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## New circumscription and segregation of *Gloxinia major* (Gesneriaceae, Gesnerieae, Gloxiniinae) an endemic species from Bolivia

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### Abstract

The genus *Gloxinia* (Gesneriaceae, Gesnerieae, Gloxiniinae) comprises four species distributed from Nicaragua to Argentina. Historically, *Koellikeria major* has been problematic to place taxonomically due to the morphological similarity with *Gloxinia erinoides*, and as a result is currently treated as a synonym. Here, we present a molecular phylogenetic and morphological study that allowed us to demonstrate that *Koellikeria major* is distinct from *G. erinoides*. As a result, we formally transfer *Koellikeria major* to *Gloxinia major*. Here *G. major* is described, illustrated, and compared to morphologically similar species. Data on its distribution, habitat, and taxonomic treatment are provided.

**Keywords:** ITS, molecular phylogenetics, morphological data, *trnL-trnF*

### Introduction

*Gloxinia* L’Héritier (1789: 331) is placed in subtribe Gloxiniinae of the Gesneriaceae (Möller & Clark 2013, Weber *et al.* 2013). The circumscription of the genus, based mainly on experimental hybridization and chromosome numbers data (Wiehler 1976, 1983), has been revised extensively, since several genera, subgenera, and sections were included under the synonym of *Gloxinia* (Araujo *et al.* 2012). However, the instability in the genus circumscription has promoted an overproduction of taxonomic names, which has also implied several nomenclatural transfers from one genus to another (i.e., Wiehler 1976, Roalson *et al.* 2005c, Araujo 2007, Araujo *et al.* 2012). Nevertheless, molecular and morphological evidence (Roalson *et al.* 2005b) indicates that *Gloxinia sensu* Wiehler (1976, 1983) (or *Gloxinia s.l.*) is polyphyletic. On the other hand, Roalson’s *et al.* (2005b) contribution shows that *Gloxinia s.s.* is segregated and synonymized by Wiehler (1976). Currently, according to the circumscription adopted by these authors, *Gloxinia* (or *Gloxinia s.s.*) is monophyletic and *Seemannia* Hooker (1848: 567) appears as sister to *Gloxinia*, which includes three species: *Gloxinia perennis* (Linnaeus 1753: 618) Fritsch (1894: 174), *G. erinoides* (De Candolle 1839: 536) Roalson & Boggan (2005a: 228), and *G. xanthophylla* (Poeppig 1840: 7) Roalson & Boggan (2005a: 228). Araujo *et al.* (2010) described a new species, *Gloxinia alterniflora* Araujo & Chautems (2010: 68), which is endemic to Brazil, increasing the number of species to four.

*Gloxinia* is distributed mainly in the Central America Andes (from Honduras to Panama), South America (from Venezuela to Argentina), and only one species, *Gloxinia perennis*, reaches the Caribbean region (Araujo *et al.* 2010, 2012, Clark *et al.* 2011). This genus shares with all the members of the subtribe Gloxiniinae features such as being terrestrial and perennial herbs with scaly rhizomes or fibrous roots (Wiehler 1983, Möller & Clark 2013, Weber *et al.* 2013), with a base chromosome number  $x = 13$  (Wiehler 1983, Weber *et al.* 2013). *Gloxinia* differs from other genera of the subtribe by the lack of uncinated trichomes, by having raceme-like inflorescences with bracteate flowers, the apical dehiscence of the capsule without splitting the hypanthium, dry capsule with prominent costae, an annular nectary, or this absent, and white, purple, pinkish or partly maroon corolla (Araujo *et al.* 2010, 2012, Roalson *et al.* 2005a).

**TABLE 1.** Comparative morphological character between *Gloxinia* species. Based on Araujo (2007), Roalson *et al.* (2005c), Araujo *et al.* (2010), Hinoshita *et al.* (2018), and observations made by the authors.

Characters	Scaly Rhizome	Inflorescence	Corolla colour and shape	Corolla insertion	Lower corolla lobe margins:	Corolla lobe, indument	Extra floral nectaries	Capsule costae	Phenology	Geographic distribution
<i>G. perennis</i>	present	bracteose	light purple or pinkish, campanulate, zygomorphic	oblique to the calyx	distinctly toothed or fimbriate	without non-glandular trichomes	absent	with prominent costae	all year long	Guatemala to Bolivia
<i>G. erinoides</i>	present	bracteose	white, pinkish, cylindrical, zygomorphic	oblique to the calyx	distinctly toothed or fimbriate	with non-glandular trichomes	annular with 5 lobes	with prominent costae	All year long	Nicaragua to Argentina
<i>G. major</i>	present	bracteose	white to pinkish, infundibulate almost actinomorphic	erect to the calyx	entire, or mildly toothed	with non-glandular trichomes	annular unlobed	with prominent costae	January to March	Bolivia (Chuquisaca and Tarija)
<i>G. xanthophylla</i>	absent	frondose	white, campanulate, zygomorphic	erect to the calyx	entire or nearly so	with non-glandular trichomes	absent	without prominent costae	June to August	Ecuador and Peru
<i>G. alterniflora</i>	absent	bracteose	white or light purple, infundibulate, zygomorphic	erect to the calyx	distinctly toothed or fimbriate	without non-glandular trichomes	absent	not seen	March	Brazil (Matto Grosso)

