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Source: *Systematic Botany*, 36(2):261-267. 2011.

Published By: The American Society of Plant Taxonomists

URL: <http://www.bioone.org/doi/full/10.1600/036364411X569462>

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A New Species of *Galeoglossum* (Orchidaceae, Cranichidinae) from Oaxaca, Mexico

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Communicating Editor: Kenneth M. Cameron

Abstract—*Galeoglossum cactorum*, a new species of Orchidaceae from the Mixteca Alta region in the state of Oaxaca, Mexico, is described and illustrated. The new species is florally similar to *G. thysanochilum* but differs from it in the linear-oblongolate leaves subtended by imbricating, dark brown cataphylls, presence of a papillose thickening near the base of the labellum, proportionately shorter, obtuse column with lateral laminar wings and complete fusion of the filament, and prominent apicule of the rostellum remnant. A cladistic parsimony analysis of DNA sequences of nuclear ITS region and plastid *matK-trnK* region showed that the three currently recognized species of *Galeoglossum* form a monophyletic group, which is sister to *Prescottia*. *Galeoglossum cactorum* is known from a single locality in an unusual habitat for orchids, i.e. a xerophilous scrub dominated by columnar cacti of the genus *Neobuxbaumia*, whereas both *G. thysanochilum* and *G. tubulosum* dwell in oak- and pine-oak forest.

Keywords—Endemism, *Galeoglossum cactorum*, *matK-trnK*, Mixteca Alta, nrITS, phylogenetics.

The genus *Galeoglossum* was proposed by Richard and Galeotti (1845) to include a single species, *G. prescottiioides* A. Rich. & Galeotti. The meagre original description and the fact that no specimens were associated to the original description by its authors made it difficult to interpret the identity of this plant for over a century and a half. Williams (1939, 1951) considered *G. prescottiioides* as a synonym of *Prescottia stachyodes* (Sw.) Lindl. and accordingly sunk *Galeoglossum* in *Prescottia* Lindl. However, recently Salazar (2009) resurrected the genus *Galeoglossum*, selected a lectotype for *G. prescottiioides*, and transferred to *Galeoglossum* the two species previously known as *Prescottia tubulosa* (Lindl.) L. O. Williams and *Pseudocranichis thysanochila* (B. L. Rob. & Greenm.) Garay, treating *G. prescottiioides* as a synonym of *G. tubulosum* (Lindl.) Salazar & Soto Arenas. The two species included in *Galeoglossum* by Salazar (2009), namely *G. thysanochilum* (B. L. Rob. & Greenm.) Salazar and *G. tubulosum*, have been shown to be sisters in several recently published molecular phylogenetic analyses (Figueroa et al. 2008; Álvarez-Molina and Cameron 2009; Salazar et al. 2009). Despite the gross morphological differences between these species, as pointed out by Álvarez-Molina and Cameron (2009), their sister relationship is supported by the following unique synapomorphies: apically lobed labellum with incurved sides, column with two lateral receptive stigmatic areas separated by a sterile central area, and two hairpin-like pollinia (Salazar 2009; Salazar et al. 2009). In contrast, in all species of *Prescottia* Lindl., the sister genus of *Galeoglossum*, the labellum lacks apical lobing and is conspicuously calceolate; there is a single receptive stigmatic surface located on the ventral surface of the column, and the four pollinia are ovate (e.g. Ackerman 2003; Azevedo 2009).

The geographical distribution and habitat preferences of the two hitherto known species of *Galeoglossum* only partially overlap. On the one hand, *G. tubulosum* is widespread in most mountain ranges of Mexico and adjacent Guatemala, inhabiting a variety of soil types in oak- and pine-oak forests on both volcanic and limestone substrates, and occasionally found epiphytically on oaks. On the other hand, *G. thysanochilum* is endemic to the Mixteca region and adjacent Tehuacán-Cuicatlán valley in Oaxaca; it is also found in

oak and pine-oak forests but apparently it prefers chalky soils on a limestone substrate (Soto and Salazar 2004; Salazar et al. 2006). Because of its restricted geographical distribution and sparse populations, *G. thysanochilum* is included in the list of rare and threatened Mexican plants (as *Pseudocranichis thysanochila*) under the category of “subjected to special protection/endemic” (SEMARNAT 2002). In at least one locality, both *G. thysanochilum* and *G. tubulosum* have been found living side by side, and in such circumstance reproductive isolation seems to be promoted by differences in the flowering phenology and, likely, use of different pollinators, as suggested by the fact that the flowers of *G. thysanochilum* emit a pleasant diurnal fragrance whereas those of *G. tubulosum* produce a strong, disagreeable nocturnal odor that has been described as reminiscent of naphthalene or some insecticide (Salazar 2009).

