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We take the opportunity to acknowledge our authors, reviewers and readers, who help us making a better scientific journal.

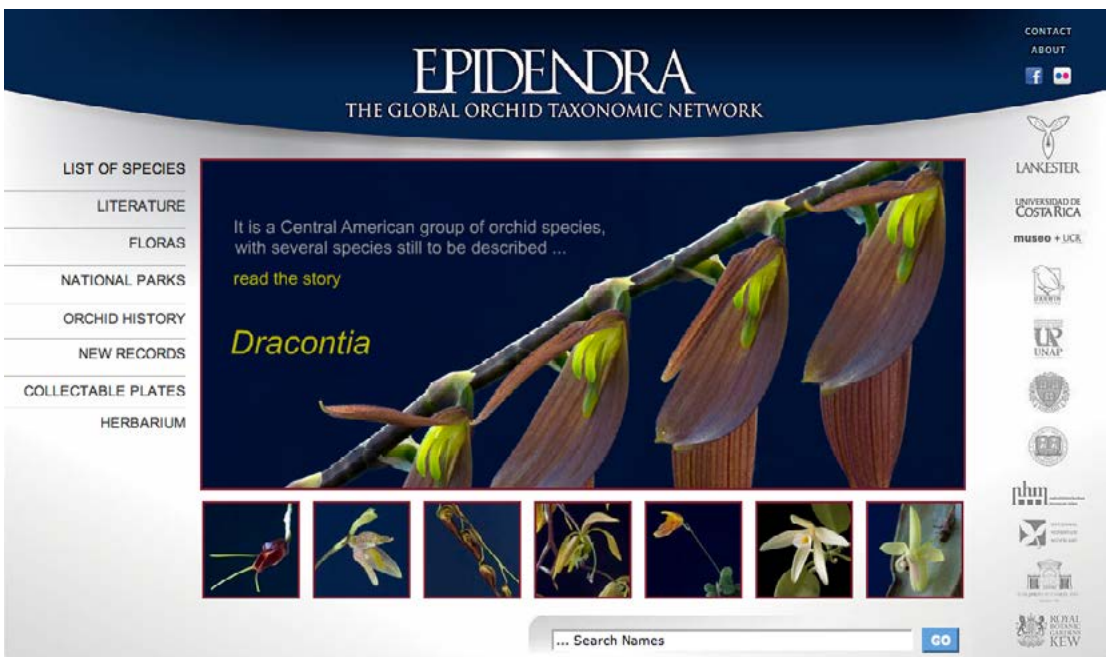
The editors

The Global Orchid Taxonomic Network at a click

www.epidendra.org

Now with a new user interface, the online database on taxonomic information by Lankester Botanical Garden includes more than 7,000 orchid names, completely cross-referenced and with evaluated synonymies.

The electronic file on each one of the names accepted by the taxonomists at the research center includes *free, immediately downloadable* protologues, type images, illustrations of the original materials, historical and modern illustrations, photographs, pertinent literature and, when available, digital images of species pollinaria.



An index (under the button "List of species") allows the users to search for any published name, independently if it is accepted or not by the taxonomic compilers. Synonyms are linked to their accepted name, where additional materials (including images) are available for download.

Hundreds of new species names and documents (mostly protologues), images (including high-res files), publications and other materials relative to orchid systematics, distribution and history are added to the database on a monthly basis (new entries can be searched by clicking on the "New records" button).

Since March, 2012, *new pages* are devoted to the orchid species recorded in the rich system of national parks and other protected areas in Costa Rica ("National Parks" button), updated checklists of the orchid floras of Central American countries ("Floras" button) and to interesting aspects of orchid history.

Under the button "Collectable plates", the research staff at Lankester Botanical Garden makes available to the public the most detailed images of orchids from the collections at the Center, organized in a series of collectable plates that can be *downloaded for free*. New ones are added each week.

Supported by the University of Costa Rica and the Darwin Initiative, *Epidendra, The Global Orchid Taxonomic Network* counts with the collaboration of respected taxonomists and leading botanical institutions worldwide.

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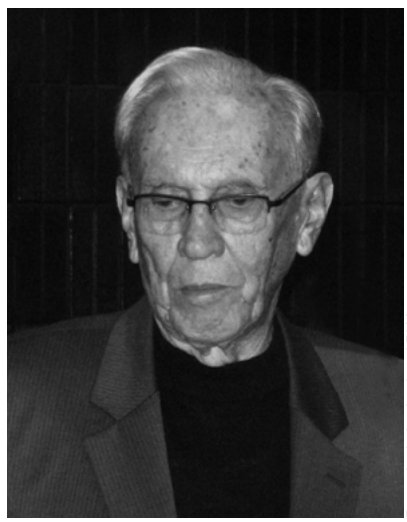
O B I T U A R I O

PADRE PEDRO ORTIZ VALDIVIESO, S. J.

(1926—2012)



El Padre Pedro era un hombre de pocas palabras: pero cada frase que pronunciaba estaba llena de significado. Era como si hablara solamente con aforismos. Tal vez su dominio de lenguas pretéritas y contemporáneas le hizo entender bien el sentido de cada término, y su verdadera utilidad.

Conocí al Padre Pedro precisamente por su dominio de lenguas que algunos llaman muertas, como parece ser hoy el caso aún del latín, que fue excluido hace apenas unos meses como lengua de referencia en la taxonomía biológica.

Hace 4 años, Alma Nohra Miranda, subdirectora del Archivo Histórico Javeriano, nos lo refirió como la persona que podría ayudarnos en la transcripción y traducción de un manuscrito inédito en latín que reposaba en el Archivo, y que Jaime Bernal y yo consideramos podría ser original de José Celestino Mutis, precursor de la botánica en nuestro país en el siglo XVIII. Como la tarea era dispendiosa, y el Padre Pedro era en ese momento director de la Biblioteca de Teología y Filosofía, le pedimos solamente que nos ayudara a contactar a algún estudiante que pudiera hacer la tarea: en un gesto de inmensa generosidad, él mismo se ofreció de inmediato.

Ese trabajo conjunto fue la base del primer libro que publicamos con él en el año 2009, bajo el título "*Filosofía Natural Mutisiana*", segundo de una trilogía que cerramos con la obra "*Academia Mutisiana*", también con la colaboración del Padre Pedro.

Pero esto no fue lo único que hicimos con él en estos 4 años que para nosotros serán inolvidables: en medio de ires y venires, mientras el Padre resultó visitándonos más a nosotros en el Instituto que nosotros a él en la Biblioteca, surgió una línea de investigación interdisciplinar que es buen modelo de las virtudes de la Universidad. A partir de un encuentro fortuito en un dominio transdisciplinario como el que configuró el que nosotros, genetistas, estuviéramos indagando en los prodigiosos fondos del Archivo Javeriano, nació un proyecto de investigación insospechado. El padre Pedro, en una de sus primeras visitas al Instituto de Genética, sacó de su maletín la tarea cumplida: era la Oración Inaugural leída por Mutis en el año 1764 en Santafé, titulada "*Oratio pro philosophia newtoniana contra peripateticos*", transcrita y traducida, con la cual pudimos iniciar la redacción de la segunda obra mutisiana. Al finalizar la reunión, con la discreción que lo caracterizó, sacó también una "USB" que cargaba siempre con él, y nos pidió si la podíamos ver en algún computador. Al abrir uno de sus archivos, apareció una orquídea.

Esa bellísima flor, que él mismo bautizó "*Santanderella amado-rinconiana*", y que quería, nos dijo, clasificar con las herramientas moleculares más allá del fenotipo, fue el inicio de una línea de investigación que ya tiene entre sus productos una tesis de pregrado en Biología (de la estudiante Laura Mazo), 3 artículos científicos y más de 30 registros publicados en el *Gene Bank*, gracias al juicio de Sonia Quintanilla que nos ha acompañado en los últimos 2 años atendiendo a cada inquietud del Padre Pedro sobre la taxonomía de las Orquidáceas.

El Padre Pedro, a quien registramos como naturalista emblemático de la Compañía de Jesús en nuestra obra "*Scientia Xaveriana*" — que trata sobre los jesuitas y el desarrollo de la ciencia en Colombia en los siglos XVI a XX —, se convirtió para nosotros en eje más esencial en la botánica de lo que nosotros fuimos para él en la genética molecular.

Y es que el Padre Pedro Ortiz Valdivieso representa para la historia de la ciencia un nuevo y brillante eslabón en la cadena de personajes históricos que se inició precisamente con Mutis, y se continuó en el tiempo con su paisano santandereano Eloy Valenzuela, luego Francisco Javier Matis, José Jerónimo Triana, Ezequiel Uricoechea, Florentino Vezga, Enrique Pérez Arbeláez, Lorenzo Uribe Uribe y que termina en él, en lo que tiene que ver con la Compañía de Jesús.

Autor de la colección "*Orquídeas de Colombia*", publicada por Colciencias y ya con tres ediciones (una de ellas por reimpresión), editor de uno de los tomos en gran formato de la "*Flora de la Expedición Botánica*" publicados en Madrid, principal aportante de ejemplares "tipo" del herbario de la Universidad Javeriana y referencia mundial en el campo de las orquídeas (soy testigo del aprecio que le tuvo Mark Chase, director de los Laboratorios Jodrell en los *Kew Gardens* en Londres, y de la admiración que le demostró el más renombrado botánico disciplinar en Colombia, Santiago Díaz-Piedrahita), el Padre Pedro deja una huella muy honda en la ciencia mundial. Esperemos que algún miembro de la comunidad tenga el rigor y la capacidad de seguir la línea.

Por mi parte, debo agradecer el haber tenido la oportunidad de conocerlo en vida.

Alberto Gómez Gutiérrez
Instituto de Genética Humana, Facultad de Medicina
Pontificia Universidad Javeriana
Bogotá - Colombia

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O B I T U A R Y

FATHER PEDRO ORTIZ VALDIVIESO, S. J.[1]

(1926—2012)

Father Pedro was a man of few words: but every phrase that he spoke was full of significance. It was as though he spoke only in aphorisms. Perhaps his dominion of ancient and contemporary languages allowed him to understand well the meaning of each word and its true utility.

I knew Father Pedro precisely through his dominion of ancient languages that some call dead, as in the case now of Latin, that was excluded only a few months ago as reference languge in biological taxonomy.

Four years ago, Alma Nohra Miranda, subdirector of the Javeriana Historical Archive, referred Father Pedro to us as the person who could help us in the copying and translation of an unpublished manuscript in the Archive, that Jaime Bernal and I thought might be an original of José Celestino Mutis, precursor of botany in our county in the 18th Century. As the task was expensive, and Father Pedro was at that time director of the Library of Theology and Philosophy, we asked him only to help us find a student who could take on the task. In a gesture of immense generosity, he offered to do it himself.

The combined work was the basis of the first book that we published with him in the year 2009, with the title "*Filosofia Natural Mutisiana"* (Mutisian Natural Philosophy), the second of a trilogy that we closed with the work "*Academia Mutisiana*" (Mutisian Academy), also with the collaboration of Father Pedro.

But this was not the only thing that we did with him in those 4 years that were unforgettable: in the middle of coming and going, while Father Pedro visited us more in the Instituto than we visited him in the Biblioteca, a new line of interdisciplinary investigation appeared that is a good model of the virtues of the University. Starting with a chance encounter in a transdisciplinary domain such as that we geneticists were researching in the prodigious holdings of the Javeriana Archives, an unsuspected research project began. Father Pedro, in one of his first visits to the Institute of Genetics, took from his briefcase the finished task: it was the inaugural speech read by Mutis in the year 1764 in Santafé, with the title "*Oratio pro philosophia newtoniana contra peripateticos*," written and translated, with which we could begin the writing ot the second mutisian work. At the end of the meeting, with the discretion that was typical of him, he took out a USB memory stick which he always carried, and asked if we could see it in some computer. On opening one of his archives, there appeared an orchid.

That lovely flower, that he himself baptised "*Santanderella amado-rinconiana*," and which he told us he wanted to classify with molecular tools, rather than just based on the phenotype, was the beginning of a line of investigation that has among its products an undergraduate thesis in biology (by student Laura Mazo), three scientific articles and more than 30 records published in Gene Bank, thanks to Sonia Quintanilla, who accompanied us en the last 2 years, dealing with Father Pedro's worries about orchid classification.

Father Pedro, whom we consider an emblematic naturalist of the Compañia de Jesús in our work "*Scientia Xaveriana*" (Javerian Science) — which treats the jesuits and the development of Science in Colombia in the 16th to 20th century —, has become for us the essential axis in botany just as we were for him in molecular genetics.

And it is that Father Pedro Ortiz Valdivieso represents for the history of science a new and brillant link in the chain of historic persons that started precisely with Mutis, and continued in time with his santanderian countryman Eloy Valenzuela, then Francisco Javier Matis, José Jerónimo Triana, Ezequiel Uricoechea, Florentino Vezga, Enrique Pérez Arbeláez, Lorenzo Uribe Uribe and that ends in him, in what has to do with the Company of Jesus.

Author of the collection "*Orchids of Colombia*", published by Colciencias and now with three editions (one of them to be reprinted), editor of one of the tomes in large format of the "*Flora of the Botanical Expedition*" published in Madrid, main contributor

[1] Translated by R. L. Dressler.

of "type" specimens of the Javeriana University and worldwide reference in the field of orchids (I have seen how much Mark Chase, director of the Jodrell Laboratories in Kew Gardens in London, respects him, and of the admiration shown for him by the most renowned botanist in Colombia, Santiago Díaz-Piedrahita), Father Pedro leaves a very deep print in world science. We hope that some member of the community has the energy and the capacity to follow in his footsteps.

For my part, I must thank having had the opportunity to know him in life.

Alberto Gómez Gutiérrez
Institute of Human Genetics, Faculty of Medicine
Pontificial Javeriana University
Bogotá - Colombia

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LANKESTERIANA 12(2): 81—83. 2012.

ORCHIDACEAE ORTIZIANAE

El nombre del Padre Pedro Ortiz Valdivieso, S.J., queda permanentemente consignado al ámbito de la botánica y la orquideología neotropical, a través de los 105 taxones que llevan su autoría y de las especies de la familia Orchidacea que fueron dedicadas a su persona.

The name of Father Pedro Ortiz Valdivieso, S.J. will remain permanently associated with the botany and the orchidology of the Neotropics, through the 105 taxa that bring his autorship and the species of the Orchidaceae dedicated to him.

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Se presenta, ordenado por fechas de publicación, un listado de la publicaciones del Padre Pedro Ortiz Valdivieso, S.J., relativas a la botánica y la orquideología. *We present here, ordered by publication date, a checklist of the publications by Father Pedro Ortiz Valdivieso, S.J., dealing with botany and orchidology*.

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THREE NEW SHOWY BUT ENDANGERED *CYRTOCHILUM* SPECIES (ONCIDIINAE: ORCHIDACEAE) FROM PERU

STIG DALSTRÖM[1,4], GUIDO DEBURGHGRAEVE[2] & SAUL RUÍZ PEREZ[3]

[1] 2304 Ringling Boulevard, unit 119, Sarasota FL 34237, USA
Research Associate, Lankester Botanical Garden, University of Costa Rica, Cartago, Costa Rica
and National Biodiversity Centre, Serbithang, Bhutan
[2] Meersstraat 147, 1770 Liedekerke, Belgium
[3] Allamanda 142, Surco, Lima 33, Peru
[4] Corresponding author: stigdalstrom@juno.com

ABSTRACT. Three new *Cyrtochilum* species from Peru that are endangered by habitat destruction, are here described, illustrated and compared with similar species.

KEY WORDS: *Cyrtochilum*, endangered species, Orchidaceae, Oncidiinae, new species, Peru, taxonomy

The genus *Cyrtochilum* Kunth has gone through quite a taxonomic turmoil during its two centuries long history. The trouble has mainly been caused by difficulties in defining the genus based on floral morphology alone, and to separate it from genera *Odontoglossum* Kunth (considered as *Oncidium* by some) and *Oncidium* Sw. Other species have been placed in various smaller genera, such as *Buesiella* C.Schweinf., *Neodryas* Rchb.f., and *Rusbyella* Rolfe. Thanks to molecular work with DNA sequencing (Williams *et al*. 2001a, 2001b) we now know a lot more about how these plants are related to each other, although the controversy of how to treat them taxonomically probably will remain for some time yet. The three large flowered species described in this paper, however, are most certainly considered as "typical" cyrtochilums by most people, so little controversy should arise from their descriptions.

TAXONOMIC TREATMENT

Cyrtochilum deburghgraeveanum Dalström & S.Ruíz, *sp. nov*.

TYPE: Peru, Amazonas, Jumbilla, Florida, Gualulo, alt. ca 2200 m, collected by S. Ruíz and G. Deburghgraeve, Nov. 2010; *S. Dalström 3498* (holotype, USM). FIG. 1.

