





PLEUROTHALLIS MARKGRUINII, A NEW PLEUROTHALLIDINAE (ORCHIDACEAE) FROM NORTHWESTERN ECUADOR

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Pleurothallis markgruinii, a particularly tall species for the genus, is described as new to science and an illustration provided. The new species is most similar to *Pleurothallis gargantua* and shares with that species its large size, reaching 1 m tall, but differs in the much smaller flowers with a deeply concave dorsal sepal, narrower petals, and a synsepal with strongly revolute margins that form an elongated, triangular shape. Comprehensive field research has shown *Pleurothallis markgruinii* to have a restricted geographical range in the northwest Andes of Ecuador, and it is assessed as Critically Endangered.

Keywords. Carchi, Dracula Reserve, new orchid species, Reserva Youth Land Trust.

Pleurothallis markgruinii, una especie con plantas particularmente largas dentro del género se describe e ilustra aquí como nueva para la ciencia. La nueva especie es similar a *Pleurothallis gargantua* y comparte con esta las grandes plantas que alcanzan 1 m en longitud, pero se diferencia en las flores mucho más pequeñas con el sépalo dorsal muy cóncavo, pétalos más estrechos, y un sinsépalo con márgenes fuertemente revolutos que le dan una forma alargada y triangular. Investigación de campo exhaustiva muestra que *Pleurothallis markgruinii* tiene una distribución restringida, al noroeste de los Andes en Ecuador y es evaluada como en peligro crítico.

Palabras clave. Carchi, minería, nueva especie de orquídea, Reserva Dracula, Reserva Youth Land Trust.

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Introduction

The genus *Pleurothallis* R.Br. was established in 1813 by Robert Brown when he separated *Pleurothallis ruscifolia* (Jacq.) R.Br. from *Epidendrum* L., in which it was originally described (Brown, 1813). It is a Neotropical genus with 550–600 species (Chase *et al.*, 2015; Karremans, 2016), which are distributed from Mexico to Brazil and central Bolivia, with the

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greatest diversity in Colombia and Ecuador (Luer, 2005). Plants of this genus grow mainly as epiphytes but may also grow terrestrially in a variety of ecosystems, from sea level up to 3500 m elevation (Dodson, 2003).

As *Pleurothallis* is one of the largest genera in subtribe *Pleurothallidinae*, various attempts have been made to reclassify the large number of species attributed to it, with many transferred to other sections, genera, subtribes and tribes (e.g. Lindley, 1859; Cogniaux, 1896; Luer, 1986, 1998; Szlachetko & Margonska, 2001; Luer, 2005). Molecular studies (Pridgeon *et al.*, 2001; Wilson *et al.*, 2011, 2013) support the recognition of a subsection *Macrophyllae–Fasciculatae* within the genus *Pleurothallis* rather than *Acronia*, and do not support raising the subsection to genus level (Wilson *et al.*, 2016).

Species of *Pleurothallis* subsect. *Macrophyllae–Fasciculatae* are characterised by sessile, usually cordate leaves held by well-developed ramicauls; single flowers produced successively or simultaneously from the apex of the ramicaul in a fascicle of peduncles/pedicels at the base of the leaf; connate lateral sepals fused into a synsepal that forms a bivalved flower with the dorsal sepal; 3-veined lip with a glenion; short, wingless column; and apical, transverse or transversely bilobed stigma (Luer, 2005). It is currently estimated that in Ecuador, where this new species was collected, there are currently around 150 species in *Pleurothallis* subsect. *Macrophyllae–Fasciculatae* (Zambrano *et al.*, 2017).

Within *Pleurothallis* subsect. *Macrophyllae–Fasciculatae*, there are a few species, namely *P. imperialis* Luer, *P. gargantua* Luer, *P. lutheri* (Luer) J.M.H.Shaw, *P. marthae* Luer & R.Escobar, *P. reginae* Garay and *P. teaguei* Luer., that we here call the Gargantua informal group. These have very tall ramicauls (up to 1 m), large leaves (13–55 × 4–20 cm) and large flowers (5–10 × 3–7.8 cm) with a concave dorsal sepal projecting outwards, forming an angle with the synsepal. The petals are laterally bent 90° towards the synsepal and are convex to revolute and obtuse to rounded at the apex, and are facing each other underneath the lip. The lip is oblate, suborbicular to ovate, heavily reflexed at the apex, and the lateral margins form a deeply convex structure that projects outwards. All the species in this informal group are geographically restricted, and except for *Pleurothallis aguirrei* are distributed in the Western Andes of southern Colombia and northern Ecuador.

In accordance with the general species concept (de Queiroz, 2007), we describe and illustrate a new and threatened species of *Pleurothallis* subsect. *Macrophyllae–Fasciculatae*.

Materials and methods

Morphological analysis

During botanical expeditions in 2016 to characterise orchid diversity near the Dracula Reserve in Carchi province, the authors (M.F.M., L.E.B.) observed plants of the new species, without flowers. On successive expeditions to the area, plants of the new species were

observed in flower by the authors (M.F.M., L.E.B., G.A.I.). Material of the new species was collected under Environmental Research Permit no. MAATE-DBI-CM-2021-0187, issued by Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador. Vegetative parts were dried as herbarium material, and flowers were preserved in 70% ethanol and 1% glycerol. Floral and vegetative structures were photographed *in situ* and *ex situ*, using a Nikon D5100 camera with an AF-S Micro Nikkor 60 mm f/2 lens (both Nikon, Tokyo, Japan), and a Canon EOS T6 camera with a Canon EF-S 35/28 Macro lens (both Canon, Tokyo, Japan). Fertile material (dried and spirit) and photographs of the new species were compared with specimens and images of all known species in the genus.

A morphological review of all very tall, large-flowered species of *Pleurothallis* subsect. *Macrophyllae–Fasciculatae* was carried out to establish if the new collection was indeed an undescribed species.

Botanical descriptions follow Lindley (1951) and Luer (1986) to standardise botanical terms, including colours. Figures and the composite digital line drawing were prepared, based on the type specimen images, using Adobe Photoshop 2019 (Adobe, San Jose, CA, USA). Conservation assessments were made in accordance with the IUCN criteria (IUCN Standards and Petitions Subcommittee, 2019) and using the GeoCAT tool (Bachman *et al.*, 2011).