In the course of botanical exploration conducted recently in the Mixteca Alta region of the Sierra Madre del Sur, state of Oaxaca, Mexico, an additional, previously undescribed species assignable to *Galeoglossum* was discovered. In the following we describe and illustrate the new species for the first time and compare it morphologically to its congeners. We also assess the phylogenetic relationships among the species of *Galeoglossum* by means of a cladistic analysis of plastid (*matK-trnK* region) and nuclear (nrITS) DNA sequence data.

MATERIALS AND METHODS

Morphological Observations—The morphological description of the new species was based on examination of several live plants, one of which flowered in cultivation and was subsequently pressed to serve as the holotype. Measurements of vegetative and floral parts were thus made on fresh material. Fresh flowers were dissected under a stereomicroscope (Stemi SV 6, Carl Zeiss) and photographed using a digital camera (Nikon Coolpix 5200).

Taxonomic Sampling—Individual exemplars of *Galeoglossum thysanochilum*, *G. tubulosum* and the new species described herein, as well as 13 species of nine additional genera of Cranichidinae s. l. (including five species of *Prescottia*) and seven species and genera of Spiranthinae, were analyzed to assess their phylogenetic relationships. Representatives of all other subtribes of Cranichideae were used as outgroups, following previous phylogenetic studies (Salazar et al. 2003, 2009; Figueroa et al. 2008; Álvarez-Molina and Cameron 2009). A list of the taxa studied with

information on voucher specimens and GenBank accessions is given in Appendix 1. The aligned data matrix is available from TreeBASE (study number 10653).

Molecular Methods—Extraction, amplification, and sequencing of DNA of the new species were carried out from fresh leaf tissue using standard protocols explained in Salazar et al. (2003) and Figueroa et al. (2008). All other sequences were previously generated by us for previous works (Salazar et al. 2003, 2009; Figueroa et al. 2008). Bidirectional sequencing reads were obtained for both DNA regions and the chromatograms were edited and assembled with Sequencher v. 4.8 (GeneCodes Corp., Ann Arbor, Michigan). Sequence alignment was carried out using the E-INS-i iterative strategy (Katoh et al. 2005) available in the online submission version of the program MAFFT version 6 (Katoh et al. 2009), with minor subsequent manual adjustments. No data were excluded from the analyses due to unambiguous alignment, and individual gap positions were treated as missing data.

Cladistic Analysis—Our previous phylogenetic studies on Crani- chideae have shown that the nrITS and *matK-trnK* regions recover similar patterns of relationships with no conflicting groups supported by the bootstrap, and their combination usually enhances both resolution and internal clade support (Salazar et al. 2003, 2009; Figueroa et al. 2008; Salazar and Dressler in press). Accordingly, in the present work we analyzed both data sets in combination using cladistic parsimony. The analysis was conducted with the computer program PAUP* version 4.02b for Macintosh (Swofford 2002) and consisted of a heuristic search with 1,000 replicates of random taxon addition for the starting trees and tree rearrangements using tree bisection-reconnection (“TBR”) branch swapping; the option “MULTREES” was activated to allow for multiple trees and no limits to the number of most parsimonious trees were imposed. All characters were treated as unordered and had equal weights (Fitch 1971). Internal support for clades was assessed by nonparametric bootstrapping (Felsenstein 1985), for which 300 bootstrap replicates were performed, each with 20 replicates with random taxon addition and TBR branch swapping, keeping up to 20 most-parsimonious trees from each replicate.

RESULTS

Cladistic Analysis—The aligned matrix of the nrITS and *trnK-matK* regions resulted in 2,717 characters, of which 965 (35%) were variable and 601 (22%) were potentially parsimony informative. The heuristic search found a single most parsimonious tree with a length of 2,216 steps, consistency index (excluding uninformative characters) = 0.5 and retention index = 0.63 (Fig. 1). Subtribes Spiranthinae and Cranichidinae s. l. are both monophyletic and they are strongly supported as sisters to one another. Within Cranichidinae, a “core Cranichidinae” clade (after Salazar et al. 2009), consisting of *Pterichis*, *Ponthieva*, and *Cranichis*, is the sister of a clade formed by all the genera formerly included in Prescotttiinae, which obtained bootstrap support (BS) < 50. The last group consists of two subclades: a strongly supported one (BS 100) including the high-Andean genera *Stenoptera*, *Altensteinia*, *Gomphichis*, *Porphyrostachys*, and *Aa*, and a weakly supported one (BS 68) encompassing *Galeoglossum* (BS 100) sister to *Prescottia* (BS 98). Within *Galeoglossum*, *G. tubulosum* is the sister of a weakly supported clade formed by the new species (referred to hereafter as *G. cactorum*; see Taxonomic Treatment, below) and *G. thysanochilum* (BS 59).

