclassified. Most fungal isolates were found in roots of up to two different orchid species; however, *Coprinellus radians* was found in the roots of all sampled orchids. The potential of *C. radians* to promote germination of orchid seeds was tested in a separate assay using seeds from two orchid species different than those found in the experimental forest plots. Of the two *C. radians* isolates tested, one improved germination in the two orchids evaluated to a level about half of that observed in seeds germinated in nutrient-rich medium (Phytamax) and above the null germination observed in plates without the fungus. Together, these results revealed a generalist relationship between *C. radians* and all the studied epiphytic orchids and the potential role of this fungus as a promoter of orchid seed germination.

Resumen. Las orquídeas establecen interacciones complejas con hongos endófitos a lo largo de su ciclo de vida, particularmente durante la germinación de sus semillas limitadas de reservas de nutrientes. Caracterizar interacciones generalistas y específicas entre las orquídeas y sus hongos asociados es clave para apoyar esfuerzos para su conservación. En este trabajo se estudiaron los hongos endófitos asociados a raíces de orquídeas epífitas de dos bosques montanos del sur del Ecuador. Se colectaron raíces de orquídeas de diez parcelas de ~500 m² distribuidas en dos bosques cercanos. Los hongos endófitos presentes en estas raíces fueron aislados, cultivados e identificados usando marcadores de ADNr. De un total de 10 especies de orquídeas se obtuvieron 52 aislados. Estos aislamientos se clasificaron en nueve grupos taxonómicos identificados y un grupo de hongos endófitos sin clasificación taxonómica. La mayoría de los grupos taxonómicos aislados estuvieron presentes en no más de dos especies de orquídeas diferentes, no obstante, *Coprinellus radians*, fue aislado a partir de raíces de todas las orquídeas colectadas. Adicionalmente, en una prueba separada, se evaluó el potencial que tiene *C. radians* como potenciador de la germinación de semillas de dos orquídeas no presentes en los bosques muestreados. De los dos aislamiento de *C. radians* probados, uno incrementó la germinación de ambas orquídeas a niveles cercanos a la mitad de lo registrado en semillas cultivadas en medio rico en nutrientes (Phytamax), lo cual fue mayor a la nula germinación observada en semillas cultivadas en placas en ausencia del hongo. Nuestros resultados revelan la asociación de hongos del género *Coprinellus* en todas las orquídeas epífitas estudiadas y un potencial rol para *C. radians* como promotor de la germinación de semillas de orquídeas.

Key Words / Palabras clave: Agaricales, *Epidendrum*, germinación simbiótica, symbiotic germination

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Introduction. Under natural conditions, orchid seeds depend on their interactions with a variety of fungi such as *Ceratobasidium* spp., *Tulasnella* spp., *Rhizoctonia* spp., *Epulorhiza* spp., and *Ceratorhiza* spp. to obtain phosphorus, carbon, and nitrogen from the environment to support germination and initial development (Rasmussen 1995, Sathiyadash *et al.* 2020, Smith & Read 2010). Understanding these orchid-fungus interactions is key to supporting conservation efforts because orchids produced using alternative propagation methods, such as *in vitro* culture, show low survival rates after re-introduction into native or rehabilitated habitats and slow growth thereafter (Chen, Wang & Guo 2012, Herrera *et al.* 2019, Swarts & Dixon 2009). Members of Tulasnellaceae and Ceratobasidiaceae are among the most frequently reported mycorrhizal fungi associated with roots of epiphytic orchids (Sathiyadash *et al.* 2020, Suárez *et al*. 2006), and thus, have received most of the attention in orchid germination studies. However, orchid seed germination in nature likely involves other, and perhaps more complex, interactions with non-mycorrhizal fungi (Meng *et al.* 2019), and even other types of organisms (Rasmussen *et al.* 2015). Although the interactions between orchids and non-mycorrhizal microorganisms have been characterized concerning nutrient acquisition, growth stimulation, and pathogen protection processes (Rasmussen *et al.* 2015, Strobel 2002, Yuan, Chen & Yang 2009), the role of this group of microorganisms during orchid seed germination remains less understood. The objectives of this study were: 1) to isolate culturable endophytic fungi from roots of ten epiphytic orchids in two montane forests of Southern Ecuador, and 2) to evaluate their possible beneficial role during the germination stage.

Materials and methods

Fungal endophytes associated with orchids in two Andean montane forests

Study area and sample collection.— Samples were collected at Mazán and Llaviuco forests, both located in the Macizo del Cajas Biosphere Reserve. Mazán is located 10 km west of the city of Cuenca in the province of Azuay, Ecuador (02°52’12” S, 79°06’55” W). The forest covers ~1050 ha and it is located within the very humid tropical montane forest life zone, according to Holdridge’s classification (Holdridge 1987). Air temperature can exceed 20°C on sunny days, and it can approach freezing on cold nights. Additionally, precipitation is distributed throughout the year, with lower intensity between July and August. The presence of clouds is frequent, especially in the months of greatest precipitation (February and April). The most abundant vascular plant families in the forest are Asteraceae, Melastomataceae, Solanaceae, Rosaceae and Ericaceae (Minga Ochoa 2000). Orchids are a very diverse group with around 40 species reported within the forest (V. Fleming, *unpubl. data* 1987). Llaviuco forest is located 17 km northwest of the city of Cuenca (02°50’40” S, 79°08’33” W), in a valley next to the Mazán forest. It is very similar to Mazán in most environmental characteristics and its vegetation composition and structure.

In each forest, five study plots were established following the methodology of Gradstein *et al.* (2003). Briefly, in each plot, a dominant tree was selected and used as a center to delineate a ~500 m² circular plot with a 12.7 m radius. Within this plot, orchids were sampled from ground level up to the first branch on all the trees with a diameter at breast height ≥ 10 cm. The sampling stratum was constantly under the shade of the dominant trees, and its air temperature averaged about 10°C. The canopy at the sampling sites reached 15 m and the understory was dominated by herbs and young trees. When many specimens of the same orchid were present in a single phorophyte, up to three specimens of each orchid were sampled. Samples, which were only collected from adult orchids, consisted of 5 cm live root segments containing the root tip. Additionally, fertile specimens and photographs were taken to expert orchid taxonomists for identification.

Root samples were placed in zip-lock bags with their respective identification, transported on ice to the laboratory, and kept refrigerated until processing within 24 h.

Isolation of endophytic fungi.— Endophytic fungi were isolated from root samples as in Zettler, Sharma & Rasmussen (2003), with some modifications. First, samples were carefully washed with water to avoid damaging the tissue and were then transferred to a laminar flow hood to continue the process under sterile conditions. Roots were surface sterilized by immersion in a solution of 4.8% ethanol and 0.25% sodium hypochlorite, followed by three rinses in sterile distilled water to remove residues of the disinfectant solution. Clean and intact (no tissues excluded) 2 cm-long root segments were individually transferred to sterile Petri dishes and cut into very small fragments to release hyphae of endophytic fungi. Root fragments were then spread on fungal isolation medium, which was supplemented with 300 mg L^{-1} streptomycin sulfate to reduce bacterial contamination. The plates were sealed with parafilm and incubated at 27°C in the dark. After two days, each hypha that had emerged from root fragments was isolated and subcultured on potato dextrose agar (PDA) on a fresh plate. Isolates were not classified or grouped based on morphological or growth characteristics prior to molecular identification. All cultures were maintained at 27°C until processed for molecular analysis.

Identification of endophytic fungi.—DNA was extracted from pure fungal cultures using the PureLink Genomic DNA extraction kit (Invitrogen, Carlsbad, CA, USA). DNA integrity was checked by electrophoresis on 1% agarose gels. Afterwards, a fragment of the ITS region was amplified by nested PCR using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and TW14 (5'-GCTATCCTGAGGGAAACTTC-3') (Cullings 1994, White *et al.* 1990) for the first amplification, and then primers ITS1 and NLB4 (5'-GGATTCTCACCCTCTATGAC-3') in the nested reaction (Martin & Rygiewicz 2005). The amplified fragments were purified and sequenced by an external service provider (Macrogen Inc., South Korea). The sequences were then compared to those deposited in the GenBank databases of NCBI (National Center for Biotechnology Information) using BLAST (Altschul *et al.* 1997). The identity of each isolate was assigned based on the GenBank accession with the most similar sequence identity.

In vitro germination of orchid seeds in co-culture with *Coprinellus radians*

Species selection and seed quality check.— After noticing from initial results that *Coprinellus radians* was present in all collected roots, we conducted an assay to determine whether this fungus could be involved in the promotion of seed germination. For this test, *Epidendrum dalstromii* Dodson and *E. nocturnum* Jacq. were selected. These two orchids had not been present in the study sites in Mazán and Llaviuco forests but were selected due to the availability of their seeds in the University of Cuenca orchid germplasm collection. *Epidendrum dalstromii* is reported as endemic to Southern Ecuador and is currently listed as an endangered species (León-Yánez *et al*. 2011). *Epidendrum nocturnum* is a species widely distributed over the Americas and is currently not listed as threatened in most of its range. Seeds had remained under cold storage for at least one year before the experiment. The viability of each seed lot was evaluated using the tetrazolium test. Briefly, seeds were immersed in a 1% sodium hypochlorite solution containing one drop of Tween 20 for 15 minutes, and they were then submersed in water for 48 hours in the dark. Seeds were drained and immersed in a 1% tetrazolium salt solution for 24 hours at 30°C. Seeds stained after incubation in tetrazolium were considered viable and used for estimating the viability of the seeds. The viability of the *E. dalstromii* seeds was 90%, whereas it was 20% for *E. nocturnum*.

Germination test.— Seeds were surface-sterilized by immersion in 1% sodium hypochlorite followed by three successive washes in sterile distilled water. Seeds were then resuspended in sterile distilled water to a concentration of ~60 seeds/ml and 500 µl of seed suspension were spread on the surface of sterile Petri dishes containing oatmeal-agar. Afterward, a 0.5 cm^2 block of PDA containing *Coprinellus radians* mycelia was placed in the center of each plate. Two *C. radians* isolates were tested: *C. radians 1* and *C. radians 2*, hereafter. These two isolates showed different morphology although they were classified within the same taxon based on sequencing results. For additional comparisons, seeds were sown on oatmeal agar (a nutrient-poor medium) and Phytamax™ Orchid Maintenance Medium (Sigma-Aldrich P6668, Saint Louis, MO, USA) (pH 5.6) (a nutrient-rich medium), both without fungus inoculation. Ten replicate plates were prepared for each of the conditions. Plates were incubated at 20°C in darkness for 10 days. After this period, the plates were incubated for 16 weeks under a 16/8 h light/dark photoperiod. Germination was evaluated at the end of week 16 using the scale of Zettler & McInnis (1993) where: Stage 0 = hydrated seeds; Stage 1 = rupture of the testa due to embryo lengthening; Stage 2 = appearance of rhizoids; Stage 3 = leaf tip emergence; Stage 4 = leaf 0 emergence; and Stage 5 = leaf blade elongation. The number of seeds in each developmental stage was recorded and compared against counts from uninoculated oatmeal-agar and Phytamax™ plates. Seeds were considered to be germinated when rhizoids emerged (Stage 2). The colonization of fungi inside the tissues was not verified. Differences in germination between treatments could not be statistically tested due to the complete absence of germination in the negative control (oatmeal agar), which resulted in statistical test assumptions not satisfied.

Results

Epiphytic orchid diversity.— A total of 612 orchids were identified within the experimental plots. These orchids were classified within six genera and 10 species, namely: *Epidendrum fruticosum* Pav. ex Lindl., *E. geminiflorum* Kunth, *Epidendrum* sp.1, *Epidendrum* sp.2, *Epidendrum* sp.3, *Lepanthes* sp., *Frondaria caulescens* (Lindl.) Luer, *Pleurothallis coriacardia* Rchb.f., *Odontoglossum* sp. and *Stelis* sp. The most abundant species was *Stelis* sp. with 272 specimens. *Epidendrum* was the most represented genus with five species identified. Each forest showed different orchid diversity indices, whereby Llaviuco generally showed higher diversity than Mazán (Table 1). Specimens of *E. fruticosum*, *E. geminiflorum*, *F. caulescens*, and *Lepanthes* sp. were present only in plots located in the Llaviuco forest.

Endophytic fungal diversity associated with epiphytic orchid roots.— Fifty-two pure isolates were recovered from the orchid roots. These isolates were classified as *Coprinellus radians*, *Trametes* sp., *Meyerozyma guilliermondii*, *Penicillium chrysogenum*, *Penicillium rubens*, *Fusarium* sp., *Botryobasidium* sp. and Lepidiotaceae (Table 2). An additional isolate was classified at the division level as a mycorrhizal Basidiomycete, and one isolate remained taxonomically unclassified, although its sequence has been reported to belong to an endophytic fungus colonizing purple loosestrife (*Lythrum salicaria*) (David *et al.* 2016). Forty-one of the 52 isolates were classified as *C. radians*, making this species the most abundant in orchid roots from both forests; all other species were represented by only one or two isolates each. *Coprinellus radians*, *Trametes* sp., and *P. chrysogenum* were isolated from both forests, whereas the other fungi were found exclusively in either the Mazán or Llaviuco forest.

TABLE 1. Orchid diversity in the sampling plots at Llaviuco and Mazán Forests.

	Llaviuco	**Mazán**	**Both forests**
Specimens			
Stelis sp.	211	61	272
Epidendrum sp1	64	4	68
Epidendrum sp2	23	4	27
Epidendrum sp3	114	18	132
Epidendrum geminiflorum	14	0	14
Frondaria caulescens	1	0	1
Lephante sp.	4	0	4
Odontoglossum sp.	17	18	35
Pleurothallis coriacardia	11	1	12
Epidendrum fruticosum	47	0	47
Total	506	106	612
Species richness	10	6	10
Shannon's H´	1.67	1.21	1.64

When analyzing how the diversity of endophytes was distributed across the diversity of orchids, no more than three different endophytic fungi were associated to the same orchid species (Fig. 1). In the case of *Pleurothallis coriacardia* and *Lepanthes* sp., only one endophyte was associated with their roots. Most fungal endophyte isolates (six out of 10) were recovered from the roots of only one orchid species, although three endophytes were isolated from two different orchid species. Most notably, *Coprinellus radians* was isolated from all orchid species sampled (Fig. 1 and Table 3).

Germination-promoting effects of Coprinellus radians.— Due to the presence of the fungal species *C. radians* in all sampled orchid species, a germination test was carried out with two different isolates belonging to this fungus (*C. radians 1* and *C. radians 2*). Only isolate *C. radians 2* promoted germination of both

TABLE 2. Identification of ITS sequences of endophytic fungi isolates from orchid roots in Mazán and Llaviuco forests.

Isolate	Max identity (%)	Assigned identity based on most similar GenBank accession	GenBank accession	Source
UC-2ll	96%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-4ll	99%	*Fungal endophyte*	KT291127	David *et al.* (2016)
UC-5ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-6ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-7ll	85%	*Fungal endophyte*	KT291127	David *et al.* (2016)
UC-8ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-12ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-13ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-15ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-16ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-17ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-18ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-19ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-20ll	89%	*Penicillium chrysogenum*	JF834167	Guo *et al.* (*Unpub. data*)
UC-23M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-24M	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-25M	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-26M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-27M	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-28M	99%	*Trametes* sp.	KJ831923	Gazis *et al.* (*Unpub. data*)
UC-29M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-30M	99%	*Fusarium* sp.	KU974301	Moretti *et al.* (2011)
UC-31M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-32ll	98%	*Coprinellus radians*	FJ185160	Badalyan et al. (2011)
UC-34ll	98%	*Penicillium rubens*	LT558978	Guevara Suarez *et al.* (2016)
UC-35ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-36ll	99%	*Meyerozyma guilliermondii*	KJ451706	Herkert (*Unpub. data*)
UC-37ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-40ll	96%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-42ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-43ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-47ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-53ll	89%	*Trametes* sp.	KF578082	Maza *et al.* (2014)
UC-55ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-56ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-59ll	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-60ll	94%	*Mycorrhizal Basidiomycete*	AB176570	Yamato *et al.* (2005)
UC-61ll	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-63M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-64M	97%	*Botryobasidium* sp.	KU194318	Ding & Gu (*Unpub. data*)
UC-65M	99%	*Penicillium chrysogenum*	KF011475	Wicklow (2013)

Table 2 *continues*

UC-66M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-68M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-69M	99%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-70M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-75M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-76M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-77M	75%	*Lepidiotaceae*	AF079745	Mueller *et al.* (1998)
UC-78M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-80M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-81M	98%	*Coprinellus radians*	FJ185160	Badalyan *et al.* (2011)
UC-83M	99%	*Penicillium chrysogenum*	KF011475	Wicklow (2013)

Epidendrum dalstromii and *E. nocturnum* seeds (Table 4). The germination rate in co-culture with *C. radians 2* was low in absolute terms (2.9% and 13.3% for *E. nocturnum* and *E. dalstromii*, respectively); however, no seeds germinated in the plates containing oatmeal agar without fungi, indicating germination promoting effect of this fungus. Nonetheless, this germination promoting effect was not as strong as the one observed in nutrient-rich Phytamax™ (7.3% and ~24.4% for *E. nocturnum* and *E. dalstromii*, respectively).

Discussion

Fungi and orchids form both specialist and generalist relationships. These relationships often arise from specific ecological roles, although in many cases these roles have not yet been elucidated (Favre-Godal *et al.* 2020, Selosse 2014). The genera of six of the 10 fungal endophytes identified in our study have been previously reported as orchid endophytes; two of them have also been reported to form mycorrhizal

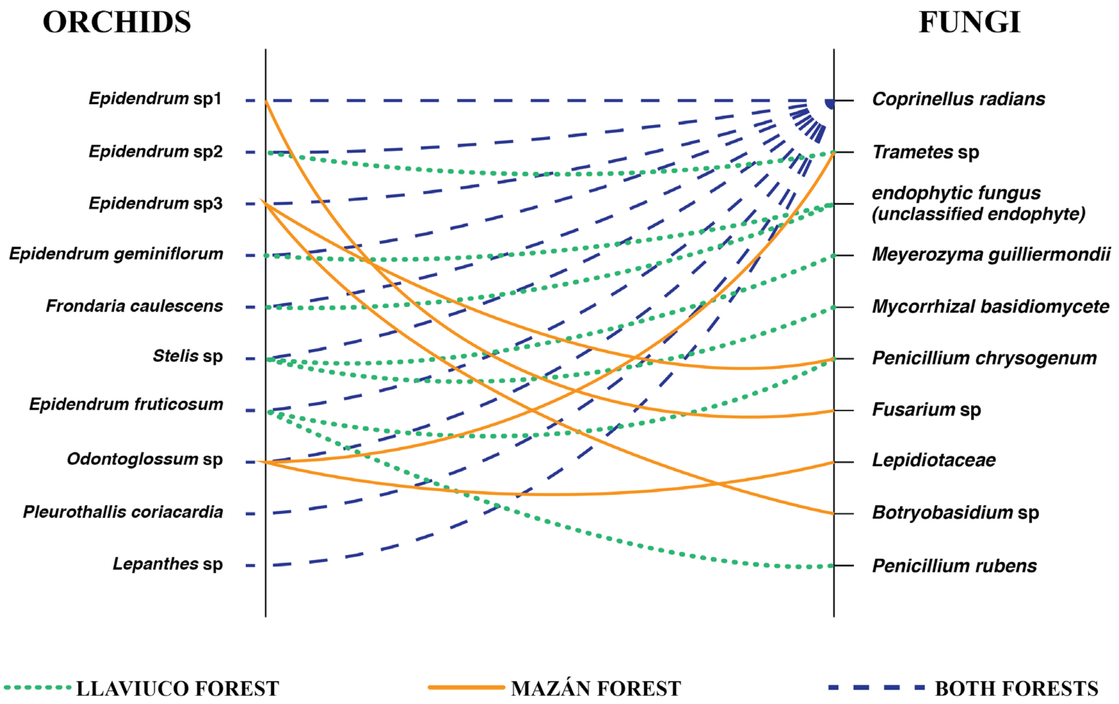

Figure 1. Endophytic fungal diversity associated with epiphytic orchid roots in two Andean montane forests. Solid, dotted, or dashed lines represent isolates from orchids present at Mazán, Llaviuco, or both forests, respectively.

Table 3. Endophytic fungal diversity associated with roots of orchids from the sampling sites at Llaviuco and Mazán Forests.

	Orchid host									
Putative isolate identity	*Stelis* sp.	*Epiendrum* sp1	*Epidendrum* sp2	*Epidendrum* sp3	*Epidendrum eminiflorum*	*Frondaria caulescens*	*Lephante* sp.	*Odontoglossum* sp.	*Pleurothallis coriacardia*	*Epidendrum fruticosum*
Coprinellus radians	7	3	1	10	1	1	1	7	2	6
Endophytic fungus	-	-	-	-	1	1	-	-	-	-
Meyerozyma guilliermondii	1	-	-	-	-	-	-	-	-	-
Mycorrhizal Basidiomycete	1	-	-	-	-	-	-	-	-	-
Penicillium chrysogenum	-	-	-	-	-	-	-	-	-	1
Trametes sp.	-	-	1	-	-	-	-	1	-	-
Fusarium sp.	-	1	-	-	-	-	-	-	-	-
Penicillium rubens	-	-	-	-	-	-	-	-	-	1
Botryobasidium sp.	-	-	-	1	-	-	-	-	-	-
Penicillium chrysogenum	-	-	-	2	-	-	-	-	-	-
Lepidiotaceae	-	-	-	-	-	-	-	1	-	-
Total number of isolates per orchid species	9	4	2	13	2	2	1	9	2	8
Fungal isolate richness per orchid species	3	2	2	3	2	2	1	3	1	3
Shannon's H´	0.68	0.56	0.69	0.68	0.69	0.69	0	0.68	0	0.73

Table 4. Number of seeds germinated in co-cultures of *Epidendrum nocturnum* and *Epidendrum dalstromii* seeds with two *Coprinellus radians* isolates on oatmeal-agar (OA) after 122 days (n=10 plates). Seeds germinated on Phytamax™ medium (P) were included for comparison of the responses from nutrient rich medium.

Orchid	Germination conditions	Seeds sown per plate	[a] Unchanged	Stage 0	Stage 1	Stage 2	Germinated (%)
Epidendrum nocturnum	OA + *C. radians 1*	26.3 ± 4.01	19.7 ± 3.63	6.5 ± 0.71	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	OA + *C. radians 2*	34.2 ± 6.21	26.0 ± 5.7	3.4 ± 0.22	3.8 ± 0.35	1.0 ± 0.14	3.32 ± 0.1
	Negative (OA)	27.0 ± 7.36	18.4 ± 2.22	8.4 ± 0.45	0.2 ± 0.20	0.0 ± 0.0	0.0 ± 0.0
	Positive (P)	35.6 ± 5.21	27.1 ± 4.64	1.0 ± 0.29	4.9 ± 0.45	2.6 ± 0.54	7.06 ± 0.65
Epidendrum dalstromii	OA + *C. radians 1*	27.4 ± 2.17	4.2 ± 0.85	22.3 ± 1.59	0.9 ± 0.23	0.0 ± 0.0	0.0 ± 0.0
	OA + *C. radians 2*	32.3 ± 1.74	4.2 ± 0.64	13.6 ± 1.09	10.2 ± 0.87	4.3 ± 0.49	13.46 ± 1.66
	Negative (OA)	31.1 ± 1.08	5.9 ± 0.62	25.2 ± 1.21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Positive (P)	31.2 ± 2.05	4.5 ± 1.08	2.2 ± 0.24	16.9 ± 1.79	7.6 ± 1.15	24.39 ± 3.22

[a] Germination stages: Unchanged = the seed has not changed from its original state at sowing; Stage 0 = hydrated seeds; Stage 1 = rupture of the testa due to embryo lengthening; Stage 2 = appearance of rhizoids

associations with orchids (Yamato *et al.* 2005, Yukawa *et al.* 2009). For instance, *Penicillium* spp. (Bayman *et al.* 1997, Tremblay 2008, Yuan *et al.* 2009, Sudheep & Sridhar 2012) and *Fusarium* spp. (Bayman *et al.* 1997, Behera, Tayung & Mohapatra 2013, Jiang *et al.* 2019, Yuan *et al.* 2009) have been isolated from the roots of epiphytic orchids and have demonstrated growth-promoting effects on their hosts (Jiang *et al.* 2019, Ovando *et al.* 2005). Likewise, similar effects have been reported for *Meyerozyma* sp. (Pecoraro *et al.* 2012), *Botryobasidium* sp. (Ogura-Tsujita *et al.* 2012), and *Trametes* sp. (Cueva 2014). Most studies on the relationships between orchids and endophytic fungi have focused on the potential role of the latter in pathogen defense, improved nutrient acquisition, or stress tolerance (Ordoñez Castillo 2012, Yuan *et al.*

2009), with other potential ecological roles remaining mostly unexplored. Although less frequently studied, the germination enhancing the effects of endophytic and saprophytic fungi, such as *Fusarium* spp. or *Mycena* spp., have also been reported (Meng *et al.* 2019). In this study, most of the isolated endophytes were associated with a limited number of orchid species, suggesting potential specific interactions between these endophytes and their hosts. However, the isolation of *Coprinellus radians* from the roots of all sampled orchids represented a striking exception and suggested a wide generalist relationship between this fungus and orchids.