Cyrtochilo cordato (Lindl.) Kraenzl. *similis, sed lobulis lateralibus et callo labelli recedit.*

Epiphytic *herb*. *Pseudobulbs* caespitose or slightly creeping on a bracteate rhizome, oblong ovoid, *ca.* 10 × 5 cm, distantly bifoliate (terminal leaf *ca*. 2 cm above lower leaf), surrounded basally by 7-8 distichous sheaths, the uppermost foliaceous. *Leaves* subpetiolate, conduplicate, narrowly elliptic to slightly obovate, narrowly acute to broadly acuminate, 30-45 × 1.5-2.5 cm. *Inflorescence* axillary from the uppermost sheaths, erect, then wiry and flexuous, to *ca.* 160 cm long panicle, with widely spaced 3-5 flowered side branches. *Bracts* appressed, involute and cucullate, 10-15 mm long. *Pedicel* with *ovary*, 20-35 mm long. *Flower* stellate to slightly campanulate, showy; *dorsal sepal* brown with white to yellow edges and apex, spathulate with basal auricles, then laminate, ovate, obtuse to acute, undulate, 13-24 × 11-12 mm; *lateral sepals* similar in color, spathulate with basal auricles, laminate, elongate ovate, obtuse to obliquely acute, 25-30 × 9-10 mm; *petals* similar in color, broadly spathulate, ovate, obtuse to acute, oblique, undulate, 23-24 × 10-13 mm; *lip* white with pale brown front-lobe, rigidly attached to the base of the column, cuneate, trilobate with erect, broadly linear and elongate, obliquely rounded side-lobes, and a narrowly triangular, ligulate, apically slightly convolute, obtuse to acute, recurved front-lobe, 22 × 22 mm; callus white, with three low and flattened, fleshy, longitudinal keels emerging from the base and extending to the front-lobe, then spreading, with the lateral pair ending in erect, laterally flattened angulate keels, and with an additional emerging pair of erect,

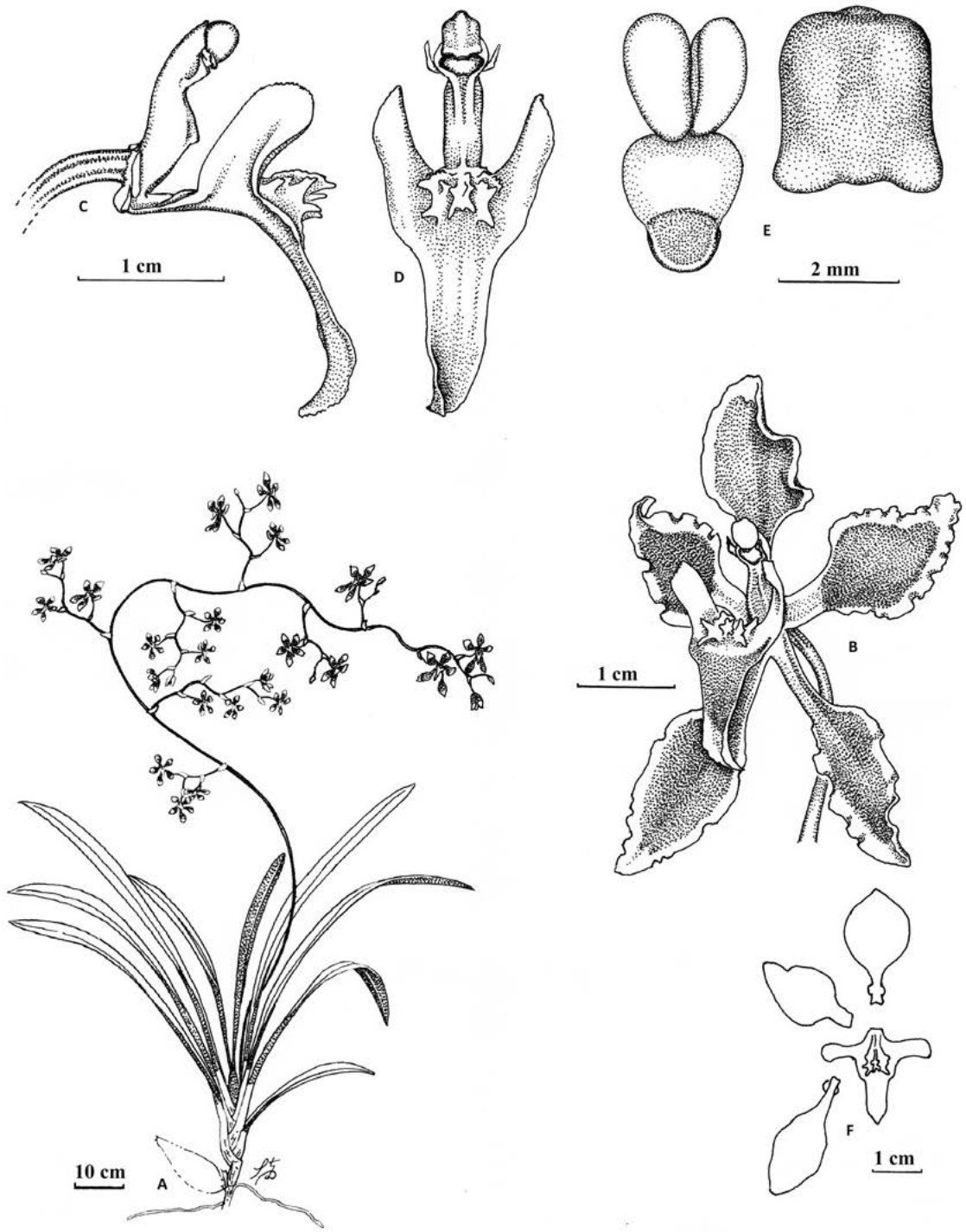

FIGURE. 1. *Cyrtochilum deburghgraeveanum*. A — Plant habit. B — Flower. C — Column-lip lateral view. D —Column-lip frontal view. E — Pollinarium and anthercap. F — Flower dissected. Drawn from the holotype by Stig Dalström.

angular, laterally flattened, denticulate keels that are placed on each side of the rectangularly angulate central keel; *column* basally white with pale yellow on the ventral side, then brown, erect in a ca 90º angle from the base of the lip, slender and clavate, slightly sigmoid, with a slight swelling near the middle on the ventral side, with two parallel longitudinal keels below the stigma, where a pair of lateral, erect and falcate, slightly unequally bilobed to digitate and pointed wings emerge, *ca.* 11 mm long; *anther cap* yellow to purplish brown, campanulate with a pale green dorsal lobule; *pollinarium* of two pyriform, folded pollinia on a broadly obovate, slightly concave, *ca.* 1 mm long stipe, on a pulvinate viscidium.

Eponymy: Named in honor of Guido Deburghgraeve of Liedekerke, Belgium, whose support and contributions to the knowledge of Oncidiinae orchids has been substantial for many years.

Cyrtochilum deburghgraeveanum is similar to *C. cordatum* but differs in the rounded and white side-lobes of the lip versus pointed and brown to purple ones, bordered with white for *C. cordatum*. The callus of *C. cordatum* is also more elaborate with additional lateral teeth next to the main central structure.

Cyrtochilum deburghgraeveanum is only known from the heavily deforested montane area of northern Peru, where its existence is severely threatened by senseless destruction of the natural habitats and the depletion of the country's rich biodiversity.

Cyrtochilum ruizii Dalström & Deburghgraeve, *sp. nov*.

TYPE: Peru, Cajamarca, Incahuási, alt. ca 2700 m, S 06º 26,700'; W 079º 01,177', collected by Saul Ruiz, May 23, 2011, *S. Dalström 3495* (holotype, USM). Fig. 2.

Cyrtochilo cordato (Lindl.) Kraenzl. *et Cyrtochilo deburghgraeveano* Dalström & S.Ruíz *similis, sed colore petalibus, lobulis lateralibus et callo labelli differt.*

Epiphytic or terrestrial *herb*. *Pseudobulbs* caespitose, ovoid, unifoliate or bifoliate, surrounded basally by distichous, foliaceous sheaths. *Leaves* subpetiolate, conduplicate, obovate, obtuse to acuminate (no vegetative parts were included in the type specimen, diagnosis based on observations only). *Inflorescence* axillary, from the base of the uppermost sheaths, erect, then wiry and flexuous, to more than 1 meter long panicle (tip broken off on type specimen, but much longer inflorescences have been observed), with widely spaced few-flowered side branches. *Bracts* appressed, involute, cucullate, 10-12 mm long. *Pedicel* with *ovary* 20-30 mm long. *Flowers* stellate to slightly campanulate, sometimes irregular but showy; *dorsal sepal* brown with whitish edges and apex, spathulate with basal auricles, then laminate, broadly ovate, obtuse, widely undulate, 22-28 × *ca.* 15 mm; *lateral sepals* similar in color, spathulate with basal auricles, then laminate, broadly ovate, obtuse, widely undulate and slightly oblique, 27-28 × *ca.* 13 mm; *petals* basally whitish to pale pink, then with brown irregular mottling and a white apical third, shortly and broadly spathulate, then laminate, broadly ovate, obtuse, widely undulate, 21-23 × *ca.* 15 mm; *lip* yellowish brown with yellow around the callus, rigidly attached to the base of the column, cuneate, then hastate to cordate, trilobate with erect, obovate, weakly serrate and oblique side-lobes, and a narrowly triangular, ligulate, apically slightly convolute and recurved, acute to slightly acuminate front-lobe, *ca.* 22 × 22 mm; callus yellow, of three low, longitudinal keels, emerging from the base and extending to near the frontlobe, spreading, the lateral pair ends in raised, blunt, angulate keels, while the central keel continues with two new emerging, lateral, spreading keels on each side of the angular, nose-like apex; *column* basally pale yellow then brownish with brown wings with yellow edge, erect in an almost 90º angle from the basal part of the lip, slender and clavate, slightly sigmoid, with a slight swelling and two parallel short keels at the middle of the ventral side below the stigma, and with a pair of falcate, digitate wings on each side of the stigma, *ca.* 13 mm long; *anther cap* reddish brown, campanulate, with a pale green dorsal lobule; *pollinarium* of two pyriform, folded pollinia on a broadly rotund and slightly concave, *ca.* 1.5 mm long stipe, on a ovate, pulvinate viscidium.

Additional specimens seen: Ecuador, Loja, "Rio Zenen", color transparency by R. Thompson (Dalström photo archives).

Eponymy: Named in honor of Saul Ruíz Perez, a resident of Lima who collected the type plant and

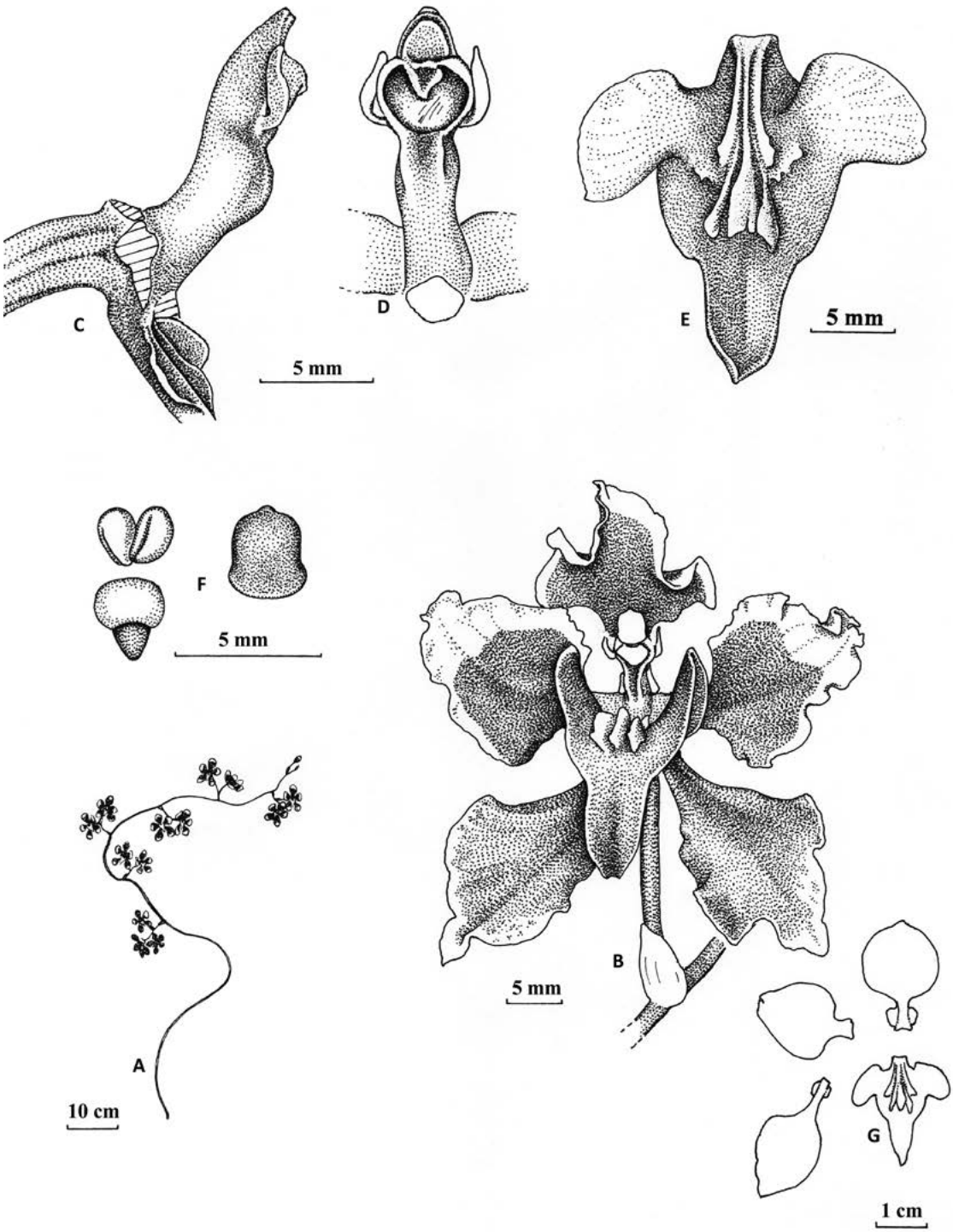

FIGURE 2. *Cyrtochilum ruizii*. A — Inflorescence. B. Flower. C — Column lateral view. D — Column frontal view. E —Lip flattened. F — Pollinarium and anther cap. G — Flower dissected. Drawn from holotype by Stig Dalström.

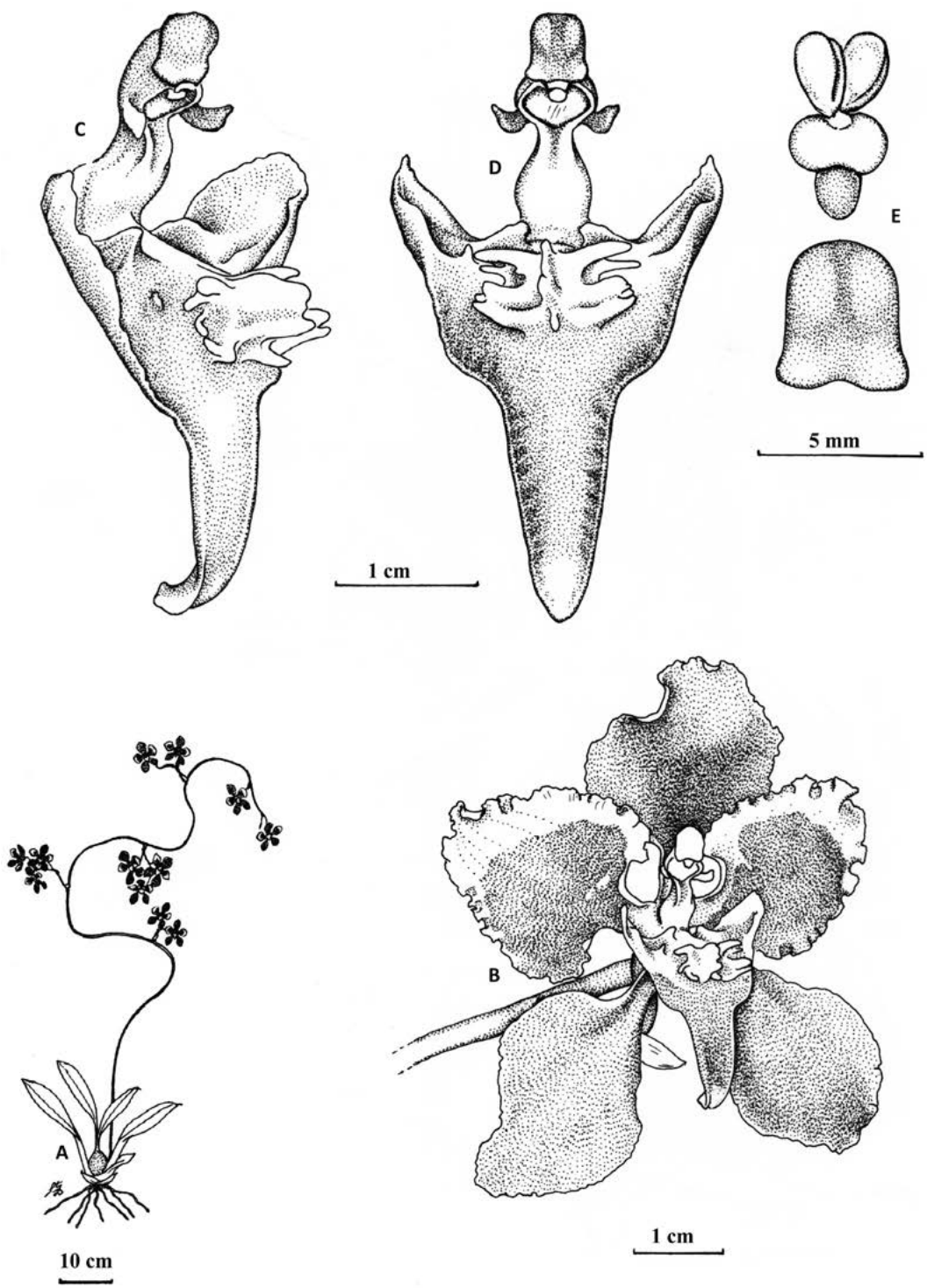

FIGURE 3. *Cyrtochilum xanthocinctum*. A — Plant habit. B — Flower. C — Column-lip lateral view. D — Column-lip frontal view. E — Pollinarium and anther cap. Drawn from holotype by Stig Dalström.

who tirelessly continues to contribute knowledge of Peruvian orchids.

Cyrtochilum ruizii differs from both *C. deburghgraeveanum* and *C. cordatum* by the large and widely rounded, slightly spreading, unicolored brown lateral lobes of the lip, and by the attractive white petals, covered basally by a large brown blotch.

This apparently very rare species was previously known only from a color transparency, photographed some twenty-five years ago in a rather heavily deforested area north of Loja, Ecuador. No plant material ever surfaced. The second observation, and first known collection, constitutes the type of this species, which was found in a similarly deforested montane area in northern Peru. Only fractions of the rich and lush cloud forest that once covered the Peruvian Andes remain today, particularly in the northern regions. These remnants will, no doubt, be altogether gone within a few years. Fortunately, a few individual plants have been secured and artificial propagation will soon ensure the survival of this highly endangered and attractive orchid.

Cyrtochilum ruizii is similar to *Cyrtochilum cordatum* (Lindl.) Kraenzl., and *Cyrtochilum deburghgraeveanum* but is most easily distinguished by the brightly patterned petals, and the solid brown lip with a yellow callus.

Cyrtochilum xanthocinctum Dalström & S.Ruíz, *sp. nov*.

TYPE: Peru, Amazonas, Chachapoyas, Molinopampa, alt. ca 2400 m, collected by S. Ruíz in March 2011, *S. Dalström 3450* (holotype, USM). Fig. 3.

Cyrtochilo macrantho Lindl. *similis, sed colore petalibus, lobulis lateralibus et callo labelli differt*.

Epiphytic *herb*. *Pseudobulbs* caespitose, broadly ovoid, ca 7.5 × 4.5 cm, bifoliate, surrounded basally by 4 to 6 distichous, sheaths, the uppermost foliaceous. *Leaves* subpetiolate, conduplicate, obovate, acute, 13-17 × *ca.* 3.5 cm. *Inflorescence* axillary, from the uppermost sheaths, erect then wiry, to ca 150 cm long. *Bracts* large and conspicous, involute cucullate, *ca.* 10-15 mm long. *Pedicel* with *ovary ca.* 5 cm long. *Flower* stellate and showy; *dorsal sepal* brown, spathulate with basal auricles, then cordate laminate, broadly ovate, obtuse and apiculate, widely undulate, *ca.* 28 × 23 mm; *lateral sepals* similar in color, spathulate with basal auricles, then cordate laminate, ovate, rounded obtuse, widely undulate, 35 x 20 mm; *petals* basally white, then with a large brown blotch, bordered with yellow, broadly and shortly spathulate, then cordate laminate, ovate, rounded obtuse, densely undulate, 27 × 22 mm; *lip* basally pale lilac, then white, and then brown with a lighter apex, rigidly attached to the base of the column, cordate, trilobate with a pair of basal, erect and slightly incurved denticles, and widely spreading semi-erect and slightly convcave, angular side-lobes, with or without additional erect minor denticles, and a narrowly triangulate, ligulate, slightly recurved, acute frontlobe, 23 × 20 mm; callus whitish, of a fleshy central, longitudinal, low keel turning into an erect structure near the base of the front-lobe, similar to some strange aircraft with spreading double pairs of wings; *column* basally yellow then brown, stout, erect in a ca 90º angle from the base of the lip, slightly sigmoid, with a pair of parallel longitudinal ventral keels below the stigma, and with lateral, oblique obdeltoid, wine-red spreading wings, *ca.* 12 mm long; *anther cap* brown, campanulate, with a slight, purple dorsal lobule; *pollinarium* of two pyriform/globose cleft (or folded) pollinia on a broadly rotund, slightly concave *ca.* 1.5 mm long stipe on an oval, pulvinate viscidium.