Molecular analysis

Genomic DNA was isolated from fresh leaf samples following the rapid extraction procedure of Kasajima *et al.* (2004). PCR amplification was performed using 7.5 μ L of GoTaq Green Master Mix 2X (Promega, Madison, WI, USA), 3 μ L of extracted DNA, 1 μ L of each primer (0.75 μ M), and 7.5 μ L of ultrapure water (primer and specimen information is shown in Tables 1 and 2, respectively). PCR conditions were standardised at 95°C for 2 min, 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, 35 cycles, and a final extension of 72°C for 15 min. PCR products were purified and Sanger-sequenced in an ABI 3500xL Genetic Analyzer, Applied Biosystems (Waltham, MA, USA). Three sequences for each species were concatenated using Geneious Prime 2022.1. The consensus sequences were aligned using the Geneious alignment tool (using default parameters) for final comparison.

Table 1. Primers used in the present study

Gene	Primer	Sequence	Reference
rITS	ITS1	TCCGTAGGTGAACCTGCGG	Vijayan & Tsou (2010)
	ITS4	TCCTCCGCTATTGATATGC	Vijayan & Tsou (2010)
<i>matK</i>	56F	ACTTCCTCTATCCGCTACTCCTT	Williams <i>et al.</i> (2005)
	2.1a-Fw	ATCCATCTGGAAATCTTAGTTC	Vijayan & Tsou (2010)
	2.1a-Rv	GTTCTAGCACAAAGAAAGTCG	Vijayan & Tsou (2010)

Table 2. Voucher and GenBank accession numbers for the *Pleurothallis* specimens used in the present study

Taxon	Voucher no.	Herbarium	GenBank accession no.	
			rITS	matK
<i>P. markgruinii</i>	MFM246	QCNE	OR141753	OR142643
<i>P. gargantua</i>	MFM239	QCNE	OR141754	OR142640
<i>P. aff. imperialis</i>	MFM243	QCNE	OR141752	OR142639
<i>P. aff. marthae</i>	GI-LB-2306.1	QCNE	OR141755	OR142642
<i>P. teaguei</i>	GI-LB-2306.2	QCNE	OR141756	OR142641

Biogeographical analysis

The type and additional collections for the eight species of the *Gargantua* group (including the new species) were mapped to understand the distribution of this group of species. The extent of occurrence and the area of occupancy of the new species were calculated and mapped using ArcGIS (GIS software version 10.8; Environmental Systems Research Institute, Inc., Redlands, CA, USA, <https://www.esri.com/>).

Results

Morphological and molecular data support the recognition of the new collections as a distinct and, until now, undescribed species.

Morphological analysis

The species of *Pleurothallis* subsect. *Macrophyllae–Fasciculatae* that are particularly tall, including our new species, have five morphological features that seem to be constant phenotypic expressions: the overall shape of the leaves and basal lobes of the leaves; the number of simultaneous flowers; the length of the flower peduncles; the folding at the margins of petals and sepals; and pigmentation patterns (colours, contrast and saturation) in sepals and petals (see [Table 3](#)). In the new species, the leaf is ovate with deep conspicuous basal lobes (commonly one overlapping the other), there are few (frequently two) simultaneous flowers, the flower peduncles are short, the synsepal is always heavily folded in a revolute manner, the dorsal sepal always has a slightly revolute margin, and the pigmentation pattern is constant, with a darker synsepal compared with clearer dorsal sepal, petals and lip.

Molecular analysis

Molecular data based on the genes rITS and *matK* show a difference between our new species and the morphologically and geographically most similar species, *Pleurothallis gargantua*. The new species forms a distinct lineage from the rest of the species of

the Gargantua group and is sustained by several lines of evidence, in particular a four-base difference in its rITS and a lack of a duplication of nine bases in *matK* present in *Pleurothallis gargantua* (as well as *P. teaguei* and *Pleurothallis* aff. *marthae* but not *Pleurothallis* aff. *imperialis*) (Figure 1).

Biogeographical analysis

All the species in the informal Gargantua group have limited, rather highly endemic distributions, occurring between southern Colombia and northern Ecuador in the western Andes (except *Pleurothallis aguirrei*, from the southeastern Andes of Colombia). Fieldwork in Ecuador over the past 15 years focusing on this group of *Pleurothallis* species has found no evidence of sympatry, with none of the species found growing next to each other (see Figure 6). The most geographically distant species within the Gargantua group are *Pleurothallis marthae* (the most northern species in the group) and *P. lutheri*, growing farther south. More than 50 individual plants of our new species have been found growing between these two localities.

Among the populations of *Pleurothallis gargantua* and our new species, the much more modestly sized *Pleurothallis* aff. *imperialis* has been found nearby (see Figure 6). Populations of our new species and *Pleurothallis gargantua* are isolated, and *Pleurothallis* aff. *imperialis* is distributed between both species. No plants of any of these three species have been found intermixed in the same forest. The new species is vegetatively similar to *Pleurothallis gargantua*, *P. lutheri* and *P. reginae*. The broadly cordate leaves and smaller flowers of *Pleurothallis marthae* immediately distinguishes it from the new species. *Pleurothallis reginae*, with its glandulose-puberulent sepals at the adaxial side,