Associations between orchids and members of Psathyrellaceae, to which *Coprinellus* spp. belong, have been reported as beneficial to orchid growth and development (Terashita & Chuman 1987, Yagame *et al.* 2013, Yamato *et al.* 2005, Yukawa *et al.* 2009). Furthermore, *Coprinellus* spp. have been reported to promote germination in the terrestrial orchid *Epipogium roseum* and increase the growth of its rhizomes and tubers (Yagame *et al.* 2007, Yagame *et al.* 2008). Xiaoya and collaborators (2015) have confirmed that *Coprinellus* spp. establish generalist mycorrhizal associations with terrestrial orchids beginning at the seed stage. Here, we found *C. radians* associated with the roots of all orchids sampled, supporting the findings of Xiaoya *et al.* (2015) that this fungus can establish generalistic relationships with diverse orchid species. Further, we confirmed the potential role of *C. radians* in promoting germination in two epiphytic orchids, although only one of the tested fungal isolates showed this effect. While the association of *C. radians* to terrestrial orchids is not new (Terashita & Chuman 1987, Yagame *et al*. 2013, Yamato *et al.* 2005, Yukawa *et al.* 2009), our results report for the first time the association of *C. radians* to epiphytic orchids and reveal a potential ecological role for this endophyte. Saprophytic members of Coprinaceae have been reported as potential intermediary providers of organic carbon from decaying wood to the mycothropic orchid *Epipogium roseum* (Sathiyadash *et al.* 2020). Carbon remobilized by fungi from decaying substrates could serve as energy source for germinating orchid seeds and favor the establishment of orchid-endophyte associations. Such interactions between orchids, saprophytic fungi, and decaying substrates have also been suggested for *Epidendrum* spp. in the same forests studied by us (Herrera *et al.* 2019). Considering the large diversity of orchids present in tropical montane forests, describing and characterizing the interactions between orchids and their endophytes is relevant for a better understanding of the ecology of these plants and the ecosystems in which they grow. In this study, we described a small portion of the endophytic fungal community and explored a potential ecological role for one of the endophytes. However, investigating the more complex interactions that also exist, such as with other endophytes (e.g., bacteria) and the phorophytes that support epiphytic orchids, could contribute to a more thorough description of orchid ecology. Here, we found a widespread association between *C. radians* and all the orchids studied, which demonstrates a generalistic association of this fungus with the orchid community in the Tropical Montane forests used for this study. In contrast, the presence of all the other isolates was restricted mainly to only one orchid species which could suggest a more specific type of interaction between these fungi and their orchid hosts. Nonetheless, this study was not designed to test for specialistic relationships and cannot conclude on this point based on our data.

Our data suggest that *Coprinellus radians* could be an important component of the endophytic community of epiphytic orchids in the Andean montane forests. In addition, our data showed that at least one of these *C. radians* isolates promotes orchid seed germination. Together, our results contribute to scientific understanding of the relationships between orchids and their associated organisms and how these interactions can be used to design effective conservation strategies in the future.

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THE GENUS *CORYBAS* IN VIETNAM, WITH TWO NEW RECORDS

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Abstract. An overview of the orchid genus *Corybas* in Vietnam is presented. It was known to be represented by a single species, *C. annamensis*. In this article, we report two new records, *C. himalaicus,* and *C. geminigibbus*. Detailed descriptions, synonyms, type data, information on ecology and phenology, distribution and color plates are provided for all three species along with the key to their identification.

Key Words: *Corybas annamensis*, *Corybas geminigibbus*, *Corybas himalaicus*, Orchidaceae, Orchidoideae

The genus *Corybas* Salisb. (Salisbury 1807) comprises about 120 terrestrial and lithophytic orchid species, widely distributed in India, South China, Taiwan, Thailand, Vietnam, the Malesian region, Australia, New Zealand and the western Pacific (Pridgeon *et al*. 2001, Chung & Hsu 2008, Chantanaorrapint & Chantanaorrapint 2016, Govaerts *et al*. 2019). All species of this genus are small plants with underground tubers bearing single cordate leaf and a solitary flower with a pair of spurs at the base of the labellum.

This genus was first included in the orchid flora of Vietnam, in 2007 with the discovery of *Corybas annamensis* Aver. (Averyanov *et al*. 2007). Recently, from separate surveys in Fansipan Mountain and Chu Mu Mountain, two more species of *Corybas* have been discovered. Based on available literature and consultation of herbarium specimens at K, P, LE, these two species were identified as *C. geminigibbus* J.J.Sm. and *C. himalaicus* (King & Pantl.) Schltr. and these are hitherto new records for the orchid flora of Vietnam. Detailed description, synonyms, type data, information on ecology and phenology, distribution and color photographs are provided for all three species along with the key to identification of these species. Voucher specimens were collected and preserved in 70% ethanol and stored at VNM. Photographs were taken with a Canon 600D fitted with an EF–S 60mm f/2.8 Macro USM lens. Terminology for the morphological description follows Beentje (2012).

Key to the species of *Corybas* in Vietnam

1′ Spurs of labellum poorly developed or very short (<2 mm long); apical part of the labellum irregularly fimbriate .... ***C. geminigibbus***

1″ Spurs well developed (>3mm long), horn–shaped; labellum apex acute, straight or slightly curved 2

2′ Margin of labellum ciliate in apical part and erose at base, without swollen callus at mouth of throat .... ***C. annamensis***

2″ Margin of labellum dentate in apical part and entire at the base, swollen callus at mouth of throat present .... ***C. himalaicus***

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Taxonomic treatment

Corybas annamensis Aver. OrchideenJ. 14: 97. 2007 (Fig. 1).

TYPE: Vietnam. Lam Dong Province: Lac Duong District, Da Chais Municipality, elevation 1600 m, 19 October 2005, *T.T.T.Tramg, T.V.Thao, N.T.Vinh, HLF 5317* (*LE01059678*-Holotype!).

Small, terrestrial *herbs* with underground tubers. *Tubers* spherical, up to 7 mm in diameter, warty, covered with short hairs. *Stem* erect, terete, 2.5–4.5 mm long, 1.0–2.5 mm wide, tinged green to white; with a sheathing bract 0.8–1.2 cm long, 0.2–0.3 mm wide, finely haired at the base. *Leaf* solitary, sessile, cordate, surface slightly plicate, 1.5–3.5 cm long, 1.0–2.8 mm wide, membranous, dull greyish-green, irregularly wavy at the margin, apex acute. *Inflorescence* terminal, single flowered. *Flower* 1.2–1.6 cm in diameter, dorsal sepal dark crimson–red to purple, lateral sepals and petals white tinged with crimson towards the base, labellum white with red stripes and red margin. *Dorsal sepal* ovate or spathulate, emerging from the narrow base, strongly bend upwards, 2.0 – 2.5 cm long, 0.8 – 1.2 cm wide, with around 11 white stripes, sepals along with labellum forming a helmet like structure. *Lateral sepals* filiform, free, erect, 5.0–5.5 cm long, 0.8–1.0 mm wide. *Petals* similar to lateral sepals in shape, size. *Labellum* immobile, ovate, inconspicuously 3-lobed, 1.6–2.2 cm long, 1.4–1.8 cm wide, concave, arcuate, bent downwards, apex acute, surface finely warty, with hairs on the margin, surrounding the column towards the base, white with crimson–purple margin and 3–5 dark crimson-purple longitudinal bands on the lateral lobes and towards the margin, spur 0.4–0.6 cm long. *Column* short, 3–4 mm long, 2.0–2.5 mm wide, erect at the tip, with protruding round wings. *Pollinia* 4 in 2 pairs. *Pedicel* slender, gradually extending up to 12 cm during fruit maturity. *Capsule* ovate to cylindric, 1.0–1.5 cm long, 0.4–0.6 cm wide.

Ecology and phenology: This species was found growing at around 1400–1600 m in elevation in primary humid broad-leaved evergreen montane forest. Very rare.

Flowering: June–July.

Distribution: Endemic to Vietnam (Bi Doup-Nui Ba National Park).

Specimen examined: Bidoup National Park, Da Chais Commune, Lac Duong District, Lam Dong Province, *Truong Ba Vuong, Truong Quang Cuong*, 2 August 2018, *BV 382* (*VNM 00023640*) (VNM!).

Corybas himalaicus (King & Pantl.) Schltr., Repert. Spec. Nov. Regni Veg. 19: 19. 1923; Pearce & Cribb, Orch. Bhutan 118–120. 2002. ≡*Corysanthes himalaica* King & Pantl., J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 65: 128. 1896. ≡*Calcearia himalaica* (King & Pantl.) M.A.Clem. & D.L.Jones, Orchadian 13: 444. 2002. (Fig. 2).

TYPE: India. Sikkim: *Pantling 385* (CAL-Holotype); *K000859038*, *K000859039*, *K000859040* (K-Isotypes!); *P00372553* (P-Isotypes!); (*BM000958057*, *BM000032565* (BM-Isotypes!).

Small, terrestrial or lithophyte *herbs* with underground tubers. *Tuber* ovoid, *ca*. 2–5 mm in diameter. *Stem* erect, terete, *ca*. 5–40 mm long, divided into 2 parts, separate by a node covered by a sheath *ca*. 2.5 mm long, tubular, basal part of stem with white papillose hairs, stem glabrous above the sheath. *Leaf* sessile, cordate, *ca*. 72–12 mm long, 8–11 mm wide, apex mucronate. *Inflorescence* terminal, single flowered, floral bract 4–5 mm long, 1.0–1.4 mm wide, apex acuminate, as long as or slightly longer than ovary and pedicel; pedicel (with ovary) *ca*. 4.7–5.0 mm long. *Flower* dorsal sepal reddish-white at base, petals and lateral sepals reddish-white at base, labellum white with red to dark red spots. *Dorsal sepal* hooded, oblanceolate, *ca*. 1.0–1.5 cm long 0.4–0.5 mm wide, clasping the basal part of labellum, margin entire. *Lateral sepals* filiform, connate at base *ca*. 5 mm long. *Petals* similar to lateral sepals, slightly longer than lateral sepals, *ca*. 6 mm long, connate with lateral sepals at base. *Labellum* margin inrolled forming a tubular basal, curving downward, *ca*. 4 mm long; apical part extended, 3.2–3.5 mm long, *ca*. 7 mm wide, orbicular (flattened) in outline shape; disc with reddish white callus, in front of callus dark crimson papillose; margin erose, irregular dentate only at apical part, basal two spurs at base of labellum, *ca*. 2.2 mm long, 1 mm wide, apex obtuse, pointing forward. *Column* winged at apex *ca*. 2.5 mm long. *Pollinia* 4 in 2 pairs. *Capsule* not seen.

Figure 1. *Corybas annamensis* Aver. **A–D**. Plants in habitat showing morphological characters. Photographs by Nguyen Van Canh.

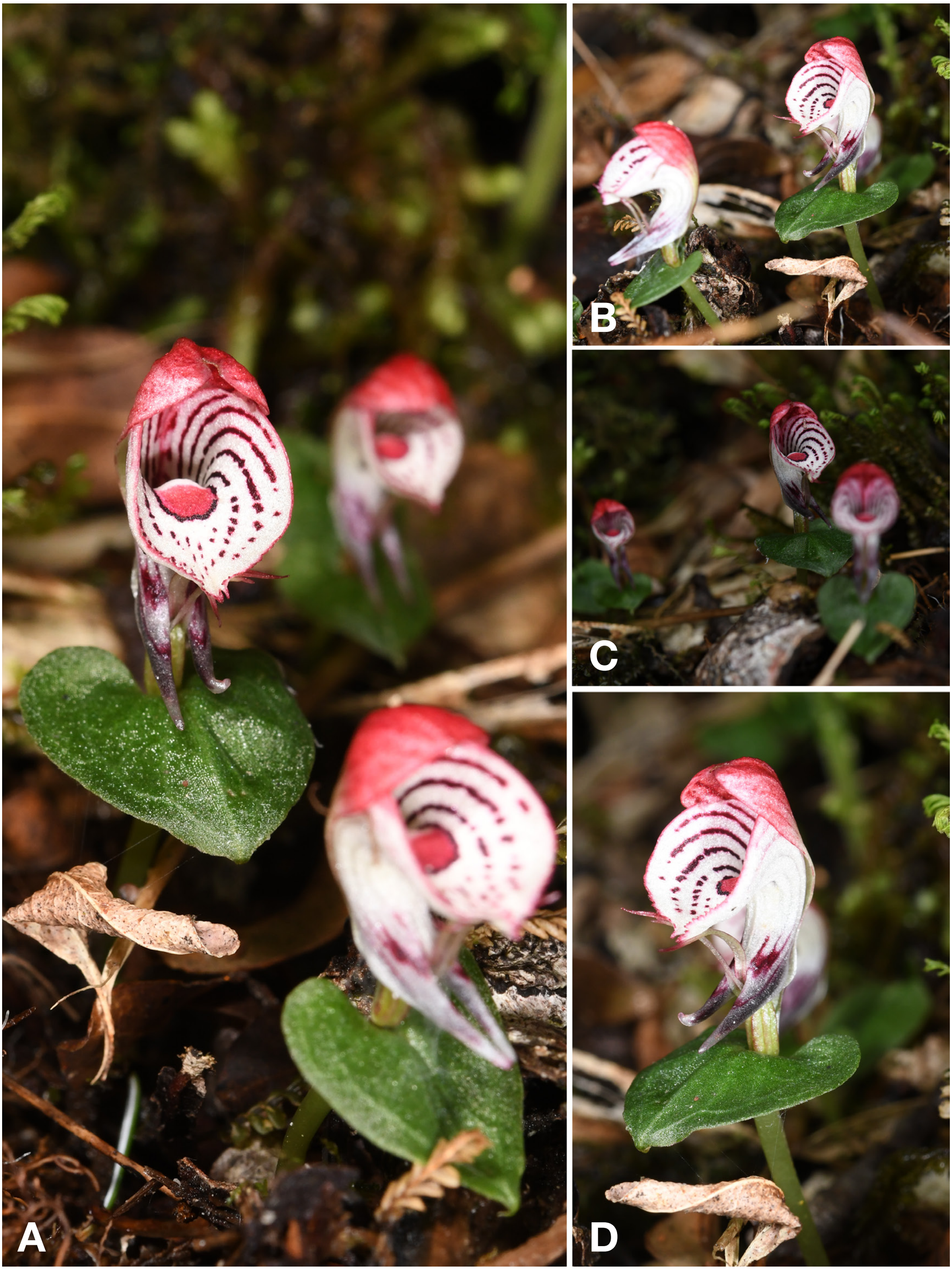

FIGURE 2. *Corybas himalaicus* (King & Pantl.) Schltr. **A–D**. Plants in habitat showing morphological floral characters. Photographs by Nguyen Thanh Luan.

ECOLOGY AND PHENOLOGY: This species was found growing as a lithophyte among mosses around 2500 m a.s.l. in primary evergreen mixed broadleaved–coniferous forest, with *Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth as the dominant tree species. Very rare.

FLOWERING: June.

DISTRIBUTION: Vietnam (Fansipan Mountain), Bhutan, India (Sikkim) and China (Taiwan).

SPECIMEN EXAMINED: Lao Cai Province, Sapa Pistrict, Fansipan Mountain, *Truong Ba Vuong, Nguyen Thanh Luan*, 22 June 2019, *BV 381* (*VNM 00023641*) (VNM!); Lao Cai Province, Sapa District, Fansipan Mountain, *Truong Ba Vuong, Nguyen Thanh Luan*, 23 June 2019, *LE 01066674* (LE!), *LE 01061397* (LE!)

NOTE: The Vietnamese specimens slightly differ from the type in somewhat shorter lateral sepals and petals.

Corybas geminigibbus J.J.Sm., Mitt. Inst. Bot. Hamburg 7: 13. 1927; Dransfield *et al*.: 593. 1986; Seidenfaden & Wood: 121. 1992. (Fig. 3).

TYPE: Indonesia. Borneo: West Kalimantan, Mt Mulu, *Winkler 496* (*HBG500940*) (HBG-Holotype).

Small, terrestrial or lithophyte *herbs* with underground tubers. *Tuber* ovoid, *ca*. 2–5 mm in diameter. *Stem* short, erect, pale green, *ca*. 6 mm, basal part covered by a tubular sheath with acuminate apex clasping the stem, *ca*. 3 mm long, with white papillose hairs at base. *Leaf* single, green with white markings on adaxial surface, green to pale red on abaxial, sessile, broadly ovate to cordate, *ca*. 10–12 mm long, 7 mm wide (at widest part), apex shortly acuminate. *Inflorescence* terminal, single flowered, floral bract pale green *ca*. 3–4 mm long, apex acuminate; pedicel (with ovary) *ca*. 3 mm long. *Flower ca*. 6 mm in diameter, dorsal sepal white at base and dark purple in apical part, lateral sepals and petals whitish with dark purple, labellum white with dark purple marks towards margin. *Dorsal sepal* hooded, strongly curved downward, oblanceolate, *ca*. 8.0–8.5 mm long, 3 mm wide, apex mucronate. *Lateral sepals* free, filiform, *ca*. 15–17 mm long. *Petals* similar to lateral sepals but shorter, *ca*. 11 mm long. *Labellum* margin inrolled forming a tubular basal part, curving downward *ca*. 4 mm long; apical part expended *ca*. 7 mm long, 6 mm wide (at the widest point), margin irregularly fimbriate-ciliate; apex acute or retuse; spur 2, small, *ca*. 1.5– 2.0 mm long, poorly developed, broad, apex rounded. *Column* short, *ca*. 1 mm long, shortly winged at apical part. *Pollinia* 4, in 2 pairs. *Capsule* ellipsoid, *ca*. 6 mm long.

ECOLOGY AND PHENOLOGY: Lithophyte, associated with mosses, at around 1800–1900 m a.s.l. in Chu Mu mountain, and at 1400–1500 m a.s.l. in Bidoup–Nui Ba National Park. Very rare.

FLOWERING: July.

DISTRIBUTION: Vietnam (Vong Phu and Bidoup–Nui Ba National Park), Thailand (Nakhon Si Thammarat-Khao Ramrom Mountain), Peninsular Malaysia (Kedah), Indonesia (Borneo), Philippines (Palawan).

SPECIMENS EXAMINED: Dak Lak province, M'Drak Disctrict, Cu Mroa Commune, Chu Mu Moutain, *Truong Ba Vuong, Nguyen Thanh Luan*, 22 June 2019, *BV 381* (*VNM 00023642*) (VNM!).

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FIGURE 3. *Corybas geminigibbus* J.J.Sm. **A–D**. Plants in habitat showing morphological characters. Photographs by Nguyen Van Canh (A, C, D), and Truong Quang Cuong (B).

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THE ORCHIDS OF JOHN HENRY LANCE (1793-1878)

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ABSTRACT. John Henry Lance, a British barrister, spent a ten-year term in Surinam as Judge appointed to the 'Mixed Court' in Paramaribo, a post created to supervise the compliance of the Dutch authorities with a treaty signed between the Netherlands and England in 1818 prohibiting the slave trade in the Dutch colonies. During his term in Paramaribo, Lance, a friend of Bateman and Lindley, collected several new orchid species. However, his collection of watercolors depicting plants from Surinam, many of them orchids, would appear to be more important. Some of these were painted by himself, others by the Surinamese artist Gerrit Schouten. The orchids of this collection, never published, were supplied by the Lindley Library of the R.H.S. and are reproduced here with its kind permission.

KEY WORDS: botanical illustration, history of botany, Orchidaceae, Surinam

"I beg leave to inform you, that the schooner 'Snow', captured by the Netherlands sloop-of-war, the 'Kemphaan', was condemned as prize on the 6th instant. Inclosed I have transmitted an abstraction of her case:

One of the Negro women taken in her died since her arrival, and after she had been landed in this colony: the remaining Slaves, forty-nine in number, have received their certificates of freedom, and have been placed by the Government under the care of that very useful and respectable religious body, the Moravians; to the end that, when sufficiently instructed in the truth of the Christian religion, they may all be baptized; after which, it is the intention of the Governor, to employ them as free laborers.

I am sorry to be under the necessity of informing you that one of the sailors found on board of the slave-schooner, named William Askens, is a British subject. […] *I conceived it to be my duty to claim the above-mentioned William Askens of the Governor, for the purpose of having him sent by the earliest opportunity to some British settlement, with a copy of the sentence* [---] *that he may take his trial under the Act. 51 Geo, III, c, 23, for being engaged in the Slave Trade"* (Lance, 1823, extract of a letter to George Canning, British Foreign Secretary, on May 11, 1823).

The letter cited above gives a good idea of the duties imposed on John Henry Lance (1793–1878) (Fig. 1)**,** who in 1822 had been appointed as Judge in the Mixed Court of Justice in Paramaribo for 10 years (1823-1833). On May 1, 1807, after a long campaign by active abolitionists in and outside Parliament (Fig. 2), the British Parliament approved the Abolition Bill, containing a definitive prohibition of slave trading. It was England who, after the final victory over Napoleon, brought the question of slavery to the Congress of Vienna in 1814.

A condition of England's recognition of the "new" Netherlands was that King Willem I should also prohibit slave trading. Both parties signed an agreement in 1818: [...] *for preventing Their respective Subjects from engaging in any Traffic of Slaves...* To ensure that the Netherlands would comply with this prohibition, so-called 'Mixed Courts of Justice' were established in Sierra Leone, on the West Coast of Africa, and at Paramaribo, Surinam (Fig. 3–4). [...] *And it was by the said Treaty further stipulated and agreed, that said Courts should judge the Causes submitted to them according to the terms of said Treaty, without appeal, and according to the Regulations and Instructions annexed to the said Treaty* […]*, and Whereas it was, by the said Regulations annexed to the said Treaty, that the said Mixed Courts of Justice* […] *should be composed in the following manner, that is to* say: *that the Two High Contracting Parties should each of them name a Judge and an Arbitrator* (Ferrier 1983: 4, 6).

When Thomas Sherard Wale, the first English arbitrator appointed to the Mixed Court of Paramaribo,

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FIGURE 1. John Henry Lance (1793–1878). In Ferrier (1893: 5).

died suddenly in 1819, J.H. Lance was appointed as his successor.

Surinam, like most other European colonies in the Caribbean area, based its economy on the plantation system (mostly sugar cane and coffee),and heavily depended on slave labor (Fig. 5). It is therefore understandable that the Dutch plantation owners tried to circumvent the conditions of the treaty, under which they could not increase the number of slaves through new 'imports', and had to employ hired labor instead.

It was under these circumstances that John Henry Lance arrived in Paramaribo on January 7, 1823. Lance was born in 1793 at Netherton, near Andover. His father, a clergyman, took much interest in giving his only male child the best education. Thus, J.H. Lance attended Eton College from 1810 to 1812, and in 1815 was admitted to the Middle Temple, one of the four Inns of Court entitled to call their members to the English Bar as barristers. In 1820 he was appointed to the Degree of the Utter Bar of the Inner Temple. Letters from his tutors and professors underlined his qualities as a talented and hard-working student with an exemplary character (Ferrier 1983: 8).

FIGURE 2. *Am I Not a Man and a Brother?* 1787 medallion designed by Josiah Wedgwood for the British anti-slavery campaign.

In Surinam, *Lance's days were filled with opportunities to absorb the values of the planter class. He spent nearly half his time on various estates, "sometimes on business & sometimes not"* (Ben-Ur 2016: 6) (Fig. 6). A close friend of James Bateman and John Lindley, Lance shared their passion for plants, especially orchids, and his excursions from one plantation to the other gave him plenty of opportunities to collect orchids, of which he sent a number of new species to the nursery of George Loddiges.

Cycnoches loddigesii Lindl. (Fig. 7A)*, Pleurothallis lanceana* Lodd., and *Schomburgkia marginata* Lindl. (Fig.7B) were described amongst Lance's collections in Surinam.

A few others of his plants were named in his honor: *Oncidum lanceanum* Lindl. (Fig.7C), and *Brassia lanceana* Lindl. (Fig.7D).

By the end of his term in Surinam, John Henry Lance had become well adapted to life in the Dutch colony. Notwithstanding his position in the Mixed Court, he had no problem in accepting slavery in society as it was. He envied the proprietors he

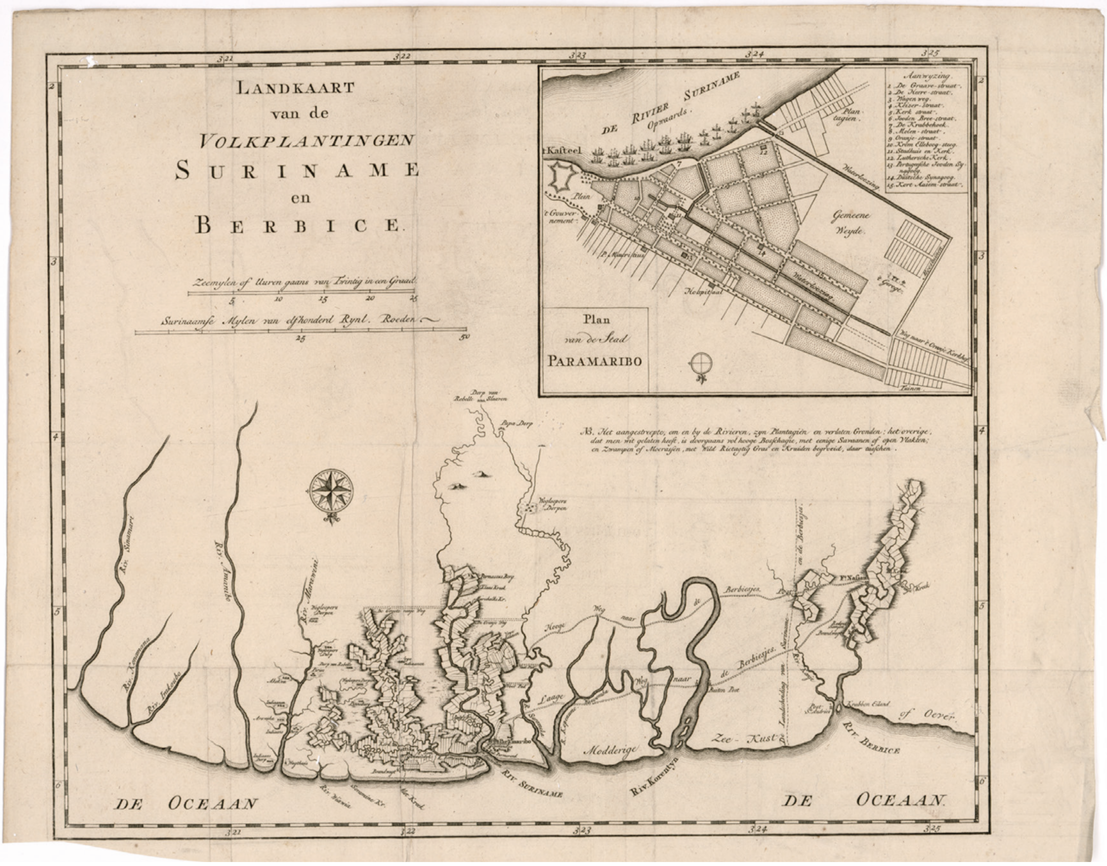


Figure 3. Map of the colonies of Surinam and Berbice. 18th century. Unknown author.