**TABLE 2.** Gesnerieae samples included in this study (the new accessions are indicated with an asterisk).

Species	Collector and number, herbarium code	Accesion n° (GenBank) ITS, <i>trnL-trnF</i>
Subtribe Gloxiniinae		
<i>Achimenes candida</i> Lindl.,	Skog 7840 (US)	AY047065, AY047124
<i>Achimenes cettoana</i> H.E.Moore	Skog 7991 (US)	AY047066, AY047125
<i>Achimenes misera</i> Lindl.	Skog 7903 (US)	AY047067, AY047126
<i>Amalophyllon divaricatum</i> (Poepp.) Boggan, L.E.Skog & Roalson	Perret & Chautems 89 (G)	KU991233, JX195796
<i>Amalophyllon ecuadoranum</i> J.L. (Wiehler)	Clark & Skog 8039 (US)	AY047078, AY047137
<i>Chautemsia calcicola</i> A.O.Araujo & V.C.Souza	Araujo et al. 500 (ESA)	GQ344506, GQ383543
<i>Diastema comiferum</i> (DC.) Benth. ex Walp.	Skog 8259 (US)	KY858320, AY702398
<i>Diastema racemiferum</i> Benth.	Skog 8052 (US)	AY047069, AY047128
<i>Diastema vexans</i> H.E.Moore	GRF 840 306 Skog 8260 (US)	KY858321, AY702401
<i>Eucodonia andrieuxii</i> (DC.) Wiehler	Skog 7620 (US)	AY047060, AY047119
<i>Eucodoni verticillata</i> (M.Martens & Galeotti) Wiehler	Skog 7714 (US)	AY047061, AY047120
<i>Gloxinella lindeniana</i> (Regel) Roalson & Boggan	without voucher	AY702361, AY702405
<i>Gloxinia erinoides</i> (DC.) Roalson & Boggan	Skog 7596 (US)	AY047073, AY047132
1- <i>Gloxinia perennis</i> (L.) Fritsch.	Araujo et al. 536 (ESA)	GQ344516, GQ383551
2- <i>Gloxinia perennis</i> (L.) Fritsch.	Skog 7723 (US)	AY047071, AY047130
<i>Gloxinia xanthophylla</i> (Poepp.) Roalson & Boggan	Skog 8140 (US)	AY047074, AY047133
<b><i>Gloxinia major</i> (Fritsch) C.A.Zanotti &amp; Lizarazu</b>	<b>Zuloaga and Deginani 15804 (SI)</b>	<b>(*MT125605, *MT125606)</b>
<i>Gloxiniopsis racemosa</i> (Benth.) Roalson & Boggan,	Skog 8258 (US) Araujo et al. 548 (ESA)	AY702364, GQ383549
<i>Goyazia petraea</i> (S.M.Phillips) Wiehler	Araujo et al. 457 (ESA)	(GQ344517, GQ383552).
<i>Goyazia rupicola</i> Taub.	Araujo et al. 503 (ESA)	GQ344526, GQ383558
<i>Kohleria peruviana</i> Fritsch	Skog 8145 (US)	KY858347, AY702418
<i>Kohleria tigridia</i> (Ohlend.) Roalson & Boggan	Clark 6041	AY702352, AY702396
<i>Kohleria villosa</i> (Fritsch) Wiehler	Skog 8041 (US)	AY047076, AY047135
<i>Kohleria warszewiczii</i> (Regel) Hanst.	Chautems & Perret 11-110 (G)	KY858352, AY702422
<i>Mandirola ichthyostoma</i> (Gardner) Seem. ex Hanst.	Skog 8219 (US)	AY702360, AY702404
<i>Monopyle flava</i> L.E.Skog	Cairampoma & Martel 94, Skog 8055, (US)	KY858354, AY702424
<i>Monopyle macrocarpa</i> Benth.	Cairampoma & Martel 117 Clark 5638	KY858355, AY702425
<i>Moussonia septentrionalis</i> (D.L.Denham) Wiehler	Skog 8045 (US)	AY047068, AY047127

.....continued on the next page

TABLE 2. (Continued)

Species	Collector and number, herbarium code	Accesion n° (GenBank) ITS, <i>trnL-trnF</i>
<i>Niphaea oblonga</i> Lindl.	Skog 5336(US)	AY047064, AY047123
<i>Nomopyle dodsonii</i> (Wiehler) Roalson & Boggan.	GRF 9769	KY858400, GQ383565
<i>Pearcea abunda</i> (Wiehler) L.P.Kvist & L.E.Skog	Skog 8019 (US)	AY047077, AY047136
<i>Seemannia gymnostoma</i> (Griseb.) Toursark.	Araujo <i>et al.</i> 549 (ESA)	AY702359, GQ383569
<i>Seemannia nematanthodes</i> (Kuntze) K.Schum.	Araujo <i>et al.</i> 550 (ESA)	AY702362, GQ383568
<i>Seemannia purpurascens</i> Rusby	Chautems & Perret 97-019 (G) Skog 7839 (US)	KY858422, AY047131
<i>Seemannia sylvatica</i> (Kunth) Hanst.	Skog 7760 (US)	AY702365, AY702408
<i>Smithiantha aurantiaca</i> Wiehler	Skog 7834 (US)	AY047063, AY047122
<i>Smithiantha canarina</i> Wiehler	Skog 7684 (US)	AY047062, AY047121
<i>Solenophora calycosa</i> Donn.Sm.	Forther <i>et al.</i> 10474	AY702392, AY702435
Subtribe Gesneriinae:		
<i>Gesneria humilis</i> L.	without voucher	AY047051, AY047110
<i>Rhytidophyllum tomentosum</i> (L.) Mart.	Skog 7865 (US)	AY047056, AY047115
<i>Rhytidophyllum vernicosum</i> Urb. & Ekman	without voucher	AY047059, AY047118

When Fritsch described *Koellikeria major* Fritsch (1914: 398), he considered that the species was morphologically similar to *Koellikeria argyrostigma* (Hooker 1845: 4175) Regel (1848: 250) (currently synonym of *G. erinoides*), but differed in having subequal lobes of the corolla almost actinomorphic (*G. erinoides* presents the corolla zygomorphic, the two upper lobes are markedly smaller than the three lower lobes). Nevertheless, Araujo (2007) and Araujo *et al.* (2012) placed *K. major* in synonymy with *G. erinoides* considering the relative size of the plant and the flowers. These authors did not consider the corolla lobes as a character to distinguish these two species.

During fieldtrips to Bolivia (Departament Tarija), one specimen of *Gloxinia* (Zuloaga & Deginani 15804, SI) was found, determined and analyzed morphologically by the second author of this manuscript. This specimen has a high morphological similarity to *G. erinoides*, except that the corolla lobes are all about the same size as originally described in *Koellikeria major*.

The aim of this study was to analyze the taxonomic position of this specimen based on morphology and molecular data to test if *Koellikeria major* is distinct from *Gloxinia erinoides*.