Additional specimens seen: Only a color transparency of a cultivated plant has been seen of this elusive and attractive species. The plant apparently died shortly after the photo was taken.

Etymology: In reference to the attractive yellow borders of the petals.

Cyrtochilum xanthocinctum is only known from the heavily deforested area near the town of Molinopampa in northern Peru. Only small patches remain of the forest and the fate of this beautiful orchid is gloomy to say the least. Two plants were rescued from a scrubby patch of previously burnt vegetation and hopefully artificial propagation will ensure this spectacular species survival.

Although morphologically similar to the sympatric *Cyrtochilum macranthum* (Lindl.) Kraenzl., *Cyrtochilum xanthoxinctum* is easily recognized by

the brown petals with strikingly contrasting yellow borders. Also the lateral callus keels on the lip are more laterally spreading in our new species.

Acknowledgments. The authors thank the staff at the Instituto Recursos Naturales (INRENA), and Betty Millán at the Universidad de San Marcos, Museo de Historia Natural, Lima, for aiding in providing the necessary permits. We thank Wesley Higgins and Franco Pupulin for assistance with the manuscript, Ricardo Fernández for assistance at the USM herbarium, and a gracious thank you to Manolo Arias, his family and staff for generous logistic support. All plant material involved in this article was acquired in accordance with collecting permit 0283-2010-AG-DGFFS-DGEFFS.

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TWO NEW SMALL FLOWERED *CYRTOCHILUM* SPECIES (ORCHIDACEAE: ONCIDIINAE) FROM VENEZUELA

GILBERTO MORILLO[1] & STIG DALSTRÖM[2,3]

[1] Departamento de Botánica, Facultad de Ciencias Forestales y Ambientales, Universidad de Los Andes, Mérida 5101 A, Venezuela

[2] 2304 Ringling Boulevard, unit 119, Sarasota FL 34237, U.S.A.
Research Associate: Lankester Botanical Garden, University of Costa Rica, Cartago, Costa Rica, and National Biodiversity Centre, Serbithang, Bhutan

[3] Corresponding author: stigdalstrom@juno.com

ABSTRACT. During ongoing research on the genus *Cyrtochilum* Kunth in the Venezuelan Andes, two new species have been found, which are described, illustrated and compared with similar species here.

KEY WORDS: *Cyrtochilum*, new species, Oncidiinae, Orchidaceae, taxonomy, Venezuela

The number of new orchid species that are being discovered and scientifically described every year is impressive. Some of these are the result of explorations into previously unknown habitats of isolated regions. Often enough though, new species are found in herbaria where they have been hiding under a misapplied name or simply as an undetermined species, sometimes for a century or more. Two such "new" species are described here.

TAXONOMIC TREATMENT

Cyrtochilum dunstervilleorum G.Morillo & Dalström, *sp. nov.*

TYPE: Venezuela. Táchira, headwaters of Río Quinimari, on felled tree in rainforest clearing on trail to Alto de Tierra Negra, at ca 2500 m, March 1968, *G. C. K. Dusterville 1064* (holotype of flowers in alcohol, SEL; dried specimen unknown but illustration at K). Fig. 1.

Cyrtochilum dunstervilleorum is similar to *C. megalophium* (Lindl.) Kraenzl., but differs in having larger flowers spotted and marked with dark brown, versus uniformly light yellow flowers for *C. megalophium*. The lip of *C. dunstervilleorum* is rather uniformly cordate versus a distinctly trilobate lip for *C. megalophium*, which also has a much simpler callus.

Epiphytic *herb*. *Pseudobulbs* more or less caespitose to repent on a bracteate woody rhizome, ovoid and slightly ancipitous, bifoliate, to 8 × 4 cm, surrounded basally by up to 8 distichous, foliaceous sheaths. *Leaves* subpetiolate, conduplicate, fairly rigid with a midvein that is distinctly sulcate above and carinate below, elliptic to obovate, narrowly attenuate obtuse, to 24 × 4 cm. *Inflorescence* axillary from the uppermost sheath, erect, to at least 60 cm long panicle with strongly fractiflex side-branches and 3- to 4-flowered fractiflex branchlets. *Bracts* appressed and scale like (dimensions unknown). *Pedicel* with *ovary* 16-22 mm long. *Flower* slightly campanulate; *dorsal sepal* light brown, heavily overlaid with dark brown markings, unguiculate, basally sulcate, lamina broadly elliptic, obtuse, slightly apiculate, 9-11 × 4 mm; *lateral sepals* similar in color, spathulate, basally slightly sulcate, lamina elliptic and slightly oblique, obtuse to acute, 12-13 × 4 mm; *petals* similar in color, slightly spathulate, lamina weakly oblique, obtuse to acute, 9.0-10.0 × 3.5 mm; *lip* bright yellow with some dark spots basally, rigidly attached to the base and gradually curved away from the column, cordate to truncate, laminate, obtuse, slightly undulate and microscopically papillate, with a weakly involute apex, 8.0-9.0 × 5.5 mm; callus bright yellow, of two erect, fleshy, parallel longitudinal keels emerging at the base and abruptly truncate near the middle of the lamina, with multiple, spreading variably sized denticles in front; *column* light brown with large dark brown marks on the basal part, then velvety

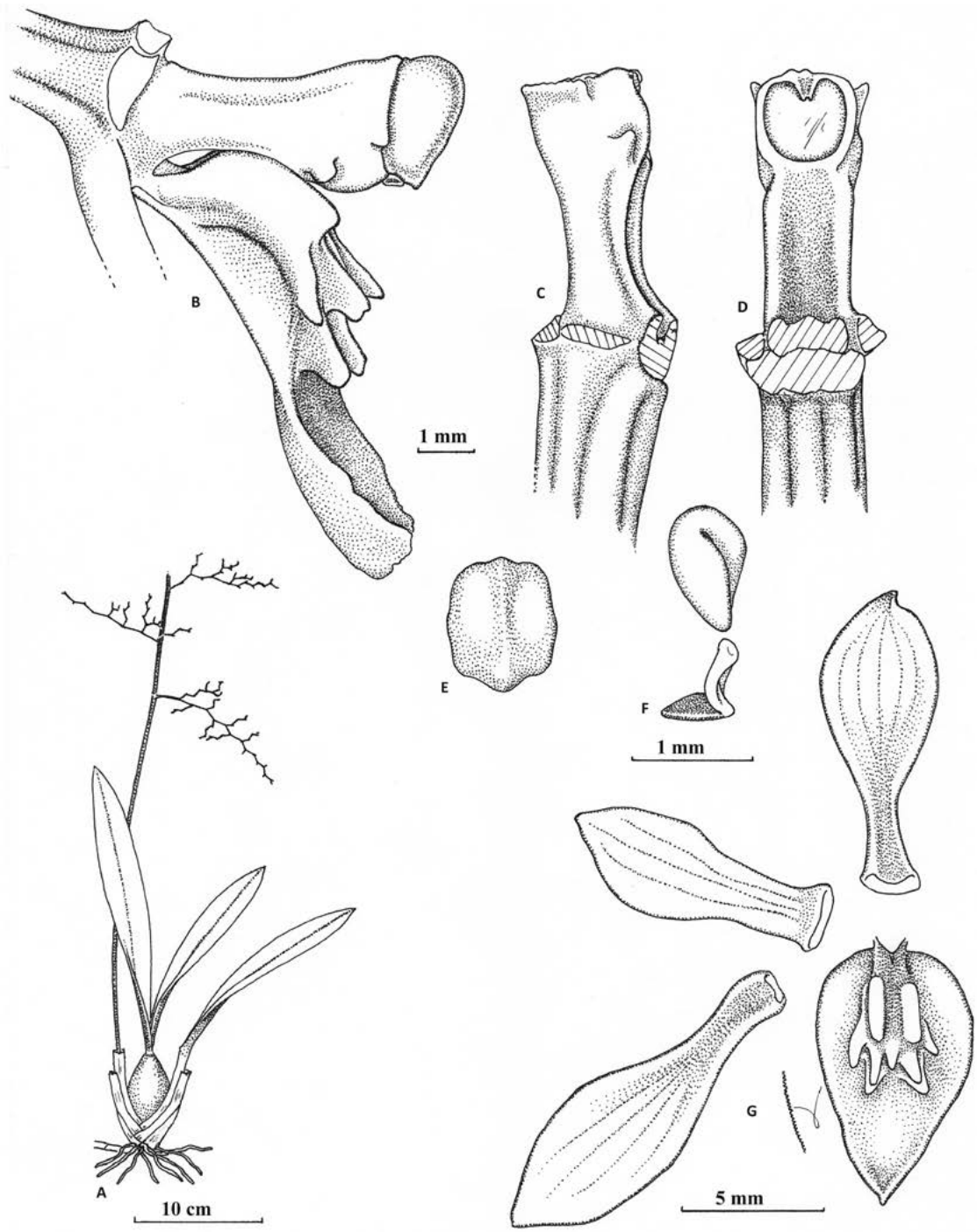

FIGURE 1. *Cyrtochilum dunstervilleorum* A — Plant habit based on Dunsterville's drawing at Kew. B — Column-lip, lateral view. C — Column lateral view. D — Column ventral view. E — Anther cap, dorsal view. F. Pollinarium with one pollinium. G — Dissected flower. Based on *G. C. K. Dunsterville 1064* (SEL). Flower details drawn from holotype by Stig Dalström.

dark brown, straight, clavate, ventrally canaliculate (or sulcate), with a small, blunt, thumb-like wing on each side of the stigma, apically truncate with a pair of lateral, short and projecting angles, *ca.* 6 mm long; *anther ca*p brown with maroon marks, campanulate and weakly rostrate; *pollinarium* of two pyriform, folded pollinia on a laminate, *ca.* 0.5 mm long stipe, on an ovoid, pulvinate, ca 0.5 mm long viscidium.

Additional specimens seen: A plant of unknown origin on a color transparency (*G. Escobar 147*, Dalström photo archives) presumably from Colombia. Collections have also been made by C. Fernández et al., near Andrés Bello in Táchira at 2500 m, but the whereabouts of any preserved specimens is unknown (color photo in "Orquídeas nativas del Táchira, by César Fernández; *C. Fernández 450*).

Etymology: Named in honor of Ellinor and Galfrid Clement Keyworth 'Stalky' Dunsterville, for their immense contribution to the knowledge of Venezuelan orchids.

Cyrtochilum dunstervilleorum is only known from the upper elevation Andean region in western Venezuela, and most likely eastern Colombia (*G. Escobar 147*).

Cyrtochilum fernandezii G.Morillo & Dalström, *sp. nov.*

TYPE: Venezuela. Táchira: El Portachuelo, 3080 m, N08 10,817, W071 54,298. Jan.-Feb. 2010, *C. Fernández s.n.* (holotype, MER). Fig. 2.

Cyrtochilum fernandezii appears most closely related to *C. ramosissimum* (Lindl.) Dalström and *C. densiflorum* (Lindl.) Kraenzl. It differs from the former by the smaller flowers with forward projecting petals and a finely pubescent apical lip callus, and from the latter species by slightly larger and differently colored flowers with a finely pubescent and more elaborate callus on the lip. *Cyrtochilum fernandezii* is also morphologically similar to *C. leucopterum* (Rchb.f.) Dalström, but differs in having a lip callus that is glabrous near the base and finely pubescent apically, and through a club-shaped column without distinct lateral lobes or ridges, which makes it narrower at the base than at the apex, versus a callus that is pubescent throughout and a column that has broad lateral-ventral column lobes for *C. leucopterum*.

Epiphytic *herb*. *Pseudobulbs* caespitose to slightly creeping on a bracteate rhizome, ovoid, ca 7 × 3 cm, surrounded basally by several distichous, foliaceous sheaths, unifoliate or bifoliate. *Leaves* subpetiolate, conduplicate, elliptic, obtuse, apiculate, ca 28.0-38.0 × 2.0-2.5 cm. *Inflorescence* axillary from the uppermost sheaths, erect to arching, weakly flexuous multiflowered panicle (cut off on type), with spreading almost straight to flexuous 3-7 flowered side branches. *Bracts* appressed, scale-like 5-15 mm long. *Pedicel* with *ovary ca.* 10-15 mm long. *Flower* pale yellowish to whitish with purple to brown markings basally on all segments; *dorsal sepal* spathulate, lamina elliptic, obtuse, apiculate, ca 10 × 4 mm; *lateral sepals* elongate sub-spathulate, lamina obovate, rounded obtuse and broadly apiculate, *ca*.18.0-20.0 × 3.5-4.0 mm; *petals* broadly truncate, linear to slightly obovate and slightly oblique, obtuse, forward projecting, *ca*. 8.0 × 3.5 mm; *lip* fused rigidly to the base of the column, then forward projecting basally and parallel with the column, then abruptly recurved near the middle, cordate, weakly trilobate with erect and concave lateral lobes and a broadly obtuse, apically canaliculate front lobe, *ca.* 8-9 × 4-5 mm; callus of two fleshy longitudinal, glabrous, erect, rounded keels, ending in a digitate denticle, with a finely pubescent fleshy intermediate ridge, ending in multiple and variable, finely pubescent spreading denticles; *column* straight, stout and clavate, sulcate ventrally with a low median ridge, truncate, with a minute lateral denticle on each side of the stigmatic surface, ca 4 mm long; *anther cap* globular; *pollinarium* of two folded pyriform pollinia on a *ca.* 0.5 mm long linear stipe, on an equally long, ovoid, pulvinate viscidium.

Additional specimens seen: Venezuela. Merida, Km. 52 from La Victoria to La Grita, just below the top of Páramo la Negra. On large *Podocarpus* tree by roadside, in a fairly isolated position, elevation close to 3300 m (10,000 ft), June 1962, *G. C. K. Dunsterville 706* (dried specimen unknown, illustration and flowers at K).

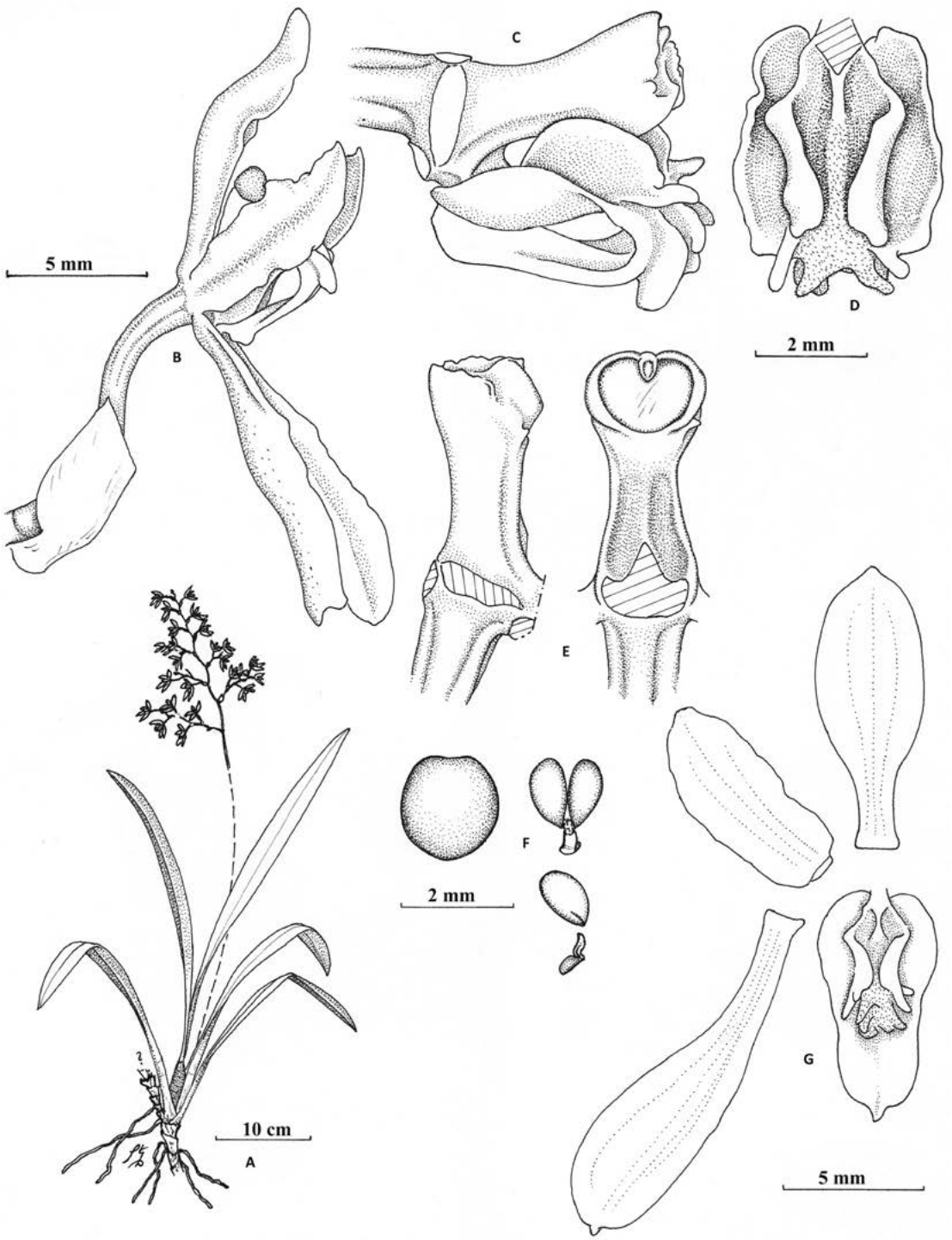

FIGURE 2. *Cyrtochilum fernandezii* A — Plant habit. B — Flower, lateral view. C — Column and lip, lateral view. D — Lip dorsal view. E — Column lateral and ventral views. F — Anther cap and pollinarium. G — Dissected flower. Based on holotype, *Fernández s.n.* (MER). Drawn by Stig Dalström.

Etymology: Named in honor of César Fernández, who has contributed substantially to the knowledge of the orchid flora in his home state of Táchira, Venezuela.

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THREE NEW *LEPANTHES* (ORCHIDACEAE: PLEUROTHALLIDINAE) FROM SOUTH-EAST COSTA RICA

DIEGO BOGARÍN[1,2,5], ADAM P. KARREMANS[1,3] & FRANCO PUPULIN[1,4]

[1] Jardín Botánico Lankester, Universidad de Costa Rica, P. O. Box 302-7050 Cartago, Costa Rica.