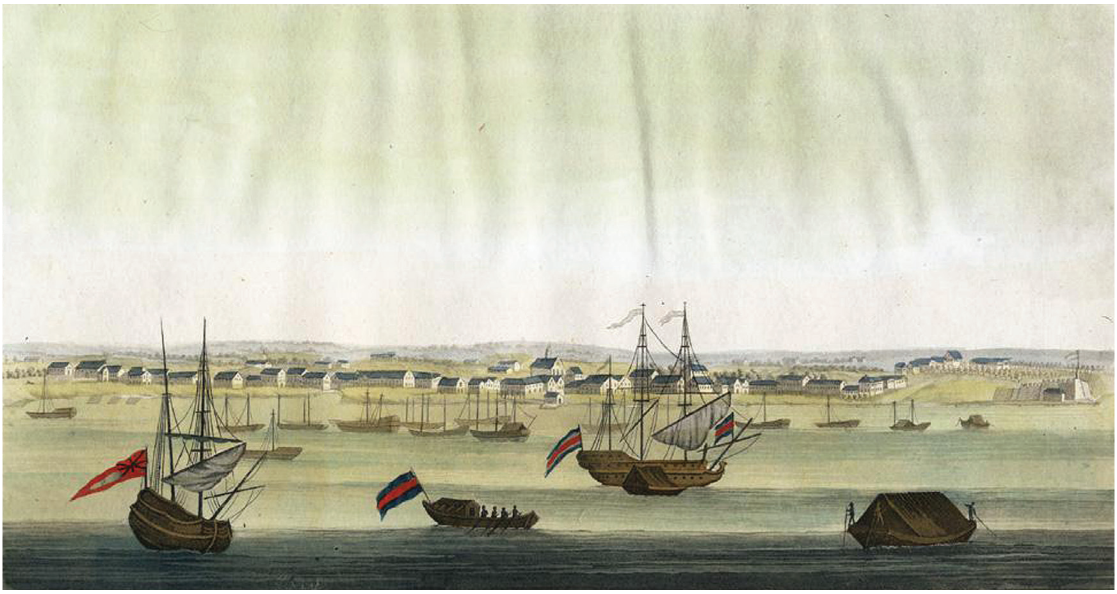


Figure 4. View of Paramaribo, Surinam (1827) as it appeared during the Dutch colonial period. With ships and the fort Zeelandia on the right. Print by Giulio Ferraio.

Figure 5. Slave trade in Surinam, early 19th century. Unknown artist.

Figure 6. View of the plantation Marienbosch at the Taparoepikanaal in Surinam. Oil on canvas by Willem de Klerk, ca. 1825.

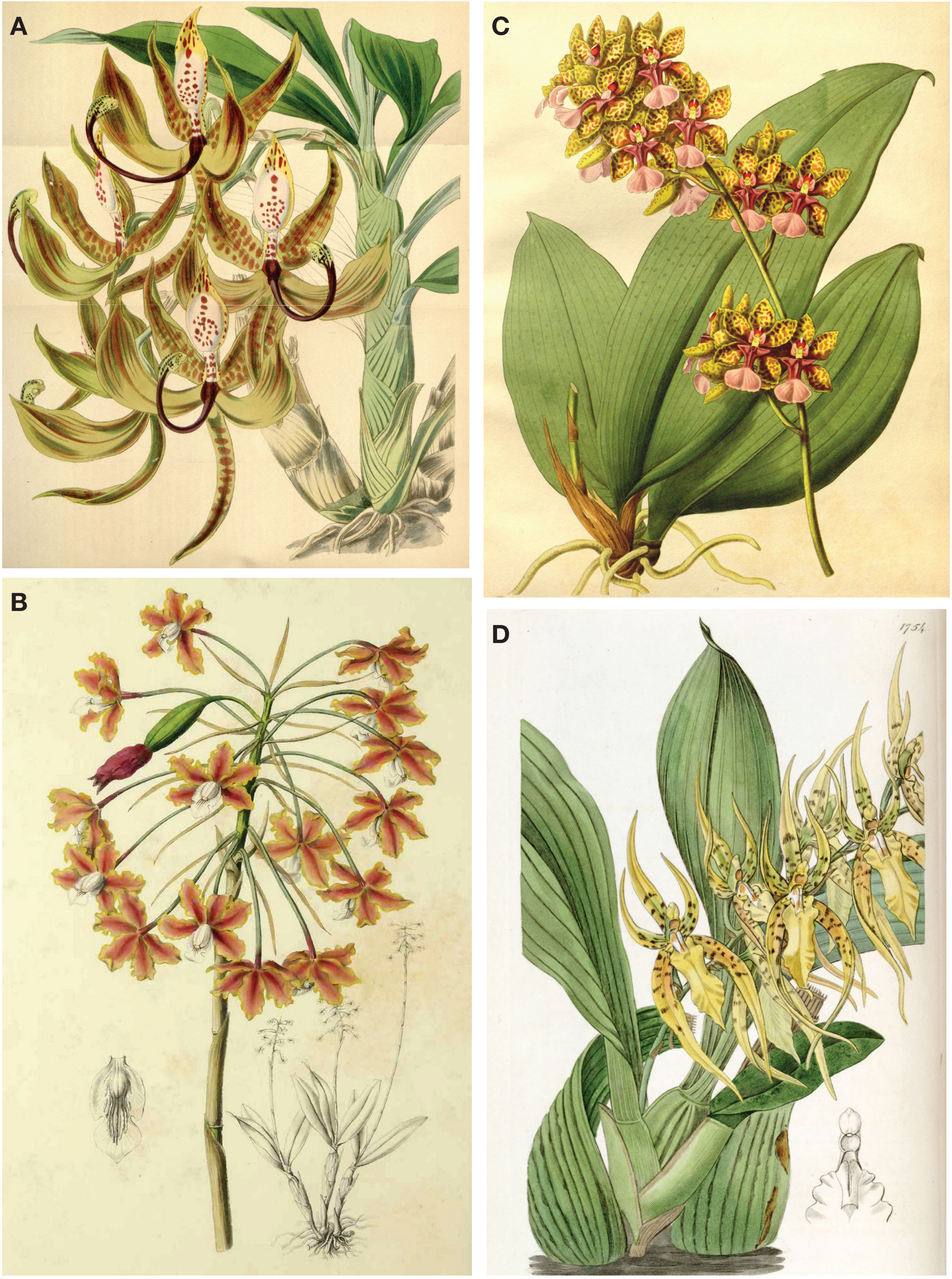


Figure 7. A, *Cycnoches loddigesii,* from Hooker (1846). B, *Schomburgkia marginata* Lindl., from Lindley (1838). C, *Oncidium lanceanum* Lindl.from Lindley (1842). D, *Brassia lanceana* Lindl., from Lindley (1836).

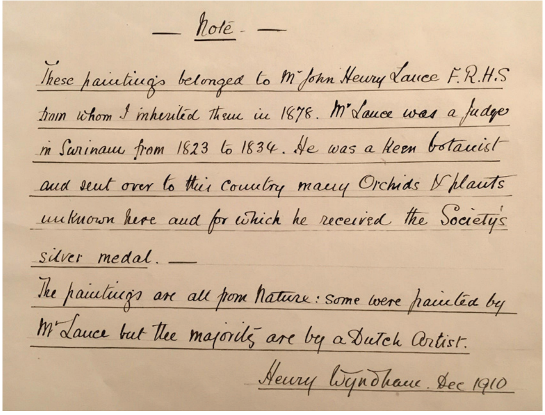
described, particularly the Dutch. Three quarters of them lived "quietly in Holland…doing nothing but receive their remittances which after all the usual rascality of their agents are still considerable." In 1829, Lance was still trying to convince his father to become a co-investor. To assuage his "qualms… about being a holder of slaves," Lance assured him that the slaves were "as happy & contended a race of people in this colony as your parishoners." (Ben-Ur 2015: 7). When he embarked for his return to England, he had in his company two manumitted female slaves, and it is said that he once cracked a joke about his father acquiring a "Black" daughter-in-law.

Well-known as a plant collector, Lance's talent as a botanical illustrator went largely unknown during his time. It was not until Lindley, in 1838, described his *Schomburgkia marginata* from a plant collected by Lance in Surinam (Fig. 14) that illustrations of orchids by the hand of Lance were mentioned for the first time. "*When Schomburgkia crispa was published a few months since in this work, mention was made of a second species of the genus, of which I had received specimens from Mr. Schomburgk. I have since been so fortunate as to find a beautiful coloured drawing of this curious epiphyte, among a valuable collection of figures of Surinam plants, made by direction of my friend John Henry Lance, Esq., during his residence in that colony. From these materials I have been allowed to prepare the accompanying figure, corrected from specimens in my herbarium"* (Lindley 1838: under plate XIII). Sixty years later, in 1898, Robert A. Rolfe, in his obituary for James Bateman, mentions these illustrations again. Rolfe mentions a letter which he received in 1892 from Bateman, in which the latter wrote: *Mr. Lance (after whom Oncidium Lanceanum is called), and who discovered Cycnoches Loddigesii in Surinam has not been dead many years.* […] *I remember going to his rooms in the Temple, together with Mr. Huntley (hence the genus Huntleya), who was a friend of his, where we feasted our eyes on a large portfolio of drawings (by Mr. Lance himself) which he brought with him from Surinam. Lindley, in a letter to me, describes his first visit to them in these words: 'Oh! I have just seen such drawings of such things of Surinam – beautiful beyond description, and nearly all new!!* (Rolfe 1898: 56–57).

Lance's drawings would have remained largely unknown were it not for a fortunate accident. While searching for images about Lance and his plants on the Internet, the author found on the web page of the Lindley Library of the Royal Horticultural Society a beautiful illustration of a specimen of *Passiflora laurifolia* with the following title: *Watercolour on paper of Passiflora laurifolia, by John Henry Lance (c.1793–1878). From volume I of Surinam Orchids Etc. from Nature*. The Lindley library was immediately consulted and soon digital images were received of all orchids contained in this portfolio. In addition, two notes in volume II of Lance's work had been copied. The first of them read:

> *These paintings belonged to Mr. John Henry Lance F.R.H.S. from whom I inherited them in 1878. Mr. Lance was a judge in Surinam from 1823 to 1834. He was a keen botanist and sent over to this country many orchids & plants unknown here and for which he received the Society's Silver Medal. The paintings were all from Nature: some were painted by Mr. Lance but the majority are by a Dutch Artist.*
>
> *Henry Windham. Dec. 1910.* (Fig. 8)

Wyndham's note reveals an important fact: many of the flowers drawn in Lance's work were not painted by him, but by a Dutch artist by the name of Gerrit Carl François Schouten (1779-1839). His relationship to Lance is not clear. However it seems probable that Schouten was engaged by Lance to paint plants for him. Schouten, a Surinamese born in Paramaribo and son of a Dutch government clerk and a local black woman, taught himself to paint and



— Note —

These paintings belonged to Mr John Henry Lance F.R.H.S
from whom I inherited them in 1878. Mr Lance was a Judge
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unknown here and for which he received the Society's
silver medal. —
The paintings are all from Nature: some were painted by
Mr Lance but the majority are by a Dutch Artist.
Henry Wyndham. Dec 1910

FIGURE 8. Note in volume II of Lance's *Surinam orchids from nature*.. Courtesy and with permission of the Lindley Library, R.H.S.

Figure 9. Dancing slaves. Diorama by Gerrit Schouten..

Figure 10. Government Square in Paramaribo. Diorama by Gerrit Schouten.

had become famous for his painted papier-maché dioramas of Surinamese life. These are flat boxes containing perspective, three-dimensional painted depictions of slave dances and city and plantation views among other themes. These dioramas, preserved today at Amsterdam's Rijksmuseum, form a significant source of historical and topographical information about Surinam in the early 19th century (Fig. 9–10).

An additional note in volume II of Lance's work reads: *These 2 volumes are presented to the R.H.S. by Henry Wyndham, Thornton Heath, Surrey. January 1878.*

As to the paintings themselves, a few explanatory notes supplied by Charlotte Brooks, Art Curator of the Lindley Library, are of interest:

- Most of the artworks are by Gerrit Carl François Schouten (1779-1839), and as such as signed '*G Schouten fecit'* in ink, in the lower right or lower left corners.
- A few paintings are signed '*John Henry Lance fecit'*, these tend not to be of the same quality as those by Schouten.
- There are several paintings that are unsigned, but it may be possible to attribute these to Lance, judging them by the quality of the painting
- There are other paintings that feature the initials '*JHL'* in the lower right or left hand corner, in graphite pencil. This cannot be assumed to be a signature, though, as they appear on works signed by Schouten and unsigned paintings. It is more likely this was just an indication that the artworks belonged to Lance.

A total of 12 watercolors depicting Surinam orchids are preserved at the Lindley Library. Of these, seven were painted by Lance and six by Gerrit Schouten.

The species painted by John Henry Lance were: *Brassavola angustata* Lindl.**,** *Catasetum macrocarpum* Rich. ex Kunth., *Cycnoches ventricosum* Bateman, *Epidendrum schomburgki* Lindl., *Oncidium lanceanum* Lindl., *Schomburgkia marginata* Lindl., and *Sobralia sessilis* Lindl. (Fig. 11–17).

By the hand of Gerrit Schouten were the illustrations of *Aspasia variegata* Lindl, *Ionopsis utricularioides* (Sw.) Lindl., *Pescatoria violacea* (Lindl.) Dressler, *Prosthechea* cf. *crassilabia* (Poepp. & Endl.) Carnevali & I.Ramírez, *Rodriguezia secunda* Kunth. and, *Stanhopea grandiflora* (Lodd.) Lindl. (Fig. 18–23).

A final anecdote: in 1834, Bateman sent a collector to Surinam named Thomas Colley. Several orchid species were published in the *Botanical Register* from Colley's expedition. One of them was *Oncidium lanceanum,* of which John Lindley wrote in his *Sertum Orchidaceum*: […] *John Henry Lance, Esq., upon his return to England from Surinam, where he had been residing several years, brought with him a considerable collection of orchideous epiphytes, which he presented to the society. Among other interesting species was the subject of the following memorandum; a plant than which a more acceptable addition to the hothouses of this country has seldom been made* (Lindley 1838: 238). Bateman, full of pride, wrote that *Colley had found a tree covered with this species and, knowing that Francis Henchman, another 'traveler' working for Low's nursery was not far behind, stripped the tree of all the orchids. Bateman later said, without regret, that the species was not found before or since. He says rather boastfully, 'Everyone was prepared to go down on their knees… offering their greatest treasures in exchange.' This sad commentary reveals the unsustainable way that orchids were collected in Victorian times, even by those who were supposedly educated.* (Siegel 2013:19).

ACKNOWLEDGEMENTS**.** To the Royal Horticultural Society Lindley Collections for supplying the digital images of the orchid paintings by J.H. Lance and G. Schouten. To Crestina Forcina, Digital Images Assistant and Charlotte Brooks, Art Curator, both of the RHS Lindley Library, for all their kind help in this matter. All images of these paintings are reproduced by permission of the RHS Lindley Collections. Finally, to Franco Pupulin for the determination of the species painted by Lance and Schouten and to Mark Budworth, for the philological revision of the text.

Figure 11. *Epidendrum schomburgki* Lindl. Plate 101 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

Figure 12. *Cycnoches ventricosum* Bateman. Plate 102 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

FIGURE 13. *Sobralia sessilis* Lindl. Plate 104 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

FIGURE 14. *Schomburgkia marginata* Lindl. Plate 115 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

Figure 15. *Oncidium lanceanum* Lindl. Plate 125 of *Surinam orchids from nature*, by J.H. Lancee. With permission and under © of the Lindley Library, R.H.S.

Figure 16. *Catasetum macrocarpum* Rich. ex Kunth. Plate 124 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

Figure 17. *Brassavola angustata* Lindl. Plate 120 of *Surinam orchids from nature*, by J.H. Lance. With permission and under © of the Lindley Library, R.H.S.

Figure 18. *Aspasia variegata* Lindl. Plate 072 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

FIGURE 19. *Pescatoria violacea* (Lindl.) Dressler. Plate 080 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

FIGURE 20. *Ionopsis utricularioides* (Sw.) Lindl. Plate 031 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

Figure 21. *Rodriguezia lanceolata* Ruiz & Pav. Plate 049 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

FIGURE 22. *Stanhopea grandiflora* (Lodd.) Lindl. Plate 055 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

Figure 23. *Prosthechea* cf. *crassilabia* (Poepp. & Endl.) Carnevali & I.Ramírez. Plate 080 of *Surinam orchids from nature*, by G. Schouten. With permission and under © of the Lindley Library, R.H.S.

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THE ENDANGERED DAFFODIL ORCHID *IPSEA SPECIOSA* (ORCHIDACEAE): POPULATION STATUS IN UNPROTECTED AREAS IN THE CENTRAL HIGHLANDS, SRI LANKA

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Abstract. The Daffodil Orchid, *Ipsea speciosa*, is a rare endemic terrestrial orchid species found in the highland grasslands of Sri Lanka. Due to the restricted distribution range, this species is considered as an endangered taxon. This study aimed to assess the present state of *I. speciosa* populations outside of protected areas. During field investigations of nine selected grasslands, the abundance of *I speciosa* and accompanying plant species composition was determined. The abundance of *I. speciosa* varied from 2 to 23 flowering plants per site. Species composition in study sites consisted of 41 plant taxa that included 12 invasive species. Habitat deterioration, intentional burning, clearing of vegetation, and spread of invasive alien plant species were found to be the most significant threats to highland grasslands and the orchid. During the survey, we observed the rapid spreading of invasive species in most of the sites which suggests that the terrestrial orchid population could shrink further. Apart from intentional burning, other serious threats to the orchids are various anthropogenic activities such as illegal collection for medicinal purposes and establishing timber plantations that negatively impact native grassland flora including terrestrial orchids. Our results suggest that a continuous monitoring program for *I. speciosa* should be initiated. Additionally, monitoring of potentially adverse anthropogenic activities is considered to be vital for the conservation of valuable grasslands and native flora of Sri Lanka.

Key Words: Epidendroideae, grasslands, invasive species, native flora, terrestrial orchids, threatened species

Introduction. Sri Lanka, with a total land area of 65,610 km^2 is a tropical island located in the Indian Ocean bearing a rich and unique biodiversity. The Central Highlands complex is situated in the south-central part of the island and is considered as a super biodiversity hotspot (Unesco 2010). More than 50% of the endemic vertebrates, 50% of the endemic flowering plants and more than 34% of the endemic trees, shrubs, and herbs are reported from these diverse montane rainforests and associated grassland areas (Gunawardene *et al*. 2007, Ministry of Environment 2012). Orchidaceae is among the largest families of flowering plants in the country with 189 known species, belonging to 78 genera, including 55 endemic species (Fernando 2013). More than any other plant family, Orchidaceae has a high proportion of threatened genera with most containing threatened species (Swarts & Dixon 2009). Considering the Sri Lankan orchids, 70.6% of the species including 84% of the endemics are categorized as threatened. Within this 8.6% of the species are Critically Endangered (CR), 29.3% are Endangered (EN) and 32.6% of species are Vulnerable (VU). Further, 2.2% of the species are Critically Endangered Possibly Extinct [CR(PE)], 14.1% are Near Threatened (NT) (Ministry of Environment 2012).

The distribution and abundance of orchid populations depend on a suite of biological and ecological factors, including pollinator specialization, seed production and dispersal, limited germination rates, the viability of mycorrhizal fungi (Otero & Flanagan 2006) and appropriate environmental conditions (McCormick & Jacquemyn 2014). Besides, temperature, altitude, and soil pH are the main factors affecting the distribution and abundance of terrestrial orchid species (Djordjević *et al.* 2016). Sustainability of orchid populations is determined by changes in the number of individuals within a population, the degree of completeness in the ontogenetic spectrum of species,

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population age and sex structure, etc (Valuiskikh & Teteryuk 2013, Khapugin *et al.* 2016).

Numerous studies have indicated the importance of certain vegetation types in determining the distribution and abundance of orchids (Landi *et al.* 2009, Djordjević *et al.* 2016, Khapugin *et al.* 2017). Grasslands, wet meadows, bogs, marshes, and montane forests represent important ecosystems that host many orchid species. In Sri Lanka, the lower montane zone (900–1500 m a.s.l.) and montane zone (above 1500 m a.s.l) has recorded the highest wild orchid diversity (Fernando 2013). The measure of the orchid species diversity also provides an insight into the health and the complexity of the ecosystem that they are living in (Fernando 2013, Khapugin *et al.* 2016).

Among terrestrial orchid species, *Ipsea speciosa* Lindl. has been identified as an endemic and endangered species (Ministry of Environment 2012) with medicinal properties (Kumari *et al.* 2006). It is hard to distinguish this species among grasses when there are no flowers as it does not bear leaves during the flowering season. *Ipsea speciosa* represents a formerly common species, occurring in grasslands of the Central Highlands of Sri Lanka (Jayaweera 1981). However, the number of sites of orchid species including *I. speciosa* started to decline due to various anthropogenic activities, such as intentional burning, illegal collection from the wild for medicinal purposes etc. (Fernando 2012). Therefore, the present study aimed to determine the population status and the factors affecting the distribution of *I. speciosa* in isolated populations of unprotected areas in the Central Highlands, Sri Lanka.

Materials and Methods

Study species.— *Ipsea speciosa*, popularly called the Daffodil Orchid, is an endemic and endangered terrestrial orchid species occurring in wet grasslands in the Central Highlands of Sri Lanka (Ministry of Environment 2012). It is easily distinguished by its large bright yellow flowers among the grasses of the patana lands in the montane zone (915–1829 m a.s.l). This terrestrial herb has 2–3 cm broadly ovoid pseudobulbs, long filiform roots from their bases. Leaves are usually single, 15–25 x 0.5–2.2 cm, narrowly or lanceolately linear. It produces one or two (rarely three) large, 5.0–6.6 cm across, bright golden-yellow flowers in a tall, erect, sheathed, 15–40 cm long peduncle. The flowering season is from September to February (Jayaweera 1981).

Study sites.— Information on the possible habitats of *I. speciosa* was compiled from published information and literature (Jayaweera 1981, Vlas & Vlas 2008, Fernando & Ormerod 2008, Fernando 2013). Based on compiled information, the surveying area was focused on the western slope of the Central Highlands, starting from Nawalapitiya in Kandy district (600 m a.s.l.) to a higher elevation in Ohiya in Badulla district (1850 m a.s.l.). The mean annual temperature of the Central Highlands is 16 °C, with January as the coldest month with respect to mean monthly temperature, and April and August being the warmest months (Department of Meteorology 2019). This region receives relatively high rainfall throughout the year and contains a diversity of edaphic environments, particularly montane forests, tea plantations and grasslands. The grasslands habitats including banks of the streams of the Mahaweli River, abandoned areas in tea (*Camellia sinensis*) plantations, *Eucalyptus* plantations, banks and rock outcrops along railway line were investigated.

Based on preliminary survey, there were nine isolated populations of *I. speciosa* identified. Sites 1–7 formed a single cluster which was located approximately 20 km away from each other in a mid elevational range (800–1200 m a.s.l.) (Fig. 1). The area where the sites 1–7 were located received highest rainfall (above 3200 mm per annum) during the monsoon periods (Department of Meteorology 2019). Site 8 was observed near to human settlement which was located more than 50 km away from other sites, whereas site 9 was observed near to the montane cloud forest (1850 m a.s.l.) (Fig. 1). Both sites were in higher elevation (above 1500 m a.s.l.) in the western slope of the Central Highlands.