DISCUSSION

Salazar (2009) noted several unique structural attributes shared by *Galeoglossum thysanochilum* and *G. tubulosum*. These include the apically lobed labellum, the similarly shaped column, the peculiar stigma consisting of two lateral receptive areas separated by a central sterile surface, and the hairpin-like pollinia. *Galeoglossum cactorum* shares all these structural features (Figs. 2, 3; note that in Fig. 2N the two pollinia are broken at their apices, for which they look as if there were

four pollinia) and florally it is similar to *G. thysanochilum* (Fig. 2B-L), in agreement with their sister-species relationship found in our cladistic analysis (Fig. 1). However, *G. cactorum* differs from the two other species of the genus in the linear-oblancoolate leaves subtended by imbricating, dark brown cataphylls (Fig. 2A), presence of a papillose thickening near the base of the labellum (Fig. 2F), proportionately shorter, obtapezoid column with lateral laminar wings and complete fusion of the filament (Fig. 2G-J), and prominent apicule of the rostellum remnant (Fig. 2K). The pollinarium is as in *G. thysanochilum* (Fig. 2L; compare with Fig. 2I in Salazar 2009). The main features allowing for distinction of the three species we currently accept in *Galeoglossum* are summarized in Table 1.

TAXONOMIC TREATMENT

Galeoglossum cactorum Salazar et Chávez-Rendón, sp. nov. (Figs. 2, 3).—HOLOTYPE: MEXICO. Oaxaca: Distrito Tlaxiaco, municipio San Bartolomé Yucuañe, Cerro Jacaba, 6 km al sur del poblado sobre el camino de San Bartolomé Yucuañe a La Paz Tilantongo, 1708 m elev., collected 15 Aug 2008, flowered and pressed in cultivation 1 Oct 2008, *Chávez-Rendón, Avendaño & Sánchez 1604* (MEXU!).

Galeoglossum thysanochilo (B. L. Rob. & Greenm.) Salazar simile, a quo cataphyllis ad rosulae basem imbricatis, foliis lineari-oblancoolatis, labello prope basin excrescentia papillosa instructo, columna proportione brevior, obtapezoidea, aliis lateralibus decurvatis provista, filamento toto adnato et apiculo reliqui rostellis magis prominenti differt.

Geophytic, acaulescent herb ca. 18 cm in height above ground including the inflorescence. Roots fasciculate, fleshy, terete, dull yellow, to 5 cm long, 5–7 mm in diameter. Leaves 4–5, forming a rosette, present and functional at flowering time but subtended by several imbricating, brown, papery cataphylls that apparently represent the sheathing bases of the leaves of the previous growing season; functional leaves consisting of a sheathing basal portion and a blade; leaf sheaths white, imbricating, forming a “neck” covered by the cataphylls, to ca. 5.5 cm long; leaf blades shiny pale green above, opaque grayish green with three darker longitudinal veins below, somewhat fleshy, narrowly oblancoolate, acute, with the midvein somewhat prominent on the underside, 6–11 cm long, 8–18 mm wide. Inflorescence erect; peduncle ca. 11 cm long from the top of the rosette of leaves, ca. 2 mm in diameter, pale green, glabrous, with three tubular, loosely adpressed bracts, these pale green with white margins, glabrous, acute, 7–21 mm long; raceme dense, ca. 4 cm long and 2.5 cm in diameter, our specimen with 16 flowers that start opening successively, eventually there being three or four open at the same time. Floral bracts glabrous, ovate, acuminate, slightly concave, pale green with three darker veins and white margins, 7–10 mm long. Flowers nonresupinate, ascending, campanulate, white, with two yellowish green calli and a short green line in between on the distal one-third of the labellum (the intensity of the color of the calli is variable among flowers of the same inflorescence, probably depending on age), without a perceptible fragrance during the day. Sepals free. Dorsal sepal slightly concave at base, otherwise somewhat convex, lanceolate, rounded, 3-veined, 6.3 mm long, 3 mm wide. Lateral sepals slightly concave, obliquely lanceolate, acute, somewhat conduplicate at apex, 3-veined, 6.2 mm long,