[2] Herbario UCH, Universidad Autónoma de Chiriquí, P.O. Box 0427, Chiriquí, Panamá.

[3] NCB Naturalis - NHN Universiteit Leiden, The Netherlands.

[4] Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts, U.S.A.; Marie Selby Botanical gardens, Sarasota, FL, U.S.A.

[5] Corresponding author: diego.bogarin@ucr.ac.cr

ABSTRACT. Three new species of *Lepanthes* from south-east Costa Rica are described and illustrated. *Lepanthes erubescens* resembles *L. limbellata*, from which it can be distinguished by the pendent plants, with inflorescences that borne above the leaf, the upper petal lobe ovate, acute, 1,3 mm wide, larger than the lower lobe, and the ovate lip. *Lepanthes sandiorum* resembles *L. dolabriformis*, however it can be distinguished by the smaller plants, the smaller flowers, the narrower petals, which are filiform, with the upper lobe oblong, rounded, entire and glabrous. *Lepanthes sanjuanensis* is similar to *L. elegans,* but it is distinguished by the green-purple, un-reticulated leaves, the petals with the upper lobe oblong, rounded, with the margins red, the lower lobe oblong, rounded, divergent at apex, and the pinkish or orange-red lip. Additional comments on the species are provided.

KEY WORDS: Orchidaceae, Pleurothallidinae, *Lepanthes erubescens, Lepanthes sandiorum, Lepanthes sanjuanensis,* new species, taxonomy

Lepanthes comprises about 120 species in Costa Rica. The taxonomic history of the genus in the counry began with the arrival of the explorers A. Oersted and H. Wendland in the second half of the nineteenth century. They collected *Lepanthes* between 1855 and 1857, mostly at El Desengaño and Cartago, along the mountains of the Valle Central. H.G Reichenbach *f.* described 8 new species from among their collections (Reichenbach 1866). From that same year and up to 1874, botanist Auguste R. Endrés thoroughly explored Costa Rica, documenting almost half of the known species of *Lepanthes* now reported from the country. Endrés gathered more than 250 specimens, corresponding to 63 species and prepared 201 illustrations (Pupulin *et al.* 2011). Unfortunately his work was never published and at least 55 species of *Lepanthes* remained undescribed until Luer (1995) studied his material kept at W.

Between 1912 and 1923, R. Schlechter published 18 species based on material collected in Costa Rica. The material was gathered mostly by G. Acosta, the brothers A. and C. Brade, A. Brenes, H. Pittier, A. Tonduz and C. Wercklé (Schlechter 1923). Contemporarily, O. Ames, who received material from C. Lankester, also added about 22 species to the genus (Ames 1923). After the pasing of O. Ames and R. Schlechter, the description of new orchid species in Costa Rica declined considerably. No *Lepanthes* species were described during the next 53 years.

Luer (1983) began to describe new *Lepanthes* species based on his own field work activities since 1981, but mainly from studying Endrés' material that remained forgotten for over a century. Indeed, even with the contributions of Reichenbach, Schlechter and Ames, the diversity of *Lepanthes* was far from being understood. Luer (1996) added 70 species (almost doubling the number of species known to date) to the flora of Costa Rica. About 21 of them were based on the collections by Endrés (Luer 1995).

After Luer (1995, 1996), and with the turn of the century, research at Lankester Botanical Garden (JBL) has revealed some 22 new species of *Lepanthes*. Those studies mostly covered areas not visited by the traditional collectors mentioned previously (Pupulin

et al. 2010). The area that comprises the Southern Pacific watershed of the Talamanca mountain range, from the foothills of Cerro de la Muerte and Chirripó, neighboring the Valle de El General, Fila Costeña and up to the border with Panama, in the Costa Rican south-east, is very rich in *Lepanthes* species.

Here we propose three new species from this region of Costa Rica:

Lepanthes erubescens Bogarín, Pupulin & Karremans, *sp. nov.*

Type: Costa Rica. San José: Pérez Zeledón, Santa Elena de El General, Quebrada Los Granados, 710 m, collected by Vicente Juárez-Pérez, 17 March 2002, flowered in cultivation at Jardín Botánico Lankester, 29 June 2002, *F. Pupulin 4027* (holotype, JBL). Fig. 1, 4A.

A Lepanthes limbellata Endrés ex Luer similis, sed planta pendula, inflorescentiis folii portatis adaxialibus, lobo superno petalorum ovato acuto latiore lobum inferum superantibus et lobulis labelli ovatis.

Plant epiphytic, caespitose, pendent, up to 15 cm tall. *Roots* slender, flexuous, to 0.5 mm in diameter. *Ramicauls* slender, pendent, 4.5-12 cm long, enclosed by 8 minutely ciliate, lepanthiform blackish sheaths, the ostia minutely ciliate, ovate, acute, adpressed. *Leaves* coriaceous, elliptic to ovate or suborbicular, acute to subacuminate, emarginate, with a short apiculus, purplish-green, 2.5–4.0 × 1.5–2.6 cm, the rounded base narrowing into a petiole less than 3 mm long. *Inflorescence* racemose, distichous, glabrous, successively flowered, born above the leaf, shorter than the leaves, up to 2.7 cm long, peduncle 1.9 cm long, rachis 0.8 cm long. *Floral bracts* ovate, acuminate, conduplicate, membranaceous, 1 mm long, muriculate. *Pedicels* 2 mm long, persistent. *Ovary* to 2 mm long, glabrous. *Flowers* with yellowish-orange sepals, the petals scarlet red, the upper lobe with a yellow stain, the lip reddish-pink, the column scarlet. *Dorsal sepal* ovate, acute, entire, dorsally with three keels, connate to the lateral sepals for about 0.6 mm, 3.6 × 2.2 mm. *Lateral sepals* narrowly ovate to elliptic, acute, entire, dorsally with three ciliate keels, connate for 0.8 mm, 3.6 × 1.8 mm. *Petals* transversely bilobed, entire, 5.0 × 1.3 mm, the upper lobe ovate, acute, the lower lobe ovate, narrowly triangular, narrower and shorter than the upper lobe. *Lip* bilobate, adnate to the column, the blades ovate with rounded ends, embracing the column 1.3 × 1.7 cm, the connectives terete, oblong, to 0.3 mm long, the body thick, oblong, rounded, connate to the base of the column, the appendix thick, oblong, pubescent, cylindric. *Column* cylindric, to 2 mm long, mucronate, the anther apical and the stigma ventral. *Pollinia* two, ovoid. *Anther cap*, triangular, cucullate.

Paratypes: Costa Rica. Puntarenas: Buenos Aires, Volcán, 09°13'N, 83°26'W, ca. 450 m, bosque muy húmedo premontano transición a basal en bosque secundario muy alterado a orillas de un riachuelo, 17 abril 2012, *A.P. Karremans 5312, J. Cambronero & J. Gemmel* (JBL-Spirit!) Same locality and date, *A.P. Karremans 5314* (JBL-Spirit!). San José: Pérez Zeledón, El Alto de San Juan, ca. 1300 m, collected by Jeremy Quesada Gonzalez, flowered in cultivation in the collection of Daniel Jiménez at Paraíso de Cartago, 12 Julio 2012, *J. Quesada s.n.* (JBL-Spirit!).

Distribution: known from south-east Costa Rica, on the Pacific watershed of the Talamanca mountain range.

Habitat and ecology: epiphytic in secondary forest in premontane wet forest, basal belt transition, premontane wet forest and premontane moist forest, at 450-1150 m of elevation.

Eponymy: from the Latin *erubescens* "reddening, blushing" in reference to the scarlet red color of the petals.

Lepanthes erubescens resembles *L. limbellata* Endrés ex Luer. However, it can be distinguished mainly by the the pendent plants (vs. erect), with inflorescences that is borne above the leaf (vs. behind), the upper lobe ovate, acute, 1.3 mm wide, larger than the lower lobe (vs. narrowly ovate-triangular, narrowly obtuse, 0.6 mm wide, equal in length to the lower lobe) and the ovate lip blades (vs. narrowly oblong).

Lepanthes sandiorum Bogarín & Karremans, *sp. nov.*

Type: Costa Rica. Puntarenas: Coto Brus, Sabalito,

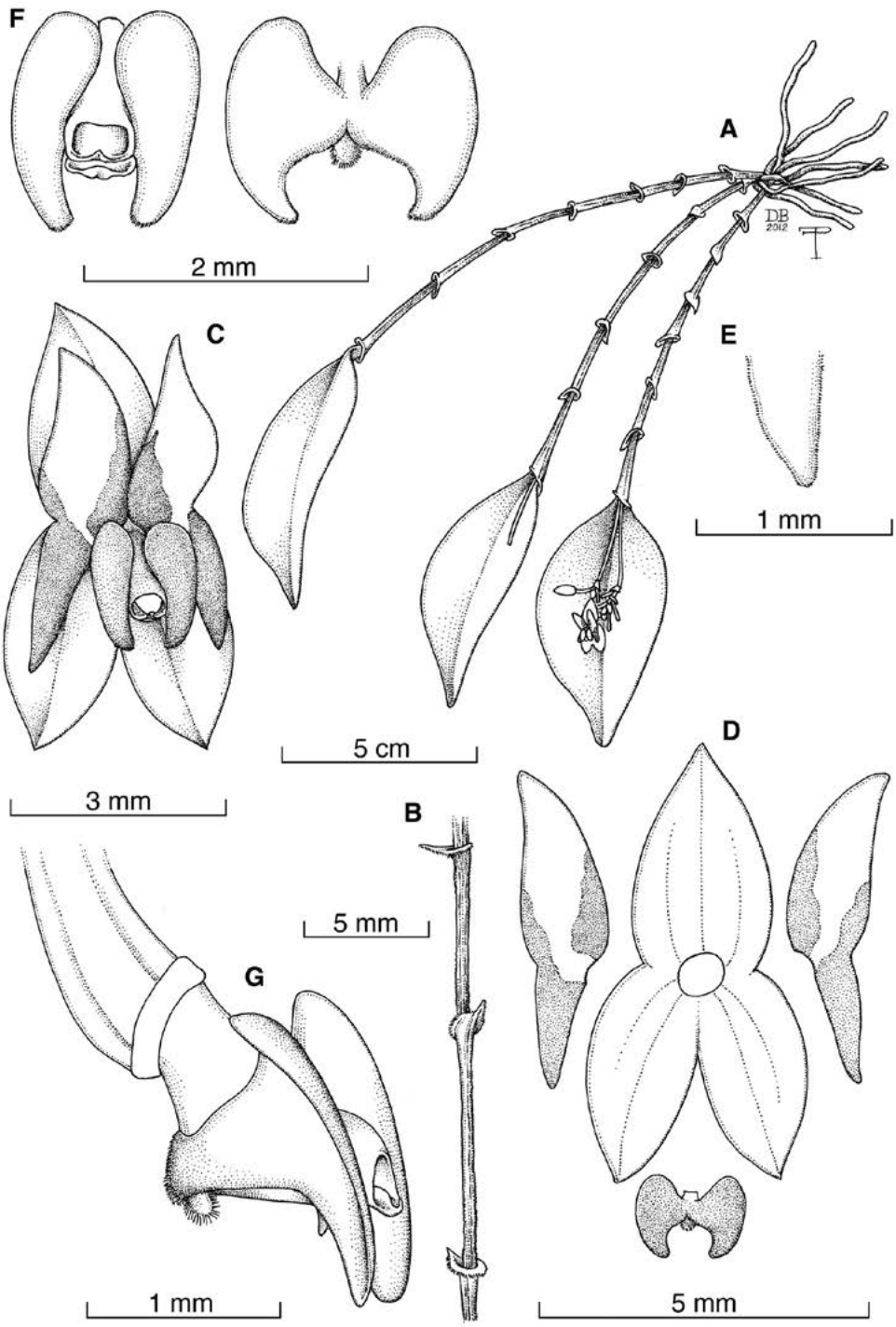

Figure 1. *Lepanthes erubescens* Bogarín, Pupulin & Karremans. A — Habit. B — Ramicaul. C — Flower. D — Perianth FLATTEN. E — Apex of petal. F — Lip, front view with the column and spread without the column. G— Lip and column, lateral view. Drawn by D. Bogarín and F. Pupulin from the holotype.

Zona Protectora Las Tablas, 13 km al noreste de Lucha, Sitio Coto Brus, entre Río Surá y Quebrada Sutú, Finca de Miguel Sandí, 8°56'46.1" N 82°44'30.9" W, 1778 m, bosque pluvial montano bajo, epífitas en potreros arbolados, 6 junio 2010, *D. Bogarín 7773 & A.P. Karremans* (holotype, JBL-Spirit!). Fig. 2, 4B.

Species Lepanthi dolabriformi Luer *similis, distincta habito perparvo, floribus in diametro brevioribus, petalis angustis filiformis et lobo superno petalorum oblongo, rotundato, glabro.*

Plant epiphytic, caespitose, suberect, up to 5.5 cm tall. *Roots* slender, flexuous, to 0.5 mm in diameter. *Ramicauls* slender, erect, 2.3–3.5 cm long, enclosed by 7 glabrous, lepanthiform sheaths, the ostia minutely ciliate, ovate, acute, adpressed. *Leaves* coriaceous, elliptic to ovate or suborbicular, obtuse, emarginate, with a short apiculus, purplish beneath, 1.3–1.7 × 0.6–1.4 cm, the rounded base narrowing into a petiole less than 1 mm long. *Inflorescence* racemose, distichous, glabrous, successively flowered, born above the leaf, exceeding the leaves, up to 3 cm long, peduncle 1.5 cm long, rachis 1.5 cm long. *Floral bracts* ovate, acuminate, conduplicate, membranaceous, 1 mm long, muriculate. Pedicels 2 mm long, persistent. *Ovary* to 1 mm long, glabrous. *Flowers* with orange sepals, the petals scarlet red with the margin yellow, the lip scarlet with the apex yellow, the column red. *Dorsal sepal* ovate, acute, denticulate, slightly reflexed, dorsally with three keels, connate to the lateral sepals for about 0.4 mm, 2.6 × 1.4 mm. *Lateral sepals* narrowly ovate, acute, denticulate, dorsally with three ciliate keels, connate for 0,4 mm, 2.6 × 1.3 mm. *Petals* transversely bilobed, entire, glabrous, 0.4 × 2.3 mm, the upper lobe oblong, filiform, 1.7 mm long, rounded, the lower lobe smaller than the upper lobe, to 0.6 mm long, ovate, obtuse. *Lip* bilobate, adnate to the column, the blades oblong with rounded ciliolate ends, embracing the column 1.5 × 1.2 cm, the connectives terete, oblong, to 0.4 mm long, the body thick, oblong, rounded, connate to the base of the column, the appendix thick, oblong, cylindric. *Column* cylindric, to 2 mm long, mucronate, the anther dorsal and the stigma apical. *Pollinia* two, ovoid, basally filiform. *Anther cap*, oblong, cucullate.

Paratypes: Costa Rica. Puntarenas: Coto Brus, Sabalito, Zona Protectora Las Tablas, 13 km al noreste de Lucha, Sitio Coto Brus, entre Río Surá y Quebrada Sutú, Finca de Miguel Sandí, 8°56'46.1" N 82°44'30.9" W, 1778 m, bosque pluvial montano bajo, epífitas en potreros arbolados, 20 abril 2012, *A.P. Karremans 5350 & J. Gemmel* (JBL-Spirit!). Same locality and date, *A.P. Karremans 5381 & J. Gemmel* (CR!). Coto Brus, Sabalito, Zona Protectora Las Tablas, 13 km al noreste de Lucha, Sitio Coto Brus, entre Río Surá y Quebrada Sutú, Finca de Miguel Sandí, 8°56'46.1" N 82°44'30.9" W, 1778 m, bosque pluvial montano bajo, epífitas en potreros arbolados, 6 junio 2010, *D. Bogarín 7786* (JBL-Spirit!). Coto Brus, Sabalito, Zona Protectora Las Tablas, 13 km NE of Lucha, Sitio Coto Brus, finca Sandí "El Capricho", 8°56'46.1" N 82°44'30.9" W, 1778 m, epiphytic, mostly on *Quercus* sp. in pastures and along the river Sutú, wet premontane forest, 6 October 2010, *F. Pupulin et al. 7929* (JBL -Spirit!). Same locality and date, *F. Pupulin et al. 7930* (JBL -Spirit!). Same locality and date, *F. Pupulin et al. 7934* (JBL -Spirit!).Same locality and date, *F. Pupulin et al. 7951* (JBL -Spirit!).

Distribution: Endemic to Costa Rica, however, most probably found also in Panama, as the large populations were found growing very close to the border. It is known only from the south-east Costa Rica, on the southernmost portion of the Pacific watershed of the Talamanca mountain range.

Habitat and ecology: plants were found growing epiphytically in disturbed lower montane rain forest, at around 1800 m elevation.

Eponymy: dedicated to Miguel Sandí and his family, pioneers in the region of Las Tablas and owners of the farm where this species was found.

Lepanthes sandiorum resembles *L. dolabriformis* Luer, however it can be distinguished mainly by the smaller plants, less than 5.5 cm tall (vs. up to 7.5 cm), the smaller flowers with sepals 2.6 × 1.4 mm (vs. 4.0 × 2.5 mm), the narrower petals, which are filiform, 2.3 mm (vs. 3.5–3.75 mm) with the upper lobe oblong, rounded, entire, glabrous (vs. obtusely angled with a hatchet-shaped appearance, minutely ciliate, pubescent). Both species are related to *L. blepharistes* Rchb.f. but they can be easily distinguished by the petals with the lower lobe smaller than the upper lobe (vs. equal in length).