Measurement of ecological parameters.— Two square plots (2m x 2m) were established in each study site to survey the abundance of flowering individuals of *I. speciosa* during the blooming peak (November-December) in two consecutive years (2017 and 2018). The peak flowering period was determined based on previous literature (Jayaweera 1981, Vlas & Vlas 2008). Each peduncle above the ground was the accounting unit being conditionally treated as an individual for this study. The numbers of flowering individuals in the

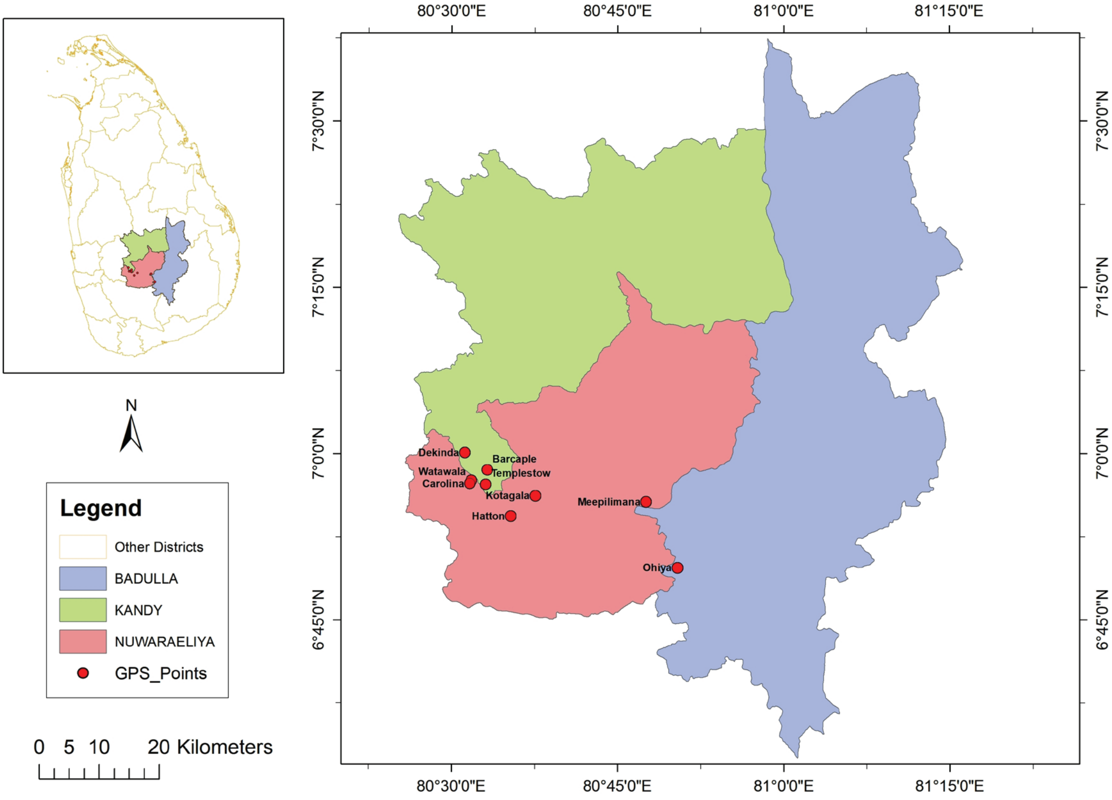

Figure 1. Geographical location of the nine *Ipsea speciosa* populations found and surveyed at the western slope of the Central Highlands, Sri Lanka.

optimum period of flowering were used for statistical analysis.

The vegetation type and the composition of the accompanying flora were recorded in each plot for each site for further interpretation. The relative abundance of accompanying flora was calculated as follows: [Number of individuals of particular species within the two plots / Total number of all individuals of all species in the two plots] × 100 . The nomenclature and the threatened status of these species were obtained from The National Red List of Sri Lanka (Ministry of Environment 2012). Recently, several invasive species were observed in the montane ecosystems, especially grasslands habitats; therefore, the abundance of invasive plant species and their potential threats were also recorded. The data set consisted of the species data, and included abundance of *I. speciosa*, number of accompanying flora, threatened/endemic species, and invasive species. Geographic coordinates in each site were used to make distribution map.

Soil samples were collected 10 cm in depth within the orchid root zone in each plot and bulked. Electrical conductivity (EC) and pH were determined using an EC-meter and a pH-meter in saturation extract, respectively. The percentage of organic matter (OM%) was determined following the protocol of the Department of Agriculture (1997). Total potassium (K) and phosphorus (P) contents were analyzed using the spectrophotometer and flame photometer, respectively. Anthropogenic disturbance such as intentional burning, clearance of vegetation, collection of firewood, lopping grasses for animal feed, among others, were annotated in each site.

Data analysis.— Study sites were mapped following geographic information system procedures using ArcGIS 10.5. The Shannon diversity index (H'), Simpson index (1-D) and Fisher alpha were used to measure of species abundance, richness and evenness to quantify diversity of the accompanying flora in

TABLE 1. Abundance of *Ipsea speciosa* and status of accompanying flora of nine locations found at the western slope of the Central Highlands, Sri Lanka.

Site	Locations	*Ipsea speciosa*	No. of accompanying plant species	No. of endemic/ threatened plant species	No. of invasive plant species	pH	EC	P (ppm)	K (ppm)	OM (%)	Habitat/ecosystem
Site 1	Barcaple	13	17	3	5	5.25	10.21	8.9	77.0	4.21	Grassland near a stream
Site 2	Templestowe	10	15	3	6	5.62	16.85	11.0	98.0	4.50	Grassland in a cemetery
Site 3	Watawala	21	21	3	7	5.43	11.01	12.0	84.0	2.43	Grassland adjoining tea land
Site 4	Carolina	3	14	2	4	5.41	21.51	10.7	92.0	3.25	Grassland in an Eucalyptus plantation
Site 5	Dekinda	4	13	4	6	5.23	11.62	5.5	74.0	2.76	Banks of railway line
Site 6	Hatton	23	10	1	5	5.12	42.20	10.0	232.0	6.07	Banks of railway line
Site 7	Kotagala	11	14	2	5	5.34	24.08	7.7	114.0	4.75	Banks of railway line
Site 8	Meepilimana	15	7	1	3	5.31	25.89	9.1	132.0	5.83	Grassland in a cemetery
Site 9	Ohiya	2	16	7	4	5.20	12.05	5.8	75.0	3.34	Banks of railway line

study sites. Similarity between plant communities in different study sites was explored using the Sorensen Similarity Index (SI). The SI is vary from 0 where the assemblages differ totally to 1 where they are identical ($SI = 2c/(a + b)$, where c is the number of species shared by the two sites, and a and b are the total number of species at each site).

A principal component analysis (PCA) and cluster analysis were performed to determine the main contributory factors associated with the grouping of habitats based on vegetation/floristic composition. Ecological parameters such as total number of *I. speciosa*, accompanying flora, threatened species, invasive species, pH, EC, P, K and organic matter were used for PCA and Cluster analysis. Principal component analysis (PCA) ordination diagram of habitats was generated using first two Principle Components. Statistical analyses were carried out using PAST 3 statistical software.

Results and Discussion. All the study sites were located in grassland habitat with various plant communities. There were four sites investigated near the railway line and two grasslands identified as cemetery sites, the rest of the grasslands belong to tea lands, *Eucalyptus* plantation, and stream bank habitat (Table 1). The abundance of *I. speciosa* varied from 2 to 23 individuals among nine investigated sites. A large population was observed in site 6 (near Hatton), followed by site 3 (near Watawala), whereas in site 4 (near Carolina) and site 9 (near Ohiya) registered 3 and 2 individuals, respectively (Table 1). In Sri Lanka, several restricted populations of *I. speciosa* have been documented in protected areas such as Peak Wilderness Protected Area, Horton Plains National Park, and Hakgala Strict Nature Reserve (Kumar & Manilal 1987, Gunatilleke & Pethiyagoda 2012). Unfortunately, all sites investigated during the present study were not entering protected areas.

Accompanying flora.— The composition of flora accompanying *Ipsea speciosa* includes 42 species of plants classified in 36 genera (Table 2). The highest number of plant species were recorded in site 3, whereas the lowest numbers were recorded in site 8. Site 3 is adjoining to a tea plantation without large trees. The most species-rich genera were *Osbeckia* L. (Melastomataceae) with 3 taxa, followed by *Mimosa* L. (Fabaceae), *Panicum* L. (Poaceae), and *Rubus* L. (Rosaceae), with two taxa each. There were six endemic, two endangered, one vulnerable and three near threatened species in the accompanying flora of *I. speciosa* (Table 2).

Table 2. List of flora accompanying *Ipsea speciosa* and their relative abundance in each site surveyed at the western slope of the Central Highlands, Sri Lanka. Designations: EN: endangered, VU: vulnerable, NT: near threatened, In: invasive species, * indicate the endemic species.

Species	**Sites**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ageratina riparia^{In}	-	-	6.8	8.2	-	-	13.3	38.1	14.8
Aristea ecklonii^{In}	-	-	-	-	-	-		28.6	6.2
Arundina graminifolia	5.7	4.1	2.7	3.1	-	2.6	1.9	-	-
Austroeupatorium inulifolium^{In}	4.8	3.1	5.5	6.1	6.6	12.8	10.5	11.9	9.9
Blechnum orientalis	7.6	-	6.2	-	10.5	-	9.5	-	-
Chamaecrista auricoma	-	-	-	-	-	-	-	-	6.2
Clidemia hirta^{In}	11.4	3.1	-	-	11.8	12.8	9.5	-	-
Clusia rosea^{In}	8.6	10.3	8.2	-	10.5	-	-	-	-
Crotalaria pallida	-	-	-	-	-	-	1.0	-	-
Cyanotis thwaitesii^{NT}	-	-	-	-	-	-	-	-	1.2
Cymbopogon confertiflorus	11.4	11.3	12.3	15.3	13.2	15.4	13.3	-	14.8
Erigeron karvinskianus	-	-	-	-	-	-	-	-	6.2
Ecucalyptus grandis	5.7	-	3.4	-	-	-	-	-	-
Exacum trinervium^{*NT}	1.9	1.0	1.4	-	-	-	-	-	-
Gleichenia linearis	7.6	9.3	6.2	8.2	-	6.4	8.6	-	4.9
Hedyotis fruticosa	4.8	4.1	5.5	-	-	-	-	-	-
Hypochoeris radicata	-	-	-	-	-	-	-	7.1	-
Ipomoea indica^{In}	-	-	3.4	-	-	5.1	3.8	-	-
Lantana camara^{In}	-	-	4.1	-	-	-	-	-	-
Liparis sp.	-	-	0.7	-	-	-	-	-	-
Litsea longifolia*	-	-	-	-	1.3			-	-
Lobelia nicotianifolia	2.9	1.0	-	4.1	5.3	6.4	4.8	-	-
Lycopodiella cernua	4.8	-	3.4	4.1	-	-	6.7	-	-
Miconia calvescens^{In}	-	-	-	-	3.9	-	-	-	-
Mimosa invisa^{In}	-	8.2	6.8	-	15.8	17.9	-	-	-
Mimosa pudica	-	-	1.4	2.0	-	-	-	-	-
Osbeckia octandra*	3.8	5.2	2.1	3.1	5.3	-	2.9	-	4.9
Osbeckia parvifolia^{EN}	-	-	-	-	-	-	-	2.4	1.2
Osbeckia rubicunda^{EN}	-	-	-	-	-	-	-	-	4.9
Panicum maximum^{In}	4.8	10.3	7.5	15.3	11.8	16.7	10.5	-	-
Panicum repens	0.0	11.3	3.4	13.3	-	-	-	-	-
Persicaria capitata	-	-	-	-	-	-	-	-	4.9
Pinus caribaea	1.9	-	-	-	-	-	-	-	
Pteridium aquilinum^{In}	-	-	-	-	-	-	-	-	6.2
Rhododendron arboretum*	-	-	-	-	-	-	-	-	4.9
Rubus ellipticus	-	-	3.4	-	-	-	-	9.5	-
Rubus indicus	-	-	2.7	-	-	-	-	-	-
Satyrium nepalense^{NT}	-	-	-	-	-	-	-	-	2.5
Sphagneticola trilobata^{In}	7.6	12.4	-	11.2	-	-	-	-	-
Torenia cyanea^{*VU}	-	-	-	-	1.3	-	-	-	-
Wendlandia bicuspidata*	4.8	5.2	2.7	6.1	2.6	3.8	3.8	-	6.2

TABLE 3. Species richness and diversity indices of different habitats surveyed.

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
No. of plant species	18	16	23	14	14	11	15	7	17
Individuals	118	107	167	101	80	101	116	56	83
Shannon index (H')	2.771	2.6	2.934	2.456	2.444	2.193	2.544	1.674	2.648
Simpson index (1-D)	0.931	0.918	0.937	0.902	0.903	0.871	0.914	0.784	0.918
Evenness	0.887	0.841	0.817	0.832	0.823	0.815	0.849	0.762	0.831
Fisher alpha	5.918	5.213	7.227	4.411	4.913	3.142	4.589	2.112	6.473

TABLE 4. Sorensen index pairwise of the accompanying flora species richness among study sites.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Site 1	1								
Site 2	0.813	1							
Site 3	0.660	0.650	1						
Site 4	0.660	0.710	0.628	1					
Site 5	0.600	0.640	0.457	0.461	1				
Site 6	0.590	0.720	0.5	0.609	0.609	1			
Site 7	0.713	0.622	0.611	0.741	0.593	0.75	1		
Site 8	0.087	0.095	0.143	0.211	0.105	0.125	0.2	1	
Site 9	0.240	0.323	0.263	0.414	0.276	0.307	0.4	0.273	1

Our results indicate that site 3 is a diverse habitat that maintains the highest species richness, recording 23 species that represent 54.8% of all species recorded in the survey (H'=2.934, Fisher alpha=7.227, Simpson index (1-D)=0.937) (Table 3). Site 1 was the next important habitat recording 18 species (H'=2.771). Site 8 showed the lowest diversity among all habitats (H'=1.674, Fisher alpha=2.112). Several causes could explain variations in the degree of diversity between the sites of the study area: soil physical and chemical properties, rainfall pattern, anthropogenic action, land use pattern, etc. Site 8 was categorized as a cemetery and several anthropogenic activities such as regular slashing, clearing, and burning were observed. Although site 9 was located outside the Horton Plains National Park, the highest abundance of endemic and nationally threatened species was observed in the grassland of this site, which could be due to a less anthropogenic disturbance away from human settlement and restricted movement along the railway line.

Among 41 accompanying flora, only one species (*Austroeupatorium inulifolium*) was shared among all the sites. Two species (*Cymbopogon confertiflorus* and *Wendlandia bicuspidata*) were found in eight sites, except in site 8, while *Hypochoeris radicata* was unique to site 8. Sites 1 and 2 shared more common species (SI=0.813, Table 4). Site 8 recorded the lowest values for the SI, indicating the most distinct site. Conversely, sites 6 and 8 recorded the lowest richness of threatened species. During the study period, flowering of limited distribution threatened species such as *Cyanotis thwaitesii* Hassk. (Commelinaceae), *Exacum trinervium* (L.) Druce (Gentianaceae), and *Osbeckia parvifolia* Arn. were also observed in few locations.

In addition to the Daffodil Orchid (*Ipsea speciosa*), three terrestrial orchid species were observed at the study sites. *Arundina graminifolia* (D.Don) Hochr. (Fig. 2B) was commonly found at sites 1–7 in mid-elevation, except site 6. *Satyrium nepalense* D.Don (Fig. 2C) was recorded only in site 9 with a few individuals. One orchid species observed in site 3 without flowers and pods, may belong to the genus *Liparis* Rich. based on leaf characters. The results of the principal component analysis (PCA) (Fig. 3) and hierarchical cluster analysis (Fig. 4) exhibited that the selected vegetation parameters strongly affect the grouping of sampled

Figure 2. **A**. Blooming of Daffodil Orchid, *Ipsea speciosa*, in grassland. **B**. *Arundina graminifolia* blooming at site 2. **C**. *Satyrium nepalense* blooming at site 9. Photographs by C. Mahanayake (A) and J. D. Kottawa-Arachchi (B–C).

grasslands. The first two principal components (PCs) of floristic compositions of investigated sites account for 99.3% of the total variability (Fig. 3). The total number of accompanying plants and the abundance of *I. speciosa* are the most contributing factors for the clustering of habitats. Following both, the principal component analysis (PCA) and hierarchical cluster analysis (Fig. 4), locations studied were grouped into three main clusters. Sites 1–5 located within 100 km^2 and the rest of the sites are scattered geographically. Out of the nine locations, sites 7 and 8 grouped in 1st cluster. Cluster II comprised six sites (1, 2, 3, 4, 5 and 9) and biplot showed scatter distribution of those sites suggesting the distance relationship each other. Sites 4, 5 and 9 which are representing few individuals of *I. speciosa* and critical conservation measures should be required to protect those small populations. Only site 6 is separate from the rest of the sites representing a relatively higher number of *I. speciosa* and accompanying species. The grassland habitats in two

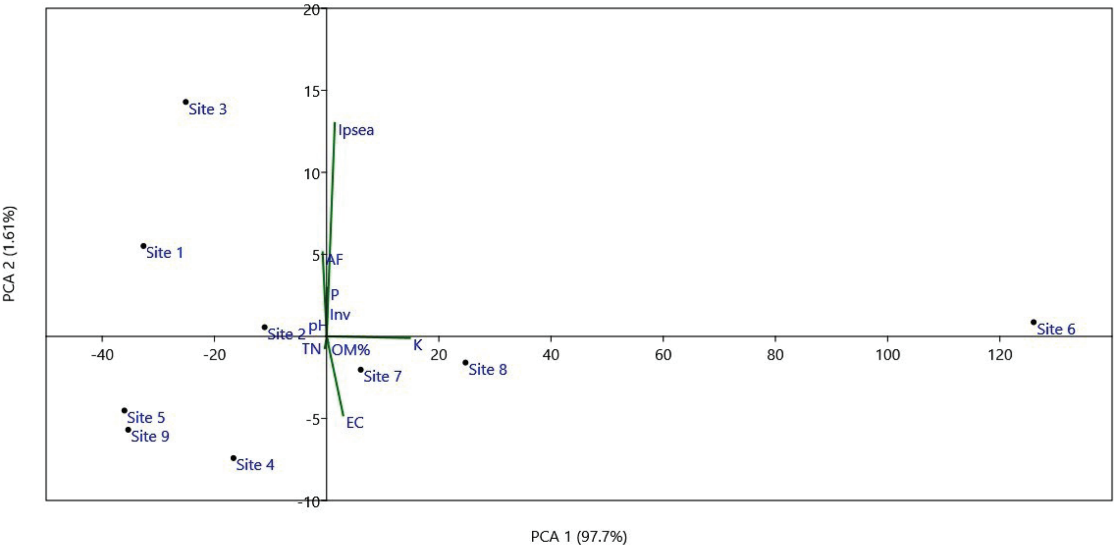

FIGURE 3. Principal component analysis (PCA) ordination diagram of sites with presence of *I. speciosa* based on vegetation parameters. Designations: Ipsea: abundance of *I. speciosa*, AF: total numbers of accompanying flora, TN: endemic/threatened species, Inv: invasive species. EC: electric conductivity, P: phosphorus content, K: potassium content, OM: organic matter.

sites (5 and 9) recorded higher threatened species. These sites are about 2–3 km away from human settlements which could consider as less disturbed sites than the rest of the locations. The clusters representing a close association in biplot could be explained by similar vegetation communities and positions within clusters.

Factors determining distribution and abundance of Ipsea speciosa.— The results of this study show that soil properties such as EC, K, and P effectively influence the distribution and abundance of orchid species within sampled grasslands. The pH of soil samples varied from 5.12 to 5.62. Organic matter (OM) content on the soils investigated was between 2.43% and 6.07% (Table 1). The OM was higher than 3% for 7 sites. Therefore, much of the investigated soils of orchid growing areas could be classified into high organic matter soils. Djordjević & Tsiftsis (2020) reported that a significant number of species of orchids that prefer acidic soils grow in high-altitude areas. Tsiftsis *et al*. (2008) revealed that altitude represents a complex variable related to climatic factors and it is positively correlated with the organic matter content of the soil. Furthermore, they mentioned that soil acidity was the most important

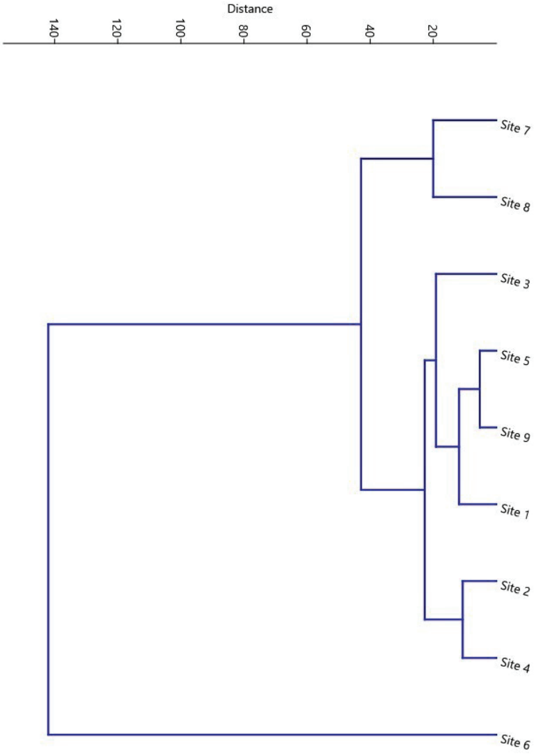

FIGURE 4. Hierarchical cluster analysis of nine locations based on ecological parameters (total number of *I. speciosa*, accompanying flora, threatened species, invasive species, pH, EC, P, K and organic matter).

factor in determining orchid distribution. The EC values of the soils were found to be between 10.21 dS m^{-1} and 42.20 dS m^{-1}. The highest K concentration (232.0 ppm) was detected in site 6, followed by sites 8 and 7. Regular intentional fires occurring at sites 4, 6, 7 could be the reason for detecting the high concentration of K in the soil.

The results of this study show that vegetation type and soil properties effectively influence the distribution and abundance of *I. speciosa* within sampled grasslands. Similarly, Tsiftsis *et al.* (2008) found that the most important gradients that govern orchid distribution in northeastern Greece are altitude, soil acidity, and specific habitat types. Furthermore, recent studies suggested that orchid richness is regulated by habitat size, altitude, and climate at large scales (Acharya *et al*. 2011, Zhang *et al*. 2015).

Although very little has been published on the life cycle of *I. speciosa*, Kumari *et al*. (2006) reported the successful artificial propagation of seeds under *in vitro* conditions. According to the report, it takes approximately 10 weeks for a fruit/capsule to mature to the point when the seeds are brown, and then take 60 days to germinate. The germination period would be altered due to particular weather factors such as temperature, moisture, etc. Furthermore, it is challenging to locate seedling orchids in dense vegetation, yet identifying the presence of early plant stages (such as protocorms) in soil subplots near mature plants is critical for a future demographic investigation.

Threat and conservation.— Habitat deterioration and degradation, clearing of vegetation, intentional forest fires, and the spread of invasive alien species are significant threats to highland grasslands and wetlands in Sri Lanka (Kotagama & Bambaradeniya 2006, Ministry of Environment 2010). In the montane and submontane areas, forests and grasslands are cleared for vegetable cultivation, this being the main agriculture-based threat. The excessive use of agrochemicals is believed to have a considerable impact on the survival of the orchid populations. Besides, forest felling for firewood, encroachments and illegal settlements, intentional forest fire, and garbage dumping are the main habitat-related threats to native orchid survival (Fernando 2012).

With the increasing demand for agricultural products, abandoned lands, especially grasslands, are vulnerable to encroachment. Unfortunately, these encroachments are established permanently, leading to significant loss of natural vegetation (Kottawa-Arachchi 2017). Furthermore, fragmentation of habitats also has a detrimental effect on small populations, especially rare and threatened species.

Spreading of invasive alien species.— Invasive alien plants have been widely recognized to exert a significant negative impact including superior competitors for limited resources in comparison with their native counterparts (Vila *et al.* 2011). In addition to the native flora, 12 invasive plant species were recorded as accompanying flora. The highest numbers of invasive species were observed in site 3 (near Watawala). Among the invasive species, the Neotropical shrub *Austroeupatorium inulifolium* (Kunth) R.M.King & H.Rob. (Asteraceae) was recorded at all the sites, and *Panicum maximum* Jacq. (Poaceae) was observed at sites 1–7 as dominant or common species. During the survey, we observed rapidly growing invasive species *Ageratina riparia* (Regel) R.M.King & H.Rob. (Asteraceae) and *Mimosa invisa* Mart. ex Colla (Fabaceae) populations in five and four locations, respectively (Table 2). Numerous studies have shown that invasive plant species impose greater effects than dominant native species on the growth and reproduction of native residents (Vila *et al.* 2011, Kuebbing & Nunez 2016). The presence of invasive species has a negative effect on native plants, indicating that invasive species may displace native species such as the terrestrial orchids *Ipsea speciosa*, and *Satyrium nepalense*. The rapid spreading of *A. riparia* and *P. maximum* was observed in site 8 and site 3, respectively. The current growth of the invasive species mentioned above is indicative of the future shrinkage of the populations of native terrestrial orchids. In addition to orchids, many threatened grassland species such as *Exacum trinervium* (L.) Druce (Gentianaceae), *Osbeckia parvifolia* Arn., and *Torenia cyanea* Atton (Linderniaceae) could be lost because native plants are unlikely to be self-sustaining in the competition with invasive plants.

FIGURE 5. Set fires in Kotagala site. Photo by J. D. Kottawa-Arachchi, captured on 26th December 2017.

Habitat deterioration.—Fire disturbance is serious when soil is overheated, and most of the grasslands in Central Highlands set fires during the dry period, especially from January to April. Incidences of intentional fires in study sites, especially Watawala, Hatton, and Kotagala, have been observed in past decades. Unfortunately, set fires were observed in Kotagala (Fig. 5) and Hatton sites after this study. Repeated burning might be deleterious and could also negatively impact existing protocorms, seedlings, and non-flowering mature plants, as well as the reproductive parts such as flowers, capsules, and seeds, and could even alter the mycorrhizal community, hence, lead to the loss of terrestrial orchids and native flora from their habitats. Out of nine habitats, four populations were recorded at banks of railway lines which are regularly cleared by the workers of the Department of Railwayv. This could be a negative impact on restricted populations. Therefore, immediate and continuous management is required to prevent further loss of remnant populations of *I. speciosa* in such valuable grasslands.

Orchids with showy flowers and medicinal properties encounter an added disadvantage due to over-collection from the wild. *Ipsea speciosa* is subjected to removal from the wild for medicinal purposes and due to various mythological beliefs connected to its tuber (Fig. 6) (Fernando 2012). Kumari *et al.* (2006) developed an *in-vitro* protocol for mass propagation of the species and rhizome tips were found to be suitable as a source of explant. This suggested approach could be useful to generate an adequate number of plants hence minimize the illegal collections from the wild.