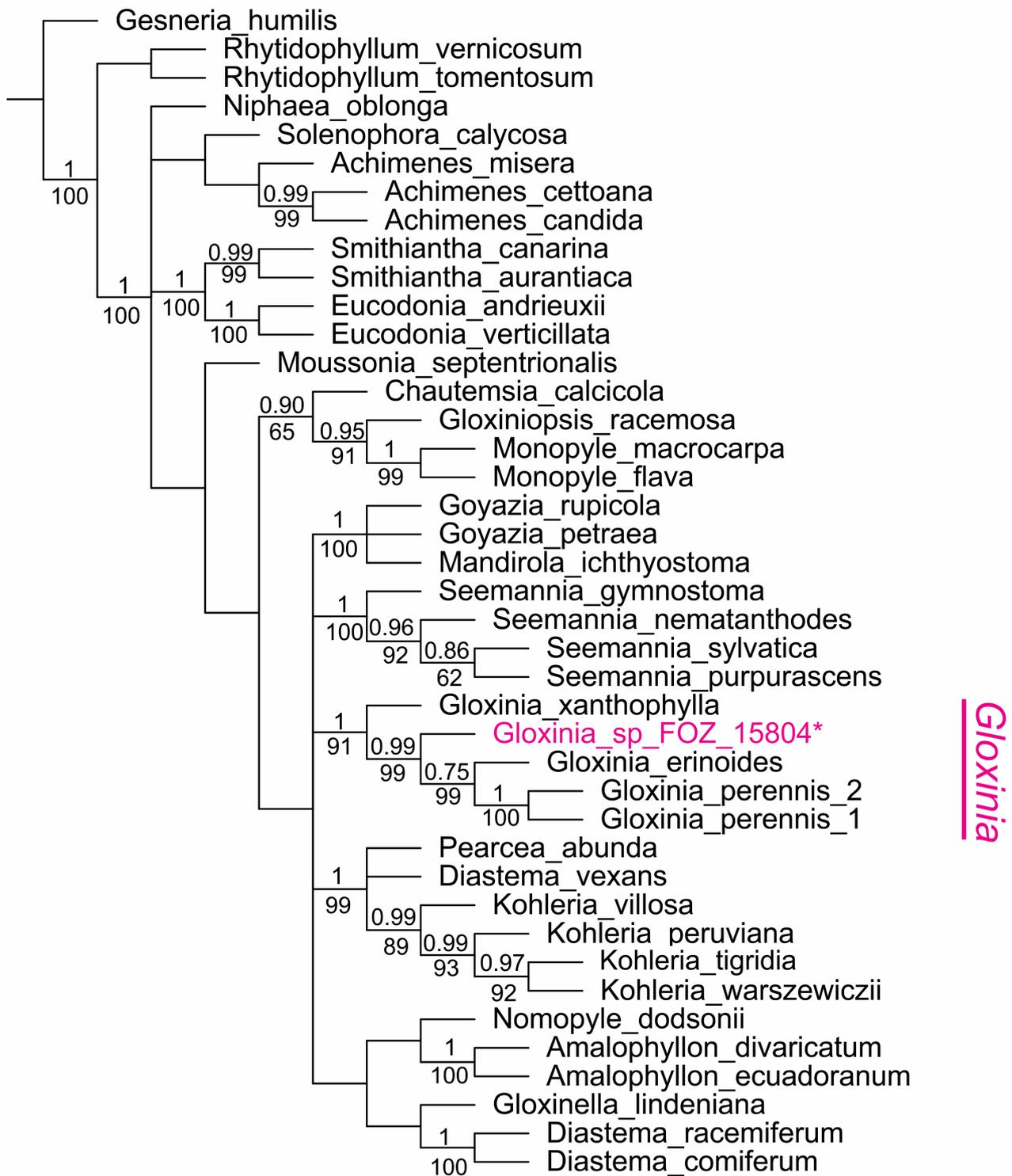
## Materials and methods

**Taxonomy**—Herbarium specimens of *Gloxinia* deposited in the Darwinion Institute (SI) and fresh material collected during fieldtrips in Bolivia by F.O. Zuloaga, were the basis for this study. High resolution images of type specimens of *Koellikeria major* were seen from F, G, and GH herbaria. The taxonomic description is based on dried and hydrated material and illustrations are based on dried material as well as photographs of fresh material. The distribution map was prepared in DIVA-GIS. Google Earth was used as a geographic information system to georeference the location data provided in herbarium labels (see appendix 1).

**Morphology**—The morphological characters (vegetative and reproductive) were taken from herbarium specimens and the literature, based on Araujo (2007), Roalson *et al.* (2005c), Araujo *et al.* (2010) and, Hinoshita *et al.* (2018); Table 1.