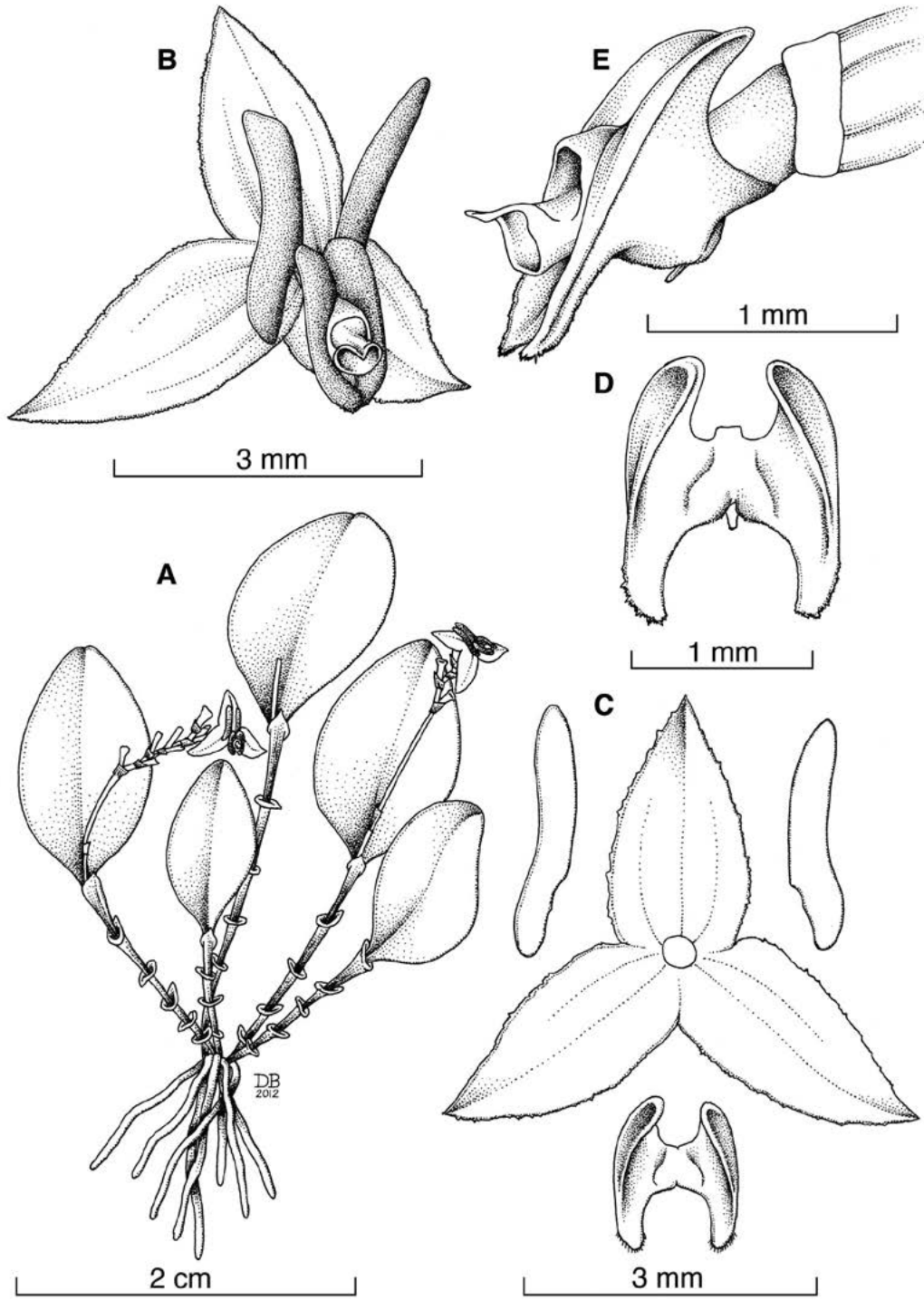

FIGURE 2. *Lepanthes sandiorum* Bogarín & Karremans. A — Habit. B — Flower. C — Perianth flatten. D — Lip, spread. E — Column and lip, lateral view. Drawn by D. Bogarín from the holotype.

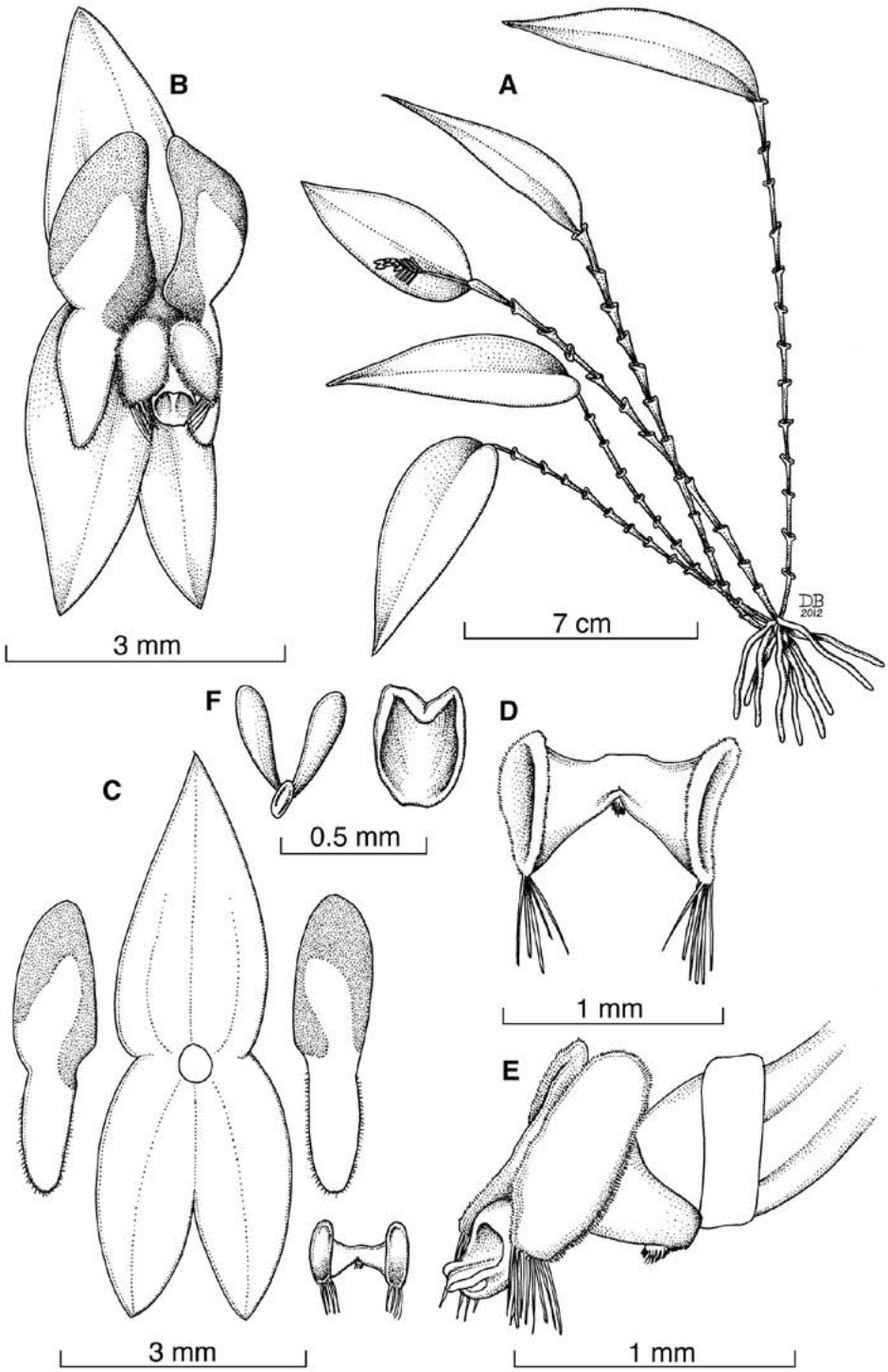

FIGURE 3. *Lepanthes sanjuanensis* Bogarín & Karremans. A — Habit. B — Flower. C — Perianth flatten. D — Lip, spread. E — Column and lip, lateral view. F — Pollinarium and anther cap. Drawn by D. Bogarín from the holotype.

Figure 4. Flowers of the new species of *Lepanthes*. A— *L. erubescens* (*F. Pupulin 4027*). B — *L. sandiorum* (*F. Pupulin 7929*). C — *L. sanjuanensis* (*D. Bogarín 7773*). Photographs by F. Pupulin (A, B) and D. Bogarín (C).

Lepanthes sanjuanensis Bogarín & Karremans, *sp. nov.*

Type: Costa Rica. San José: Pérez Zeledón, San Isidro de El General, carretera a Dominical, desvío hacia el Alto de San Juan, 2 km al noroeste de la antena, 9°20'36.8"N 83°46'07.8"W, 1187 m, bosque húmedo premontano, sobre árboles en bosque secundario y primario, 3 setiembre 2011, *D. Bogarín 9234 & A.P. Karremans* (holotype, JBL!; isotype, CR!). Fig. 3, 4C.

Species habitu cum L. elegans Luer *optime congruens, sed foliis purpureis-viridis non reticulatis, lobulis supernis petalorum oblongis, rotundatis, marginibus petalorum rubris, lobulis inferis oblongis, rotundatis, in apice divergentibus et labello rubro-aurantiaco differt.*

Plant epiphytic, caespitose, pendent or suberect, up to 30 cm tall. *Roots* slender, flexuous, to 1 mm in diameter. *Ramicauls* slender, suberect, 5–19 cm long, enclosed by 7–12 minutely ciliate, blackish, tightly adpressed lepanthiform sheaths, the ostia minutely ciliate, ovate, acute, not dilated. *Leaves* coriaceous, ovate to elliptic, acute to acuminate, emarginate with a short apiculus, conduplicate, adaxially purplish, abaxially purplish-green, not reticulate, 6.0–7.5 × 3.0–3.4 cm, the rounded base narrowing into a petiole less than 3 mm long. *Inflorescence* racemose, distichous, glabrous, successively flowered, born beneath the leaf, shorter than the leaves, up to 4 cm long, peduncle 2–2.5 cm long, rachis 1.0–1.5 cm long. *Floral bracts* ovate, acuminate, conduplicate, membranaceous, 1 mm long, muriculate. *Pedicels* 5 mm long, persistent. *Ovary* to 2 mm long, glabrous. *Flowers* with yellow sepals, stained, the petals yellow stained with red along the margin of the upper lobe, the lip pinkish, the column purple-pink basally, yellow apically. *Dorsal sepal* ovate, acute, entire, dorsally with three keels, connate to the lateral sepals for about 1 mm, 1.9 × 3.7 mm. *Lateral sepals* narrowly ovate to elliptic, acute, entire, dorsally with three ciliate keels, connate for 1 mm, 3.2 × 1.2 mm. *Petals* transversely bilobed, 1.1 × 3.5 mm, the upper lobe oblong, rounded, entire, the lower lobe oblong, rounded, ciliate. *Lip* bilobate, adnate to the column, the blades oblong, ciliolate with rounded ends, the apex of each lobe provided with conspicuous bristles, 0.8 × 1.2 cm, the connectives terete, oblong, to 5 mm long, the body thick, oblong, rounded, connate to the base of the column, the appendix pubescent, inconspicuous. *Column* cylindric, to 1.5 mm long, mucronate, the anther dorsal and the stigma ventral. *Pollinia* two, pyriform. *Anther cap*, oblong, cucullate.

Distribution: only known from south-east Costa Rica, on the Pacific watershed of the Talamanca mountain range.

Habitat and ecology: a population was found epiphytically in premontane moist forest in secondary vegetation, at around 1000-1200 m of elevation.

Etymology: named after the type locality of Alto de San Juan, along the road from San Isidro de El General to Dominical.

Lepanthes sanjuanensis is similar to *L. elegans* Luer. Both are characterized by the relatively large plants up to 30 cm tall, the flowers are small compared to the plant habit, produced below the leaf, and the lip is minute with conspicuous long-ciliate apexes. However, *L. sanjuanensis* is mainly distinguished by the green-purple, not reticulated leaves (vs. reticulated), the petals with the upper lobe oblong, rounded, with the margins red (vs. obliquely ovate, with a red blotch at the middle of the upper lobe), the lower lobe oblong, rounded, divergent at apex (vs. obliquely triangular, acute, converging) and the pinkish or orange-red lip (vs. yellow).

Acknowledgements. We are thankful to the Ministerio del Ambiente, Energía y Telecomunicaciones de Costa Rica (MINAET) and Sistema Nacional de Áreas de Conservación (SINAC) of Costa Rica for issuing the Scientific Passport N. 1862 under which wild specimens treated in this study were collected. To Daniel Jiménez for sharing material and knowledge of the species described. This paper is part of the project "Flora Costaricensis: Taxonomía y filogenia de la subtribu Pleurothallidinae (Orchidaceae) en Costa Rica", 814-BO-052, sponsored by the Vice-Presidency of Research, University of Costa Rica.

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A NEW *TELIPOGON* FROM MEXICO CLOSE TO *TELIPOGON STANDLEYI* (ORCHIDACEAE: ONCIDIINAE)

DIEGO BOGARÍN

Jardín Botánico Lankester, Universidad de Costa Rica. P.O. Box 302-7050 Cartago, Costa Rica, A.C.
Herbario UCH, Universidad Autónoma de Chiriquí, P.O. Box 0427, Chiriquí, Panamá.
diego.bogarin@ucr.ac.cr

ABSTRACT. A new *Telipogon* from Mexico, previously confused with *T. standleyi* from Costa Rica, is described and illustrated. *Telipogon amoanus* is recognized by the pink-purplish flowers, the obliquely ovate, subfalcate sepals, the elliptic, entire petals, the triangular sagittate lip, densely pilose column with short basal hairs, and the pollinarium with the stipe obtrullate. *Telipogon standleyi* is illustrated based on Costa Rican material to facilitate species comparison. Additional comments on both species are provided.

RESUMEN. Se describe e ilustra un nuevo *Telipogon* de México, previamente confundido con *T. standleyi* de Costa Rica. *Telipogon amoanus* se reconoce por las flores de color rosa-púrpura, los sépalos oblicuamente ovados, subfalcados, los pétalos elípticos, enteros, el labelo triangular sagitado, densamente piloso, la columna densamente pilosa, con pelos cortos en la base y el estípite del polinario obtrulado. Para facilitar la comparación entre especies se ilustra *Telipogon standleyi* basado en material de Costa Rica. Se proporcionan comentarios adicionales sobre ambas especies.

KEY WORDS: *Stellilabium, Telipogon amoanus,* new species, taxonomy

Williams *et al.* (2005) evaluated the phylogenetic relationships of *Telipogon* Kunth and related genera by using molecular datasets. Their results showed that *Stellilabium* Schltr. was embedded within *Telipogon,* which consists of one Central American and two South American clades. Traditionally, *Stellilabium* was recognized as having small flowers with a simple column and a tendency to leaflessness, often lacking leaves when the plants are in flower. According to Williams *et al*. (2005) that condition has arisen at least twice, once in South America and once in Central America. *Stellilabium* was treated taxonomically by Braas and Lückel (1982), Garay and Romero-González (1998) and Dressler (1999).

Telipogon ranges from Mexico, through Central America and the Caribbean to Bolivia, but the highest species diversity is found in the Andes. In Central America the majority of species are concentrated in Costa Rica and Panama, with few members from Mexico to Nicaragua. Only two species were recorded in Mexico, both classified under the former *Stellilabium*. Salazar & Hágsater (1991) published the first record as *Telipogon* (=*Stellilabium*) *standleyi* Ames from Guerrero. The second record is *Telipogon helleri* (L.O. Williams) N.H.Williams & Dressler, recently documented by Solano *et al*. (2011) from Chiapas.

Oakes Ames described *T. standleyi* from a plant collected by Paul Standley in 1924 in Costa Rica between Tarbaca and Aserrí, southeast of San José (Ames 1925). In 2008, during a field trip near the type locality of *T. standleyi*, a plant in flower was collected for documentation and cultivated at Lankester Botanical Garden.

In revising the specimen reported by Salazar and Hágsater (1991), I realized that it markedly differs from the Costa Rican specimens. The Mexican species is treated here as a new to science and it is described hereafter:

Telipogon amoanus Bogarín, *sp. nov.*

TYPE: MÉXICO. Guerrero: Camino Chilapa-Hueycatenango km 22. Preparado de material cultivado, 15 de noviembre de 1975, *E. Hágsater 4641* (holotype, AMO). FIG. 1.

Telipogon standleyi aemulans, differt floribus roseis, sepalis oblique ovatis subfalcatis, petalis

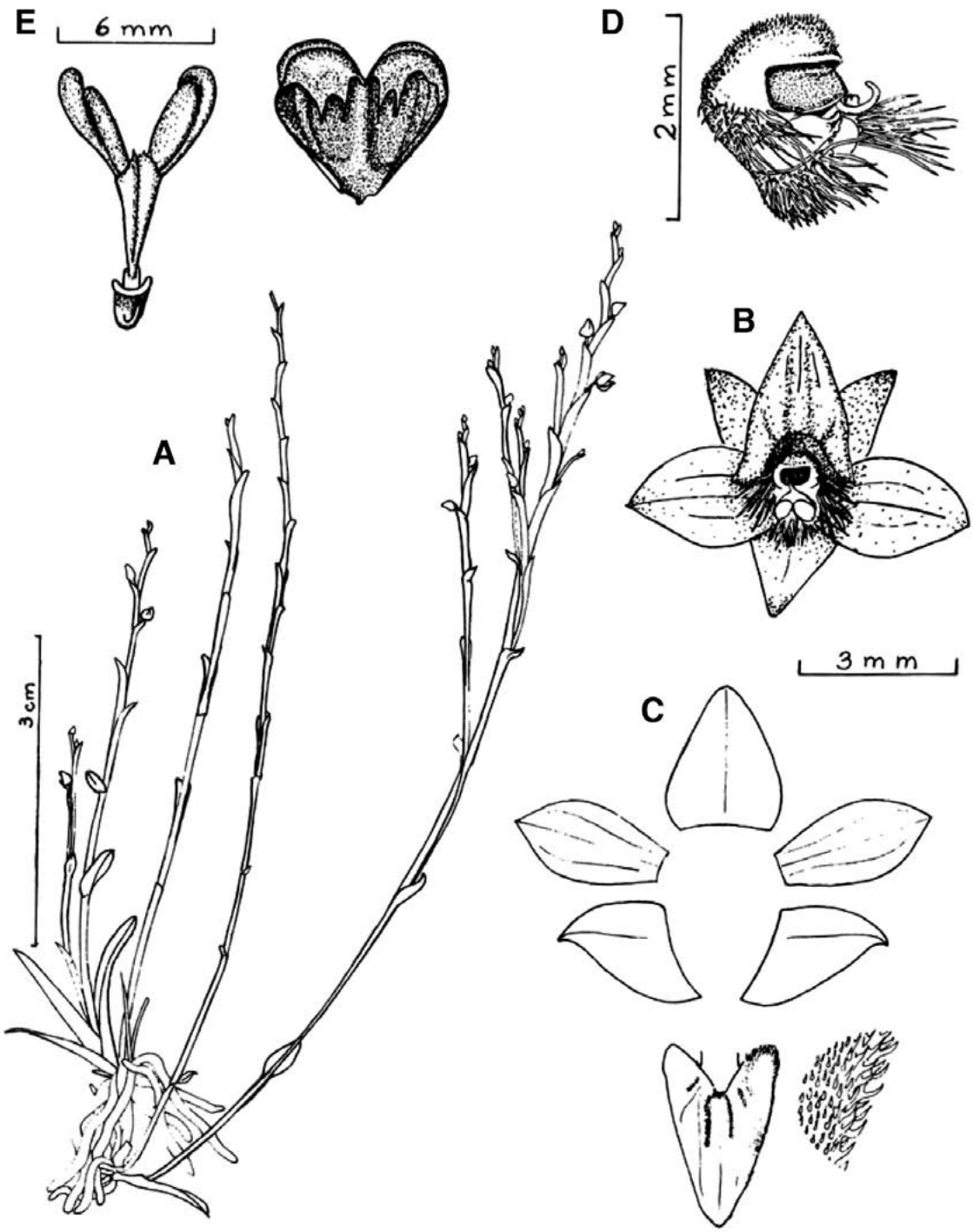

FIGURE 1. *Telipogon amoanus* Bogarín. A — Habit. B — Flower. C — Perianth flatten. D — Column. E — Pollinarium and anther cap. Drawn by E. Hágsater from the holotype.

ellipticis integris, labello triangulato sagittato dense piloso, columna confertim pilosa basaliter pilis brevis vestita, stipite pollinarii obtrullato.