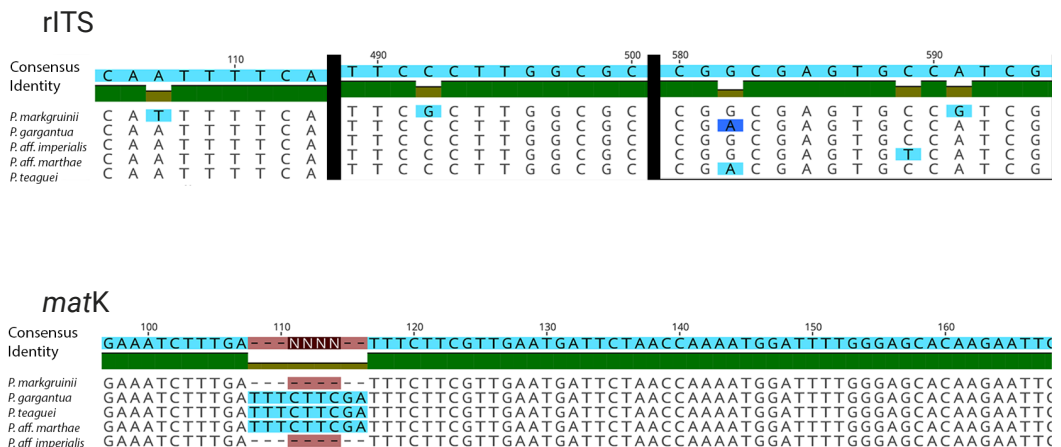


Figure 1. Comparison of sequences of rITS and *matK* between the new species and closely related species, including the morphologically most similar species *Pleurothallis gargantua* Luer.

and its distant geographical distribution from that of the new species, makes it easily distinguishable.

Taxonomic treatment

Species description

***Pleurothallis markgruinii* Baquero & M.F. Monteros, sp. nov.**

This new species is most similar to *Pleurothallis gargantua* in the large size of the plants (up to 1 m tall) and the large flowers (among the largest in the genus, between 5.6 and 10 cm long) borne at the base of a large (up to 30 cm long) ovate leaf, but it differs from that species in its considerably smaller flowers, 6.1 cm (vs 10.5 cm long when fully extended); the deeply concave, red-brown dorsal sepal (vs slightly concave light green suffused with rose dorsal sepal); the narrower petals, 2 times the length compared with the width, 1.5 × 0.75 cm (vs the broader petals, 1.5 times the length compared with the width, 2 × 1.2 cm); the triangular synsepal with heavily revolute margins (vs ovate synsepal); and the rugulose-verrucose texture of the lip (vs glabrous-rugulose). – Type: Ecuador, Carchi, along the road from El Carmen to Chical, 0°49'39"N, 78°12'39"W, 1700 m, 4 iii 2016, *Luis E. Baquero* LB-3071 (holotype QCNE!). [Figures 2, 3A, 4](#) and [5](#).

Caespitose epiphyte over 1 m tall. Roots slender, 1.5 mm in diameter. *Ramicauls* stout, erect to suberect, up to 1 m long, with three tight, tubular sheaths borne from the base towards the apex. *Leaf* spreading, coriaceous, centrally channelled, ovate, acute, acuminate 25–30 × 14–17 cm, base sessile, deeply cordate, basal lobes 3 cm deep, margin entire. *Inflorescence* a fascicle of one (or few) simultaneous flowers with later successive, resupinate, borne on top of the leaf, flowers produced from a spathaceous bract, c.2 cm long; peduncle 1–1.5 cm long concealed within the spathe, floral bract 8 mm long; pedicel clavate, straight, c.1.2 cm long. *Flower* cream-coloured suffused with brown-red and red-purple, 5.5–6.1 × 2.5–3 cm. *Ovary* 8 mm long, slightly grooved. *Dorsal sepal* cream-coloured to yellowish green suffused with red-brown, with darker red-brown veins, glabrous, ovate, obtuse, deeply concave at the basal half, the apical half flat to slightly convex, the margins from slightly revolute at the base to markedly revolute towards the apex, 3.2–3.4 × 2.6–2.8 cm, 19-veined. *Lateral sepals* red-purple, verrucose, connate into a narrowly triangular, acute synsepal, the margins strongly revolute, 2.4–2.7 × 1.8–2 cm, 16-veined. *Petals* red-brown, verrucose-papillose, oblong, obtuse, slightly falcate at the truncate base, slightly convex, 1.5 × 0.75 cm, 7-veined. *Lip* red-brown, orange-yellow towards the base, rugulose-verrucose, transversely obovate-spathulate, convex, acute, 8.5 × 9.5 mm, with three swellings at the apex that abruptly turn into two swellings towards the mid-third, separated by a deep sulcus that gradually disappears towards the basal third, the disc with a low, yellow slightly suffused with rose, ovoid, 1 × 1.3 mm callus, the base truncate, hinged at the flattened base of the column-foot.