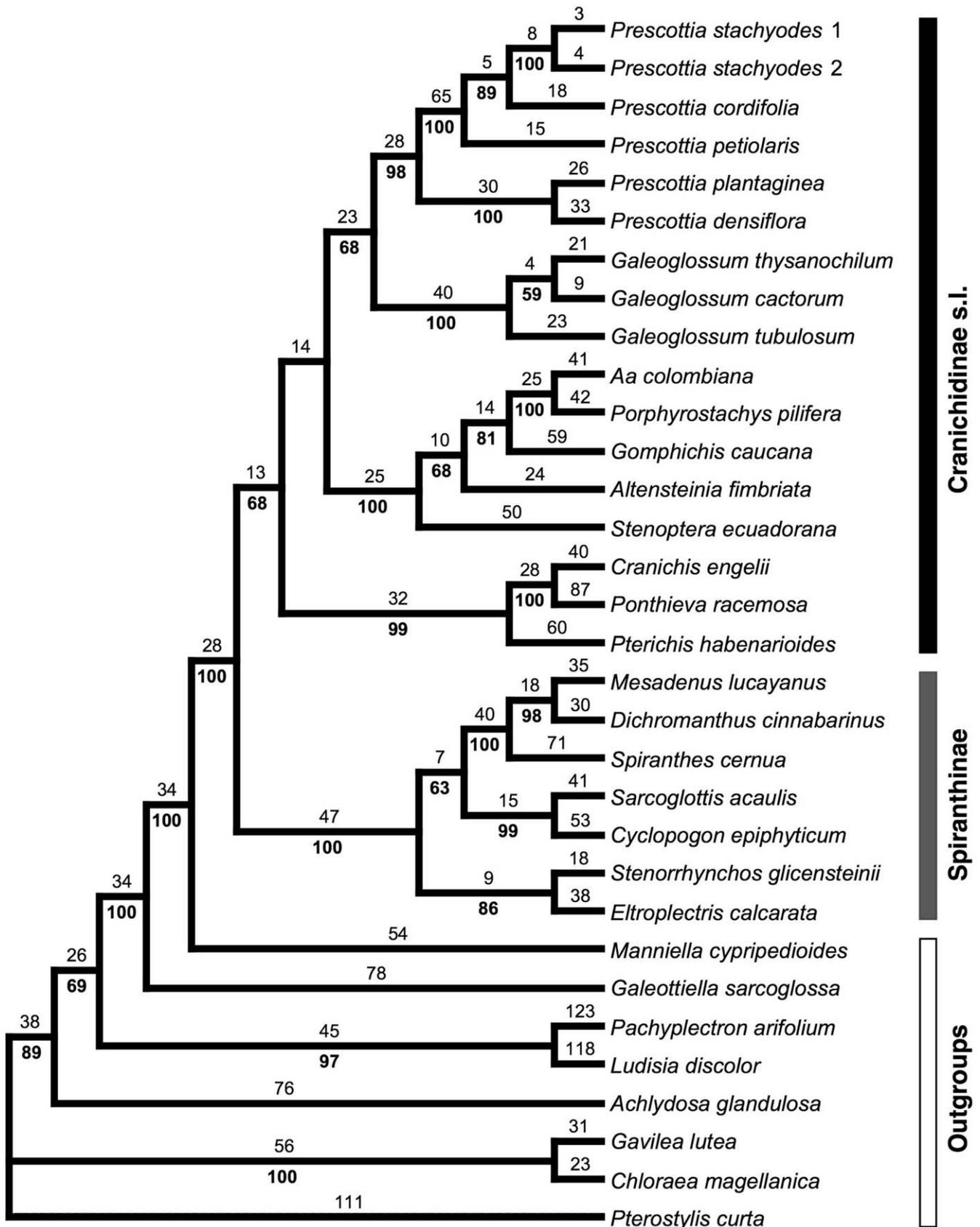


FIG. 1. Single most parsimonious tree found in the parsimony analysis of combined nrITS and plastid *matK-trnK* DNA sequences. Numbers above branches are branch lengths, numbers below branches are bootstrap percentages.