Epiphytic, acaulescent *herb*, *ca*. 9 cm tall including the inflorescences. *Roots* thick, fleshy, slightly flattened, white with green tips, *ca*. 1.5 mm in diameter. *Leaves* deciduous when flowering, erect, distichous, linear-lanceolate, acute, somewhat fleshy (very thin and translucent when dry), 7.5–22.0 × 1.5–2.0 mm. *Inflorescences* several (5 in our material), simultaneous, developed from leaf axils, simple or branched, up to 90 mm long, scape compressed, green, 0.5–1.0 mm thick, with 2-3 spaced and small, triangular-ovate, acute-attenuate, green, translucent bracts, with a thickened vein 2.4–4.5 × 0.8–1.5 mm. *Floral bracts* very small, broadly ovate-deltate, acute to shortly acuminate, green, translucent, with a thickened vein, 1.2–1.5 × 1.0–1.6 mm. *Ovary* slightly curved, terete, 2.0–2.5 mm long and 0.5–0.7 mm thick. *Flowers* small, inconspicuous, 5–7 mm in diameter, sepals and petals rose slightly yellow towards the apex, the lip pink with some yellow apical spine and purple anthers, a few scatter idioblasts containing crystalline material are evident in the floral parts. *Dorsal sepal* ovate, obtuse-rounded, concave, 1-nerved, glabrous, 2.5–3.0 × 1.6–2.0 mm. *Lateral sepals* obliquely ovate, acute, concave, 1-nerved, glabrous, 2.5-3.0 × 1.8–2.1 mm. *Petals* elliptic, obtuse to subacute, slightly concave or flat, 3-nerved, 2.8–3.5 × 1.5–2.1 mm, dense and shortly pilose internally in the basal third, the margins glabrous to conspicuously ciliate on the basal two-thirds, the cilia retrorse. *Lip* sessile, with a shallow basal groove clearly separated from the column, entire, very fleshy-thickened in basal half, triangular-sagitate, acute, the inner surface densely and shortly pilose, the margins ciliate (cilia and hairs retrorse), outer surface glabrous, 3.0–3.5 mm in total length, including the basal auricles, 2.0–2.5 mm wide between the basal auricles; basal auricles retrorse, free, triangular, subacute, 0.5-0.8 mm long. *Column* sessile, very short, lobed, 1.5–1.8 mm in diameter, densely and shortly pilose, with slightly longer hairs on the ventral surface and a bundle of very long, rigid, septate hairs at the apex of each lobe (i.e. one apical and one on each side). *Anther* cordate, apparently bilocular without obvious septa, ca. 0.8 × 1.2 mm. *Pollinia* 4, subclavate, slightly compressed and overlapping more or less dorsiventrally, in two pairs, yellow, united to a short, obtrullate, translucent stipe terminating into a large, hooked viscidium, rostellum laminar, projected on a narrowly triangular extension. *Stigma* suborbicular, slightly concave. *Capsule* not seen.

Habitat: Acccording to Salazar and Hágsater (1991), plants grow epiphytic on branches with lichens, in mixed forest of pine and oak approximately between 1500 and 2000 m elevation. Other orchids growing in the area include: *Hintonella mexicana* Ames, *Oncidium ghiesbreghtianum* A.Rich. & Galeotti, *Epidendrum marmoratum* A. Rich. & Galeotti and *Encyclia atrorubens* (Rolfe) Schltr.

Distribution: Known only from Guerrero, Mexico.

Etymology: it is a pleasure to name this species after the staff and researchers of AMO Herbarium, in recognition of their outstanding contributions to the knowledge of the orchids of Mexico and the Neotropics.

Phenology: plants flower in cultivation in November and February.

Comparison of the material collected in Mexico with living plants of *T. standleyi* from the type locality in Costa Rica lead to reconsider the application of the name *T. standleyi* for the Mexican populations (Salazar & Hágsater 1991, see picture in Hágsater *et al*. 2005, p. 246). Ames (1925) recognized *T. standleyi* as having the glandular hairs of the column much abbreviated and in being a dwarf plant with foliage present at flowering time. The flowers are yellow with a purple-brown tinged center, the base of the petals and labellum are more deeply colored than the upper half, with a deep purple column. All these characters are consistent with the material studied from the type locality and the type specimen kept at AMES (Fig. 2).

Telipogon amoanus can be recognized from *T. standleyi* by the pink-purplish flowers (rather than yellow with a dark purple or crimson center), the sepals obliquely ovate, subfalcate (rather than triangular, acute), the elliptic, entire petals (rather than ovate, ciliate), the lip triangular sagittate, densely pilose (rather than oblong, basally glandular and glabrous towards the apex), the column densely pilose with short hairs basally (rather than with few abbreviated hairs, basally glabrous) and the pollinarium with the stipe obtrullate (rather than filiform).

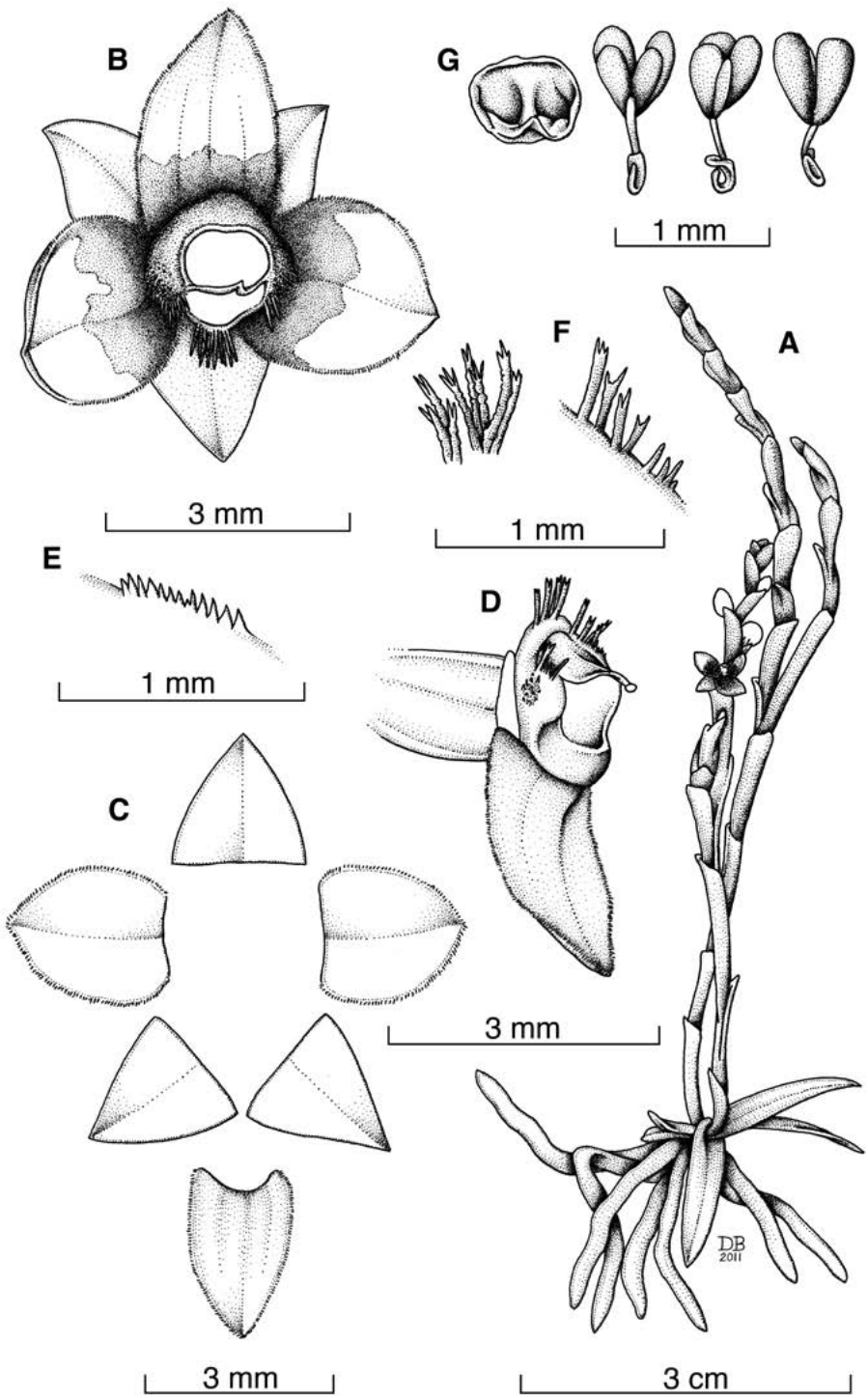

FIGURE 2. *Telipogon standleyi* Ames. A — Habit. B — Flower. C — Perianth flatten. D — Column and lip, lateral view. E — Margin of lip. F — Bristles of the column. G — Anther cap and pollinarium. Drawn by D. Bogarín based on *Bogarín 5138* (JBL-spirit).

Figure 3. Photo of: A — *Telipogon amoanus* (from Hágsater 4641). B — *T. standleyi* (from Bogarín 5138). Photographs by E. Hágsater (A) and D. Bogarín (B).

Telipogon amoanus is also similar to *Telipogon helleri*, which is distinguished by having a fringe of hairs in front of the column and lacking the hairs at the apex of the column (present in *T. amoanus* and *T. standleyi*) (Hamer 1985; Solano *et al*. 2011).

Acknowledgements. To the Ministerio del Ambiente, Energía y Telecomunicaciones de Costa Rica (MINAET) and Sistema Nacional de Áreas de Conservación (SINAC) of Costa Rica for issuing the Scientific Passport N. 1862 under which wild specimens treated in this study were collected. I am grateful to Franco Pupulin and Adam Karremans for their useful comments on the manuscript. This paper is part of the Project 814-A7-015, "Inventario y taxonomía de la flora epífita de la región mesoamericana (Orchidaceae)", sponsored by the Vice-Presidency of Research, University of Costa Rica. To Eric Hágsater for granting permission to publish his drawing and photograph.

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EXTRACTION AND AMPLIFICATION OF DNA FROM ORCHID EXSICCATES CONSERVED FOR MORE THAN HALF A CENTURY IN A HERBARIUM IN BOGOTÁ, COLOMBIA

Laura C. Mazo[1], Alberto Gómez[1,2,4], Sonia R. Quintanilla[1,2], Jaime E. Bernal[1,2] & Pedro Ortiz Valdivieso †[2,3]

[1] Instituto de Genética Humana, Facultad de Medicina, Pontificia Universidad Javeriana, Cra 7 # 40-62, edificio 32, Bogotá D.C., 110231, Colombia

[2] Iniciativa Genómica Javeriana, Pontificia Universidad Javeriana, Cra 7 # 40-62, edificio 32, Bogotá D. C., 110231, Colombia

[3] Asociación Bogotana de Orquideología, Avenida calle 63 # 68G-14, Bogotá D. C., Colombia

[4] Corresponding author: agomez@javeriana.edu.co

Abstract. Plant tissue from herbarium specimens contains DNA that has undergone *post-mortem* degradation. Only small amounts of possibly degraded genetic material free of chemicals and impurities can be extracted from these samples. The aim of the present work was to compare and determine which one of three previously published plant DNA extraction protocols would extract good quality DNA from orchid herbarium specimens stored up to 63 years, susceptible of PCR amplification and Sanger sequencing. The most effective protocol for this type of samples allowed us to obtain and sequence amplified products in 6 of the 7 samples studied with an optimal DNA / protein purity relationship.

Resumen. Los tejidos vegetales provenientes de muestras de herbario contienen ADN que ha sufrido degradación *post-mortem*. Solo pequeñas cantidades de material genético eventualmente degradado, libre de químicos e impurezas, puede ser extraído de dichas muestras. El presente trabajo buscó comparar y determinar cuál de los tres protocolos de extracción seleccionados, que habían sido publicados previamente para ADN vegetal, permitía extraer ADN de buena calidad, susceptible de ser amplificado y secuenciado, a partir de exsicados de orquídeas conservadas en herbario hasta por 63 años. De los protocolos evaluados, el más eficaz permitió obtener y secuenciar productos amplificados en 6 de las 7 muestras estudiadas, con las mejores relaciones de pureza ADN / proteína.

Key words: DNA, aDNA, Orchids, *matK*, Exsiccates, Herbarium

Introduction. The term "ancient DNA" (aDNA) refers to the DNA extracted from fossil remains and individuals preserved in musea and herbaria (Andreasen *et al.* 2009). Plant tissue from herbarium specimens contains DNA that is eventually degraded (Gugerli *et al.* 2005) with fragments not bigger than 500 base pairs (Audic *et al.* 2002). Moreover, the genetic material of those specimens can be: a- Contaminated with DNA of microorganisms such as fungi or bacteria (Deagle *et al.* 2006); b- Accompanied with precipitated chemical material, including polyphenols, terpenoids, tannins and polysaccharides (Huang *et al.* 2002), which can act as inhibitors of *Taq* DNA polymerase in plant DNA amplification protocols. A third challenge for working with ancient DNA is a process called cytosine deamination, which creates erroneous DNA sequences (Hofreiter *et al.* 2001).

Achieving adequate taxon sampling can often be challenging in order to perform molecular phylogenetic studies in plants, due to various reasons such as:

a) high costs of field work in remote locations;
b) difficulty in obtaining collection permits and access to genetic resources;
c) inaccessibility to politically unstable regions;
d) difficulty of finding the target species;
e) local or global extinction of the species; and
f) old name bearing type material lacking diagnostic characters may be included in molecular phylogenetic studies to investigate links with more recently collected specimens (Puillandre *et al.* 2012).

Collections found at herbaria are a potential source of DNA for improving taxa sampling, and thereby improve current phylogenetic understanding when there is no recent material available (Bebber *et al.* 2010). In addition, herbarium specimens are considered material with a high historical value due to the antiquity of the samples, and also to their character of taxonomic and biogeographic reference. Small amounts of tissue can be removed from the samples without causing damage to their global integrity. For this reason, the revision of extraction methods to recover as much DNA as possible from the minimum amount of tissue is crucial (Rohland & Hofreiter 2007).

A wide range of techniques have been published to date, seeking to increase the amount of DNA available and reduce the influence of PCR inhibitors. Among these, two main methods have been widely used on both fresh and herbarium samples: a- Cetyl-Trimethyl-Ammonium Bromide or CTAB (Doyle and Doyle 1987) and b- DNeasy Plant Mini Kit from Qiagen (Cota-Sánchez *et al*. 2006). Silica gels have been included by Van Geel *et al*. (2008), as a useful supplementary step in the purification of amplified ancient plant DNA. These protocols have been proposed for the extraction of ancient DNA free of chemicals and impurities from herbarium specimens, but have not been widely tested on orchids and are limited to particular groups of plants (Cota-Sánchez *et al*. 2006).

Along these lines, this study aimed to compare and evaluate three (3) modified extraction protocols, two of which were CTAB based, on the following species *Prosthechea grammatoglossa, Epidendrum secundum* and *Oncidium ornithorrhynchum*, collected between 1948 and 2011, to determine the best protocol for DNA extraction and amplification from orchid samples preserved in herbaria, according to previous reports which were applied to newly collected plants.

Material and methods

Selection of plant material. – DNA was extracted from 7 samples belonging to 3 species of orchids. Of these, 5 were dry tissue (Table 1) and the other 2 corresponded to fresh tissue samples of the species *Prosthechea grammatoglossa* and *Epidendrum secundum*. The reason why these specimens were chosen, was to include in our protocols preserved orchid samples with the longest available period of conservation, stored in the Universidad Javeriana Herbarium, to be compared with fresh material of the same species. We worked with 2 mg and 5 mg of tissue corresponding to the homogenization of subsamples of petal, stem and leaves, due to the small amount of material available.

DNA extraction. – At first, Petri dishes were washed with distilled water, sodium hypochlorite and absolute ethanol before being used and when switching from one sample to another. Then, 2 mg and 5 mg of tissue were weighed and pulverized by means of a surgical blade. Subsequently three (3) different plant DNA extraction protocols were assessed: Quintanilla´s *et al.* (2011), Cota-Sánchez´s *et al*. (2006) (Modified: Ethanol 70% and 99%, no addition of RNAse) and Jobes *et al.* (1995) (Modified: LiCl was substituted by Ammonium Acetate). The reason why these three protocols were chosen was, first, to reduce the variables to be considered, second, to employ

Table 1. Exsiccates information of the 3 species of orchids studied and preserved in the Herbarium "Lorenzo Uribe Uribe, S. J. ", of the Pontificia Universidad Javeriana (HPUJ), Bogota, Colombia.

Species	Description	Location	Elevation	Collector	Date
Epidendrum secundum Jacq	Different colors according to the land or varieties of the species	Villa blanca (Sasaima, Cundinamarca)	1400 m	C. Ortiz, S.J	17 Oct 1948
Epidendrum secundum Jacq.	Rupicola. Lilac flowers	Vereda Vancouver (Santandercito, Cundinamarca)	1900 m	P. Ortiz, S.J	3 Febr 1968
Oncidium ornithorrhynchum H.B.K.	Epiphyte with yellow flowers and brown clear spots	Hacienda la Esmeralda (Madrid, Cundinamarca)	2650 m	S. Restrepo, S.J.	30 May 1968
Oncidium ornithorrhynchum H.B.K.	Yellow flowers with brown spots	"La Rusia" (Duitama, Boyacá)	2870 m	P. Ortiz, S.J	Jun 1975
Prosthechea grammatoglossa (Rchb.f) W. E. Higgins	Yellow flowers	"La Rambla" (Santandercito, Cundinamarca)	2000 m	H. Silva	13 Jan 1949

different reagents for DNA extraction such as CTAB in conjunction with Beta-Mercaptoethanol (ME), used in Cota-Sánchez *et al*. (2006) and Quintanilla *et al*. (2011) with different periods and incubation times, and Sodium Dodecyl-Sulfate (SDS), Polyvinylpyrrolidone (PVP), Dithiothreitol (DTT), Proteinase K (PK) used in Jobes *et al*. (1995), third, to minimize the cost of DNA extractions, as supplementary purification steps such as silica columns are expensive. In addition, the protocol proposed by Quintanilla *et al*. (2011) was included as a reference in our work, as it was previously employed with success by our group on the molecular characterization of different orchids. In this later protocol, a purification step based on ammonium acetate (7.5 M) and 2-Propanol substitutes silica columns.

Spectrophotometry, amplification and sequencing – DNA quality was determined by means of a 260/280 - 260/230 purity relationship. Subsequently, a region of 640 bp corresponding to the gene *matK* was amplified using the following primers:

19Forward: 3'-CGTTCTGACCATATTGCACTATG-5'
556Reverse: 3'-GAAGAAACATCTTTGGATCCA-5'.