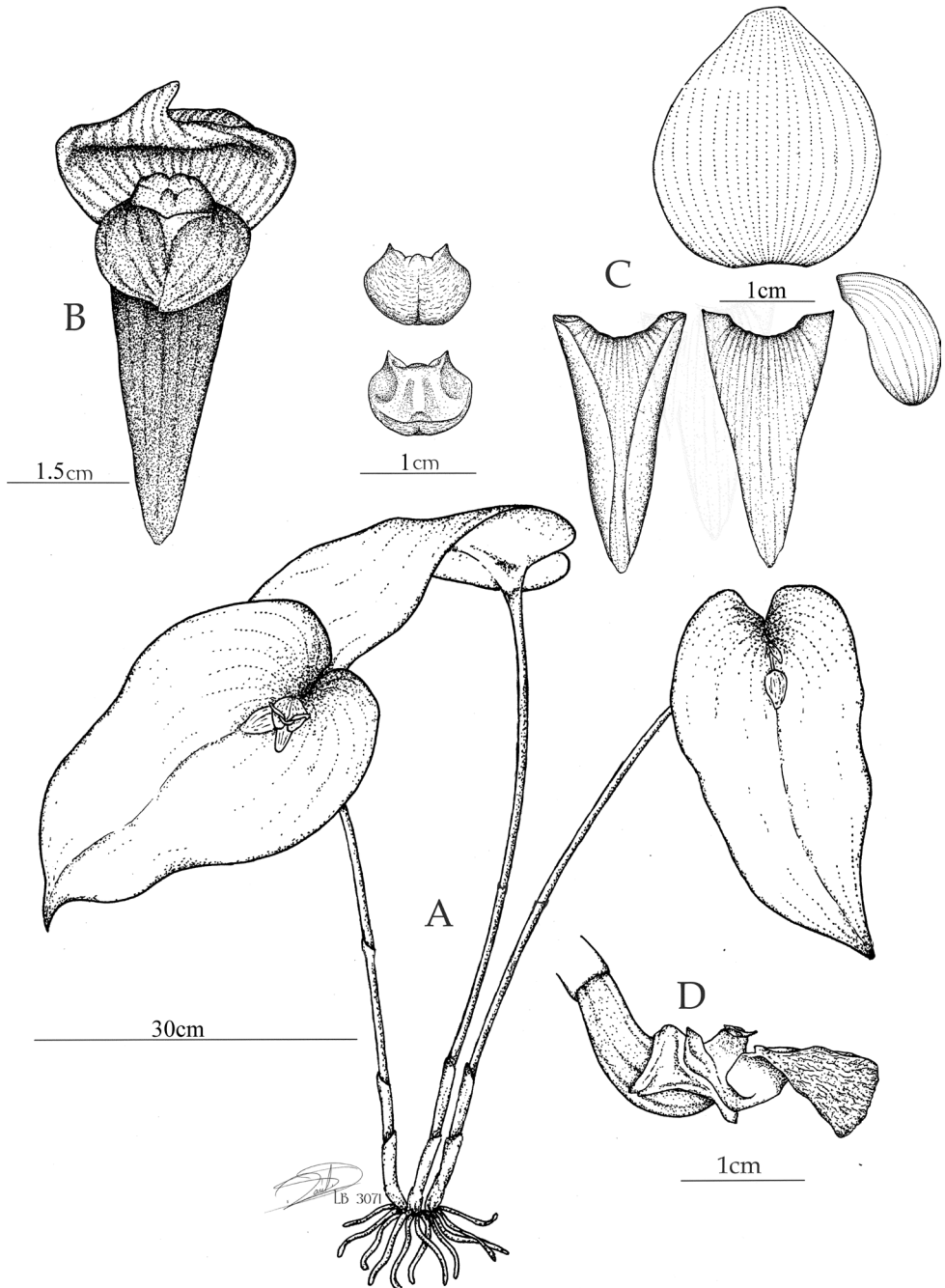


Figure 2. *Pleurothallis markgruinii* Baquero & M.F.Monteros, sp nov. A, Habit; B, flower; C, dissected perianth, including adaxial and abaxial views of synsepal and lip; D, column and lip, lateral view. Drawn from the holotype (Luis E. Baquero LB-3071, QCNE) by Raul Dueñas.

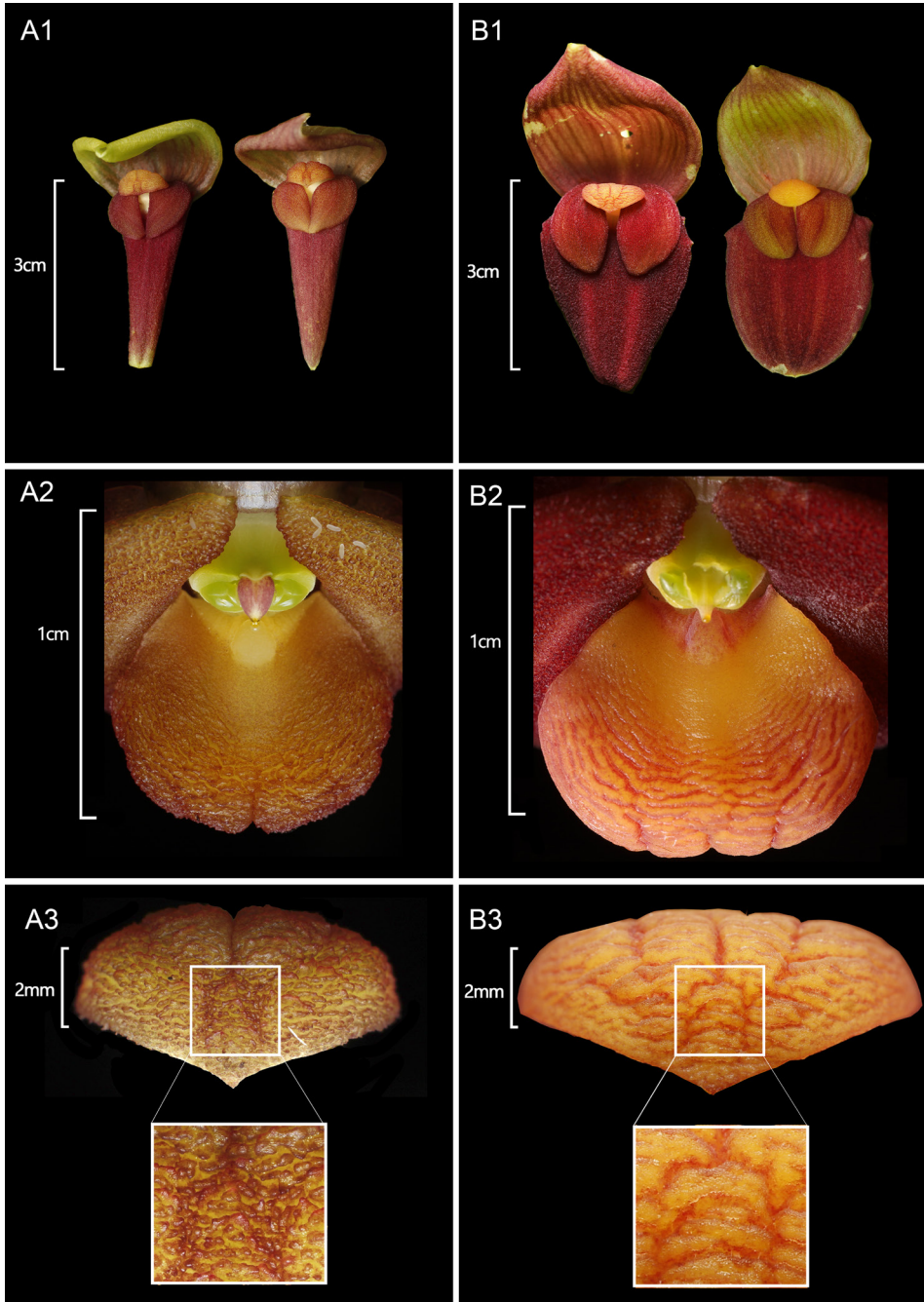


Figure 3. Comparison between *Pleurothallis markgrunii* Baquero & M.F.Monteros (A) and *P. gargantua* Luer (B). A1 and B1, Frontal view of 2-flower variant; A2 and B2, close-up of lip; A3 and B3, texture of lip. Photographs: Marco F. Monteros (A1–A3, B2 and B3), Luis Baquero (B1).