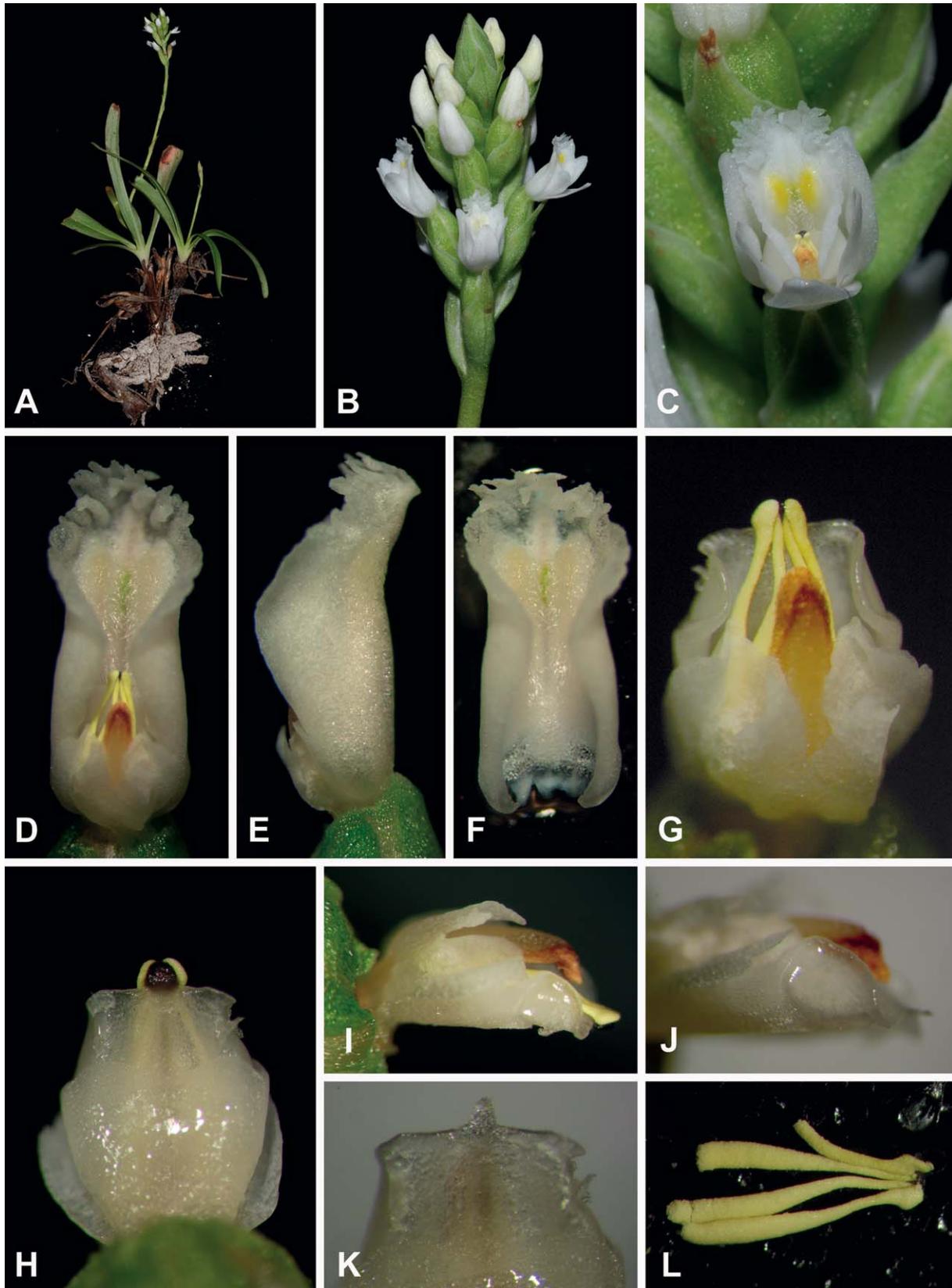


FIG. 2. *Galeoglossum cactorum*. A. Flowering plant removed from soil. B. Inflorescence. C. Flower. D. Labellum and column from above. E. Labellum and column from side. F. Labellum from above after removing the column. G. Column from above. H. Column from below. I. Column from side prior to removal of the pollinarium. J. Column from side after removal of the pollinarium, illuminated from the back to highlight the lateral flap and one of the two receptive areas of the stigma. K. Column apex from below after removal of the pollinarium showing the apiculate rostellum remnant and the nonreceptive area of the stigma. L. Pollinarium from above (the two pollinia broke at their apices during sample preparation). Scale bars: A = 10 cm; B = 10 mm; C = 5 mm; D-F = 2.5 mm; G-J, L = 1 mm; K = 0.5 mm. Photographs by G. A. Salazar from Chávez-Rendón *et al.* 1604.