This gene was previously used successfully by our group in the characterization of different species of orchids on fresh material (Quintanilla *et al.* 2011), and *matK* is reported as one of the official plant BoLD markers as follows: "The BoLD Identification System (IDS) for *rbcL* and *matK* is the default identification tool for plant barcodes and accepts sequences from the Ribulose-bisphosphate carboxylase and Maturase K genes" (BoLDsystems, 2012), and also as the reference for the CBOL Plant Working Group (Janzen 2009).

PCR reactions contained 6µl of PCR buffer (5x), 3 µl of each primer (10mM), 0.9 µl of MgCl (2.5 mM), 0.6 µl of dNTPs (200 µM), 0.3 µl of DNA Taq polymerase (1.25 units) and 14.4 µl of sterile water to complete a volume of 30 µl of each reaction. PCR cycles were programmed as follows: 3 min at 95°C initial denaturation, 39 cycles of 30 s at 95°C, 30 s at 50.4°C, 48 s at 72°C and a final extension of 5 min at 72°C. After amplification, DNA concentration was determined on 7µl of PCR product through electrophoresis in 1% agarose and 1x TBE buffer. The amplified products were Sanger sequenced (Applied Biosystems 3730XL at Macrogen, Korea) and analyzed using the programs Chromas Lite and BioEdit with 5 other sequences reported in GenBank (Table 2) and finally a phylogenetic tree was drawn through the Neighbor-Joining method.

Table 2. Sequences reported in GenBank.

Oncidium ornithorrhynchum	*H02192561, AF350645.1, AF239496.1*
Oncidium unguiculatum	*FJ563968.1*
Oncidium incurvum	*FJ565110.1*
Peristeria elata	*AF239442.1*

Statistical analysis. – A descriptive statistical report was produced by means of categorizing the results from the different indices. This was performed dividing DNA concentration values as: a) less than 10 ng (indicating low extraction, inefficient protocol; insufficient tissue quantity); b) between 11 and 30 ng (corresponding to medium extraction and an average efficient protocol) and, c) ranging from 31 to 86 ng (corresponding to an optimum extraction, a very efficient protocol and a sufficient quantity of tissue), while 260/280 and 260/230 absorbance relations were classified as: a) less than 1.8 (indicating a bad quality, and contamination by proteins); b) between 1.8 and 2.2 (good quality, free of contamination) and, c) over 2.2 (bad quality, contaminated by ARN, polyphenols and polysaccharides) (Cattaneo *et al.* 2006).

Results and discussion

Due to the limited amount of nucleic acids preserved in herbarium specimens, it is difficult to obtain an appropriate amount of molecules in the early stages of extraction. For this reason, an appropriate lysis buffer is a key factor for an optimal obtention of DNA molecules (Yang *et al.* 1997). The use of the Cetyl-Trimethyl-Ammonium Bromide (CTAB) extraction protocol was designed as an efficient method to avoid contamination with polysaccharides (Thine & Telle 2008). This method was evaluated in the protocols proposed by Quintanilla *et al.* (2011) and Cota-Sánchez *et al.* (2006), both of which include the use of reagent Beta-Mercaptoethanol (ME), which is a reducing agent that blocks the action of phenols and, when used at higher concentrations (1%), seems to be more efficient in reducing oxidation (Mittmann *et al.* 2007). Although in both Quintanilla *et al.*(2010) and Cota-Sánchez *et al.* (2006) an equivalent concentration of

TABLE 3. Data for total DNA concentration and relationships 260/280, 260/230 in samples extracted using the protocols reported by Quintanilla *et al.* (2010), Cota-Sánchez *et al*. (2006) and Jobes *et al.* (1995).

Species	Quintanilla *et al.* (2010)				Cota-Sánchez *et al.* (2006)			Jobes *et al.* (1995)		
	Tissue (mg)	ADN (ng/µl)	260/280	260/230	ADN (ng/µl)	260/280	260/230	ADN (ng/µl)	260 280	260 230
Prosthechea grammatoglossa (1949)	2	32,5	2,04	1,17	2,7	1,03	0,3	0,9	0,51	0,05
Oncidium ornithorrhynchum (1975)	2	28,7	1,98	1,8	4,2	1,32	0,45	-0,5	1,11	-1,24
Oncidium ornithorrhynchum (1968)	2	5,3	2,42	2,01	14,9	1,43	0,38	3,3	1,23	0,23
*Epidendrum secundum (*1968)	2	15,1	1,99	1,63	18,7	1,33	0,71	16,9	1,17	0,32
Epidendrum secundum (1948)	2	6,7	2,67	1,53	11,5	1,17	0,46	0,9	0,4	0,07
Prosthechea grammatoglossa (2011)	2	4,6	3,54	3,86	0,6	0,81	0,13	-1	0,85	0,91
Epidendrum secundum (2011)	2	7,4	2,22	2,62	13,7	0,99	0,51	-0,2	1,81	0,56
Prosthechea grammatoglossa (1949)	5	75,8	1,52	0,77	0,8	1,83	0,41	1,1	0,41	0,05
Oncidium ornithorrhynchum (1975)	5	85,6	1,66	0,72	25,4	1,83	1,28	6,2	0,78	0,1
Oncidium ornithorrhynchum (1968)	5	-2,9	1,51	-0,14	-0,1	0,22	0,25	5,4	0,85	0,12
*Epidendrum secundum (*1968)	5	57,2	1,91	1,01	8	1,96	0,71	0,8	0,66	0,05
Epidendrum secundum (1948)	5	28	2,02	1,68	1,2	1,68	0,27	-0,8	0,91	1,02
Prosthechea grammatoglossa (2011)	5	53,3	2,03	1,19	-1,2	0,9	1,06	2	6,64	0,02
Epidendrum secundum (2011)	5	57	1,99	1,19	-0,6	1,14	0,64	-0,4	0,56	-0,34

ME was used, there is a pronounced difference in their efficiency, as the protocol proposed by Quintanilla *et al.* (2011) results in higher quality DNA (Table 3).

In contrast, the buffer evaluated in the protocol proposed by Jobes *et al.* (1995) is composed of Sodium Dodecyl-Sulfate (SDS), Polyvinylpyrrolidone (PVP), Dithiothreitol (DTT), Proteinase K (PK) and no ME. The PVP and DTT are added in order to absorb polyphenols, which tend to co-precipitate along with DNA (Mittmann *et al.* 2007). In spite of this, it is evident that the use of ME in the lysis buffer allowed to achieve better results than the use of PVP and DTT, because there was no amplification with any amount of tissue (2 mg or 5 mg) using the protocol of Jobes *et al.* (1995) in any of the seven (7) species studied (Fig. 1-2).

Another reagent used in this buffer is proteinase K (PK), which's function is to digest proteins, and it is important to remove the Ethylene-Diamine-Tetra-Acetic Acid (EDTA) from the initial incubation steps, because this reagent inhibits the function of the PK (Cattaneo *et al.* 2006). Also, it is necessary to denature the proteinase K afterwards as this reagent may in turn inhibit the PCR (Orourke *et al.* 1996). The protocol proposed by Jobes *et al.* (1995) does not indicate any steps related to the elimination of EDTA before using the PK neither its subsequent inactivation prior to PCR. This may interfere with the action of PK during the initial incubation and that is probably the reason why there was no amplification of any of the species studied (Cattaneo *et al.* 2006).

During the DNA extraction it is important to consider steps for incubation and precipitation, as it seems unnecessary and specially inefficient to extend and increase the times and temperatures in order to obtain more DNA (Mittmann *et al*. 2007). Sablok *et al.* (2009) observed that incubation periods with temperatures above 65°C (without reaching 80 °C) resulted in loss of DNA. In the protocols proposed by Jobes *et al.* (1995) and Cota-Sánchez *et al.* (2006), the incubation temperature varied between 50°C - 56°C and in Quintanilla *et al.* (2011) between 60°C - 65°C. This contrasts with the observations made by Sablok *et al.* (2009) because at high temperatures more DNA was obtained, being evident to achieve higher quantities of DNA concentrations upon application of the Quintanilla´s *et al.* protocol (Table 3).

On the other hand, incubation of nucleic acid / salt / ethanol performed at low temperatures (i.e. -20°C) is quoted in several articles. Nevertheless, this is not necessary since nucleic acids precipitate at a temperature of -4°C in concentrations below 20 ng/ml after 15-30 minutes (Bitesize Bio). In the protocol of Jobes *et al.* (1995), four incubations were performed, three of them at -20°C for 30 minutes and the last one at

-20°C for one night, unlike Cota-Sánchez *et al.* (2006) where two incubations were performed at -20°C for two nights, and Quintanilla *et al.* (2011) where there was only one precipitation at -20°C for one night. This indicates that the smaller number of incubations and precipitations, involving a smaller number of washes, allow the obtention of higher concentration of DNA.

Another issue in the DNA extraction is the removal of polysaccharides, although high concentrations of Sodium Chloride (NaCl) appear to block the action of these polysaccharides (Mittmann *et al.* 2007). The highest concentrations of NaCl in the lysis buffer were reported by Quintanilla *et al.* (2011) and Cota-Sánchez *et al.* (2006), the first being the one that gave better results, unlike Jobes´ *et al.* protocol (1995) which did not yield any amplified product from any species (Fig. 1-2), and whose total NaCl concentration was 500 mM. Chang *et al.* (1993) state that increasing salt concentration from 1.4 M to 2.0 M in the lysis buffer, affects the solubility of the polysaccharides in ethanol, allowing optimum precipitation of nucleic acids (Mittmann *et al.* 2007) which improves the relation 260/230 (Josquin *et al.* 2006) (Table 3).

Salts such as Sodium Acetate and Ammonium Acetate, are also employed to precipitate DNA. When in solution, Sodium Acetate becomes Na^+ and $[CH_3COO]^-$, and Sodium ions positively charged neutralize the negative charge of PO_3^- of nucleic acids, then DNA precipitates more easily (BietziseBio). In the protocols of Jobes *et al.* (1995) and Cota-Sánchez *et al.* (2006), Sodium Acetate was used without obtaining good results. In Quintanilla *et al.* (2011) Ammonium Acetate was used, and showed a better performance (Fig. 1–2).

Also, several ethanol washing steps are crucial as salt residues are removed from DNA (Bitesize Bio). Regarding Cota-Sánchez *et al*. (1995) and Jobes *et al*. (1995) only one wash was performed, contrary to Quintanilla *et al.* (2011) where there were not only two washes with 70% ethanol, but also two washes with 99% ethanol, indicating that more than one wash with an alcohol leads to purest DNA, as seen in the 260 / 280 relations that are notoriously better in this last protocol than in the previous ones.

It is important to emphasize that the process of degradation and deterioration is unique in each exsiccate, and that particular chemical compounds of

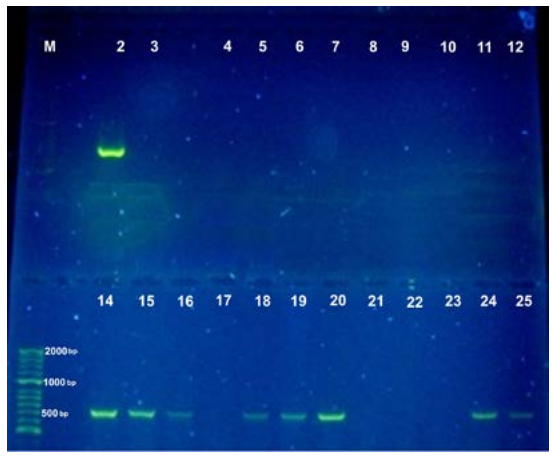

FIGURE 1. PCR products of samples from 2mg. Line 2, C + (positive control), Line 3, C-(negative control), lines 4-10, protocol Jobes *et al.,* Lines 14-20, protocol Quintanilla *et al*.; 11,12,21-25 Lines: protocol Cota-Sánchez *et al*., lines 4, 11 and 14, *Prosthechea grammatoglossa* (1949), lines 5, 12 and 15, *Oncidium ornithorrhynchum* (1975), Lines 6, 16 and 21, *Oncidium ornithorrhynchum* (1968), lines 7, 17 and 22, *Epidendrum secundum* (1968), lines 8, 18 and 23, *Epidendrum secundum* (1948), lines 9, 19 and 24, *Prosthechea grammatoglossa* (2011), Lines 10, 20 and 25, *Epidendrum secundum* (2011). M (molecular marker).

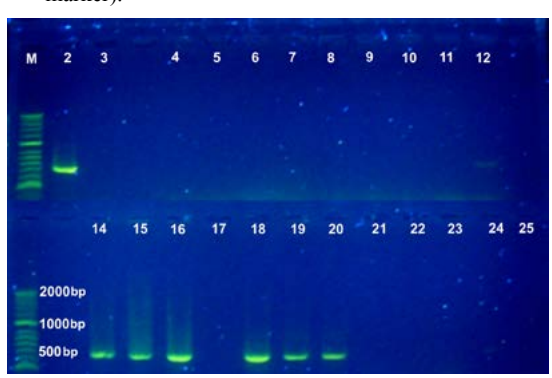

FIGURE 2. PCR products of samples from 5mg. Line 2, C + (positive control), Line 3, C-(negative control), lines 4-10, protocol Jobes *et al.*, Lines 14-20, protocol Quintanilla *et al*.; 11,12,21-25 Lines: protocol Cota-Sánchez *et al*., lines 4, 11 and 14, *Prosthechea grammatoglossa* (1949), lines 5, 12 and 15, *Oncidium ornithorrhynchum* (1975), Lines 6, 16 and 21, *Oncidium ornithorrhynchum* (1968), lines 7, 17 and 22, *Epidendrum secundum* (1968), lines 8, 18 and 23, *Epidendrum secundum* (1948), lines 9, 19 and 24, *Prosthechea grammatoglossa* (2011), Lines 10, 20 and 25, *Epidendrum secundum* (2011). M (molecular marker).

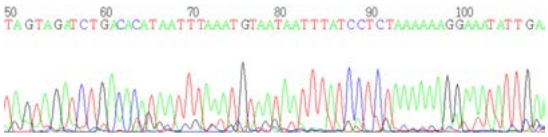

FIGURE 3. Part of the sequence of the gene *matK* of the species *Epidendrum secundum* 2011 C + (450 mg tissue).

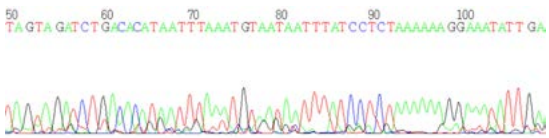

FIGURE 4. Part of the sequence of the gene *matK* of the species *Epidendrum secundum* 2011 (Quintanilla *et al.* 2010).

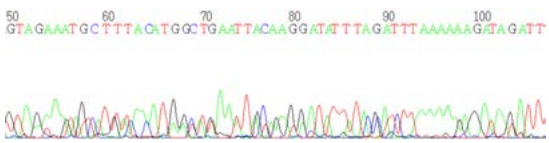

FIGURE 5. Part of the sequence of the gene *matK* of the species *Epidendrum secundum* 1948 (Quintanilla *et al.* 2010).

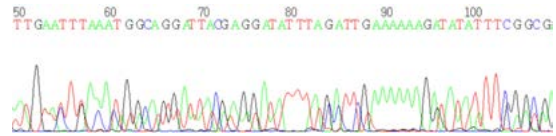

FIGURE 6. Part of the sequence of the gene matK of the species *Epidendrum secundum* 2011 (Cota-Sánchez *et al.* 2006).

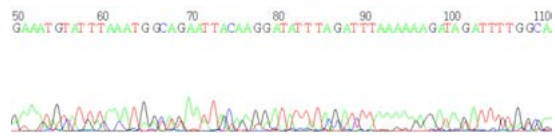

FIGURE 7. Part of the sequence of the gene *matK* of the species *Oncidium ornithorrhynchum* 1975 (Quintanilla *et al.* 2010).

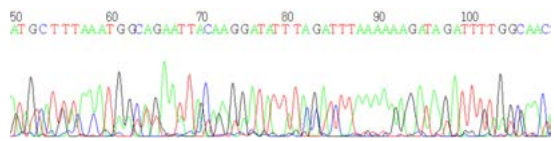

FIGURE 8. Part of the sequence of the gene *matK* of the species *Oncidium ornithorrhynchum* 1968 (Quintanilla *et al.* 2010).

each herbarium sample can either interfere or favor certain steps during the extraction process (Yang et al. 1997). That is why, in samples belonging to the same species, quite different DNA concentrations can be obtained (Table 1). This may be due to different methods (chemical -use of ethanol or formaldehyde at 30%-, and physical -drying at 70°C-) that each sample endures during its treatment after it is collected (Savolainen *et al.* 1995). The fact that these species might not be comparable in terms of the presence of particular PCR inhibiting secondary metabolites, could at least partly explain the results obtained on different specimens in our report.

Usually, the length of the sequences obtained from ancient DNA are smaller than 200 bp; in this study we observed that the sequences corresponding to the species collected in 2011, that is: *Epidendrum secundum* extracted with the protocol Quintanilla *et al.* (2011) and *E. secundum* (C+, from 450 mg of tissue), were the most readable sequences with defined peaks (Fig 3–4). The same species, when preserved for a longer time (1948) and compared with the one collected in 2011 and extracted using the protocol Cota-Sánchez *et al.* (2006), showed sequences which were not easily readable and therefore showed poor quality (Fig. 5–6). The sequences corresponding to the species *Oncidium ornithorrhynchum*, despite being poorly readable, were similar to each other (Fig. 7–8) and also coincide with the sequence previously reported by Quintanilla *et al.* (2011) which was obtained from a fresh sample (Fig. 9). Andreasen *et al.* (2009) propose that the length and quality of the amplified fragment is related to the degree of DNA degradation: the greater time of collection, the greater the DNA fragmentation. This is not observed in the species *E. secundum* collected in 1968, which although showing a high concentration of DNA, failed to be amplified in contrast to the same species collected in 1948, which was easily amplified (Fig. 1–2). This result can be influenced by the treatment that was given to each herbarium specimen when collected and preserved. However, in both cases, their corresponding data were not noted and kept, so there is no available information that would allow to perform a correlation. Hanni *et al.* (1994), suggest that inefficient amplification of the samples is due to the small amount of tissue used corresponding to 2 mg and 5 mg as, in other cases, approximately 30 mg or even 100 mg of plant tissue have been used (Lister *et al.* 2008).

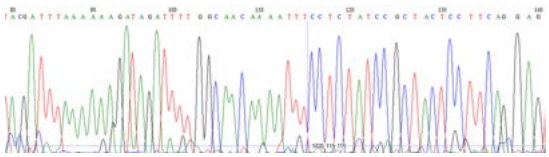

FIGURE 9. Part of the sequence of the gene *matK* of the species *Oncidium ornithorrhynchum,* reported in 2010 by Quintanilla *et al*. (H02192561).