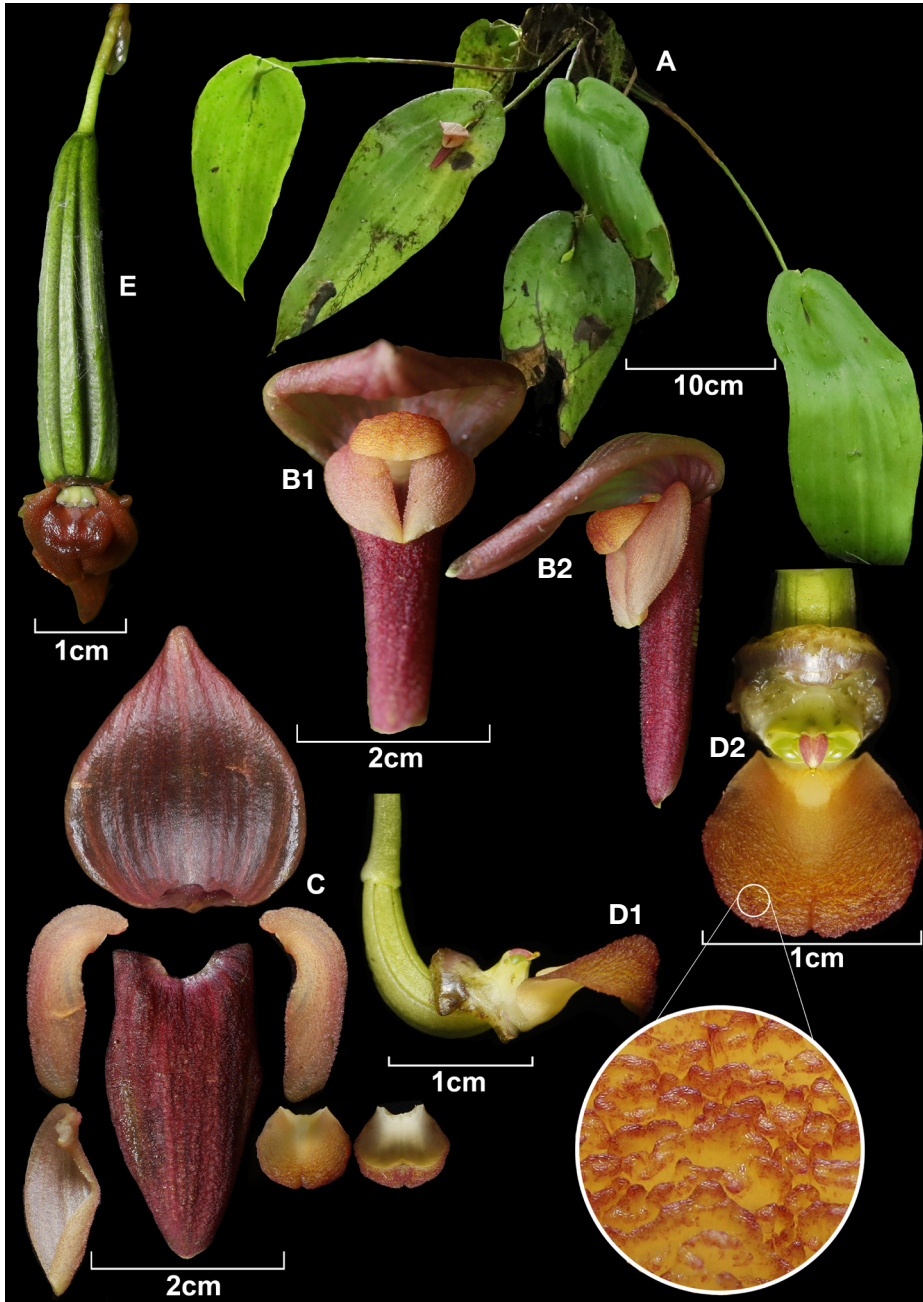


Figure 4. Composite dissection plate for *Pleurothallis markgrunii* Baquero & M.F.Monteros, sp. nov. A, Habit; B1, flower, frontal view; B2, flower, lateral view; C, dissected perianth; D1, lip and column, lateral view; D2, lip and column, dorsal view with magnified image showing the rugulose-verrucose texture of the lip; E, capsule, dorsal view. Photographs: Marco F. Monteros.