Climate change impacts the environment leading to a reduction in the distribution and abundance of species, especially endemics, which may even result in their global extinction. Climate change can also affect biota of the various ecosystems through extreme rains, changes in evaporation, modification of the microclimate, and alteration of the composition of forest-grassland flora (Reina-Rodriguez *et al.* 2017).

Although grasslands are considered as vulnerable to climate change, the long term monitoring of adaptability of plant communities and reproductive biology, which respond to extreme weather events, is essential for understanding the future impact of climate change (Kottawa-Arachchi & Wijeratne 2017).

Conclusions. The present study demonstrates that the Daffodil Orchid, *I. speciosa,* shows a restricted distribution in the Western slope of the Central Highlands, Sri Lanka. Its presence is confined to a few grassland habitats where increasing anthropogenic activities have been observed. Based on the spreading of invasive species, all sites are vulnerable to shrinkage of terrestrial orchid populations, including *I. speciosa*. Besides, intentional firing is another serious threat to the grasslands, and these anthropogenic activities affect negatively the native grassland flora, including terrestrial orchids. The present study revealed that habitat conservation is an important strategy for the protection of threatened species. To better understand the population dynamics of *I. speciosa*, our results suggest continuing monitoring and assessment of its population in selected grasslands from Sri Lanka.

Figure 6. Tuber of *Ipsea speciosa.*

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THE *CHLORAEA* FROM LIMA, A LITTLE-KNOWN SPECIES BUT DESCRIBED SEVERAL TIMES

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Abstract. During the botanical expedition carried out in Peru by Ruiz and Pavón, later by Raimondi's explorations and finally by Weberbauer's, specimens of a *Chloraea* species were collected in localities around Lima and used to describe *Chloraea pavonii*, *C. undulata* and *C. peruviana*. In this study we reviewed original materials, protologues, drawings, additional herbarium specimens and living material to clarify the taxonomic status of *C. pavonii*. A description, illustration, photographs, and distribution of the species are provided. We also point out the morphological features that distinguish *C. pavonii* from *C. densipapillosa* and *C. septentrionalis*. Lectotypes for *C. undulata* and *C. peruviana* are here designated.

Resumen. Durante la expedición botánica realizada en el Perú por Ruiz y Pavón, luego por las exploraciones de Raimondi y finalmente por las de Weberbauer, especímenes de una especie de *Chloraea* fueron colectados en localidades alrededor de Lima y usados para describir *Chloraea pavonii, C. undulata* y *C. peruviana*. En este estudio, hemos revisado los protólogos, material original, ilustraciones, material adicional de herbario y material vivo para clarificar el estado taxonómico de *C. pavonii*. Se presentan una descripción, ilustración, fotografías y distribución de la especie. También señalamos los caracteres morfológicos que permiten distinguir *C. pavonii* de *C. densipapillosa* y *C. septentrionalis*. Asimismo, se designan los lectotipos para *C. undulata* y *C. peruviana*.

Key Words / Palabras clave: *Chloraea*, ecosistema de lomas, Lima, Peru, lectotipificación, lectotypification, lomas ecosystem

Introduction. The Lomas formations are a seasonal ecosystem that occur in the desertic lowlands of the western slopes of the Peruvians Andes. Most of its vegetation (mainly herbaceous) flourish during the humid winter season (May/June to September/October), when the thick fog masses, coming from the Pacific Ocean, are intercepted by foothills near the sea (Dillon *et al.* 2011). Five orchids species have been recorded for the Lomas formations (Trujillo 2013); among them, the most conspicuous is a *Chloraea* Lindl. species (Fig. 1). Since specimens of this species were originally collected in the Lomas near Lima city, it is locally known as the "Lima Orchid". Nevertheless, this species is not restricted to Lomas formation, since some populations have been recorded in shrublands and grasslands on slopes at the highlands of the department of Lima, between 2400 and 3100 m of elevation (Bennett & Christenson 1998, Schweinfurth 1958).

The nomenclatural history of this species is a sequence of overlooked works and constant redescriptions of the same entity under different names. The first record of this *Chloraea* in Lomas was during the Royal Expedition to Peru led by Ruiz and Pavón in 1781 (Ruiz 2007). One of the specimens collected in that expedition was used to describe *Chloraea pavonii* Lindl, but citing the species for Chile (Lindley 1827, 1840). The second record of this species was done by Antonio Raimondi. Thinking that this was the first record of *Chloraea* for Peru, he described again the

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FIGURE 1. Inflorescence of *Chloraea pavonii* Lindl. Photograph by D. Trujillo.

species under the name *Chloraea undulata* Raimondi (1857). Later on, based on a specimen collected by August Weberbauer, the species was described again by Kränzlin in 1906 as *Chloraea peruviana* Kraenzl.

An additional name, but unpublished, was also used by Lindley for this Peruvian orchid: "*Chloraea macleanii*". The material was collected by John Maclean in Langa (a locality at the highlands of Lima) and sent to Sir William Jackson Hooker at Kew. The herbarium specimens were examined later by Reichenbach, who identified them as *Chloraea venosa* Rchb.f., a species described for Chile.

Schweinfurth (1958), in his work Orchids of Peru, only cited the species published by Kränzlin: *Chloraea peruviana*, because he was unaware of Lindley's and Raimondi's works. Some years later, M. N. Correa (1969), in a revision of the genus *Chloraea*, placed *C. pavonii* as a synonym of *Chloraea chrysantha* Poepp., and *C. peruviana* as a synonym of *C. venosa*. Correa had access to the specimens collected by Raimondi housed at the herbarium USM, but it seems that she did not know the publication of Raimondi; hence *C. undulata* was not cited in her publication. The following year, Schweinfurth (1970), in a supplement of the Orchids of Peru, recognized *C. undulata* and placed *C. peruviana* as its synonym. In 1998, Garay and Romero-González clarified the identity of *C. pavonii* and placed *C. venosa, C. undulata* and *C. peruviana* as its synonyms. In subsequent works done by orchid taxonomists, *C. pavonii* is referred as a correct name (Bennett & Christenson 1998, Pupulin 2012).

Recently, after 50 years without any report of *C. pavonii* from Lomas, Lleellish (2015) recorded the species for Lomas de Asia (located to the south of the department of Lima, central-western portion of Peru); although considering the species as *C. undulata* and omitting the name *C. pavonii*. In addition, Lleellish indicated that *C. undulata* [i.e. *C. pavonii*] occurs in the departments of Cusco, Cajamarca and La Libertad. The misinterpretations of Lleellish then has been followed by other authors; for instance, Leiva and collaborators (2016), who reported *C. undulata* to the district of Salpo, in La Libertad Department.

After a revision of fresh and herbarium specimens of *Chloraea*, the present contribution aims to clarify the taxonomic status of *C. pavonii* by providing morphological and ecological information of the species and pointing out some morphological characters that can help to distinguish it from the other *Chloraea* species that occur in Peru.

TAXONOMIC TREATMENT

Chloraea pavonii Lindl. Gen. Sp. Orchid. Pl. 404. 1840.

Basionym: *Asarca speciosa* Lindl., Quart. J. Sci. Lit. Arts 1: 52. 1827.

TYPE: "Habitat in Chili, Pavón (olim v. s. sp.)"; "Serapias Gavilú. Pavón in herb. Lambert", *Pavón s.n.* (holotype: BM-95631) (Fig. 2)[1].

Synonyms: *Chloraea undulata* Raimondi, Elem. Bot. (Raimondi) 143. 1857.

TYPE: [Peru], Lima, *Raimondi s.n.* [Raimondi Herbarium sheet 9904, Catalogue No 446a] (**lectotype, here designated:** USM).

Chloraea peruviana Kraenzl., Bot. Jahrb. Syst. 37(5): 528. 1906.

TYPE: Peru. Amancaes Mountains at Lima, in Loma Formation, 500 m, *A. Weberbauer s.n.* (holotype: B destroyed, photo at, AMES-38618!. Isotype: HBG-501465, photo seen, **designated here as the lectotype**).

Terrestrial *plant* about 40 to 79 cm high. *Roots* fascicled, cylindrical and fleshy. *Stem* stout, leafy. *Leaves* spirally arranged along the stem (rare compressed like a basal rosette), blade ovate, ovate-oblong or oblong-lanceolate, obtuse or acute, amplexicaul, about 6–14 [19] × 2–5 cm (Figure 3A, B). *Inflorescence* terminal, lax, 11–30 cm long, with 6 to 30 flowers. *Floral bracts* ovate to lanceolate, acute, slightly longer to shorter than the ovary, green, 1.8–3.9 × 0.7–1.8 cm (decreasing in size towards the apex). *Flowers*, resupinate, yellow with green reticulate veins (Figures 1, 3C–E). *Dorsal sepal* elliptic to oblong-elliptic, obtuse to sub-acute, with 5 main nerves, 2.8–3.5 × 0.9–1.6 cm. *Lateral sepals* elliptic to oblong-lanceolate, slightly oblique, obtuse to sub-acute, with 5 to 7 main nerves, 2.9–3.4 × 1.1–1.5 cm. *Petals* elliptic-ovate, asymmetric, obtuse-rounded, with 4 main nerves, 2.2–2.8 × 1.2–1.4 cm. *Lip* clawed, entire to obscurely 3-lobed, oblong to ovate-oblong, basal part and central disk provided with numerous short flattened keels or falcate keel-like teeth irregularly scattered along the main nerves of the lip, 2.1–2.2 × 1.2–1.4 cm. *Column* slender, slightly arcuate, apex with rounded wings, with a pair of nectaries between the column and the labellum, 1.7–1.8 cm long. *Stigma* triangular. *Ovary* 2.0–4.5 cm long (Fig. 4).

Specimens examined.– PERU. **Lima**. Flo P., cum L., Habitat in Provinciae Limae ad Amancaes et Chancay ad Jequar collibus altis inter saxa [*Ruiz & Pavón s.n.*] (MA-810745, photo seen). In Jequar Chancay in collibus altis inter saxa in Lima et Amanc alt, Flor P. Jul-Aug, [*Ruiz & Pavón s.n.*] (W-78557 Rchb. Orch. 43089, photo seen). Without a precise locality, Flor. P, Jul-Aug, [*Ruiz & Pavón s.n.*] (G-105744, photo seen). Without a precise locality, *Dombey s.n.* (P-369476, photo seen). Grows on almost inaccessible heights between Cerros de San Cristobal and de Amancaes, as well as on rocky slopes of the San Bartolomé, Jul-Aug, *Barranca & Wawra 471* (W-55114, photo seen). Without collection data [dissected flower with a copy of the original drawing on sheet number K-501998], (W-78549 Rchb. Orch. 43091, photo seen). Without collection data [Drawing] (W-78558 Rchb. Orch. 42990, photo seen). Prov. Canta, Limite de los distritos Arahuay-Lachaqui, 2650–2700 m, declive rocoso con vegetación herbácea, 10 Apr 1993, *G. Vilcapoma 2212* (USM-278407). Prov. Canta, Lachaqui, arriba del puente Romero en Camino hacia Arahuay, 2750–2900 m, ladera rocosa, 29 Mar 1993, *G. Vilcapoma 2420* (USM-278406). Prov. Canta, Collo, camino al paraje de Achupata, 2400–2500 m, ladera rocosa con predominio de Malvaceas, 16 Apr 2006, *G. Vilcapoma 7623* (USM-278405). Prov. Canta, Arahuay, Camino al lado del rio Arahuay, 2550–2650 m, vegetación dominada por *Carica candicans* "mito", 24 Mar 2008, *P. Gonzáles 97* (USM-259386). Prov. Canta, Arahuay, 2600 m, Mar 1994, *M. León 391* (MOL). Prov. Canta, Arahuay, 2635 m, 3 Apr 1994, *M. León s.n. ex Bennett 6583* (MOL-spirit). [Probably same locality] *M. León 393 ex Bennett 6583* (MOL-spirit). Prov. Canta, Arahuay, 2600 m, *without collector* (MOL-spirit). Prov. Cañete, Asia, 514 m, Ecosistema de Lomas,

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[1] Due to the extraordinary circumstances provoked by the Covid-19 pandemic, direct access to herbarium high resolution images through the curatorial staff has been largely impeded. The figures printed in this paper were therefore obtained from the images made available in the web pages of the concerned herbaria, which are individually acknowledged in the captions as to their copyright.

FIGURE 2. Holotype of *Chloraea pavonii* Lindl. Original collection by H. Ruiz and J. Pavón, hosted at the Natural History Museum, London (BM000095631). Retrieved from the web page of Natural History Museum, Data Portal Specimen Records, filed as *Gavilea pavonii*. Permanent URL: https://data.nhm.ac.uk/object/298ae096-756e-44e2-bbe1-f4345246e02a/1586390400000. Retrieved: 13 Apr 2020 15:27 (GMT). All the rights belong to the Natural History Museum.

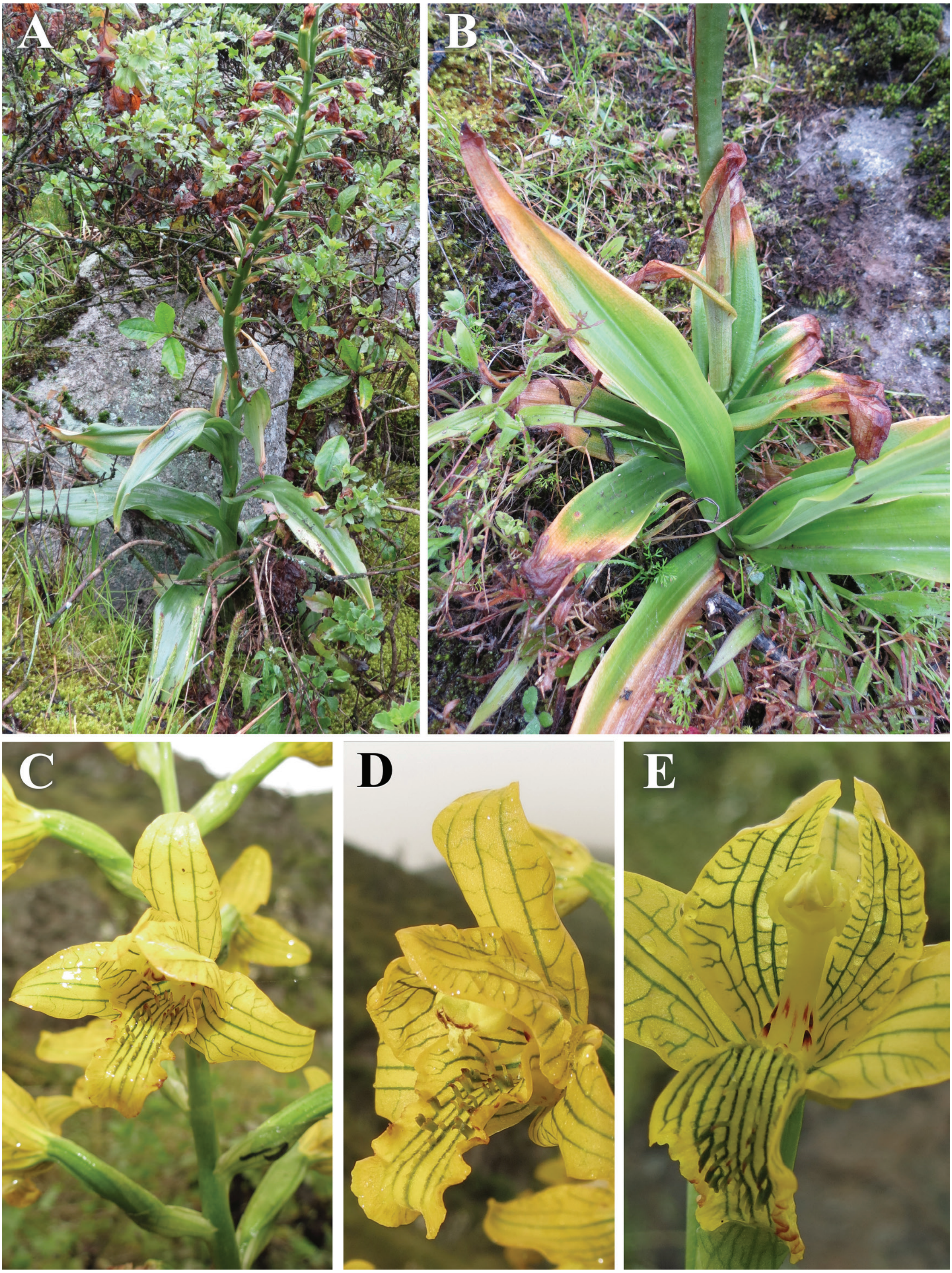

FIGURE 3. *Chloraea pavonii* Lindl. **A**. Habit. **B**. Leaves. **C**. Flower, front view. **D**. Flower, side view **E**. Flower, showing the column and the lip. Photographs taken in Lomas de Asia (Cañete) by D. Trujillo.

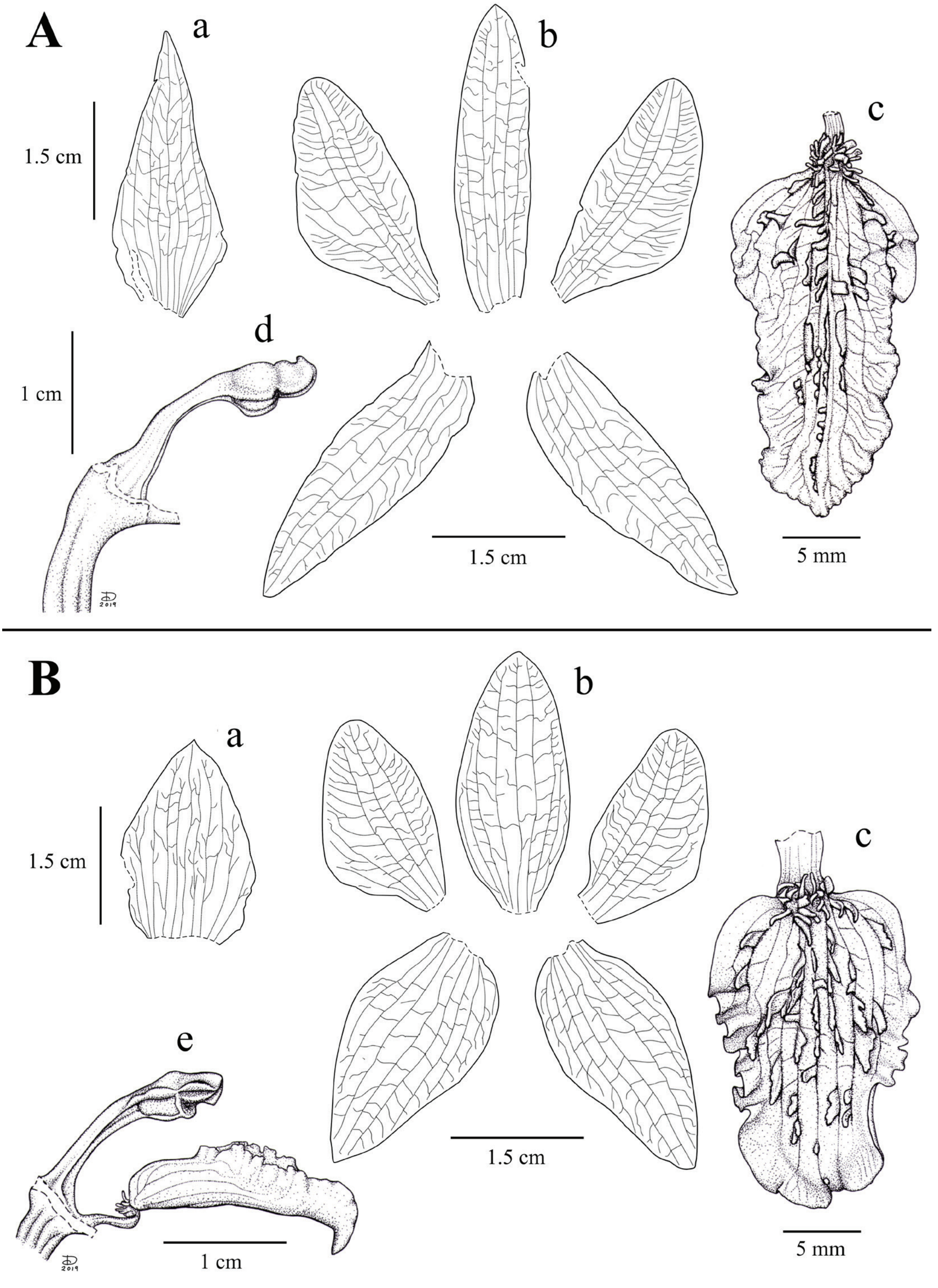

FIGURE 4. *Chloraea pavonii* Lindl. **A**. Flower of a plant from Arahuay-Lachaqui, Canta (*G. Vilcapoma 2212*, USM-278407). **B**. Flower of a plant from Bosque de Zárate, Huarochirí (*E. Morón s.n.,* USM-257683). **a**. Floral bract. **b**. Dissected perianth. **c**. Lip. **d**. Column. **e**. Column and lip lateral view. Drawing by D. Trujillo.

zona media, sobre ladera con afloraciones rocosas, 16 Sep 2017, *Paredes & Reyna 871* (USM). Prov. Chancay [Huaral], Lomas de Chancay, 350 m, hábitat rocoso, en laderas muy inclinada, 24 Set 1952, *R. Ferreyra 8748* (USM-165083). Prov. Chancay [Huaral], Lomas de Chancay, 350 m, hábitat rocoso, lomas arcillosas, 24 Set 1952, *R. Ferreyra 8748* (USM). Prov. Huarochirí, Langa, 2900 m, 22 Mar 1982, *A. Cano et al. 2123* (USM-165081). [Prov. Huarochirí], Langa, *Maclean s.n.* (K-501996, photo seen.). Same locality, *Maclean s.n.* (K-501997, photo seen). Same locality, *Maclean s.n.* (K-501998, photo seen). [Prov. Huarochirí], Langa, [drawing of K-501998], *Maclean s.n.* (W-78548 Rchb. Orch. 42983, photo seen). Prov. Huarochirí, Matucana, 8000 ft, in the moister swales of eastern hillsides, 12 Apr – 3 May 1922, *Macbride & Featherstone. 372* (F-516907, photo seen). Prov. Huarochirí, San Bartolomé, Bosque de Zárate, km 56 Carretera Central, 3100 m, bosque andino, 15 Jun 2012, *E. Moron s.n.* (USM-257683). Prov. Huarochirí, San Bartolomé, Monte de Zárate, 1400–3550 m, matorral y relicto de bosque dominado por *Oreopanax* y *Myrcianthes* entre otros, 24–26 Apr 2009, *P. Gonzáles et al. 481* (USM-256794). [Prov. Huarochirí, San Bartolomé], Cerro de San Bartolomeo, Jul 1876, *L Savatier 1449* (K-501994, photo seen). Prov. Huarochirí, Surco, 2400 m, Apr 1916, *N. Esposto s.n.* (MOL-8287). Prov. Huarochirí, Surco, cumbre de un cerro, 15 Apr 1920, *N. Esposto s.n.* (MOL-8286). Prov. Lima, San Jerónimo Uber Amancaes, 600 m, 20 Jun 1920, *K. Maisch s.n.* (USM-13659). Prov. Lima, alrededores de Lima, *A. Raimondi s.n.* [Raimondi Herbarium Sheet 9943 Catalogue No 446b] (USM). Prov. Lima, Cerros de Amancaes, cerca a Lima, *N. Esposto s.n.* [in part] (MOL-8289). Prov. Lima, Cerros de Amancaes, cerca a Lima, Ago 1910, *N. Esposto s.n.* (MOL-8288). Prov. Lima, Hills around Lima, 1862, *W. Nation s.n.* (K-501995, photo seen). [Prov. Lima,] Lima, cerro Agustino, 490 m, Set 1945, *J. Soukup 1625* (LIL-15022, photo seen).

Distribution: Known only from central western slopes of Peru. It has been recorded in five provinces of the department of Lima: Huaral, Canta, Huarochirí, Lima and Cañete, between 350 and 3100 m of elevation (Fig. 5).

Habitat and ecology: Individuals of this species were found growing in Lomas formation of rocky or stony ground in the desertic lowlands, and montane forest relict, shrublands and grasslands in the highlands. Flowering from March to June in highlands; and from August to September in Lomas formation.

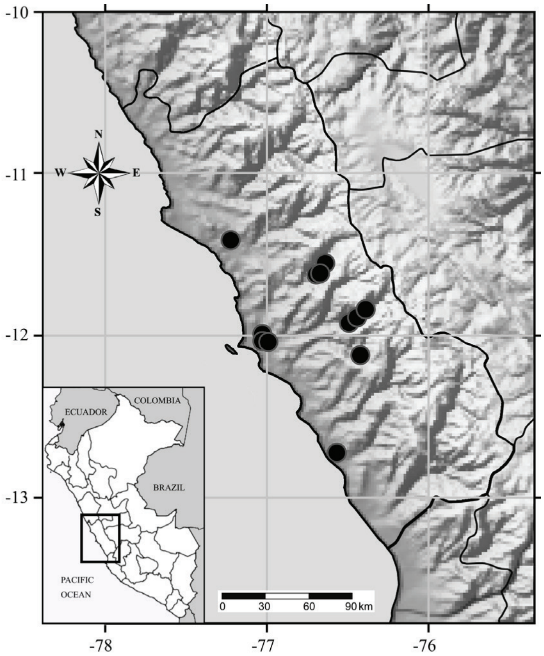

Figure 5. Distribution map of *Chloraea pavonii* Lindl. (black circles) in Peru.