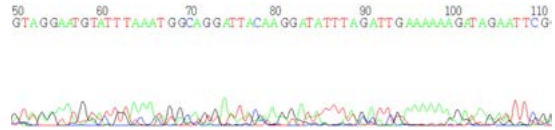

FIGURE 10. Part of the sequence of the gene *matK* of the species *Prostechea grammatoglossa* 2011 (Quintanilla *et al.* 2010).

In this study, DNA extracted using the protocol Quintanilla *et al.* (2011) and 5 mg of tissue, showed a higher concentration of Nucleic Acids than those obtained from 2 mg (Table 1). Therefore, the higher amount of available material, the greater the possibility of obtaining higher concentrations of DNA.

Finally, the Neighbor-Joining method (NJ) is frequently used in studies of "molecular barcode" (DNA barcoding) when aiming to identify unknown individuals and to generate a single phylogenetic tree with the lowest genetic distances (Saitou & Nei 1987). Thus, by reflecting the similarity between sequences, individuals belonging to the same species will integrate isolated groups due to their similarities (similarity in terms of sequences) (Peña 2011). We can then infer that the sequences of the species *Oncidium ornithorrhynchum* form an isolated group that also includes the previously reported sequences of this same species in NCBI (Fig. 11).

In conclusion, the methodology for DNA extraction on herbarium individuals with the protocol proposed by Quintanilla *et al.* (2011) is effective for samples with storage times over fifty years, as compared with the other two methodologies reported and studied in the present work on this type of specimens. The importance of prior information available from the herbarium related with the method of fixation (chemical or physical) of each individual has to be emphasized to assess how the samples will eventually work, as it was found upon completion of this study that the effect of time is not as important for DNA degradation as the initial use of formaldehyde, methanol or application of heat. We recommend: 1- Further testing different sets of primers that may generate shorter fragments, 2- DNA polymerases that have the property to correct readings, and 3- Next generation sequencing to obtain more replicates of ancient DNA sequences to reveal deamination generated errors (Metzker 2010).

ACKNOWLEDGMENTS. The authors thank the staff from the "Lorenzo Uribe, SJ" herbarium (Faculty of Science, Pontificia Universidad Javeriana), as well as the staff of the Institute of Human Genetics (Faculty of Medicine, Pontificia Universidad Javeriana), particularly our colleagues involved in the research group "Ancient DNA". We also thank CorpoGen for the use of their DNA quantification equipment. Our final manuscript includes important remarks from two anonymous reviewers, to whom we are indebted.

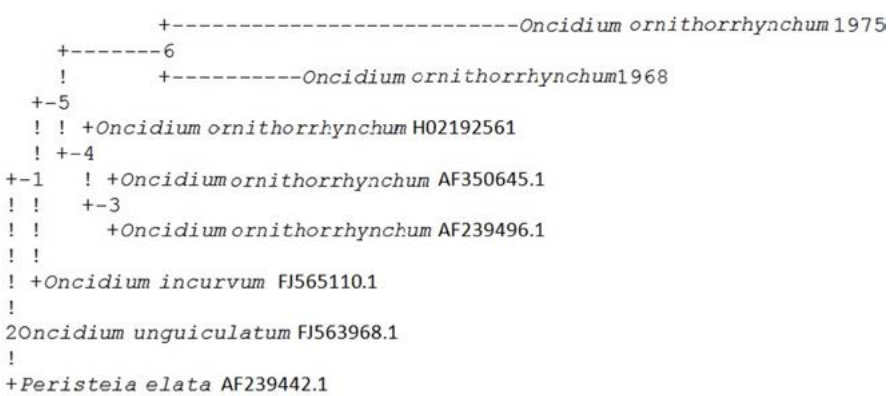

FIGURE 11. Genetic distance phylogram through Neighbor-Joining clustering of the sequences studied plus 6 previously reported sequences in GenBank.

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MICROPROPAGATION OF *DENDROBIUM AGGREGATUM* BY GREEN POD CULTURE

S. VIJAYAKUMAR[1], G. RAJALKSHMI[2] & K. KALIMUTHU[1,3]

[1] Plant Tissue Culture Laboratory, PG and Research Department of Botany, Government Arts College, Coimbatore -641 018, India

[2] Department of Biotechnology, Hindusthan College of Arts & Science, Coimbatore

[3] Corresponding author: k_kalimuthu@rediffmail.com

ABSTRACT. An efficient protocol for micropropagation of *Dendrobium aggregatum* using the axenic immature seeds, derived from green pod, was developed. The immature embryos from 120 days old capsules after pollination were germinated on Murashige and Skoog (MS) medium supplement with various concentration of BAP alone or in combination with NAA along with coconut water was used for induction, multiplication, elongation and rooting *in vitro* shoots. MS medium fortified with 3% sucrose 1.5 mg L^{-1} Benzyl amino purine (BAP) and 15% coconut water (CW) favoured the higher rate of germination, more number of protocorm bodies, production of maximum number of shoots, elongation of shoots, as well as root formation. During acclimatization, 95% of the plantlets survived after one month.

KEY WORDS: *Dendrobium,* protocorm like bodies, acclimatization, *in vitro*

Introduction. Orchids are the most pampered and occupy top position among all flowering plants valued for cut flower production as potted plants which sketch a very high price in the international market. The genus *Dendrobium* (Family: Orchidaceae) exhibits a vast diversity in vegetative and floral characteristic and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity (Jones *et al*. 1998).Though orchids produce a large number of seeds, very few (<5%) of them germinate under natural condition because the seeds are non-endospermic, minute and require a mycorrhizal association (Rao 1977). The conventional method of vegetative propagation of orchids is a time consuming and tedious process (Sagawa & Kunisaki 1984).

Dendrobium aggregatum Roxb. (= *D. lindleyi* Steud.)[1] is a mild fragrant sympodial orchid, grown in many counties especially in developed temperate countries of the world as pot plants or cut flowers (Aktat *et al*. 2007). It has graceful, pendulous racemes of medium sized flowers usually in white colour. The traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year. Orchid is well known for their exploitation as major trade in developed countries (Sagawa & Kunisaki, 1984). *In vitro* culture has proved particularly useful with groups of plants, which are difficult to propagate using conventional techniques (Fay 1994).When mass propagation of a new hybrid or a varity is needed within a short time, tissue culture is the only method (Goh *et al*. 1992).

The orchid resource of the world in general and Western-Ghats region of India in particular is depleting day by day due to habitat loss. The collection of wild *Dendrobium* continues at levels ranging from hobbyist to large-scale illegal trade. Endemic orchids of the Western-Ghats India are facing the grim possibility of extinction under intense biotic pressures like jhum cultivation, forest fires, indiscriminate wild collection and illegal trade by the local people. Hence conservation and sustainable utilization assume greater importance to save the dwindling orchids (Kishor *et al*. 2006). Satisfying the interest of the hobbyist and demand of the traders through large-scale micropropagation is one the preferable options to prevent illegal collection from wild (Sunitibala & Rajikumar 2009).

[1] The name *Dendrobium aggregatum* Roxb. (1832) is illegitimate, being a later homonym of *D. aggregatum* Kunth (1816), the basionym of the species presently known as *Ornithidium aggregatum* (Kunth) Rchb.f. As *D. lindleyi* is still amply cultivated and traded under the name of *D. aggregatum* Roxb., we have mantained the latter name throughout the article. [*Note by the Editor*]

A perusal of available literature reveals that micropropagation has been achieved using immature or mature embryos, shoot tip explants and from axenic nodel segments in *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch., *D. candidum* Wall. ex Lindl. and other hybrids of *Dendrobium* (Zhang *et al.* 1993, Liu *et al.* 1988, Shiau *et al.* 2005, Xie *et al.* 2010, Nambiar *et al.* 2012). However, there is no report on clonal propagation of *D. aggregatum* using different explant sources. In this study, authors report the development of an efficient simple and reproducible one step protocol for multiple *D. aggregatum* seedlings, rooting of the microshoots and successful transplantation.

Materials and methods. Plants of *D. aggregatum* were collected from their natural habitat and kept under shade net (75%) house environment at the campus of the Government Arts College, Coimbatore. After flowering, several flowers were hand pollinated on the second of anthesis. The pollinated flowers were bagged with butter paper for one week. Several capsules of *D. aggregatum* were harvested 120 days after pollination and brought to the laboratory for *in vitro* seed germination.

Establishment of shoot cultures by *in vitro* germination of immature embryos. The harvested capsules were soaked in aqueous solution of commercial detergent (labolene) for 10 minutes followed by 0.5 mg/L^{-1} Bavistin (Himedia) for 20 minutes. The capsules were surface disinfected in 70% (v/v) ethyl alcohol for 30 seconds followed by 0.12% (w/v) mercuric chloride solution for 10 minutes and then rinsed 3-4 times sterile distilled water before air drying in a laminar air flow chamber for 5 minutes. Green capsules were dissected longitudinally with a sterile surgical blade. The immature seeds were scooped out of the sterilized capsules and small mass of the aggregated seeds were germinated in culture bottles (60 mm × 105 mm) each containing 30 ml of full strength Murashige and Skoog (MS) basal medium. The basal medium was comprised of full strength MS medium 30 mg/ L^{-1} sucrose and gelled with 8 g/ L^{-1} Difco bacto agar (Himedia, India). It was then supplemented with different concentration and combination of naphthalene acetic acid (NAA) and benzyl aminopurine (BAP) along with coconut water (CW) at pH 5.8 (Table 1). The cultures were

Table 1.Effect of different concentration of plant growth regulators on *in vitro* development of plantlets from immature seeds of *Dendrobium aggregatum*

Concentration of BAP and NAA in MS medium + CW 150 ml/L	No. of green pods used per bottle	Capability of immature seeds forming protocorm like bodies	No. of shoots per bottle	No. of shoots with roots	Percentage of shoot forming the roots
BAP					
0.5	1	+	35	14	40.0
1.0	1	++	50	35	87.5
1.5	1	+++	75	75	100.0
2.0	1	+	41	28	68.3
2.5	1	+	32	14	36.8
3.0	1	+	20	5	25.0
BAP + NAA					
0.5 + 0.5	1	+	23	13	56.5
1.0 + 1.0	1	+	27	20	74.0
1.5 + 1.5	1	+++	37	37	100.0
2.0 + 2.0	1	++	26	15	57.8
2.5 + 2.5	1	+	20	8	40.0
3.0 + 3.0	1	+	14	3	21.4
MS Basal	1	-	-	-	-

\+ -- Very less number of protocorm like bodies formation
++ -- Less number of protocorm like bodies formation
+++ -- More number of protocorm like bodies formation

incubated at 25 ± 2ºC under cool white fluorescent light with 14 hours photoperiod.

After 15 days culture, various concentration of BAP and NAA ranging from 0.5 – 3.0 mgL-1 individually (BAP) and combined (BAP + NAA) along with coconut water effect were analyzed through the parameters such as production of amount of protocorm like bodies (PLBs), numbers of shoots and number of shoots with roots.

Subculture of protocorm like bodies and shoots. Shoots and protocorm like bodies were transferred to the same media composition produced more protocorm like bodies, multiple shoots and roots.

Hardening. Well rooted shoots were removed from culture vessels and thoroughly washed with tap water to remove residual medium and transferred to plastic pots containing a mixture uniform, small charcoal pieces and brick pieces (1:1). They were then kept in the shade house 25% light and mist irrigated.

Experiment design and data analysis. Experiments were set up in completely randomized design. Each treatment had 10 replicates. Significance of treatment effects was determined using DMRT analysis.

Results and discussion. The seeds taken from the green pods were sown on the MS medium (Table-1) containing various concentrations of two plant growth regulators, namely BAP and NAA along with CW. Invariably all the embryos transferred to the MS medium with various concentrations of BAP, NAA and CW germinated within two weeks. Swelling and glistering of the embryos were first noticed within 10 days. The swelling of the embryo was followed by pigment synthesis. The embryos turned from yellow to yellowish green and finally becoming green as they grew.

Due to the non-endospermic nature of the seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful in *in vitro*. The production of orchid seedling from seed involves, sequential phases of germination, protocorm formation and seedling development. In the present investigation also same sequence of seedling development was observed when the selected orchid, *D. aggregatum* was grown on the medium. As the embryos development into globouse protocorms, seed coat (testa) got ruptured and rhizoids and shoot initials were getting formed. Among the six different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l-1) individually in combination with NAA (0.5, 1.0 1.5, 2.0, 2.5 and 3.0 mg l-1) along with CW was used. MS medium contain BAP + CW (1.5 mg/l + 15% in combination) was found to be the most suitable, which supported higher rate of germinations, more number of protocorm bodies, shoots and roots. Shoots and protocorm like bodies were transferred to same media composition produced more protocorm like bodies, multiple shoots and roots. Incorporation of coconut water to basal medium induces and enhances early differentiation of PLBs. Earlier Talukdar (2001) reported similar observation in *D. aphyllum*. Leetham (1974) reported that a plant growth hormone like cytokinin is present in coconut water. PLBs developed from the germinating seeds after 45-50 days of culture were allowed to differentiate in the same medium. The pear shaped PLBs with tiny leaf sprouting were developed after 20-25 days of germination.

Auxin was the first plant growth hormone added to the seed culture. In majority of the cases auxins (mostly NAA, IAA and IBA) enhanced the germination and seedling growth (Nasiruddin *et al.* 2003). In the present study BAP and CW stimulated shoot and root growth in *D. aggregatum* as reported in *D. microbulbon* A. Rich. (Urvashi Sharma *et al.* 2007). In the present study more number of protocorm like bodies were produced by the medium, which contained 1.5 mgl^{-1} BAP with CW and 1.5 mg/l + 1.5 mg/l BAP and NAA with CW. Very poor result was observed in the medium contained 3.0 + 3.0 $mg1^{-1}$ BAP and NAA. (Table-1). In the present investigation the seedling development of *D. aggregatum* was best on the MS medium supplemented with 1.5$mg1^{-1}$ BAP + 15% CW. These finding are in agreement with Urvashi Sharma 2007, who observed that BAP induced better shoot and root growth in *D. microbulbon*.

A well-developed cluster were selected and transferred to second subculture for root induction (Fig. 1). These subculture were grown using the same concentration and combination of the same plant growth regulators. These cultures media were used to study their stimulatory effect of the number of shoots and roots per shoot. The number of shoots and roots were counted. After maintaining for 2-3 passages on the

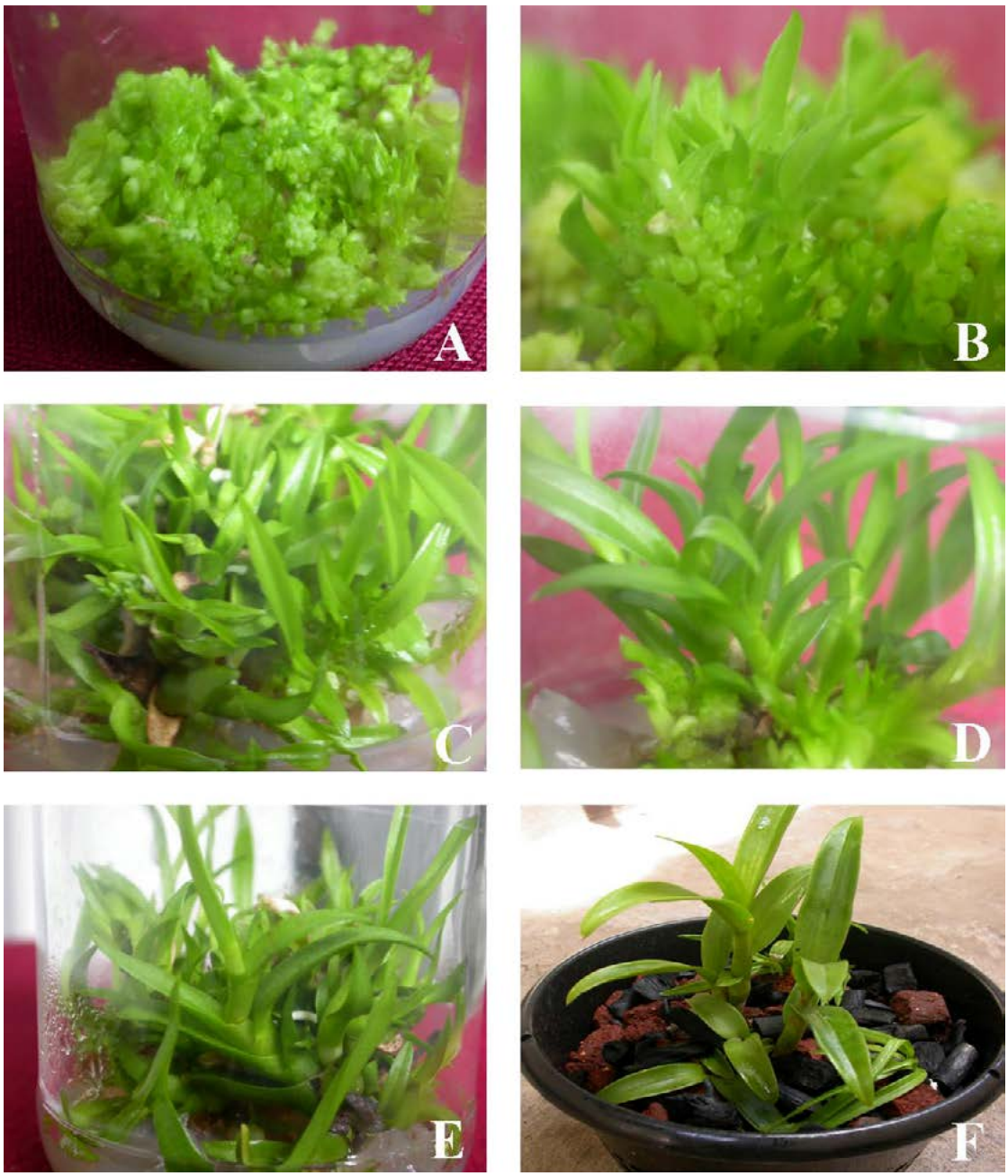

FIGURE 1. Different stages of immature seed germination and regeneration of plants of *Dendrobium aggregatum* on MS medium. A — PLBs. B — PLBs and young plants. C, D — Developed plantlets. E, F — Potted plant.

medium, the plants were taken out, washed thoroughly to remove traces of agar and transplanted to perforated plastic pots containing pieces of charcoal and bricks (Fig. 1). About 95% of the potted plants survived after one month in the shade house. The transplanted plants were acclimatized in the shade house for 1-2 months and transferred to the environmental condition.

The present investigation revealed that the MS medium supplemented with certain concentrations of the plant growth regulators influenced on seed

germination, production of protocorm like bodies, shoot multiplication and root initiation. The *in vitro* raised seedlings were successfully established in the potting medium. Further growth and development of seedlings will be observed in further.

In conclusion, a simple, efficient and commercially viable protocol for mass clonal propagation of *D. aggregatum* from green pod has been established. Using this protocol viable, uniform and healthy plants with maximum survival rate that can be used for large scale cultivation. Furthermore, the protocol may facilitate conservation of this commercial orchid from extinction in the natural population.

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