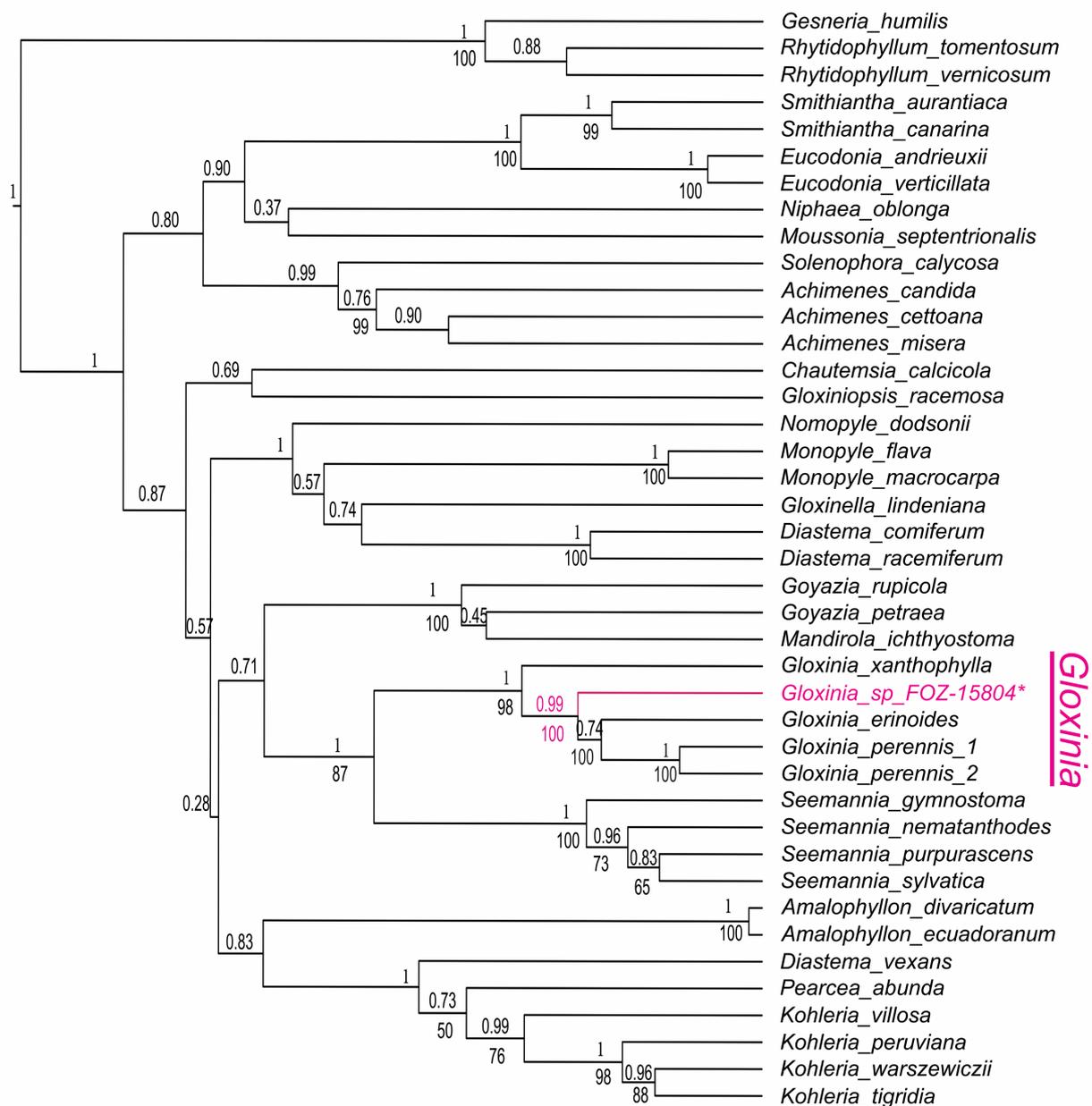
**Micromorphology**—Micromorphological observations were based on herbarium material and made through light microscopy. Herbarium materials were rehydrated in soapy water and stored in 70% alcohol or FAA (Ruzin 1999). Leaves, flowers and fruits were cleared with diluted chlorine, stained with safranin (D'Ambrogio de Argüeso 1986, Ruzin 1999), and mounted in glycerin on temporary slides. Observations were done with a Nikon Microphot-FXA,

equipped with a photographic digital camera Nikon DS-U2, and picture processing software NIC-Elements, F-package 2.30.



**FIGURE 1.** Strict consensus tree obtained with ITS sequences showing relationship of *Gloxinia* sp. FOZ 15804 (\*). Number above and below branches indicate Bayesian Posterior Probabilities (PP) and Parsimony Bootstrap values (BS), respectively.

**Taxa Selection for molecular study**—The voucher of *Gloxinia* sp. [Zuloaga & Deginani 15804 (SI)] was analyzed alongside the available sequences downloaded from GenBank: a selection of 19 out of 21 genera of the subtribe Gloxiniinae (39 species and three of which are *Gloxinia*) and three taxa of the subtribe Gesneriinae. *Gesneria humilis* Linnaeus (1753: 612) (Gesneriinae) was used to root the tree, based on Zimmer *et al.* (2002). The total number of terminals analyzed was 41.



**FIGURE 2.** Maximum clade-credibility tree of combined matrix (ITS + *trnL-trnF*) showing relationship of *Gloxinia* sp. FOZ 15804 (\*). Number above and below branches indicate Bayesian posterior probabilities (PP) and parsimony bootstrap values (BS), respectively.

The details of the species included in the analyses, the GenBank accession numbers and the voucher information of the new sequences generated are shown in Table 2.

**DNA Extraction and Sequencing**—The genomic DNA was extracted from a leaf of the herbarium specimen using the modified CTAB (cetyltrimethylammonium bromide) protocol from Doyle & Doyle (1987). The plastid *trnL-trnF* and the nuclear internal transcribed spacer (ITS) markers were PCR amplified. For the *trnL-trnF*, the pairs of primers defined as c–d and e–f by Taberlet *et al.* (1991) were used; the ITS region was amplified with ITS4 and ITS5 universal primers (White *et al.* 1990). The PCR (Saiki *et al.* 1988) was performed in 25  $\mu$ l final volume with 50 ng of template DNA, 0.2  $\mu$ M of each primer, 25  $\mu$ M of dNTPs, 5  $\mu$ M MgCl<sub>2</sub>, 17  $\mu$ M of water, buffer 10 $\times$ , and 1.5 units of *Taq* polymerase (Invitrogen, São Paulo, Brazil). Both regions were amplified as follows: one cycle of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for the *trnL-trnF* and 53°C for ITS for 1 min, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 6 min terminated the reactions. The PCR products were run out on a 1% TBE agarose gel and stained with SybrSafe (Invitrogen). Sequencing reactions were performed by Macrogen, Inc. (Seoul, Korea). Sequences were edited and aligned using the program Bio Edit version 7.2.5 (Hall 2013). The matrices can be found in Treebase: <http://purl.org/phylo/treebase/phylovs/study/TB2:S24335>.

**Data Analyses**—Data were analyzed separately and also in a combined matrix using maximum parsimony (MP), and Bayesian inference (BI) approaches. Parsimony analysis was carried out using TNT version 1.1 (Goloboff *et al.* 2008). The searches involved 1000 replicates, each of which generated a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of 10 trees in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 10000 trees. A strict consensus tree was generated from the most parsimonious trees. Branch supports were estimated with Bootstrap (Felsenstein 1985) using a total of 10000 replicates. Each replicate was analyzed using 10 Wagner trees as a starting point followed by TBR branch swapping, saving only one tree per replicate. Bootstrap values (BS) over 50% are reported. We calculated the consistency index (CI) and retention index (RI) for the most parsimonious trees (Farris 1989).