Collection and nomenclatural history: The first specimens of *Chloraea pavonii* were collected by Ruiz and Pavón during the winter of 1781 (July to September), when their expedition carried out a second trip to the Province of Chancay (currently, split into three Provinces: Barranca, Huaura and Huaral). In his diary, regarding to this trip, Ruiz refers: “During this time [we] walked the Hills, Lomas and Valleys of Chancay, Pasamayo, Jequar, Retes and Laral in which [we] described several new plants of which I described the following: […] Serapias flava, […]” (Ruiz 2007, p.183, Ms 22). Ruiz also refers that once ended this trip to Chancay, he and Pavón met with Don Toribio Bravo de Castilla, owner of the Hacienda Torreblanca who escorted them to collect plants not only in his property, but also in Lima, at the hills of Amancaes (Ruiz 2007, p.188, Ms 24).

Specimens of *C. pavonii* collected by Ruiz & Pavón are currently located at the BM, G, MA and W herbaria; a drawing of the species, prepared by Brunete (one of the painters of the expedition) is also conserved at MA (Fig. 6). Specimens housed at G (G-105744, Fig. 7) and MA (MA-810745, Fig. 8) have the original labels of the expedition. These labels bear a short plant description and notes about either the collection

Figure 6. *Chloraea pavonii* Lindl. AJB, Div. IV, 1318, tempera on paper by J. Brunete. Illustration reproduced from Pupulin (2012: Fig. 10A), in *Anales del Jardín Botánico de Madrid*.

HERBIER BARBEY-BOISSIER

Constitué par William Barbey, après la mort de son beau-père Edmond Boissier (1885); donné en 1918, par les enfants de W. Barbey, à l'Université de Genève où il fut augmenté par différents collaborateurs; transféré en 1944 au Conservatoire botanique.

Serapias Gavilu, foliis lanceolatis, floribus alternis luteo, maculatis ❓
Flor. P. floret Julio Augusto.

Epipactis. amplo flore luteo, vulgo Gavilu
Feuill. videtur

FIGURE 7. *Chloraea pavonii* Lindl. Original collection by H. Ruiz and J. Pavón, hosted at the Conservatoire et Jardin botaniques de la Ville de Genève (G00105744 - n° SIB 141746/1). Retrieved from the web page of the Conservatoire & Jardin botaniques de la Ville de Genève, Catalogue des herbiers de Genève (CHG), filed as *Chloraea pavonii*. URL: http://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=131865&lang. Retrieved: 14 Apr 2020. All the rights belong to Conservatoire et Jardin botaniques de la Ville de Genève.

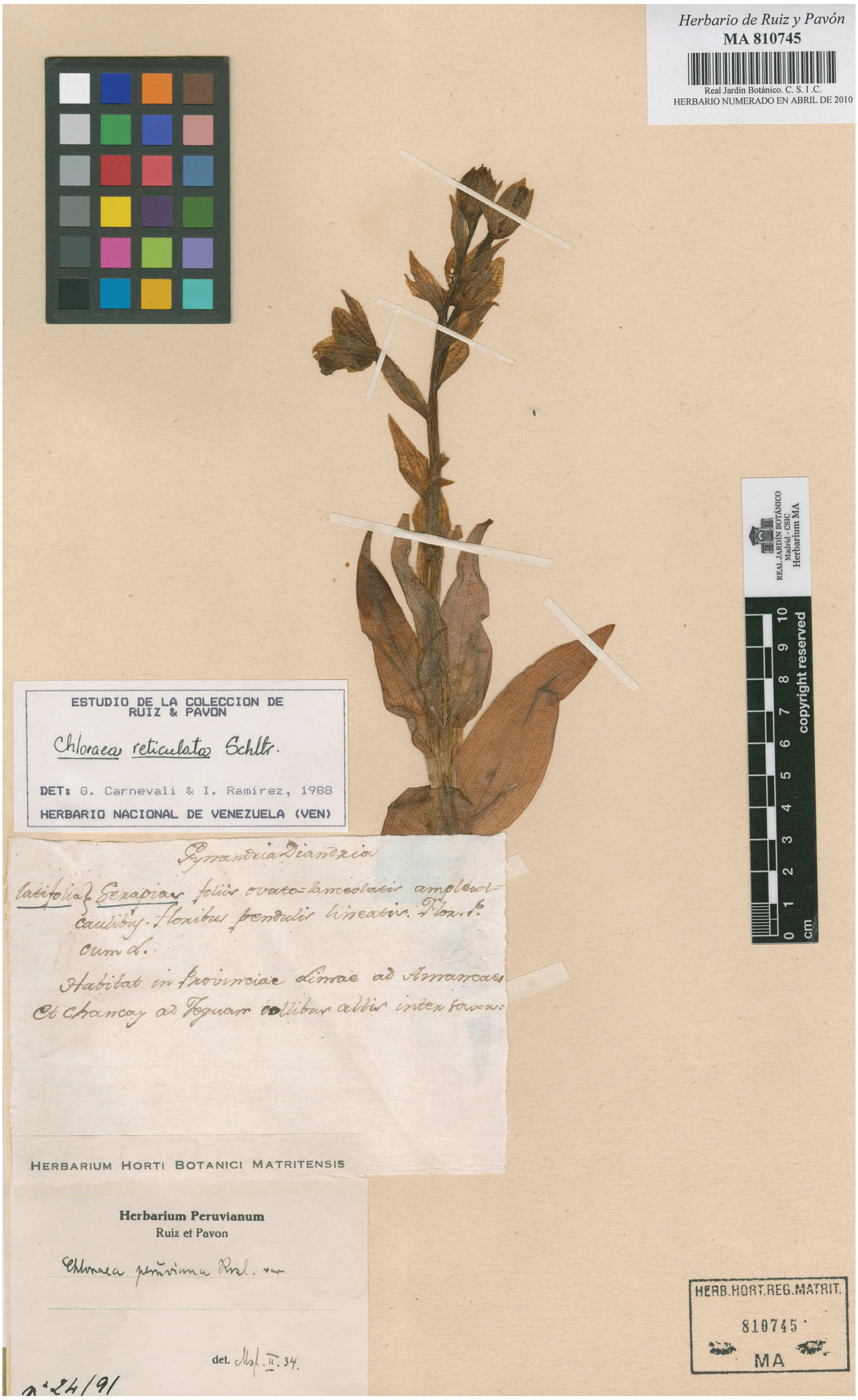

FIGURE 8. *Chloraea pavonii* Lindl. Original collection by H. Ruiz and J. Pavón, hosted at the Herbario del Real Jardín Botánico (MA-01-00810745). Retrieved from the web page of Real Jardín Botánico, filed as *Chloraea peruviana*. URL: http://colecciones.rjb.csic.es/#card.php?id_name=22431/ Retrieved: 13 Apr 2020. All the rights belong to the Real Jardín Botánico-CSIC.

localities or the flowering time. The label of the specimen at MA states the collection places: “Flor. P. Habitat in Provinciae Limae ad Amancaes et Chancay ad Jequar collibus altis inter saxa”; and the label of the specimen at G states the flowering time: “Flor. P. floret Julio-Augto”. The collection data of these herbarium specimens agree with what Ruiz wrote in his diary. The specimen housed at W belongs to the Reichenbach Herbarium (W-78557 Rchb. Orch. 43089, photo seen) and has two labels with his handwriting. One label bears similar locality data to that stated on the label of the specimen housed at MA. The second label bears the same plant description and flowering data stated on the label of the specimen housed at G.

The specimen housed at BM (BM-95631, Fig. 2) has two small labels that bears the following data: “Serapias gavilú de Chile sp. nov. ined.”, in Ruiz’ handwriting, and “Chili Herb: Pavon”, by Pavón’s hand. The specimens of *C. pavonii* currently located at BM and G are the samples that originally were acquired by Lambert from Pavón (Miller 1970). Garay and Romero-González (1998) and Pupulin (2012) designated the specimen at G as the holotype. However, the specimen used by Lindley to describe *Asarca speciosa* Lindl, later transferred to *C. pavonii* is the one housed at BM because the data on the labels agree with the protologue; therefore this specimen represents the holotype and not the one at G. Still it is uncertain whether the specimen at BM was collected in Chile (as the label indicates) or in Peru. As no additional collections of *C. pavonii* have been recorded from Chile (since the species was described), and being all the records exclusively known from Peru (even the specimens at MA and G indicate: ”Flor P”), then it seems that Ruiz could have made a mistake annotating the specimen as coming from Chile.

There is an additional specimen of *C. pavonii* housed at P (P-369476, photo seen); however, this specimen has a label of Dombey, who was the third botanist of the Royal Expedition. Since Dombey was in the Callao Port taking some sea measurements by the Viceroy’s order (Ruiz 2007, p.182, Ms. 22), he did not take part of the second trip to the Province of Chancay. Hence, it seems that the specimen of *C. pavonii* at P does not belong to the same gathering of Ruiz and Pavón in Chancay-Amancaes. Dombey could have collected it exploring alone the hills around Lima.

Chloraea undulata was described by Antonio Raimondi in his book: “*Elementos de botánica aplicada a la medicina y a la industria*” (1857: 143). Raimondi was professor of Natural History in the Medicine School of Lima between 1856 to 1873 (Quesada 1921). He published this book with the purpose to provide his students of medicine the bases of the Botany by citing species that they could observe around Lima. This way, indicating that an orchid inhabits some hills near Lima, Raimondi described *C. undulata*. In 1878, Miguel Colunga, a Raimondi’s disciple and friend, published two volumes of the book “*Lecciones de botánica*”. In the second volume (p.187), Colunga included the description of *C. undulata* but referring that Raimondi was the first who described the species.

The publication of Raimondi, as well as the one of Colunga, had a limited distribution; therefore, the description of *C. undulata* was unknown to many botanists. Schweinfurth (1970) only had access to Colunga’s publication, but not to that by Raimondi; consequently, Schweinfurth cited the book of Colunga as the original publication of *C. undulata*, but referring to it as “Text Book”: “*Chloraea undulata* Raimondi ex Colunga, Text Book. Bot. 2:187.1878”.

When Garay and Romero-González (1998) clarified the identity of *C. pavonii,* they pointed out that a material of *Chloraea* collected by Raimondi housed at W was the holotype of *C. undulata,* However, that material (W-55114, photo seen) was not collected by Raimondi but by Barranca and Wawra in 1871 during the short stay of Wawra in Lima, after his expedition to the Asian continent on board of the frigate Donau (Wawra 1873).

Two *Chloraea* specimens from Lima, determined as *C. venosa* by N. Correa, are kept in the Raimondi collection at the herbarium San Marcos (USM); they have the Raimondi Herbarium Sheet Number 9904 and 9943 (Catalogue No 446a and 466b, respectively). The specimen 9943 is still mounted on the old herbarium sheet that has a letterhead that indicate “Herbario Raymondi”. On the herbarium sheet a label indicates “No. 9943 *Chloraea peruviana* Krzl [Kränzlin]”, which is the determination label sent by the herbarium of the Botanic Garden and Botanical Museum Berlin (BGBM) that had the Raimondi collection as a loan in 1926 (Anonymous 1939, 1940). The specimen 9904, was removed from its original herbarium sheet and

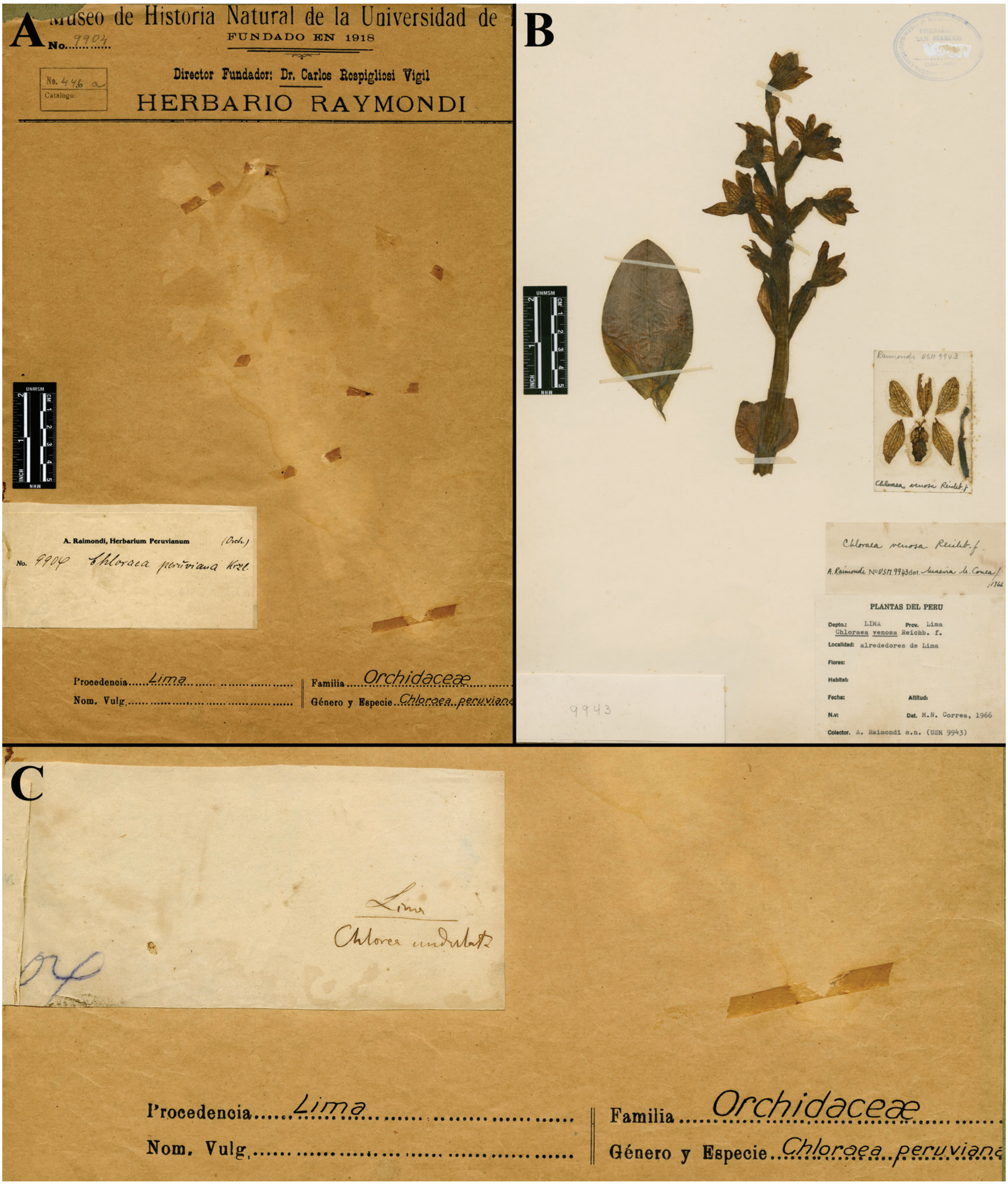

Figure 9. Lectotype of *Chloraea undulata* (=*Chloraea pavonii*). **A**. Raimondi Herbarium Sheet Number 9904 at USM, original herbarium sheet from where the specimen was removed. **B**. Herbarium specimen removed from the Raimondi Herbarium Sheet Number 9904 and mounted in a new sheet with a new label. **C**. Original label (the second one) with the Raimondi's handwriting glued on the Raimondi Herbarium Sheet Number 9904.

mounted mirroring the original position on a new sheet with a new label; on this new label the number 9943 was wrote instead of 9904 (Fig. 9). On the original herbarium sheet of 9904 two labels are still glued, one covering the other. One is the determination label sent by the herbarium of BGBM which indicates: "No

9904 *Chloraea peruviana* Krzl" (Fig. 9A). The second label bears, in Raimondi's handwriting, the following note: "Lima *Chloraea undulata*" (Fig. 9C). Both specimens (9904 and 9943) are the original material of *C. undulata*, but only the specimen 9904 has the handwriting of Raimondi; therefore, this one is here designated as lectotype (Shenzhen Code Art. 9.3, 9.12 ICN, Turland *et al*. 2018).

Lleellish (2015) in his notes on the "Lima Orchid" indicated that Raimondi´s specimen: "registro 739 USM" was collected in Lomas near Lima and used to described *C. undulata*. The specimen that Lleellish indicates – with the Raimondi Herbarium Sheet No 739-USM (=*Raimondi 343*) – was not collected in Lima but in Quebrada de Chao, La Libertad Department, as indicates the collection data on the label: "343 *Chloraea* / Bajada por el camino del peñon entre Uningambal y Palmabal, Quebrada de Chao. Mayo 1868 / Flores Amarillo-verdosas". This specimen could be referred to *C. septentrionalis* M.N.Correa (see below); but the bad condition of the single flower in the specimen prevents its correct determination.

Additional specimens of *C. pavonii* are housed at K under the name "*Chloraea macleanii*"*:* K-501996 (photo seen), K-501997 (photo seen) and K-501998 (photo seen). These specimens were collected by John Maclean in Langa (Prov. Huarochirí, Lima) and sent to Sir William Jackson Hooker between 1832 to 1854 (Desmond 1994). Lindley determined the Maclean material as *C. macleanii* but it was never published; probably because Reichenbach had access to that material in 1856 and determined it as *C. venosa* (see date annotated on a drawing glued at the sheet K-501998). *Chloraea venosa* was described by Reichenbach based on material from Chile, but without collector data (Reichenbach 1849). At the Reichenbach herbarium at W are housed two sheets with drawings based on the Maclean specimen K-501998; one bears a drawing of the inflorescence (W-78548, photo seen) and the other bears drawings of a flower and a dissected flower (W-78549, photo seen). The sheet W-78549 has been misinterpreted as type of *C. venosa* by Correa (1969)

Conservation status.– *Chloraea pavonii* is endemic to the western slope of the central Peruvian Andes. It is known from eleven locations in the department of Lima, five in the desertic lowlands and six in the highlands. For the populations that occur in the lowlands, the main threats are urban sprawl and overgrazing; three localities: Cerro El Agustino, Cerro San Cristobal and Lomas de Amancaes are today in the middle of the Lima city (capital city of Peru) and the species is considered extinct there. In Lomas de Asia, where the species was recently reported (Lleellish 2015), 18 individuals have been recorded between 2016 and 2018 (D. Paredes-Burneo, pers. obs.); the habitat of this population is being managed and protected by local people. The population that inhabits the scrubland and forest relict in the highlands is scant (P. Gonzáles, pers. comm. 2019), the main threats for these populations being habitat loss and degradation due the conversion of land for agriculture and overgrazing, and flowers harvest by the inhabitants (P. Gonzáles pers. comm. 2019). One locality, Zona Reservada Bosque de Zárate is a protected area and it is inferred that will give the adequate protection to the habitat in that area. Based on the eleven known locations, the extent of occurrence estimated by GeoCat is 6791.78 km^2, but the area of occupancy is estimated at only 60 km^2 (Bachman *et al.* 2011). The extent of suitable habitat has decreased in the last 70 years; and at present it is thought that the species occurs in eight locations. Therefore, *C. pavonii* is assessed as Vulnerable based on IUCN criteria B1 and B2 (VU, B1ab(i.iii) + B2ab(ii, iv)) (IUCN 2019).

Comments on other Peruvian *Chloraea* species

Chloraea septentrionalis occurs in northwestern Peru, in the departments of Cajamarca and La Libertad (Roque & León 2006, Trujillo 2012). Some specimens of this species have been erroneously determined as *C. pavonii* (or as its synonym *C. undulata*); for instance, *Leiva 526* (HUT-28175!) (Leiva *et al.* 2016). Both species display yellow flowers with green nerves; however *C. septentrionalis* can be distinguished from *C. pavonii* by its elongate stigma (*vs*. triangular stigma), 3-lobed lip densely covered by fleshy long warts (*vs*. entire to obscurely 3-lobed lip provided with numerous short flattened keels or falcate keel-like teeth), and elliptic petals with 5 to 7 main nerves (*vs*. elliptic-ovate, with 4 main nerves) (Fig. 4, 10, 11).

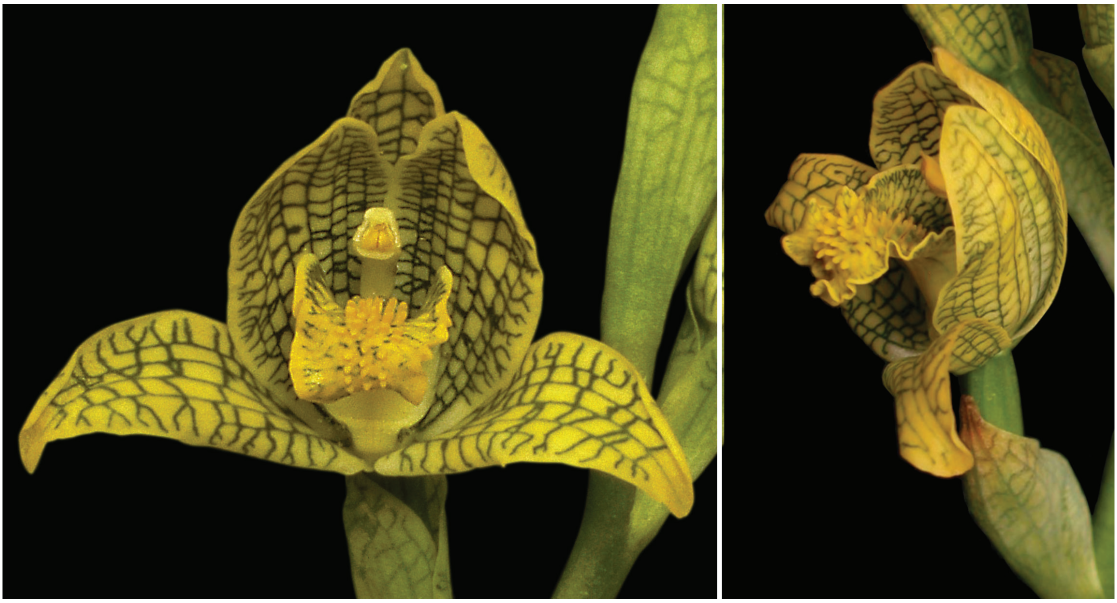

Figure 10. *Chloraea septentrionalis*. A – Flower, front view. B – Flower, side view. Photographs by D. Trujillo.

Franquemont *et al.* 1990 recorded *C. pavonii* (as *C. undulata*) for the department of Cusco (South Peru) based on the examination of the specimen *King 240* (F-1901370, photo seen). However, the floral features of the specimen agree with *Chloraea densipapillosa* C. Schweinf. This species displays a rather dense inflorescence with yellow flower without green nerves, lip markedly recurved, 3-lobed, pandurate, disc fleshy and covered by a dense pustulate ridges (Fig. 12).

Acknowledgments. We thank the staff and curators of K, LIL, MOL, USM, and W for allowing access to their collections. We also thank Paul Gonzáles for providing critical information of *C. pavonii*, to José Edquén for providing photographs of *Chloraea densipapillosa*. We would also like to thank the authorities of the "Proyecto Lomas de Asia", period 2017, especially to Iván Reyna, for supporting the observations of *C. pavonii* in the field. Author Paredes-Burneo is also grateful with the Peruvian Ministerio de Agricultura for granting a collection permit (N° 426-2017-SERFOR-DGGSPFFS). We also thank anonymous reviewers for their comments and improvements of the manuscript.

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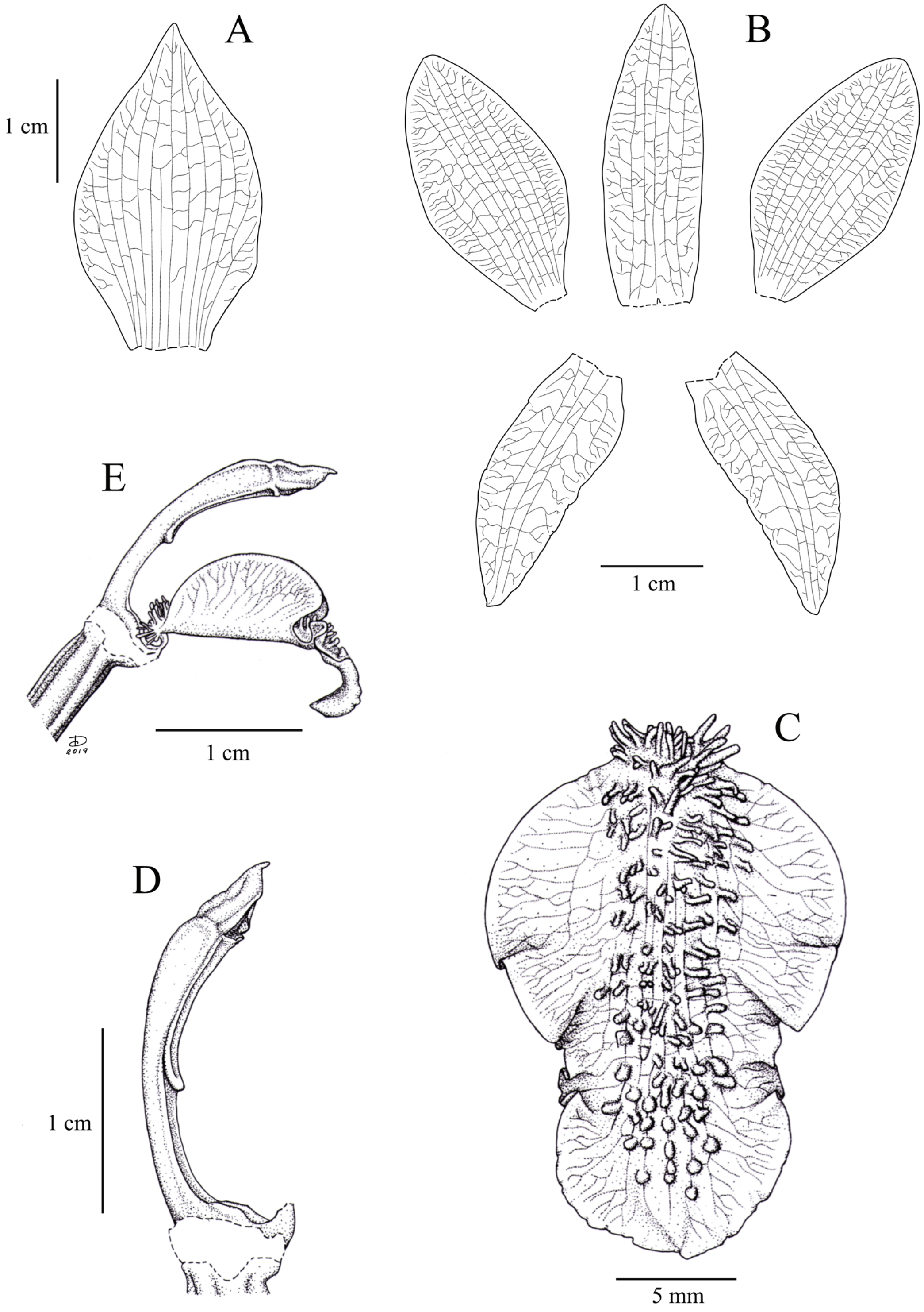

Figure 11. *Chloraea septentrionalis*. **A**. Floral bract. **B**. Dissected perianth. **C**. Lip. **D**. Column. **E**. Column and lip lateral view. Drawing by D. Trujillo based on *Trujillo 213*.