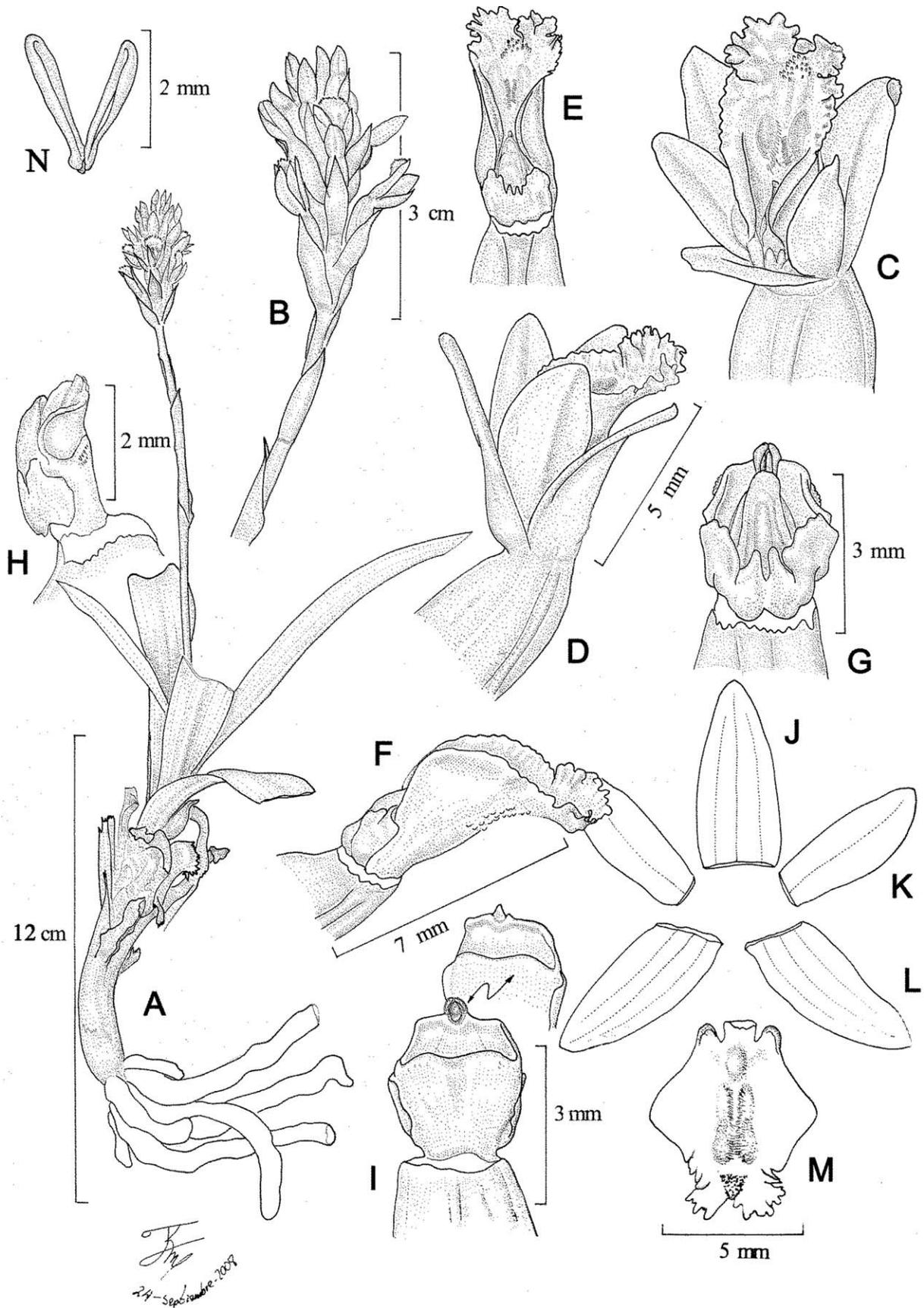


FIG. 3. *Galeoglossum cactorum*. A. Habit. B. Inflorescence. C. Flower from above. D. Flower from side. E. Labellum and column from above. F. Labellum and column from side. G. Column from above. H. Column from side. I. Column from below and detail of column apex after removal of the pollinarium, showing the apiculate rostellum remnant. J. Dorsal sepal. K. Petal. L. Lateral sepal. M. Labellum. N. Pollinarium from above. Drawn with camera lucida by Rolando Jiménez-Machorro from Chávez-Rendón et al. 1604.

TABLE 1. Comparison of the three known species of *Galeoglossum*.

Feature	<i>G. cactorum</i>	<i>G. thysanochilum</i>	<i>G. tubulosum</i>
Leaf shape	linear-oblongate	elliptic to ovate	obovate
Leaf condition at anthesis	fresh	fresh	withered or absent altogether
Flower color	white with green or yellow markings on the labellum	white with green or yellow markings on the labellum	Sepals and petals greenish, labellum yellow with base and apex whitish
Apical labellum lobe	prominent, dentate-fimbriate	prominent, dentate-fimbriate	minute, entire to denticulate
Papillose thickening near labellum base	present	absent	absent
Column	shortly obtrapezoid, with lateral decurved wings and fused filament	clavate, wingless, with partially free filament	clavate-cuneate, wingless, with fused filament
Apicule of rostellum remnant	distinctly prominent beyond the apical column margins	not prominent beyond the apical column margins	not prominent beyond the apical column margins