The Bayesian analysis was performed with BEAST Package 1.8.1 (Drummond *et al.* 2012). The appropriate model of nucleotide substitution for each partition was selected using jModelTest 2.1.4 (Darriba *et al.* 2012) following the Akaike information criterion (AIC): GTR + I + G, and SYM + I + G (*trnL-trnF* and ITS, respectively). The Bayesian analysis was conducted with settings as follow: GTR + I + G and all equal base frequencies for ITS, SYM + I + G, and estimated base frequencies for the combined matrix. The tree matrices were run under four gamma categories, uncorrelated rate variation with lognormal distribution, random starting tree, a Yule process as tree prior, auto-optimization option for all operators and default values for all other setting. Two independent runs of 10 million generations were sampled every 1000th generation. To identify when the analyses had reached stationarity, we checked the output files for convergence and effective sample size (ESS) > 200 with Tracer v1.6 (Rambaut *et al.* 2013). Based on this convergence diagnostic, the first 2500 sampled tree were discarded as burn-in from each analysis before obtaining a consensus phylogeny. Posterior probabilities (PP) of branches were calculated from a maximum clade-credibility tree (MCC) obtained with TreeAnnotator 2.4.7. The maximum clade-credibility tree showing posterior probabilities (PP) was edited in FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

## Results

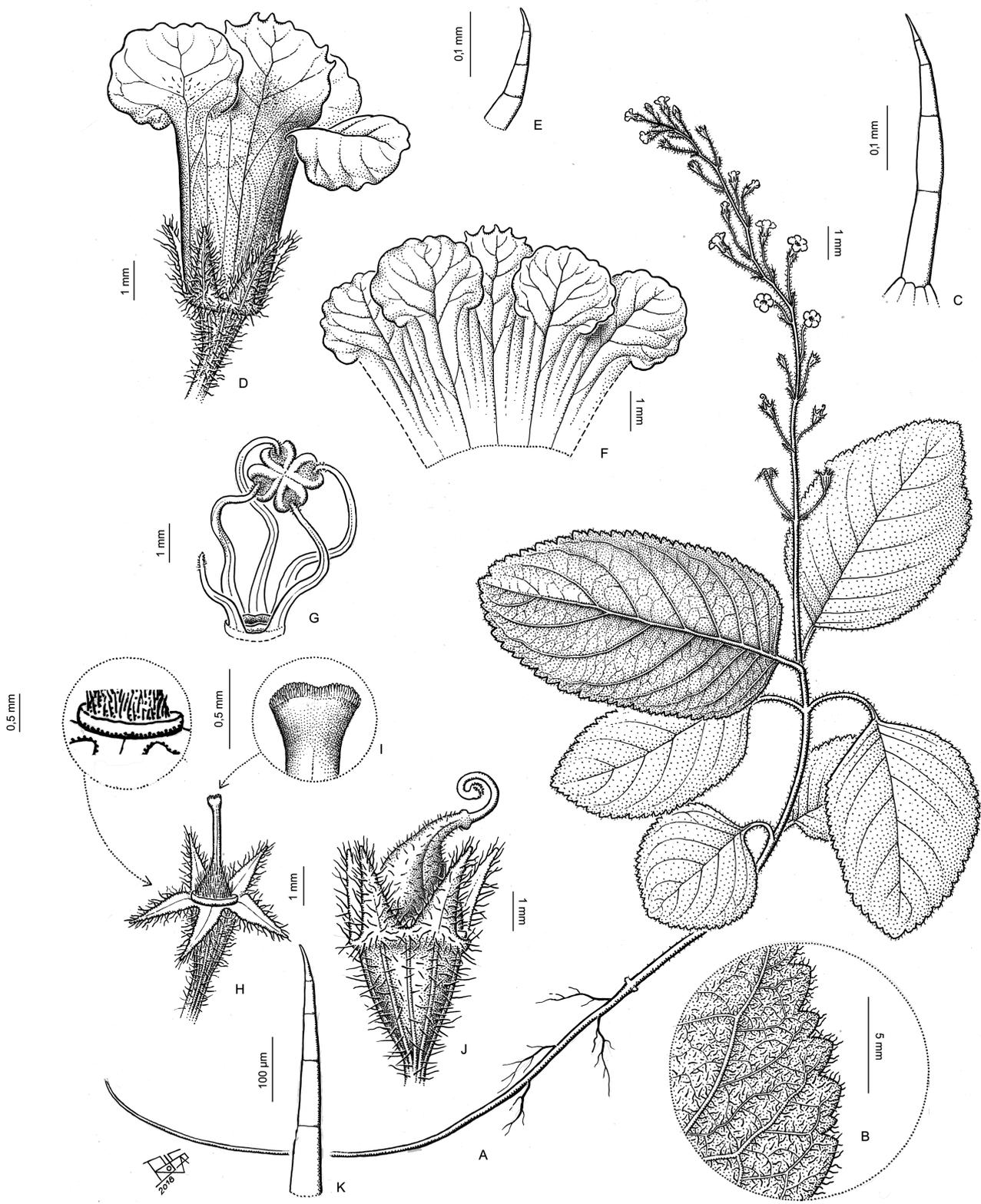
### Phylogenetic Analysis

The ITS alignment was 646 bp long, of which 193 bp (29.9%) were parsimony informative; the MP analysis resulted in 20 equally parsimonious trees of 658 steps, with CI=0.47, RI= 0.58. The *trnL-trnF* alignment included 943 base pairs of which 42 (4.5%) were parsimony informative; the MP analysis displayed 6996 trees of 68 steps, with CI= 0.58, RI= 0.76. The combined MP analysis resulted in eight trees of 748 steps, with CI= 0.57, RI= 0.60. The BI analyses recovered the same topologies of the MP analyses in combined analysis. The *trnL-trnF* analysis displayed low resolution, so the MP consensus tree of ITS and the Bayesian combined tree are shown (Fig. 1 and Fig 2, respectively).

For both the ITS and combined matrix of *Gloxinia*, *G. xanthophylla*, *G. erinoides*, *G. perennis* and the specimen of *Gloxinia sp.* (Zuloaga & Deginani 15804) were strongly resolved as monophyletic (ITS: BS = 91, PP = 1; combined: BS = 98, PP = 1) with five shared synapomorphies (positions 85, 102, 201, 449, and 603 of the ITS alignment). *Gloxinia sp.* (Zuloaga & Deginani 15804) is sister to the clade *G. erinoides-G. perennis* (BS = 99, PP = 0.99). The clade *G. erinoides-G. perennis* has one unique mutation (C to T) in the position 536 of the ITS alignment. *Gloxinia xanthophylla* (BS = 99, PP = 1) appears as sister to the rest of the species of the genus.

