

## *Chautemsia calcicola*: A new genus and species of Gloxinieae (Gesneriaceae) from Minas Gerais, Brazil

Andréa Onofre de Araújo,<sup>1</sup> Vinicius Castro Souza<sup>1</sup> & Mathieu Perret<sup>2</sup>

1 Departamento de Ciências Biológicas, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Av. Pádua Dias, 11, 13418-900, Piracicaba – SP, Brazil

2 Conservatoire & Jardin botaniques de la Ville de Genève, Impératrice I, C.P. 60, 1292 Chambésy, Genève, Switzerland

Author for correspondence: Andréa Onofre de Araújo, aonofrearaujo@yahoo.com.br

**Abstract** A new species of Gesneriaceae discovered in remnants of deciduous forests on limestone outcrops in Minas Gerais, Brazil, is described and compared with morphologically related taxa. This plant presents the diagnostic features of the tribe Gloxinieae, but a unique combination of morphological traits distinguishes this taxon from previously described genera. Its phylogenetic position was inferred based on analyzing DNA sequences variation of five loci: the *rpl16* intron, *rps16* intron, *trnL-F* intron-spacer, a portion of the plastid-expressed glutamine synthetase gene (*ncpGS*) and the ribosomal DNA internal transcribed spacer (ITS). Molecular phylogenetic analyses confirm the position of this new species in the Gloxinieae, as a sister lineage of a clade including the Brazilian genera *Mandirola* and *Goyazia*. However, tests using topological constraints do not reject the alternative relationship that places this taxon with *Gloxiniopsis* in a monophyletic group. To accommodate this species in the current generic circumscription of Gloxinieae, the new genus *Chautemsia* A.O. Araujo & V.C. Souza is created.

**Keywords** Brazil; Brazilian Atlantic forest; *Chautemsia*; Gesneriaceae; Gloxinieae; molecular phylogeny; taxonomy

### ■ INTRODUCTION

Generic boundaries in tribe Gloxinieae were reorganized by Roalson & al. (2005a) on the ground of phylogenetic analyses of molecular characters and morphological synapomorphies (Roalson & al., 2005b). Gloxinieae now include some 20 genera and approximately 170 species (Boggan & al., 2008; Roalson & al., 2005a, 2008). The recent studies showed that several genera of Gloxinieae, particularly *Gloxinia* L'Hér. as recognized by Wiehler (1983), were not monophyletic. From the latter genus, a new tribe Sphaerorrhizeae Roalson & Boggan was segregated including the new genus *Sphaerorrhiza* Roalson & Boggan. For the remaining taxa new generic names were proposed including *Gloxinella* (H.E. Moore) Roalson & Boggan, *Gloxiniopsis* Roalson & Boggan and *Nomopyle* Roalson & Boggan, whereas genera *Mandirola* Decne. and *Seemannia* Regel were resurrected.

In the framework of a detailed taxonomic survey of the Gloxinieae in Brazil, a new taxon was discovered in Minas Gerais, Brazil. The material was identified as belonging to Gloxinieae based on the presence of scaly rhizomes, but did not fit in any of the existing genera. In the present study we describe the diagnostic features of this plant and explore its affinity in Gloxinieae using sequences of the *rpl16* intron, *rps16* intron, *ncpGS*, and ITS regions and the published ITS/*trnL-F* dataset of Roalson & al. (2005b).

### ■ MATERIALS AND METHODS

**Taxon sampling and DNA sequencing.** — Leaves from live plants collected in the field or from cultivated specimens held in the greenhouses of the Conservatoire & Jardin botaniques

de la Ville de Genève (Switzerland) were sampled and immediately dried in silica gel. Twenty-four accessions representative of 20 Gloxinieae species and 14 genera (including the newly described taxon) plus two outgroups were sequenced for the *rpl16* intron, *rps16* intron, the *trnL-F* intron-spacer, a portion of the plastid-expressed glutamine synthetase gene (*ncpGS*) and the ribosomal DNA internal transcribed spacer (ITS). Two outgroup species, *Gesneria humilis* L. and *Rhytidophyllum vernicosum* Urb. & Ekman, were selected from the tribe Gesnerieae, a sister lineage of Gloxinieae according to previous phylogenetic analyses (Zimmer & al., 2002; Roalson & al., 2005b). A list of these 26 accessions and their vouchers is provided in the Appendix.