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FIGURE 12. Flowers of *Chloraea densipapillosa* C.Schweinf. Photograph by J. Edquén.

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ROOT ENDOPHYTIC FUNGI PROMOTE *IN VITRO* SEED GERMINATION IN *PLEUROTHALLIS CORIACARDIA* (ORCHIDACEAE)

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Abstract. Seeds of many orchids rely on the presence of fungi to trigger the germination process and even to initiate the full development of protocorms. While attention has been directed towards the study of mycorrhizal fungi, the diversity and functions of endophytic fungi from orchid roots remain underexplored, and few studies have verified their ecological role. This is the case of *Pleurothallis coriacardia*, an endemic green Neotropical orchid with both epiphytic and lithophytic habits growing in High-Andean montane forests. In the present study, we screened the cultivable fraction of the endophytic fungi colonizing the roots of mature plants of *P. coriacardia* using ITS rDNA markers. We also tested the potential of these endophytic fungi to improve embryo development and seed germination. Most of the isolated endophytes were classified within Psathyrellaceae. Some isolates, identified as members of the *Ilyonectria* and *Coprinellus* genera, significantly promoted embryo development *in vitro* in *P. coriacardia* seeds, a result that highlights the ecological roles these endophytic fungi may play in nature.

Resumen. Las semillas de la mayor parte de orquídeas dependen de la presencia de hongos para desencadenar el proceso de germinación e incluso el desarrollo de protocormos. Mientras la atención se ha dirigido hacia el estudio de los hongos micorrícicos con este fin, la diversidad y las funciones de otros hongos endófitos que habitan las raíces de orquídeas siguen siendo poco conocidas, y pocos estudios han verificado un rol ecológico en beneficio de la planta. Este es el caso de *Pleurothallis coriacardia* una orquídea endémica de bosques montanos de los Andes, con hábitos epífitos y litófitos. En el presente estudio, se aisló la fracción cultivable de hongos endófitos de especímenes maduros de *P. coriacardia*, mediante amplificación por PCR y secuenciación de la región ITS. Además se evaluó el potencial de 15 aislados seleccionados en ensayos preliminares como promotores de la germinación, analizando la tasa de cada estado de desarrollo de las semillas en presencia de cada hongo. Se logró identificar 134 cepas de hongos endófitos, con una elevada frecuencia de géneros pertenecientes a la familia Psathyrellaceae. En los ensayos de germinación, cepas identificadas como *Ilyonectria* sp. y *Coprinellus* sp., promovieron significativamente el desarrollo embrionario *in vitro* en semillas de *P. coriacardia*, un resultado que resalta uno de los posibles roles ecológicos que estos hongos endófitos pueden jugar en la naturaleza.

Keywords/Palabras clave: Andean montane forest, bosques montanos andinos, desarrollo embrionario, embryo development, epiphytic orchids, *Ilyonectria*, lithophytic orchids, orquídeas epífitas, orquídeas litófitas, Psathryrellaceae

Introduction. Symbiotic relationships between fungi and plants have drawn the attention of scientists since their initial description 140 years ago (De Bary 1879). Countless investigations have confirmed that most, if not all, plant species in natural ecosystems establish symbiotic relationships with endophytic microorganisms (Sun & Guo 2012). This diverse group of endophytes includes both fungi and bacteria, which are able to colonize the inner tissues of their plant hosts without causing any apparent damage (Bayman & Otero 2006, Bonfante & Anca 2009). The interactions established between endophytic microorganisms and plants can range from mutualism to saprophytism and have influenced the ecology, survival, and evolution of the host plants.

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Endophytes seem to play a very important role in maintaining the structure and diversity of the plant community and, consequently, of the entire ecosystem (Laforest-Lapointe *et al.* 2017, Tao *et al.* 2008). However, despite their potential roles as promoters of orchid germination and development, endophytes have rarely been studied (Ma *et al.* 2015). This is striking considering the diversity of this group of microorganisms, containing over 110 genera, and the potential functional roles played by these fungi, among which stand out the promotion of seed germination and protocorm development in plants (Pant *et al.* 2017, Teixeira da Silva *et al.* 2015, Tsavkelova *et al.* 2008).

Regardless of being an old and highly specialized group, the Orchidaceae family still displays diversification and speciation processes (Otero, Ackerman & Bayman 2002, Zettler, Sharma & Rasmussen 2003). Orchid development depends on the establishment of symbiotic associations with fungi, which provide a source of nutrients, particularly during seed germination—a behavior known as *mycoheterotrophy* (Kottke *et al.* 2013, Riofrío *et al.* 2013, Yoder, Zettler & Stewart 2000, Zettler *et al.* 2003).

Pleurothallis coriacardia is a green orchid found in the high Andes of South America, which shows both epiphytic and lithophytic habits. According to CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), *Pleurothallis* spp. commercialization should obey strict commerce regulations (CITES 1992). However, many of these orchid species are extracted from their natural environments and marketed illegally (Jiménez 2014, Pant *et al.* 2017), with only a few companies dedicated to their reproduction for commercial purposes. An effective alternative to avoid illegal extraction of orchids is their controlled propagation for horticultural purposes using germination media enriched with simple sugars and nutrients, such as Phytamax™ and Murashige & Skoog (1962). However, it is also possible to take advantage of the natural functions played by endophytic fungi for orchid propagation and conservation purposes (Sharma *et al.* 2002).

Information concerning *P. coriacardia* ecology, distribution, and symbiotic relationships is scarce (Suárez *et al.* 2006, 2008, Suárez & Kottke 2016). Even though no experimental results are currently available, *Pleurothallis* spp. are considered very challenging to reproduce and grow under controlled conditions. Indeed, it has been shown that *Pleurothallis* spp. are dependent on insect pollination to achieve successful fruit development and to attain high seed viability, with reports of high inbreeding depression in natural populations (Borba, Semir & Shepherd 2001) and limited sexual reproduction (CaraDona & Ackerman 2012).

Seeking to understand more about the ecological relationship between endophytes and epiphytic orchids, this work aimed to characterize endophytic fungi that naturally colonize the roots of *P. coriacardia* and to evaluate the endophytes potential as promoters of *P. coriacardia* seed germination *in vitro*.

Materials and methods

Study site.— Samples were collected in the Mazán Forest Reserve, located about 10 km east of the city of Cuenca (Azuay Province, Ecuador), at 02°50'S and 79°13'W (Fig. 1). This forest reserve occupies a total area of 2640 ha, ranging from 2800 to 3500 m in elevation. The annual precipitation in this region varies between 1000 and 2000 mm, with a temperature range between 6 and 12°C. Mazán Reserve is representative of the evergreen high montane forests and contains about 300 species of vascular plants, among which are 40 species of epiphytes, including orchids and bromeliads (Guzmán & Moreno 2014). The areas adjacent to the Mazán river are covered by both primary and mature secondary forests, with an important arboreal stratum represented by species such as "Arrayan" (*Myrcianthes rhopaloides*), "Romerillo" (*Prumnopyts montana*), "Sarar" (*Weimannia fagaroides*), "Pururug" (*Hedyosmum luteunyii*), "Tililin" (*Piper andreaum*), and "Jigua" (*Ocotea* sp.), among others. These trees foster the maintenance of a constant humidity and contribute to the absence of a marked dry season, creating a suitable place for the establishment of an important diversity of epiphytic species (Beltrán 2001).

Orchid species description.— Pleurothallis coriacardia Rchb.f. is an abundant species in Mazán Reserve, showing abundant wild populations in primary and secondary forests. Its growth habits are both epiphytic and lithophytic (Fig. 2). The most important morphological characteristics of this species, as recorded in the field include: i) sympodial growth, with unifoliar stalks covered in the base by purple bracts; ii)

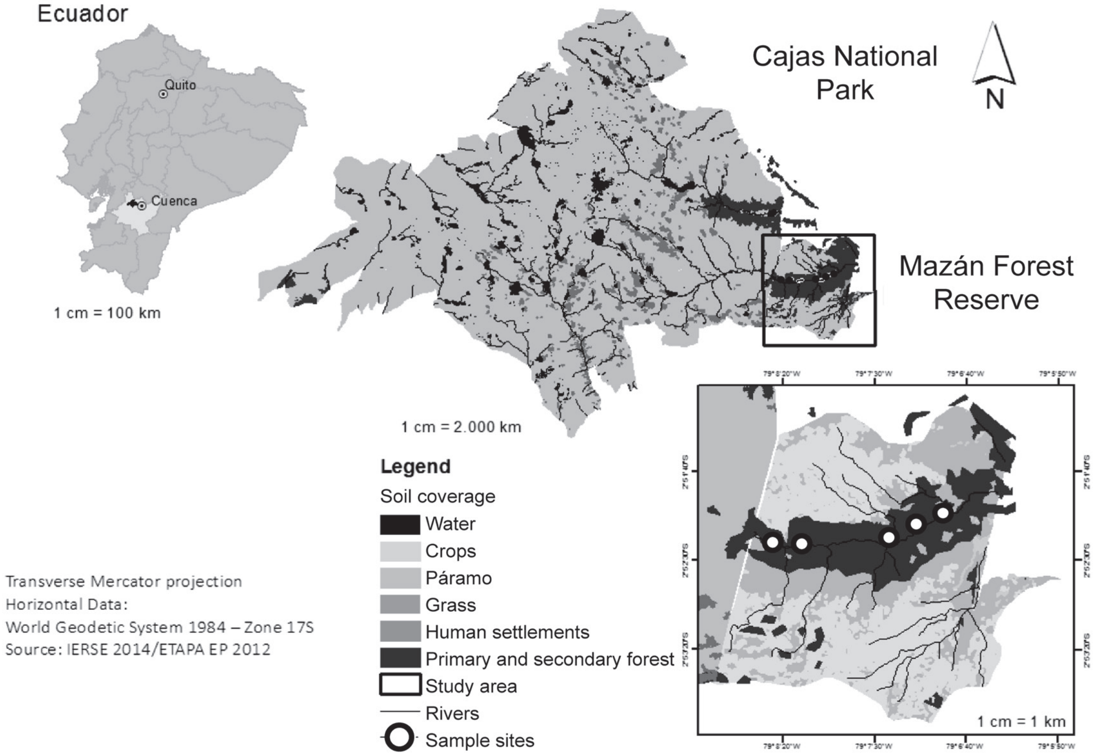

FIGURE 1. Map showing location of the study area in Mazán Forest Reserve, Cajas National Park, Ecuador.

oblanceolate and leathery leaves, sub-petiolated at the base; iii) velamentous roots exhibiting a characteristic green-translucent color at the tip (Fig. 3A); iv) apical inflorescences with up to 5 flowers—exhibiting fused lateral sepals and yellow-colored edges—per stem; v) small and elongated petals, varying from brown/reddish to yellow; vi) yellow to green lips and columns, with yellow colored pollinia (Fig. 3B); and, vii) apical capsules developing from each flower and containing thousands of pale-yellowish seeds of about 60–40 µm in length (Fig. 3C). The presence of asexual propagules emerging from the petiole, showing an abundant development of roots and new stems with no bulbs, was frequently observed (Fig. 3D). We also noticed the presence of numerous *P. coriacardia* protocorms, indicative of natural germination of the orchid seeds.

Plant material.— From February to March 2016 root samples were collected from 35 different adult individuals of *P. coriacardia* colonizing either rock substrates, live phorophytes, or decomposing arboreal substrates. Living phorophytes were mainly *M. rhopaloides*, *W. fagaroides* and *Ocotea sp.*, but also other less representative phorophytes were present. We selected five different sites, separated from each other by no less than 200 m, for collecting samples in the forest (Fig. 1). We took care to include orchids from at least one rock and three of the most representative phorophytes from each site. The samples consisted of several root fragments of approximately 3 cm in length, which included the green-translucent tips. Each sample was placed inside a sterile plastic bag and transported to the laboratory in Cuenca. Samples were refrigerated at 4°C for less than 24 hours after collection until fungal isolations were performed.

Orchid root fragments were surface sterilized following a combination of the procedures of Currah (1987) and Zettler (1997). In brief, root fragments were rinsed with tap water to remove debris, and then submerged for one minute in a solution of 2% bleach: 70% ethanol: sterile water (1:1:1), followed by several washings with sterile distilled water. Roots were then cut into small pieces (<1mm) using a sterile scalpel,

FIGURE 2. *Pleurothallis coriacardia* growth. Epiphytic (A) and lithophytic (B) habits. Mazán Forest Reserve, Cajas National Park, Ecuador. Photographs by G. P. Maldonado.

inoculated in molten fungal isolation medium (FIM) (Clements, Muir & Cribb 1986) that was supplemented with streptomycin (3 μg mL^{-1}), and incubated at 19°C. The hyphae that emerged during the next 96 hours were sub-cultured several times in potato dextrose agar (PDA) (Latalova & Balaz 2010) until their purity was confirmed. The fungal isolates were classified based on their colony morphology, taking into consideration the mycelium color, texture, and pattern of growth. Pure cultures were preserved in oatmeal-agar medium (OMA) at 4°C (Zettler 1997).

Seed were harvested from mature, dry capsules of adult *P. coriacardia* individuals in their natural habitat (Mazán Reserve) at the same time that root fragments were collected. Seeds were processed less than 24 hours after collection. For this, the capsules were opened under sterile conditions and seeds were placed in sterile vials (one for each capsule) and stored at -20°C until used (Zettler & McInnis 1993). Prior to sowing, seeds (of a single capsule) were disinfected using a protocol adapted from Zettler, Delaney & Sunley (1998). Briefly, seeds were immersed in a solution of 85% ethanol: 2.5% bleach: sterile distilled water (1:1:1) for one minute, followed by three successive rinses with sterile distilled water. Seeds were then suspended in 100 mL of sterile distilled water and transferred from the vial to the germination plates using a sterile syringe within ten minutes for the subsequent sowing in the germination plates, as described in the next section.

Selection of endophytic fungi for symbiotic germination assays.— In order to reduce the number of fungal isolates for symbiotic germination assays, we conducted a preliminary blind assay to select only those isolates showing a positive effect on the symbiotic germination of *P. coriacardia* seeds. For this, two milliliters of a suspension of disinfected seeds were spread on the surface of OMA plates. Then, in

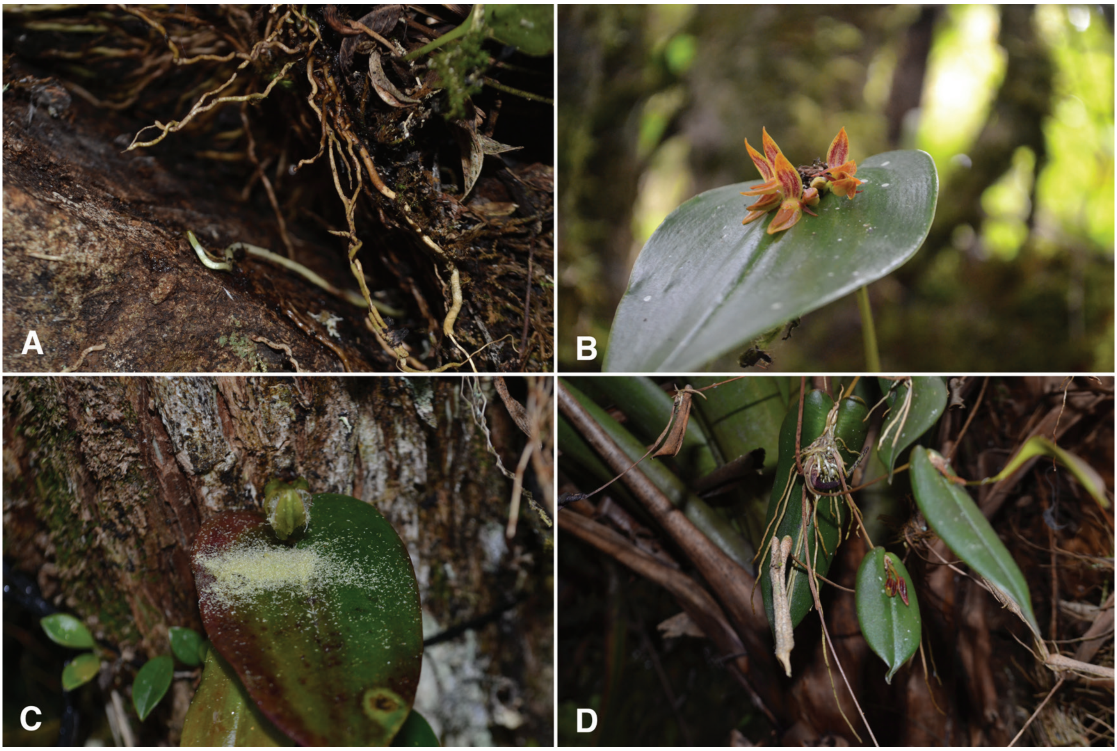

Figure 3. *Pleurothallis coriacardia* in the Mazán Forest Reserve. **A**. Roots of adult individual growing on rock substrate. **B**. Close-up of an inflorescence. **C**. Mature seed capsule of an adult individual growing on a phorophytes. **D**. Close-up of asexual propagules emerging from the stem. Photoghraps by L. A. Yarzábal (A) and G. A. Maldonado (B–D).

the center of each plate one block of agar (≈0.5 cm^3) containing the mycelium of each fungal strain was inoculated. In total, we tested all 134 isolates twice. Uninoculated OMA plates were used as negative controls for germination. Plates were sealed with Parafilm^{TM} and incubated in the dark at 19°C for two months; then, the plates were exposed to light under a 16/8 h light/dark photoperiod. Approximately ten randomly selected seeds in each plate were visually inspected with a stereo microscope (Olympus SZ61) every two weeks for five months.

The embryo development of each seed was scored according to a modification of the scale from Seaton & Ramsay (2009) as follows: 0=no germination/dead seeds; 1=seed with viable embryo; 2=swollen embryo; 3=testa rupture by enlarged embryo; 4=protocorm development; 5=appearance of first true leaf. The embryo diameters of 10 randomly selected seeds were recorded, per replicate and per treatment.

After obtaining the results of the preliminary symbiotic germination assay, genomic DNA was extracted from fungal isolates sharing the same macroscopic (color, appearance, consistency, size and shape) and microscopic (mycelium, hypha and conidia) characteristics. The 134 isolates were grouped into 76 morphotypes. DNA was extracted for polymerase chain reaction (PCR) amplification following the protocol of Cennis (1992). The ITS1-5.8S-ITS2 region was amplified by PCR using eukaryote universal primers ITS1 and ITS4 (White *et al*. 1990). The thermal profile was as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 57.4°C for 45 s and 72°C for 45 s, with a final extension step of 7 min at 72°C. The presence of amplicons was verified by agarose gel electrophoresis, using SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA). The amplicons were sent for further purification and sequencing to an external service provider.

To assign each fungal isolate to a particular taxon, we compared the obtained nucleotide sequences with those deposited in the GenBank database of the National Center for Biotechnology Information (NCBI 2017) and the UNITE fungal

ITS reference database (Version 7.1) (Kõjalg *et al*. 2005, Nilsson *et al*. 2018) using BLAST (Basic Local Alignment Search Tool) (Altschul *et al*. 1997). Following a strict criteria, we provided genus names only to those isolates whose sequences exhibited an identity >95% to the reference sequence and with an E value lower than or close to 0.001. Isolates exhibiting lower sequence identities were labelled at the order- or family-level name, or referred to as 'unknown' fungi.

Symbiotic germination assays.— Seeds were surface sterilized as previously described and spread over the surface of OMA. Fifteen selected fungal isolates were then inoculated as described above, and the embryo development was monitored every four weeks for a total period of four months. The test was performed with ten replicates per fungal isolate. For a positive control of germination, seeds treated as previously indicated were spread on the surface of Phytamax™ Orchid medium P6793 (Sigma-Aldrich) (pH 5.6), supplemented with gibberellic acid including the analysis of three different concentrations: 0, 10 and 20 µg mL^{-1} (Sigma Aldrich). This nutrient-rich medium is frequently used for asymbiotic germination of orchid seeds and was expected to provide the necessary nutrients to support germination of *P. coriacardia* seeds. The negative control of germination uninnoculated OMA as described above.

To score the seed development, we followed the same scale used in the preliminary assay, counting all seeds in each plate. To establish a relationship between seed diameter and developmental stage we only considered an average of 30 seeds, and conducted a Kruskal–Wallis non-parametric test and a sequential Bonferroni-corrected test (p=0.003) (Holm 1979). The same analysis was used to test whether there was a difference in the development rates of each stage with the different isolates and with the negative and positive control. Embryo development and seed diameter data were analyzed with XLSTAT (V. 2014.5.03) (Addinsoft 2014).

Results. In total, 134 pure fungal isolates were obtained from *P. coriacardia* roots and as previously stated, they were grouped into 76 morphotypes based on macroscopic and microscopic characteristics. Isolates were identified using BLAST searches. *Coprinellus* (Psathyrellaceae) was the most frequently occurring genus in the entire collection (20% of the isolates), followed by *Fusarium* (Nectriaceae), *Nigrospora* (Trichosphaeriales), and *Trichoderma* (Hypocreaceae) (represented by 10%, 10%, and 8% of the isolates, respectively). The isolates originated from *P. coriacardia* individuals colonizing various substrates. We found 16 isolates originated from lithophytic plants; 82 from epiphytic individuals colonizing the most representative phorophytes (7 from *W. fagaroides*; 17 from *Ocotea* sp.; 30 from *M. rhopaloides*; 28 on other less abundant phorophytes); and the remaining 36 originated from *P. coriacardia* collected from decomposing substrates (Fig. 4). Information of the entire endophytic collection are detailed in Appendix 1.

Twenty-three out of 134 fungal isolates exerted a positive effect on *P. coriacardia* embryo development in the preliminary symbiotic germination assay (data not shown). The relationship between embryo diameter and growth stage was established from the results obtained in this assay in order to confirm the suitability of the scale used to determine the stage of development. As can be seen in Fig. 5, there were significant differences between the average embryo diameter of seeds at different developmental stages. Based on these results, we selected 15 fungal strains for the final assay and discarded strains that exhibited a systematic contamination of the growth medium during germination assays.

Isolates selected from this assay were identified as *Ilyonectria* (6MF3), *Coprinellus* (18.1MF3, 22MF2.1, 19MF1.4.1, 20MF3, 21MF1, and 22MF2.3), *Nigrospora* (18.2MF2, 26MF1.4.1), *Chaetomium* (17MR4), *Fusarium* (29MF2.8), *Trametes* (33MF2.2), *Trichoderma* (3MF5), and Unidentified Endophyte (12.1MF3, 27MF3).

Three of these 15 selected isolates—6MF3, 18.1MF3, and 22MF2.1—significantly promoted embryo development after 12-weeks incubation, as compared to the negative control (Fig. 6). Noticeably, two of these fungal isolates were closely related to *Coprinellus* species. It is important to highlight that inoculation with eight of these selected isolates allowed the embryos to develop until stage 3, even though the percentages varied from 3% to 30% depending on the isolate.