### Morphological Characters

The morphological characters analyzed for all species of *Gloxinia* are shown and summarized in Table 1. *Gloxinia* comprises four species that share features such as erect herbal habit with scaly rhizomes (rhizomes absent in *G. xanthophylla* and *G. alterniflora*), opposite, elliptic, lanceolate, cordiform, and ovate leaves. Inflorescences are bracteose (only frondose in *G. xanthophylla*) with opposite or alternate bracts; the corolla is campanulate, cylindrical and infundibulate, white, pink, purple, with lobes subequal to unequal, entire to toothed or fimbriate, the nectary is absent or annular, ovary half inferior to inferior; stigma capitate to stomatomorphic; the fruit is ovoid to elliptical dry rostrate capsule with prominent costae (without prominent costae in *G. xanthophylla*), loculicidally dehiscent without splitting the hypanthium; and the seed are numerous, minute, rhombic to ellipsoid.



**FIGURE 3.** A–L. *Gloxinia major* (Fritsch) C.A. Zanotti & Lizarazu, *comb. nov.* A. Habit, B. Leaf, detail, C. Long conical eglandular trichome, leaf, D. Flower at anthesis, lateral view, E. Long conical eglandular trichome, flower, F. Dissected corolla, G. Stamens, I staminode and nectary (calyx and corolla removed), H. Calyx, nectary and gynoecium (corolla removed), I. Stigma, J. Annular nectary, K. Immature, L. Long conical eglandular trichome, calyx. [A–L from Zuloaga & Deginani 15804, SI; drawn by Francisco Rojas].



FIGURE 4. *Gloxinia major* (Fritsch) C.A. Zanotti & Lizarazu, *comb. nov.* A–B inflorescence. Photographs by F.O. Zuloaga.

### Taxonomic Treatment

*Gloxinia major* (Fritsch) C.A. Zanotti & Lizarazu, *comb. nov.* *Koellikeria major* Fritsch, *Bot. Jahrb. Syst.* 50: 398. 1914. Type:—BOLIVIA. Chuquisaca: 31 Jan 1904, K. Fiebrig 2680 (lectotype G-00365303 image!, isolectotypes: F-0060594 image!, GH-0009215 image!; lectotype designated by Araujo *et al.* 2012).

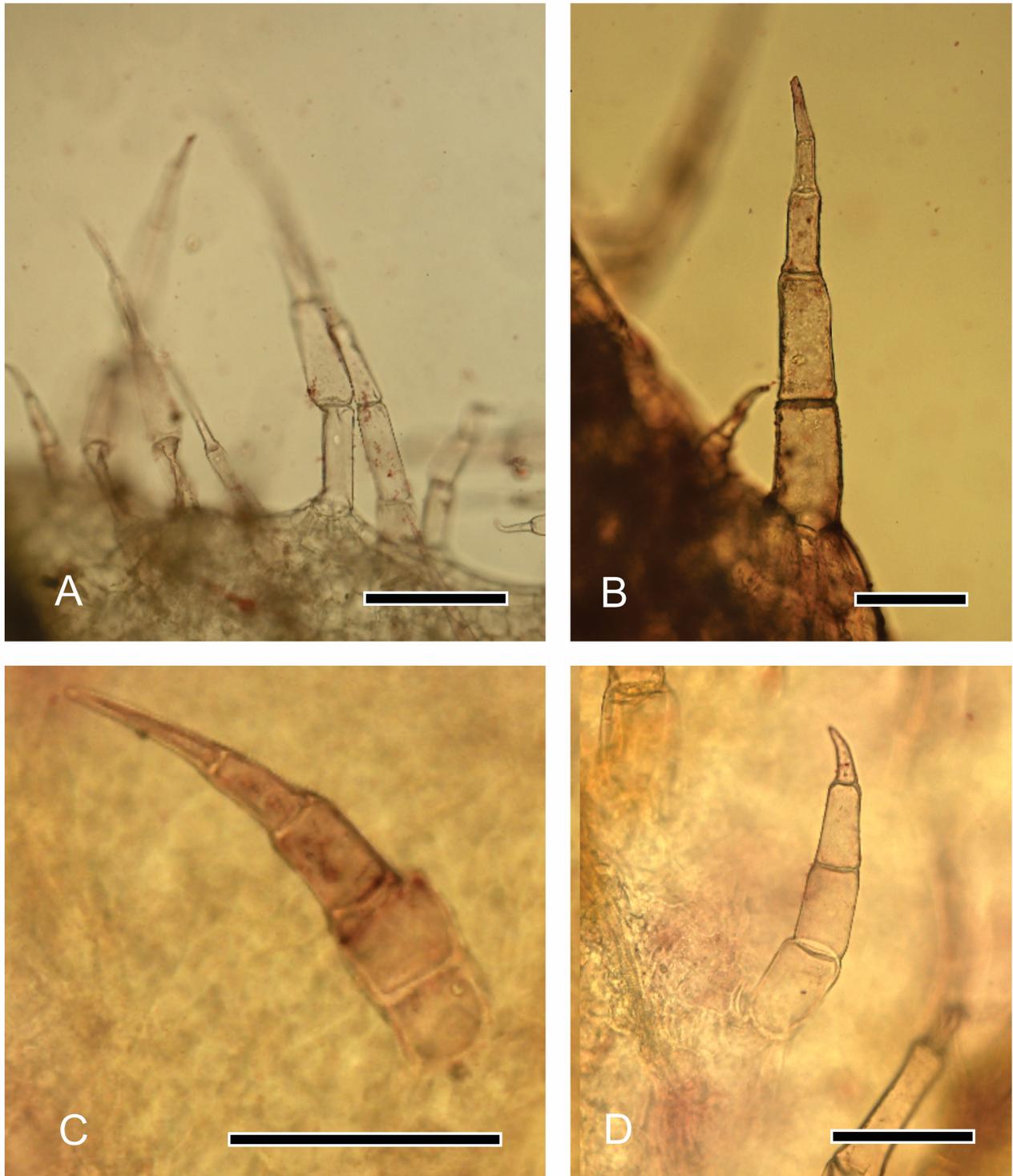
Perennial herbs, 15–25 cm high; with scaly rhizomes, with fleshy scales, without stolons. Stems terete, erect, unbranched, hispid. Leaves opposite, entire, petiole 1.3–2.9 cm long, blade margin serrate to crenate, 5–8 pairs of secondary veins, 7–12.3 × 6–6.9 cm, obovate to elliptic, apex acute to obtuse, base mildly asymmetrical, cuneate or attenuate, both surfaces pubescent and greenish. Inflorescence racemose, bracteose, raceme terminal or axillary, peduncle 3–9 cm long, hispid. Flowers almost actinomorphic, pedicel erect, (5–)12–15 mm long, hispid, bracteolate; bract linear to filiform, green, 2.5–3 × 0.5–0.7 mm. Sepals 5, free, aestivation valvate, 4–6 mm long, linear to lanceolate, green, margin entire. Corolla infundibuliform, aestivation imbricate, tube 6–7.9 × 3.4–3.6 mm, subglabrous inside, sparsely pubescent outside, with non-glandular, uniseriate long trichomes; tube white, throat white to pinkish, lobes white, slightly sinuate margins, erect or mildly oblique in the calyx, limb of 4 equal lobes, 3–4 × 3–4 mm, only one lobe is mildly toothed. Stamens 4, 1.5–2 mm long, included in the corolla, filaments glabrous, anthers coherent opening by longitudinal slits, staminode 1; annular nectary unlobed. Ovary inferior, style glabrous, 3.5–4 mm long, stigma stomatomorphic. Capsule conic, 4–5.5 × 2.5–3.5 mm, dry, apex curved, whit prominent costae, dehiscent only apically, pubescent. Seeds numerous, ellipsoid. Figs. 3–5.