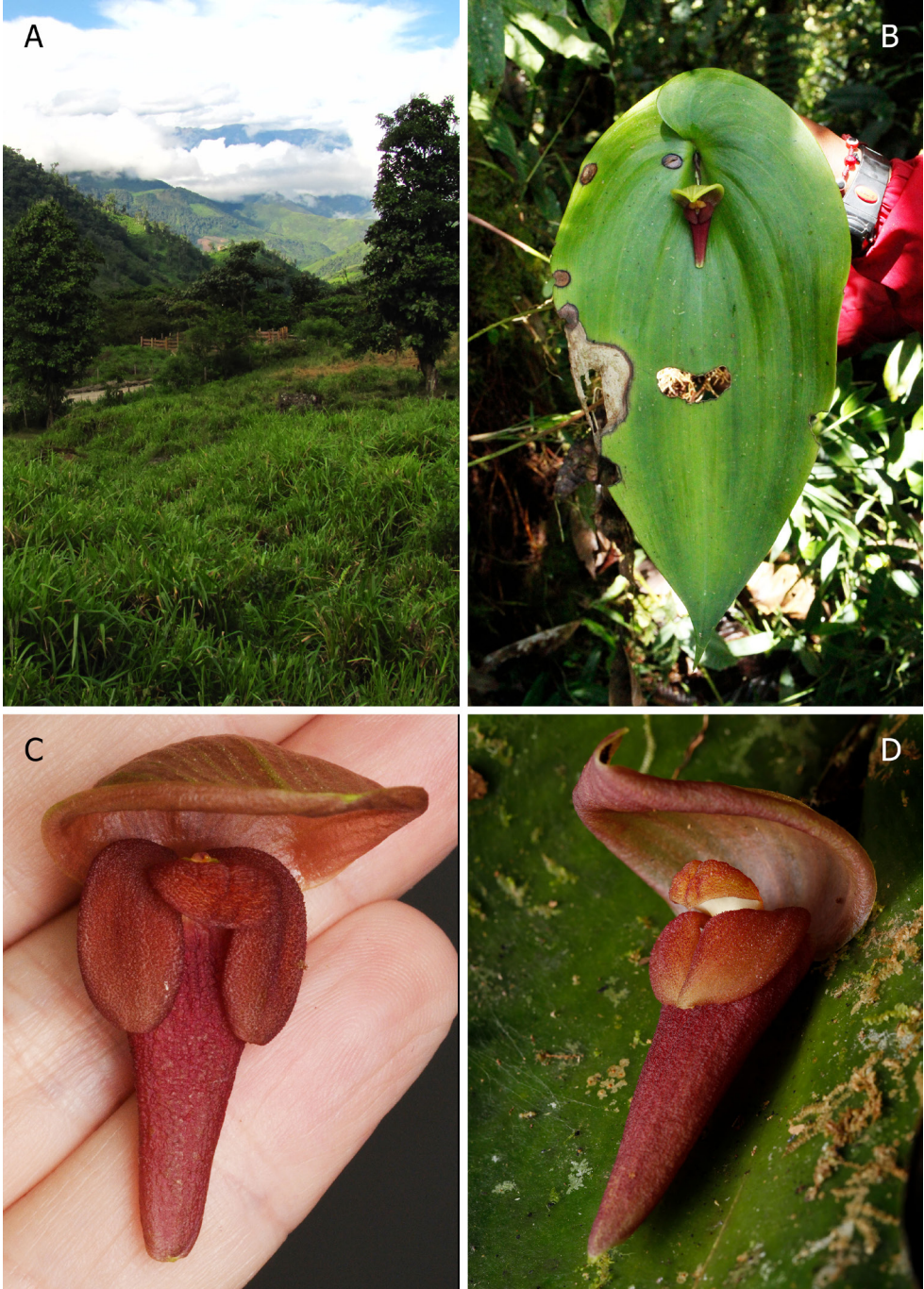


Figure 5. *Pleurothallis markgruinii* Baquero & M.F.Monteros, sp. nov. A, Habitat; B–D, flower *in situ*. Photographs: Maro F. Monteros.

Column pale yellow suffused with rose degrading stripes, stout, semi-terete, 2 × 4 mm. *Anther* cap yellow suffused with rose, cordiform, glabrous, apical stigma, bilobed. *Pollinia* 2, ovoid.

Distribution. Cloud forests of northwestern Ecuador, near the limit of Fundación EcoMinga's Dracula Reserve, around 1700 m in elevation. According to the Ministerio del Ambiente del Ecuador (2013), this ecosystem is classified as lower montane evergreen forest of the western Andes (BSBN04). So far, five populations have been found to the southwest of Cerro Golondrinas; all the plants were found growing epiphytically (Figure 6).

Habitat and ecology. Flowering *in situ* between March–April and August. Field observation of dozens of plants over more than 5 years has found this species growing as an epiphyte on branches and occasionally tree trunks (*Pleurothallis gargantua* has always been found growing on embankments or at the base of trees instead of at the above-ground level on branches or tree trunks).

Pleurothallis markgruinii shares its habitat with other Pleurothallidinae, such as *Andinia pilosella* (Rchb.f.) Karremans & S.Vieira-Uribe, *Platystele alucitae* Luer, *Platystele pamela* Baquero & Zuchan and *Trisetella vittata* (Luer) Luer, and other species of orchids, such as *Dichaea morrisii* Fawc. & Rendle, *Miltoniopsis vexillaria* (Rchb.f.) God.-Leb. and *Telipogon maldonadoensis* Dodson & R. Escobar. The forests in which it is found are poorly researched, as evidenced by the recent description of *Pleurothallis pamela* Baquero & Zuchan and the number of unidentified taxa awaiting description.

Etymology. Named in memory of Mark Gruin, horticulturist, curator at Zoo America, and benefactor at the Rainforest Trust, who devoted the bulk of his life's work to protecting nature and supporting budding conservationists.

Proposed IUCN conservation category. The extent of occurrence and area of occupancy are 7.43 and 16 km², respectively. *Pleurothallis markgruinii* is restricted to moist cloud forests where there is an immediate threat from mining activities. Although the species is found in the megadiverse Tropical Andes hotspot in the Ecuadorian Cordillera Occidental biogeographical sector (see Figure 6), it is not in a protected area, and illegal extraction of species is occurring (Guayasamin et al., 2021; Monteros et al., 2021; Yáñez-Muñoz et al., 2021). We suggest that *Pleurothallis markgruinii* is classified as 'Critically Endangered' following the criteria B1b(iii)(iv) IUCN (2019).