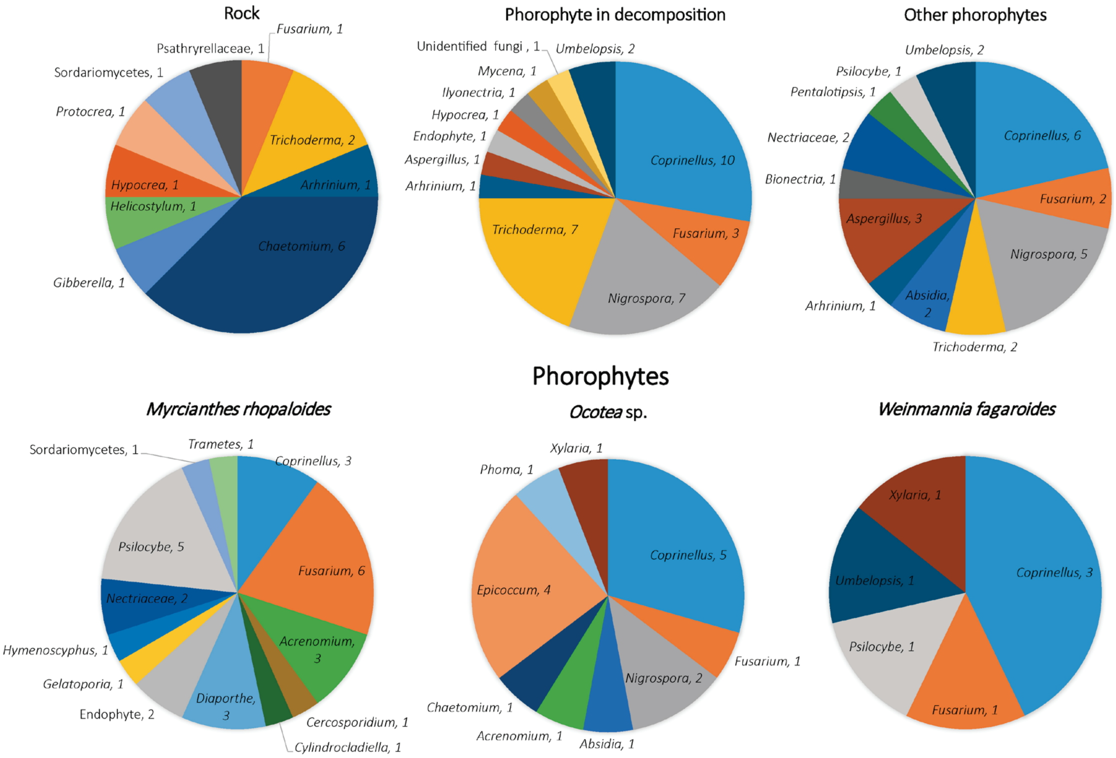

Figure 4. Distribution of endophytic fungi. In the upper panel there are three colonization substrates, in the lower panel there are three phorophytes substrates. The labels next to the fungus name represents the number of isolates.

Seeds of *P. coriacardia* sowed in GA-supplemented Phytamax™ medium (included as a positive control of germination) produced some unexpected results: after 12-weeks incubation, all seeds reached stage 1 (viable embryo) regardless the concentration of GA, but there was no further development after that point. Surprisingly, 3% of seeds incubated in OMA medium (included as negative control of germination), reached stage 2 (embryo swelling) and then stopped developing. As can be seen in Fig. 5, the average diameter of the embryo was larger in seeds incubated in OMA medium (our negative control), when compared to those incubated in GA-supplemented Phytamax™ medium (p=0.0105) (considered as positive control). We considered the experiment for 12 weeks, with a sequential revision of the plates, however, after this time some plates with developing seeds were reviewed but until week 16 we did not find evidence of changes in the development of *P. coriacardia* seeds.

Discussion. Our findings show that the cultivable fraction of endophytic fungi colonizing *P. coriacardia* roots was rich in species irrespective of the substrate type on which the orchids grew. The isolates belonging to the *Coprinellus* genus were the endophytic partners which are the most frequently associate to *P. coriacardia* roots. Furthermore, some of the isolates, such as those belonging to the *Ilyonectria* and *Coprinellus* genera, stimulated seed germination by promoting embryo development *in vitro* until testa rupture (stage 3). Endophytic fungi colonizing roots of orchids have been reported to have a crucial role in plant propagation, embryo development, seed germination, and protocorm nutrition (Rasmussen *et al*. 2015, Ma *et al*. 2015, Pant *et al*. 2017). For instance, *Tolumnia variegata* in the presence of *Ceratobasidium* have a positive influence in seed germination (Otero *et al*. 2005), *Epulorhiza* spp. promoted protocorm development until leaf production of *Cyrtopodium glutiniferum* (Pereira *et al*. 2015). Besides, *Fusarium* spp. have

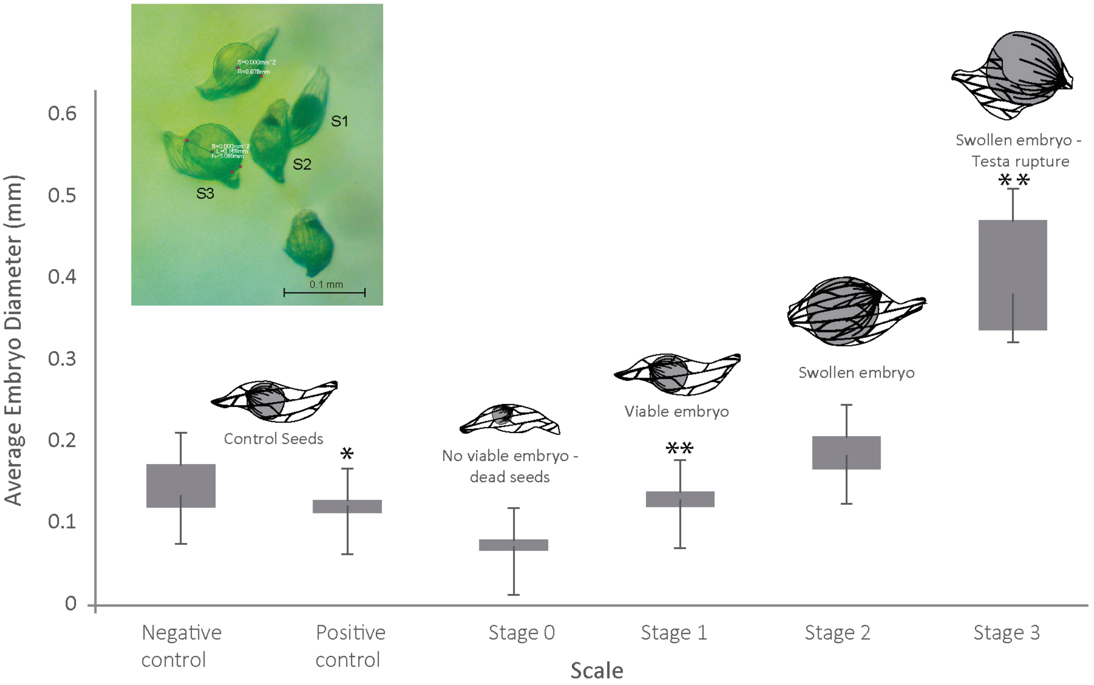

FIGURE 5. Diameter versus embryo developmental stage of *P. coriacardia* seeds. The schemes illustrate the morphology of the seeds at different stages. Inset: the picture shows seeds at different developmental stages, as seen under a stereo microscope, S1 represents Stage 1=Viable embryo; S2, Stage 2=swollen embryo; S3, Stage 3=Testa rupture. Average diameter identified with asterisks differ significantly from the negative control $p < 0.001$; the two asterisks is $p < 0.01$.

been described as active auxin- and gibberellic acid producers in *Dendrobium* sp., *Pterostylis* sp. and *Cymbidium* sp., promoting high germination rates and playing important roles in the development of protocorms (Tsavkelova *et al.* 2008).

The root fungal endophytic communities of *P. coriacardia* and other green orchids in Andean ecosystems have been poorly studied. Greater research efforts have been made using massive sequencing techniques for understanding the mycorrhizal and endophytic fungi associated with green epiphytic Andean orchids like *Odontoglosum pardinum*, *Epidendrum marsupial*, *Cyrtochilum pardinum*, *C. flexuosum*, *C. myanthum* and *Maxillaria calantha* reported for the same study site (Cevallos *et al.* 2018, Guzmán & Moreno 2014, Herrera *et al.* 2018, Herrera *et al.* 2019). However, no information is available regarding the cultivable fraction of fungi for these species. We show high incidence of root endophytic fungi in adult plants of *P. coriacardia* from Mazán Reserve. We found that the orchids growing on live phorophytes—*W. fagaroides, Ocotea* sp., and *M. rhopaloides*—and over decomposing arboreal substrates were similarly colonized by fungi customarily defined as saprophytes and pathogens such as *Coprinellus*, *Nigrospora*, *Trichoderma*, and *Fusarium*. In contrast, orchids growing on rock substrate had a different composition in which we identified morphotypes closely related to *Fusarium tricintum* and *Helicostylum*, in addition to observing a greater abundance in individuals of the genus *Chaetomium* whereas fungal isolates closely related to *Coprinellus* were completely absent.

The fact that a fungus can be isolated from a section of root tissue does not mean it is mycorrhizal. Nonmycorrhizal endophytes, saprophytes, and pathogens are commonly reported in orchid roots (Bayman & Otero 2006, Bayman *et al.* 2016, Selosse *et al.* 2010) and members of this guild have shown positive roles in orchid development from seed germination to vegetative growth. The first non-*Rhizoctonia*-like genera reported to show a symbiotic role was *Fusarium*

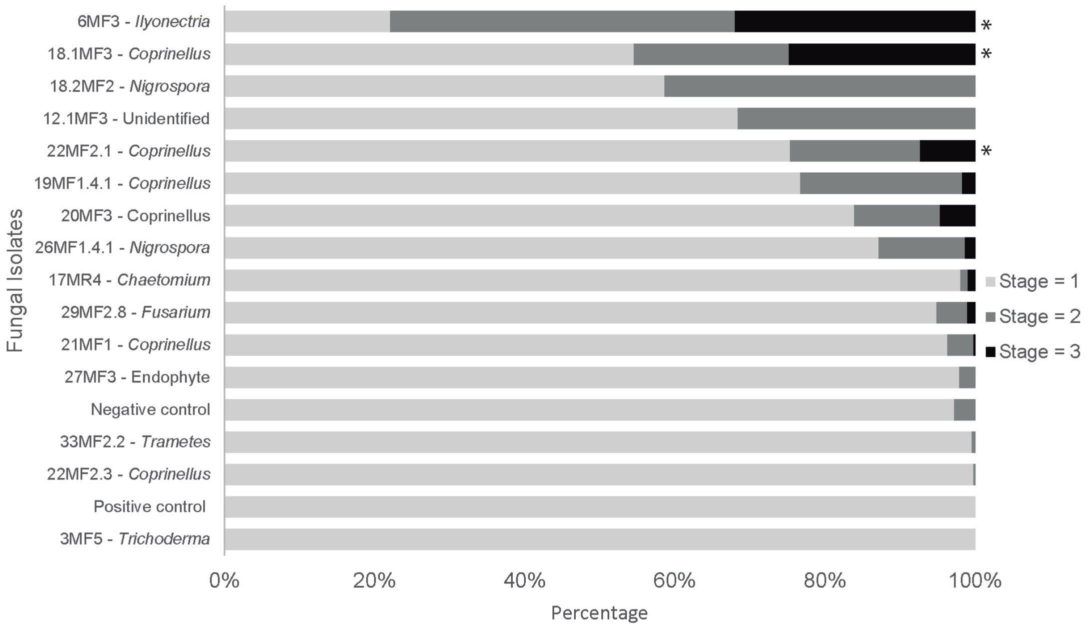

FIGURE 6. Percentage of *P. coriacardia* seeds that reached stage 1, 2 and 3 by week 12 of symbiotic germination in the presence of each fungal isolate. The percentage in each stage was calculated by dividing the number of seeds in each stage by the total number of viable seeds in each sample. The positive and negative controls of germination are also included. Treatments identified with asterisks differ significantly from the negative control $p<0.001$.

in the orchid *Cypripedium reginae* (Vujanovic 2000), with significance in seed germination. The known saprophytic fungi *Mycena*, *Trichoderma*, and *Chaetomium* have also been reported to play a specific role in orchid development (Pant *et al*. 2017); all of these genera have been reported in this study.

The genus *Coprinellus*, also saprophytic fungi, was first described as very frequently associated with the terrestrial orchid *Epipogium roseum* by Yamato *et al*. (2005), they suggest the existence of a highly specific relationship between this orchid species and members of the Psathyrellaceae family, owing to the high abundance of pelotons observed in their roots. Yagame *et al*. (2013) confirmed the mycorrhizal status of *Coprinellus* by symbiotic cultivation of seeds from *Cremastra appendiculata*, a photosynthetic terrestrial orchid, and confirmed the presence and mycorrhiza formation by *Coprinellus* in *Cremastra aphylla* (Yagame *et al*. 2018). Isolates belonging to this genus were detected very frequently in our study, a result which also confirm previous findings by Salazar *et al*. (2020). We also showed *Coprinellus*'s important role as a promotor of germination for this species, with two out of five tested isolates leading seeds to stage 3 (testa rupture) of development.

Previous studies about the germination of seeds from *Pleurothallis* orchids and symbiotic fungal associations are scarce. The only precedent to the present work is in relation to achievement for seed development of *Pleurothallis truncata* up to stage 4 after a 10-week incubation (León & Molina 2015) in Murashige & Skoog medium, supplemented with coconut water. Incidentally, the authors pointed out that attempts to achieve symbiotic germination of *P. truncata* seeds with the help of *Rhizoctonia*-like isolates were unsuccessful. On the contrary, we avoided the bias of selecting *Rhizoctonia*-like features through a preliminary blind germination assay, which probably lead to the unexpected discovery of an additional genus, *Ilyonectria*, that promoted embryo development of *P. coriacadia*.

Members of the *Ilyonectra* genus and their allied anamorphic taxa are often detected when studying rhizospheric communities of plants like *Pyrola* sp., *Populus* sp., *Enkianthus* sp., and *Alnus* sp. among others (Geml *et al*. 2014, Obase & Marsuda 2014, Unterseher, Per & Schnittler 2013). Even though there is not much information concerning the ecological role those fungi might play, recent findings support their functioning as promoters of germination of certain woody plant species (Bonito *et al*. 2014). The results presented here showing the promotion of *P. coriacardia* germination by *Ilyonectria* sp. strain 6M3 are in line with that hypothesis.

One particular result that deserves to be considered with caution was the slow rate of embryo development of *P. coriacardia* seeds in a GA-supplemented medium rich in simple sugars (Phytamax™). This is a widely used medium for *in vitro* asymbiotic germination of seeds from many orchid species, which was the reason we included it as a positive control of germination for our experiments. However, our results suggest that either Phytamax™ is not well suited for *P. coriacardia* germination *in vitro*, or the germination of this species is particularly slow. We believe that the nutrient richness of this medium may be inhibitory to germination of *P. coriacardia* seeds, as previously demonstrated with other orchid species (Rafter *et al*. 2016). We are cautious to suggest a highly specialized relationship with *P. coriacardia*, despite our results, because we only isolated fungi from adult plants, and as it has been reported, mature plants are not implicated in the symbiotic germination of orchids seeds (Ovando *et al*. 2005). Besides, for epiphytic tropical orchids a low specificity condition between orchids and the fungal guests have been well documented in comparison to their terrestrial relatives (Jacquemyn *et al*. 2010, Johnson *et al*. 2007, Stewart & Kane 2006). We consider that the isolation of fungi from natural seedlings, probably using seed baiting techniques (Rasmussen & Whigham 1993), are necessary to confirm this association. It is important to point out that in the field it was common to observe protocorms and seedlings from epiphytic orchids, near to adult populations of *P. coriacardia*, located mostly in rock substrates, where we did not register *Coprinellus* genus.

After the 16-week analysis, no further development of *P. coriacardia* seeds was observed, which may be explained by various hypotheses. Halted seed development may be due to the necessity of another nutrient source not provided by the fungal endophytes (Zettler *pers. comm.* 2017). Another hypothesis is that fungal presence improved access to water (Ovando *et al*. 2005), considering the results of the negative controls, or that the fungi were used as an initial carbon source. Accordingly, the developing seed digested the fungi until it reached a large enough size to cause the testa to rupture, but after this point other obligate symbionts may be needed for establishment of the protocorm, and/or possibly the fungi stopped acting as providers and became pathogenic, as in the case of *Ilyonectria*.

We hope our results could aid in improving descriptions of the ecology of Andean orchids and their endophytes and to support propagation programs of *P. coriacadia* both for conservation and commercial purposes which could in turn help reduce extraction from their natural habitats.

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Appendix 1. Molecular identification of endophytic fungi isolated from *P. coriacardia* roots samples in the Mazán Forest Reserve (Azuay Province, Ecuador) based on the closest match in the GenBank database.

Isolate code number		**GenBank accession number**	**Phylum**	**Possible identity**	**Best match in BLAST analysis**		**Host Substrate of *P. coriacardia***
					Closest relatives (accession number)	**% Identity**	
UCUE_Pc_M_	1MF5	MF471296	Ascomycota	*Pestalotiopsis*	AY461815.1	99	Other phorophytes
UCUE_Pc_M_	1MF1	MF471251	Ascomycota	*Nigrospora*	EU272503.1	99	Other phorophytes
UCUE_Pc_M_	1MF2	MF471234	Basidiomycota	*Psilocybe*	KC007301.1	96	Other phorophytes
UCUE_Pc_M_	1MF6	MF471289	Basidiomycota	Unknown fungi	KU761146.1	94	Other phorophytes
UCUE_Pc_M_	2MR2	MF471266	Ascomycota	*Arthrinium*	HQ385968.1	98	Rock
UCUE_Pc_M_	2MF4	MF471247	Zygomycota	*Helicostylum*	KM396375.1	95	Rock
UCUE_Pc_M_	3MR1	MF471265	Ascomycota	*Trichoderma*	KU727807.1	99	Rock
UCUE_Pc_M_	3MR3	MF471253	Ascomycota	*Hypocrea*	EU871036	99	Rock
UCUE_Pc_M_	3MR5	MF471248	Ascomycota	*Trichoderma*	JF773644	99	Rock
UCUE_Pc_M_	3MR2	MF471252	Ascomycota	Unknown fungi	KP714312.1	85	Rock
UCUE_Pc_M_	5.1MF7	MF471228	Basidiomycota	*Coprinellus*	FJ185160.1	99	Phorophytes in decomposition
UCUE_Pc_M_	5.1MF1	MF471278	Basidiomycota	*Mycena*	FJ785523.1	96	Phorophytes in decomposition
UCUE_Pc_M_	5MF2	MF471303	Ascomycota	*Trichoderma*	HM439523.1	95	Phorophytes in decomposition
UCUE_Pc_M_	6MF3	MF471236	Ascomycota	*Ilyonectria*	KF646096.1	99	Phorophytes in decomposition
UCUE_Pc_M_	7MR5	MF471241	Ascomycota	*Gibberella*	HQ630977	99	Rock
UCUE_Pc_M_	7MR4	MF471304	Basidiomycota	Psathryrellaceae	GU056021	96	Rock
UCUE_Pc_M_	8MF4	MF471270	Ascomycota	*Trichoderma*	KU727807.1	99	Other phorophytes
UCUE_Pc_M_	8MF2	MF471238	Ascomycota	Endophyte	FJ613085.1	99	Other phorophytes
UCUE_Pc_M_	8MF5	MF471268	Ascomycota	Unknown fungi	KU978069.1	94	Other phorophytes
UCUE_Pc_M_	9.1MF3	MF471245	Ascomycota	*Fusarium*	KU978069.1	99	Phorophytes in decomposition
UCUE_Pc_M_	9.3MF1	MF471246	Zygomycota	*Umbelopsis*	AB193542.1	98	Phorophytes in decomposition
UCUE_Pc_M_	9.3MF2	MF471222	Basidiomycota	*Coprinellus*	FJ185160.1	98	Phorophytes in decomposition
UCUE_Pc_M_	9.2MF2	MF471231	Ascomycota	Unknown fungi	AM999730	85	Phorophytes in decomposition
UCUE_Pc_M_	9.3MF4	MF471238	Ascomycota	Unknown fungi	FJ613085.1	80	Phorophytes in decomposition
UCUE_Pc_M_	10MF4	MF471261	Ascomycota	Nectriaceae	JN088237.1	99	Other phorophytes
UCUE_Pc_M_	10MF2	MF471242	Ascomycota	*Bionectria*	KC007301.1	96	Other phorophytes
UCUE_Pc_M_	10MF1	MF471260	Ascomycota	Unknown fungi	FR717914.1	81	Other phorophytes
UCUE_Pc_M_	12.1MF3	Unidentified	Basidiomycota	Unknown fungi	Unidentified	-	Phorophytes in decomposition
UCUE_Pc_M_	12MF3	MF471300	Ascomycota	*Xylaria*	KU743974.1	99	*Ocotea sp.*
UCUE_Pc_M_	12MF6	MF471291	Ascomycota	*Epicoccum*	HQ914878	99	*Ocotea sp.*
UCUE_Pc_M_	12MF2	MF471279	Ascomycota	*Epicoccum*	KX664321.1	99	*Ocotea sp.*

UCUE_Pc_M_	12.1MF4	MF471282	Ascomycota	*Trichoderma*	JN715591.1	98	Phorophytes in decomposition
UCUE_Pc_M_	14MF2	MF471299	Ascomycota	*Fusarium*	KU978069.1	99	*Sarar - Weinmannia fagaroides*
UCUE_Pc_M_	17MR4	MF471263	Ascomycota	*Chaetomium*	HG937119.1	98	Rock
UCUE_Pc_M_	18.1MF3	MF471240	Basidiomycota	*Coprinellus*	JN198387	99	*Ocotea sp.*
UCUE_Pc_M_	18MF4	MF471220	Ascomycota	*Phoma*	EU343130	99	*Ocotea sp.*
UCUE_Pc_M_	19MF2	MF471226	Basidiomycota	*Coprinellus*	JN198387	97	*Sarar - Weinmannia fagaroides*
UCUE_Pc_M_	19MF1.4.1	MF471221	Basidiomycota	Unknown fungi	GU055721	90	*Sarar - Weinmannia fagaroides*
UCUE_Pc_M_	20M6	MF471243	Ascomycota	*Chaetomium*	HG937119.1	99	*Ocotea sp.*
UCUE_Pc_M_	21MF2	MF471254	Basidiomycota	*Coprinellus*	FJ755223	99	*Ocotea sp.*
UCUE_Pc_M_	21MF1	MF471232	Basidiomycota	*Coprinellus*	KP216899.1	99	*Ocotea sp.*
UCUE_Pc_M_	21MF4	MF471250	Basidiomycota	*Coprinellus*	FJ755223	98	*Ocotea sp.*
UCUE_Pc_M_	22MF	MF471305	Zygomycota	*Umbelopsis*	EU490082	98	Other phorophytes
UCUE_Pc_M_	22MF2.3	MF471294	Basidiomycota	*Coprinellus*	AY461815.1	95	Other phorophytes
UCUE_Pc_M_	22MF3	MF471237	Zygomycota	Unknown fungi	AB193543.1	90	Other phorophytes
UCUE_Pc_M_	23MF3	MF471244	Zygomycota	Unknown fungi	KJ028792.1	93	*Sarar - Weinmannia fagaroides*
UCUE_Pc_M_	24MF1	MF471293	Zygomycota	*Absidia*	AY944874	96	*Ocotea sp.*
UCUE_Pc_M_	24MF2	MF471269	Ascomycota	Unknown fungi	KU978069.1	94	*Ocotea sp.*
UCUE_Pc_M_	25MF1	MF471301	Basidiomycota	*Coprinellus*	KT804053.1	96	*Sarar - Weinmannia fagaroides*
UCUE_Pc_M_	26MF1	MF471281	Ascomycota	*Hypocrea*	EF488156.1	99	Phorophytes in decomposition
UCUE_Pc_M_	27MFA	MF471259	Ascomycota	*Fusarium*	KU377445.1	99	Phorophytes in decomposition
UCUE_Pc_M_	27MF	MF471277	Basidiomycota	*Coprinellus*	KU761146.1	98	Phorophytes in decomposition
UCUE_Pc_M_	27MF3	MF471264	Ascomycota	Unknown fungi	FJ449935	92	Phorophytes in decomposition
UCUE_Pc_M_	28MFN1	MF471292	Ascomycota	*Trichoderma*	HM037962.1	99	Phorophytes in decomposition
UCUE_Pc_M_	29MF2.8	MF471274	Ascomycota	*Fusarium*	KU978069.1	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF2.3.1	MF471267	Ascomycota	*Cercosporidium*	EU543257	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF4	MF471286	Ascomycota	*Cylindrocladium*	KR780039.1	98	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF9	MF471285	Ascomycota	*Fusarium*	FJ545374.1	98	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF2.7	MF471271	Ascomycota	Sordariomycetes	FJ449913	98	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF2.2	MF471235	Basidiomycota	*Psilocybe*	AJ519795.1	98	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	29MF2.5.1	MF471306	Ascomycota	*Diaporthe*	EU272520	97	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	30MR6	MF471307	Ascomycota	*Fusarium*	GQ229075	97	Rock
UCUE_Pc_M_	30MR2.2	MF471290	Ascomycota	Sordariomycetes	JQ761706	95	Rock
UCUE_Pc_M_	31MF1	MF471262	Ascomycota	Endophyte	AY561198.1	95	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	31MF2.2	MF471288	Ascomycota	Unknown fungi	KX650836.1	81	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	32MF5	MF471264	Ascomycota	Nectriaceae	KU978069.1	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	32MF4	MF471256	Ascomycota	*Fusarium*	AY745988	99	*Arrayán - Myrcianthes rhopaloides*

UCUE_Pc_M_	32MF6	MF471280	Basidiomycota	*Coprinellus*	FJ185160.1	97	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	32MF2.1	MF471308	Ascomycota	Endophyte	HQ889707	96	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	32MF3	MF471239	Basidiomycota	*Coprinellus*	AB176569.1	91	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	33MF2.3	MF471297	Ascomycota	*Hymenoscyphus*	GU479911.1	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	33MF2.1	MF471283	Basidiomycota	*Trametes*	KX056103.1	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	33MF2.4	MF471275	Ascomycota	Nectriaceae	KU978069.1	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	33MF2.2	MF471309	Basidiomycota	*Gelatoporia*	FN907911	97	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	35MF4	MF471258	Ascomycota	*Fusarium*	AY745988	99	*Arrayán - Myrcianthes rhopaloides*
UCUE_Pc_M_	35MF1	MF471257	Ascomycota	*Fusarium*	AY745988	99	*Arrayán - Myrcianthes rhopaloides*

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    6. Manuscripts accepted for publication but not yet published: Nobody, A. B. (In press). Name of the journal or publisher. The name of the journal where the paper was accepted must be indicated, the volume number should be included if known.
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