**Distribution, habitat and phenology:**—*Gloxinia major* is endemic to Bolivia (Dept. Chuquisaca and Tarija), between 600–1000 elevation, appears in a semi-deciduous forest in the Chacoan province with precipitation up to

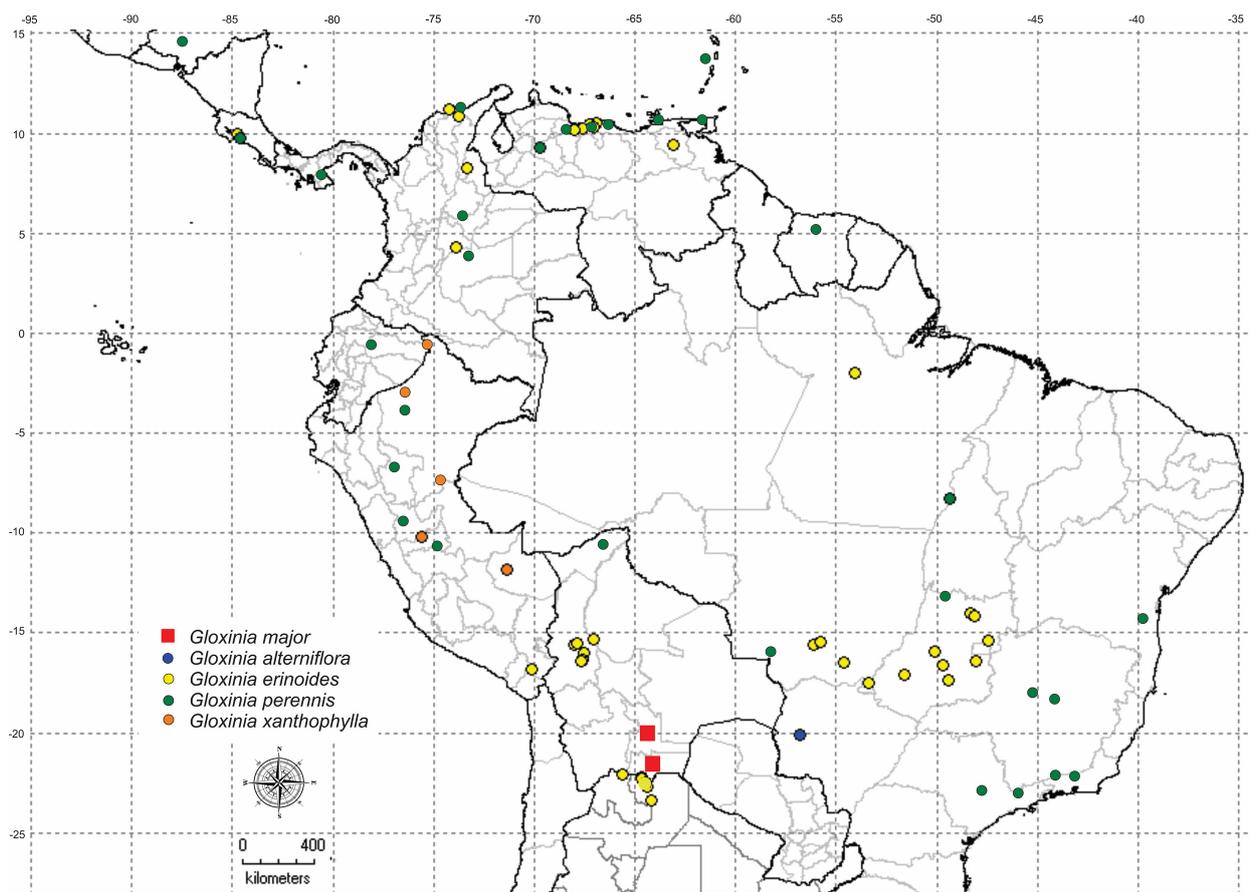
500–750 mm and annual temperatures between 18–23°C (Cabrera & Willink 1973; Fig. 6). This species is known only from two localities; it was first collected by K. Fiebrig in 1904 in Chuquisaca, and more than 100 years later (*Zuloaga & Deginani 15804*) plants of this species were found growing in a neighboring department (Tarija). Flowering and fruiting from January to March.

**Etymology:**—The name of this species was coined by Karl Fritsch (1914), probably refers to the large size of the plant and the flowers.