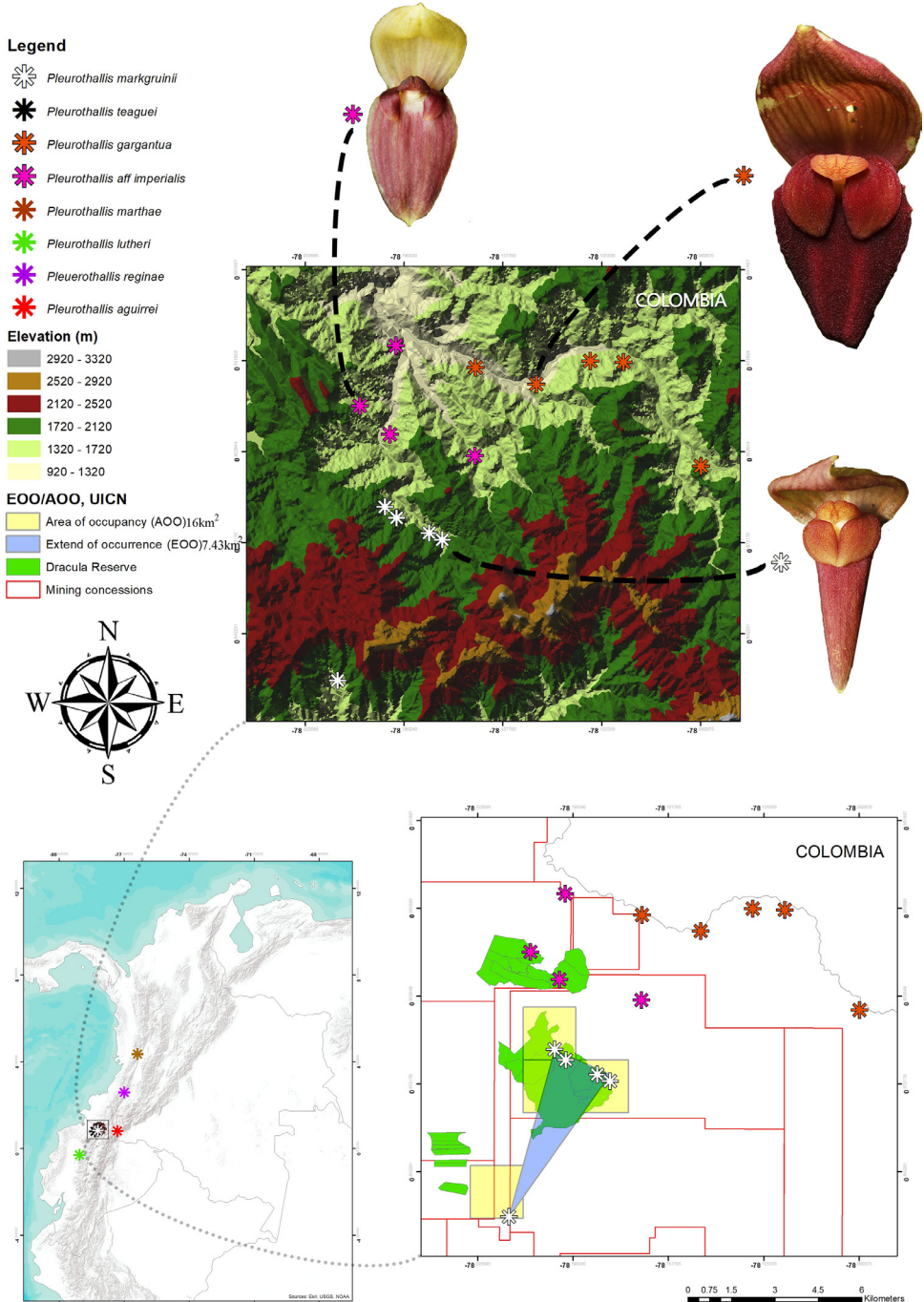
Notes. In Table 3, the characteristics of *Pleurothallis markgruinii* are presented alongside those of other members of the Gargantua group. Besides the much smaller size of the flowers, almost half as long as the flowers of *Pleurothallis gargantua*, *P. markgruinii* also differs in the colour and distribution of dark pigmentation of the flowers, magenta with red-brown, the shape of the narrowly triangular synsepal, with strongly revolute margins, and the deeply concave dorsal sepal, when compared with the red-brown, broadly ovate

Table 3. Principal characteristics distinguishing *Pleurothallis markgruinii* Baquero & M.F.Monteros, sp nov, from *P. gargantua* (to which it is most similar) and other members of the informal Gargantua group of *Pleurothallis* species

Character	<i>P. markgruinii</i>	<i>P. gargantua</i>	<i>P. aguirrei</i>	<i>P. imperialis</i>	<i>P. lutheri</i>	<i>P. marthae</i>	<i>P. reginae</i>	<i>P. teaguei</i>
Ramical	Up to 100 cm	Up to 100 cm	10–19 cm	20–55 cm	Approximately 50 cm	15–70 cm	Up to 50 cm	30–50 cm
Leaves								
Shape	Ovate, acute, acuminate, base deeply cordate	Broadly ovate, acute, base deeply cordate	Narrowly ovate, acute, base cordate	Ovate, acute, acuminate, base deeply cordate	Ovate, acute, slightly acuminate, base shallowly cordate	Broadly cordate-ovate, obtuse, shortly acuminate, base deeply cordate	Ovate-oblong, acuminate, base deeply cordate	Cordate-ovate, acute, base deeply cordate
Size (cm)	25–30 × 14–17	25–35 × 18–20	14–18 × 5–7	13–22 × 4–9	32 × 15	15–25 × 10–21	40 × 21	25–30 × 12–16
Inflorescence	One (or few) simultaneous flowers	One (few) simultaneous flowers	One (few) simultaneous flowers	One (few) simultaneous flowers	Several simultaneous flowers	Several simultaneous flowers	Several simultaneous flowers	Several simultaneous flowers
Dorsal sepal								
Size (mm)	32–34 × 26–28	50–55 × 38–38	30 × 17	26–30 × 17–20	25 × 20	18 × 13	30 × 20	28–35 × 28–30
Colour	Cream-coloured to yellowish green suffused with red-brown	Light green suffused with rose	Pale yellow	Light yellow green suffused with rose	Purple-brown	Rose	Dark brown	Dark purple
Texture	Glabrous	Glabrous	Glabrous to microscopically cellular-glandular	Glabrous	Glabrous	Glabrous	Glandulose-pubescent externally	Glabrous
Form	Ovate, obtuse, margins revolute	Ovate, subacute	Ovate	Broadly ovate, subacute to obtuse	Broadly ovate, subacute to obtuse	Broadly ovate, obtuse	Broadly ovate-lanceolate, acuminate	Broadly ovoid, obtuse
Veins	19-veined	19-veined	13-veined (faint)	Multiveined	Multiveined	15-veined	Multiveined	13- to 15-veined
Synsepal								
Size (mm)	24–27 × 18–20	45–50 × 25	30 × 15	21–30 × 15–16	25 × 15	16 × 14	25 × 17	27–32 × 18–20
Colour	Red-purple	Red-brown except green crescent at base	Light rose	Light yellow-green, suffused with rose	Purple-brown	Dark rose	Dark brown	Dark purple
Texture	Verrucose	Covered by minute blackish red papillae	Glabrous to microscopically cellular-glandular	Glabrous	Spiculate-pubescent above the middle	Glabrous externally, minutely papillose within	Glandulose-pubescent externally	Glabrous
Form	Narrowly triangular, acute, margins strongly revolute	Ovate, subacute	Ovate, acute	Ovate, subacute	Ovate, subacute	Cordate-ovate, obtuse	Broadly ovate-lanceolate, apex bidentate	Ovate, subacute