3 mm wide. Petals slightly concave, obliquely oblanceolate, obtuse, 1-veined, 6 mm long, 1.2–1.3 mm wide. Labellum with involute sides, free from the column but tightly embracing the column near its base, canaliculate and with two small elliptic areas of spherical, translucent papillae on the distal one-third of the underside; margins crenate near the middle, irregularly dentate near the apex; labellum base shortly clawed, with a rounded, concave-convex retrorse lobe at each side, each of these with a small marginal/apical thickening (nectar gland); inside the labellum at a short distance from the base there is a central, cushion-like, irregularly papillose callus, above the latter starts a central channel that ends at the base of the apical lobe between two colored calli of irregular surface; the channel is flanked along its whole length by long, translucent, retrorse hairs that reach the inner side of the two calli; labellum rhombic in outline when spread out, on the distal one-fourth distinctly narrowed and then expanded again into the apical lobe, the latter obcordate, ruffled, the margins irregularly dentate-fimbriate and sparsely papillose; whole labellum 6.5 mm long, 5.5 mm maximum width near the middle. Column white, shortly obtrapezoid, ventrally broadly channeled and covered by nectar, 2.5 mm long, 2 mm wide, provided with an inconspicuous column foot at base and a prominent, slightly downcurved flap or wing at each side covering an oblique channel; in natural position the channel accommodates the margins of the labellum and the flaps curve over them, likely adding structural strength; clinandrium with a prominent, V-shaped dorsal margin covering most of the anther. Anther dorsal, narrowly triangular-ovoid, fleshy, dull yellow with brownish margin, the filament fully fused with the column. Pollinarium ca. 2.5 mm long, formed by two hairpin-shaped, narrow and laterally compressed yellow pollinia attached at apex to a common, blackish viscidium. Rostellum truncate, upon the removal of the pollinarium having a prominent apicule. Stigma consisting of a broad, concave central dry (sterile) area and two lateral, earlike vertical receptive areas, these concave, shiny, sticky and obliquely ovoid in outline. Ovary ascending, narrowly clavate, pale green with three whitish costae and inconspicuous pedicel, ca. 10 mm long and 2.5 mm in diameter. Capsule not seen.

Etymology—The specific epithet, *cactorum*, in Latin means “of the cacti” and refers to the distinctive habitat of this species, dominated by columnar cacti of the genus *Neobuxbaumia* Backeb.

Distribution and Habitat—So far, *Galeoglossum cactorum* is known from a single limestone rocky slope with an extension of a few hectares in the Mixteca Alta region of the Sierra Madre del Sur, Oaxaca, around 1,700 m elevation. About a dozen

plants were found rooted in chalky soil in a xerophilous scrub of *Neobuxbaumia* sp. with few individuals of the palm *Brahea dulcis* (Kunth) Mart., *Hechtia* sp. (Bromeliaceae), and *Agave* sp. (Agavaceae), with an open herbaceous stratum including *Selaginella* sp. (Selaginellaceae), *Begonia* spp. (Begoniaceae), *Sedum* sp. (Crassulaceae), *Pinguicula* sp. (Lentibulariaceae), and some grasses and ferns. Two other geophytic orchid species were observed in the area, namely an as yet undescribed species of *Bletia* and an unidentified *Malaxis*. That particular habitat is not common in the area but further exploration is required to determine whether additional suitable patches in which the new species occur exist in other parts of the Mixteca region, large expanses of which remain botanically unexplored.

The single locality known for *G. cactorum* is located in the same general area in Oaxaca where populations of both *G. thysanochilum* and *G. tubulosum* occur, but the particular habitat of the former seems to differ substantially from that of the other two species. *Galeoglossum cactorum* dwells in warmer, drier conditions in a xerophilous scrub about 1,700 m, whereas its congeners inhabit moister, cooler oak and pine-oak forests above 2,000 m. The closest known localities of *G. thysanochilum* and *G. tubulosum* are about 16 and 26 air kilometers, respectively, from the site of *G. cactorum*.

ACKNOWLEDGMENTS. C. C.-R. and A. d. Á. thank the communal authorities of San Bartolomé Yucuañe for permission to conduct botanical exploration in the area, the “Patronato de las Unidades de Servicios Culturales y Turísticos del Estado de Oaxaca” for financing plant collecting by the Jardín Etnobotánico, and Roberto Avendaño and Antonio Sánchez for assistance during the collection of this species. The authors also thank Laura Márquez Valdelamar (Laboratorio de Biología Molecular, Instituto de Biología, Universidad Nacional Autónoma de México) for assistance with DNA sequencing and Fernando Chiang for translating the diagnosis into Latin, and Ken Cameron and two anonymous reviewers for useful comments on the manuscript.