**Additional specimen examined:**—BOLIVIA. Tarija: National Route 1 de Nogalitos a Bermejo, 600 m elev., 22°28'23''S, 64°28'52''W, 11 March 2017, *F.O. Zuloaga & N.B. Deginani 15804* (SI!).



**FIGURE 5.** *Gloxinia major* (Fritsch) C.A. Zanotti & Lizarazu, *comb. nov.* A. long conical eglandular trichome from the leaf, B. long conical eglandular trichome from the calyx, C–D. long conical eglandular trichome from the abaxial surface on the corolla. [A–D. *Zuloaga & Deginani 15804* (SI)]. Scale bars: A = 0.2 mm; B–D 0.1 mm.



**FIGURE 6.** Distribution of *Gloxinia*: *G. alterniflora* (blue circle), *G. erinoides* (yellow circle), *G. major* comb. nov. (red square), *G. perennis* (green circle), and *G. xanthophylla* (orange circle).

## Discussion

The results of the molecular phylogenetic analyses showed that the specimen of *Gloxinia* sp. (*Zuloaga & Deginani 15804*), here designated as the new combination *Gloxinia major*, is strongly supported as a member of the *Gloxinia* clade, and as the sister species to the clade *G. erinoides*-*G. perennis*. According to the morphological evidence, *G. major* is morphologically closest to *G. erinoides*, both sharing the presence of extrafloral nectaries (*vs.* absent in *Gloxinia perennis*, *G. xanthophylla*, and *G. alterniflora*); however, it differs from *G. erinoides* by having larger leaves and green abaxial surface, almost actinomorphic corolla, 7–9.5 mm long, annular nectary unlobed, and corolla erect in relation to the calyx (*vs.* shorter leaves and reddish abaxial surface, zygomorphic corolla, 3–5 mm long, annular nectary with 5 lobe, and corolla oblique in relation to the calyx in *G. erinoides*).

*Gloxinia major* shares a scaly rhizome with *G. perennis* and *G. erinoides* (*vs.* absent in *G. xanthophylla* and *G. alterniflora*), and also shares glandular trichomes on the corolla with *G. erinoides* and *G. xanthophylla* (*vs.* absent in *G. perennis* and *G. alterniflora*). Only *G. xanthophylla* presents the bracteose inflorescence (*vs.* absent in the rest), and the absence of the scaly rhizome and capsule with costae (present in the rest). Additional features to distinguish *Gloxinia major* from its allies are summarized in Table 1. *Gloxinia major* is a different species morphologically and molecularly to *Gloxinia erinoides*. This conclusion agrees with that of Fritsch (1914) when he proposed that *Koellikeria major* (combined here, as *Gloxinia major*) was a different taxon of *Koellikeria argyrostigma* (currently *Gloxinia erinoides*).

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## APPENDIX 1. Numerical List of Taxa and Specimens Examined.

Specimens Examined. Specimens are arranged by collector (with first initial, when known) in alphabetical order, followed by collector's. An asterisk indicates specimens bibliographic used for map (based on Araujo 2007, Araujo *et al.* 2012)

*Gloxinia alterniflora* A.O.Araujo & Chautems:

*G. Hatschbach* 74705\*

*Gloxinia erinoides* (DC.) Roalson & Boggan:

*A.O. Araujo* 501\*, 507\*; *M. Bang* 1720\*; *A.J.A. Bonpland* s.n. \*; *O. Buchtien* 715\*, 716\*; *M.T. Dawe* 337\*; *A. Ducke* s.n. \*; *A. Fendler* 784\*; *K.A.G. Fiebrig* 2707; *N. Funck* 780\*; *G. Hatschbach* 34161, 36150, 36209; *J.H. Humbert* 26023\*; *J.H. Hunziker* 12719; *J.H. Kieckbride* 3340\*; *H. Pittier* 11609\*; *O. Morrone* 4883; *L.J. Novara* 8937; *J.E. Paula* 1013\*; *Purdie* s.n. \*; *Rizzo* 10602\*; *A.E.H. Salles* 117\*; *L. Schlim* 8\*; *R. Schreiter* 11223; *H. Slevner* 3902\*; *D.N. Smith* 13993\*; *H. Smith* 1318\*; *V.G. Solis Neffa* 1059; *V.C. Souza* 29686\*; *Vargas* 1630\*; *B.M.T. Walter* 3711\*, 4026\*; *A. Weberbauer* 7929\*; *J.R.L Wood* 14265\*; *C.R. Worth* s.n. \*; *F.O. Zuloaga* 8568.

*Gloxinia major* (Fritsch) C.A.Zanotti & Lizarazu, **comb. nov.**:

*K.A.G. Fiebrig* 2680\*; *F. O. Zuloaga* 15804.

*Gloxinia perennis* (L.) Fritsch:

*A.O. Araujo* 536\*; *E. Asplund* 12064\*; *M. Barreto* 3004\*; *J.C. Biesmeijer* 217\*; *E.J.F. Campbell* s.n. \*; *J.L. Clark* 6895\*; *N.D. Cruz* 9\*; *A. Fendler* 1593\*; *J.L. Hage* 2114\*; *G. Hatschbach* 62339\*; *E.P. Heringer* 1898\*; *P.C. Hutchison* 6047\*; *J.M. Idrobo* 1337\*; *H.A.H. Kegel* 1430\*; *R. Keller* 3789\*; *G. Klug* 4214\*; *J. Linden* 1462\*; *J. Mayer* 271\*; *G.F. Mees* s.n. \*; *G. Meyer* 308\*; *D.B.J. Pickel* s.n. \*; *H. Pittier* 13057\*; *H.L. Sanchez* 2033\*; *C. Sandeman* 94\*; *M. Sazima* 35731\*; *L.L. Silva* 6\*; *S.P.C. Silva* 752\*; *J.B. Simons* 245\*; *H. Stehlé* 4336\*; *J.A. Steyermark* 127090; *L. Uribe* 4515\*.

*Gloxinia xanthophylla* (Poeppig) E.H.Roalson & J.K.Boggan:

*R.B. Foster* 10352\*, 12967\*; *P.C. Hutchison* 6040\*; *E.F. Poeppig* s.n. \*; *R. Spruce* 4400\*.