Table 3 (continued)

Character	<i>P. marigrainii</i>	<i>P. gargantua</i>	<i>P. aquirrei</i>	<i>P. imperialis</i>	<i>P. lutheri</i>	<i>P. marthae</i>	<i>P. reginae</i>	<i>P. teaguei</i>	
Veins	16-veined	18- to 20-veined	10-veined	Multiveined	Multiveined	14-veined	Multiveined	8-veined	
Petals									
Size (mm)	15 × 7.5	20 × 12	11.5 × 4.25	11–13 × 4.5–5	13 × 7.5	11 × 7.5	15 × 8–10	13.5 × 3.5 (unexpanded) Light yellow Glabrous	
Colour	Red-brown	Red-brown	Yellow	Purple-brown	Purple-brown	Rose	Not stated		
Texture	Verrucose-papillose	Covered by minute blackish red papillae	Minutely diffusely papillose	Glabrous, margins microscopically papillose	Glabrous except for spiculate medial third	Papillose on outer half, dense spiculate on inner half	With densely papillose nerves		
Form	Oblong, obtuse, slightly falcate at the truncate base, slightly convex	Thick, oblong, obtuse	Flat, broadly oblong	Oblong, convex, subfalcate, obtuse	Oblong, convex, subfalcate, apex rounded, median margins in apposition	Ovate, oblique, apex rounded, convex	Obliquely unguiculate at base, oblong-ovate, obtuse, mucronate apex	Tubular, convolute, obovate, obtuse	
Veins	7-veined	7-veined	3-veined	3-veined	7-veined	Not stated	3-veined	Not stated	
Lip									
Size (mm)	8.5 × 9.5	8.5 × 12	6.5 × 8 (unexpanded)	5–6 × 8	5–6 × 6	5 × 7	8 × 10	8 × 12	
Colour	Red-brown, orange-yellow at base	Light yellow	Yellow	Dark purple	Dark purple-brown	Rose	Not stated	Light yellow	
Texture	Rugulose- verrucose	Glabrous- rugulose	Glabrous	Glabrous	Glabrous	Transversely rugose-verrucose on convex surface above the middle, smooth below the middle	Transversely densely verrucose	Apex shallowly foveolate	
Form	Transversely obovate-spathulate, convex, acute, 3 swellings at the apex abruptly turn into 2, with a low, yellow, ovoid callus	Transversely ovate, reniform, convex, apex transverse with 2 convex, cheek-like swellings, with low, white, triangular callus	Obovate, apex broadly rounded, acutely revolute above the middle	Transversely ovate-reniform, apex broadly convex, pouch-like, involute margins, large glenion at base	Suborbicular, broadly convex, pouch-like, transversely rugose above the middle, margins involute, large glenion at base	Convex, transversely ovate, apex minutely apiculate, and acute tip deflexed	Convex, transversely the base shortly unguate, the tip acuminate	Suborbicular, transversely the base shortly unguate, the tip acuminate	Transversely obovoid, apex broadly rounded, revolute



synsepal and a light green suffused with rose and shallowly concave dorsal sepal of the flowers of *P. gargantua*. Another substantial difference is the narrower petals with 7 nerves vs the 11-nerved and rounder and broader petals of *Pleurothallis gargantua* (Figures 2–4). *Pleurothallis markgruinii* has the third largest flower of the Gargantua group of species, after *P. gargantua* and *P. teaguei*.

Considering that *Pleurothallis gargantua*, *Pleurothallis* aff. *imperialis* and *P. markgruinii* grow in similar cloud-forest ecosystems, it is possible that there may be different pollinators for each species. This would explain the genetic isolation between the three species, although more pollinator–plant studies are needed to confirm this. It is striking that no overlapping populations have been found among the three species, whose populations grow very close to each other and in very similar ecosystems (see Figure 6). After analysing many flowers from individual plants, no intermediate forms of *Pleurothallis* between *P. gargantua* and *P. markgruinii* have been found.

Additional specimens examined. Paratype: ECUADOR. Carchi, at the base of Cerro Golondrinas, 0°52'48"N, 78°11'34"W, 1729 m, 7 viii 2022, Marco F. Monteros MFM 238 (QCNE!).

Key to the species of the informal Gargantua group of Pleurothallis species

- 1a. Sepals glabrous to microscopically cellular-glandular _____ 2
- 1b. Sepals glandular-pubescent externally _____ *P. reginae*
- 2a. Dorsal sepal < 50 mm long _____ 3
- 2b. Dorsal sepal ≥ 50 mm long _____ *P. gargantua*
- 3a. Ramicaul < 70 cm long _____ 4
- 3b. Ramicaul ≥ 70 cm long _____ *P. markgruinii*
- 4a. Leaves 27.5–31 cm long _____ 5
- 4b. Leaves 13–25 cm long _____ 6
- 5a. Petals tubular, convolute, obovate, obtuse _____ *P. teaguei*
- 5b. Petals oblong, convex, subfalcate, apex rounded _____ *P. lutheri*
- 6a. Leaves narrowly ovate to ovate _____ 7
- 6b. Leaves broadly cordate-ovate _____ *P. marthae*
- 7a. Petals yellow, minutely diffusely papillose _____ *P. aguirrei*
- 7b. Petals purple-brown, glabrous, margins microscopically papillose _____ *P. imperialis*

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