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APPENDIX 1. Voucher information is listed as follows: taxon name, collector name and number (herbarium), country, GenBank accessions (*matK-trnK*, nrITS).

Aa colombiana Schltr., *Aldana* 2 (ANDES), Colombia (AM900802, AM419766); *Achlydosa glandulosa* (Schltr.) M. A. Clem. & D. L. Jones, *Clements D-285* (CANB), New Caledonia (AJ543950, AJ539525); *Altensteinia fimbriata* Kunth, *Salazar* 6789 (MEXU), Ecuador (AM900801, AM419765); *Chloraea magellanica* Hook. f., *Ryan* 1 (K, spirit), Chile (AJ543948, AJ539523); *Cranichis engelii* Rchb. f., *Schott* s. n. (K, spirit), Ecuador (AM900814, AM419779); *Cyclopogon epiphyticum* (Dodson) Dodson, *Salazar* 6355 (K), Ecuador (AJ543927, AJ539499); *Dichromanthus cinnabarinus* (Lex.) Garay, *Linares* 4469 (MEXU), Mexico (AJ543914, AJ539486); *Eltroplectris calcarata* (Sw.) Garay & H. R. Sweet, *Soares* s. n. (MEXU, photograph), Brazil (AJ519450, AJ519448); *Galeoglossum cactorum* Salazar & Chávez-Rendón, *Chávez-Rendón et al.* 1604 (MEXU), Mexico (FN645939, FN645940); *Galeoglossum thysanochilum* (B. L. Rob. & Greenm.) Salazar, *Tenorio* 17900 (MEXU), Mexico (AM900810, AM419775); *Galeoglossum tubulosum* (Lindl.) Salazar & Soto Arenas, *Salazar* 6054 (MEXU), Mexico (AJ543938, AJ539510); *Galeottiella sarcoglossa* (A. Rich. & Galeotti) Schltr., *Jiménez* 2334 (AMO), Mexico (AJ543945, AJ539518); *Gavilea lutea* (Pers.) M. N. Correa, *Ryan* 3 (K, spirit), Chile (AJ543949, AJ539524); *Gomphichis caucana* Schltr., *Díaz* 159 (ANDES), Colombia (AM900805, AM419770); *Ludisia discolor* (Ker-Gawl.) A. Rich., *Salazar* 6354 (K, spirit), tropical Asia (AJ543911, AJ539483); *Manniella cypripedioides* Salazar, T. Franke, Zapfack & Benkeen, *Salazar et al.* 6323 (YA), Cameroon (AJ543943, AJ539516); *Mesadenus lucayanus* (Britt.) Schltr., *Salazar* 6043 (MEXU), Mexico (AJ543916, AJ539488); *Pachyplectron arifolium* Schltr., *Chase* 529 (K), New Caledonia (AJ310051, AJ539522); *Ponthieva racemosa* (Walt.) C. Mohr, *Salazar* 6049 (MEXU), Mexico (AJ543936, AJ539508); *Porphyrostachys pilifera* Rchb. f., *Whalley* s. n. (K, photograph), Peru (AJ543942, AJ539514); *Prescottia cordifolia* Lindl., *Salazar et al.* 6225 (PMA), Panama (AM900807, AM419772); *Prescottia densiflora* (Brogn.) Lindl., *da Silva* 877 (MG), Brazil (AJ519449, AJ519447); *Prescottia petiolaris* Lindl., *Munich Bot. Gard.* 00/2013 (M), Peru (AM900806, AM419771); *Prescottia plantaginea* Lindl., *Salazar* 6350 (K, spirit), Brazil (AJ543939, AJ539511); *Prescottia stachyodes* (Sw.) Lindl. [1], *Salazar* 6092 (MEXU), Mexico (AM900808, AM419773); *Prescottia stachyodes* (Sw.) Lindl. [2], *Salazar et al.* 7312 (MEXU), Mexico (AM900809, AM419774); *Pterichis habenarioides* Schltr., *Aldana* 12 (COL), Colombia (AJ543937, AJ539509); *Pterostylis curta* R. Br., *Chase* 572 (K), Australia (AJ543951, AJ539526); *Sarcoglottis acaulis* (J. E. Sm.) Schltr., *Salazar* 6356 (K, spirit), Trinidad (AJ543928, AJ539500); *Spiranthes cernua* (L.) Rich., *Nickrent* 4188 (MEXU), U. S. A. (AJ543916, AJ539489); *Stenoptera ecuadorana* Dodson & C. Vargas, *Salazar* 6357 (K, spirit), Ecuador (AJ543940, AJ539512); *Stenorrhynchos glicensteinii* Christenson, *Salazar* 6090 (MEXU), Mexico (AJ543532, AJ539505).