DNA was isolated using the CTAB method of Doyle & Doyle (1987) with the addition of 1% polyphenol polyvinylpyrrolidone. The primers used to amplify and sequence the *rpl16* intron, *trnL-F* intron-spacer and the region between exons 7 and 11 of *ncpGS* are indicated in Perret & al. (2003). The *rps16* intron was amplified and sequenced using the primers *rpsF* and *rpsR2* (Oxelman & al., 1997). ITS was amplified and sequenced with primers ITS 5P and ITS 8P (Möller & Cronk, 1997). Both amplifications and sequencing reactions follow procedures described in Perret & al. (2003). All sequences were aligned by eye, insertions/deletions (indels) were not coded as separate characters. Newly acquired sequences have been deposited in EMBL/GenBank.

**Phylogenetic analyses and tests of alternative topologies.** — For parsimony analyses, all characters were equally weighted and gaps were treated as missing characters. Heuristic searches were conducted in PAUP\* v.4.0b10 (Swofford, 2002), using 1000 random taxon addition sequences with tree-bisection-reconnection (TBR) branch swapping and keeping

up to 1000 most parsimonious trees per random addition replicate. To evaluate internal support, heuristic searches were completed for 1000 bootstrap replicates (BS; Felsenstein, 1985), with TBR swapping. The number of trees saved at each replicate was limited to 1000.

Bayesian inferences were performed with MrBayes v.3.1 (Ronquist & Huelsenbeck, 2003). The combined dataset was divided into three partitions: plastidic regions, *ncpGS* and ITS. For each partition, the optimal model of DNA substitution was selected by the Akaike information criterion (AIC) using Modeltest v.3.7 (Posada & Crandall, 1998). In each case the optimal model was the General Time Reversible model, with rate heterogeneity modeled by assuming that some proportion of sites are invariable and that rate of evolution at other sites is modeled using a discrete approximation to a gamma distribution (GTR+I+ $\Gamma$ ). All the parameters values were unlinked across partitions and estimated during the Markov chain Monte Carlo (MCMC) runs. Two independent analyses were run from different random trees (Nruns = 2) with temperature of 0.15. The chains were run for 10,000,000 generations, with trees sampled every 100th generation. We discarded 25% of the samples obtained during the first 1,000,000 generations. Posterior probability (PP) and branch lengths were averaged across remaining sampled trees. To examine potential incongruences between datasets we inspected whether the topology resulting from the combined analyses of all the regions conflicts with the topologies obtained through separate analyses of the plastidic and nuclear sequences. Only conflicting nodes with 70% BS or higher were considered.

The relevance of alternative relationships between the new taxon and other Gloxinieae genera were tested using the “Shimodaira-Hasegawa test” (SH test; Shimodaira & Hasegawa, 1999; Goldman & al., 2000) implemented in PAUP\* v.4.0b8 (Swofford, 1999). Topologies that force the monophyletic relationships between *Chautemsia* and the members of the other Gloxinieae genera were built in MacClade (Maddison & Maddison, 1992). For each of these topological constraints, a new heuristic search was performed using our 5-regions matrix and the maximum parsimony criteria. Then, two subsets of trees obtained with and without constraint were compared simultaneously with the REL option of the SH test and 1000 bootstrap replicates as implemented in PAUP\* v.4.0b8 (Swofford, 1999). We used the GTR model with proportion of invariant sites (I) and gamma shape (G) parameters (six substitution types: A/C = 0.8511, A/G = 2.2387, A/T = 0.8533, C/G = 0.5712, C/T = 3.5860, G/T = 1.0000, I = 0.5094, G = 0.6706, freqA = 0.3041, freqC = 0.2038, freqG = 0.1995, freqT = 0.2926). The model and all parameters were selected based on the results of analyses using Modeltest v.3.7 and AIC (Posada & Crandall, 1998).

To test the influence of taxon sampling on the phylogenetic position of *Chautemsia*, newly generated sequences for this taxon were added to the ITS/*trnL-F* dataset of Roalson & al. (2005b). New sequences for *Gloxiniopsis racemosa* (Benth.) Roalson & Boggan, *Goyazia petraea* (S.M. Phillips) Wiehler were also added to this dataset. Accessions for which either ITS or *trnL-F* sequences were not available were excluded from the dataset (i.e., *Bellonia spinosa* Swartz, *Gloxinia*

*planalta* Wiehler and *Solenophora tuerckheimiana* J.D. Sm.) The resulting matrix includes an extensive sampling in the tribe Gloxinieae with 59 species representing all the 19 genera recognized by Roalson & al. (2005a). Phylogenetic analyses of this combined ITS/*trnL-F* dataset were performed using MrBayes v.3.1 with each region treated as a single data partition and following the procedures defined above.

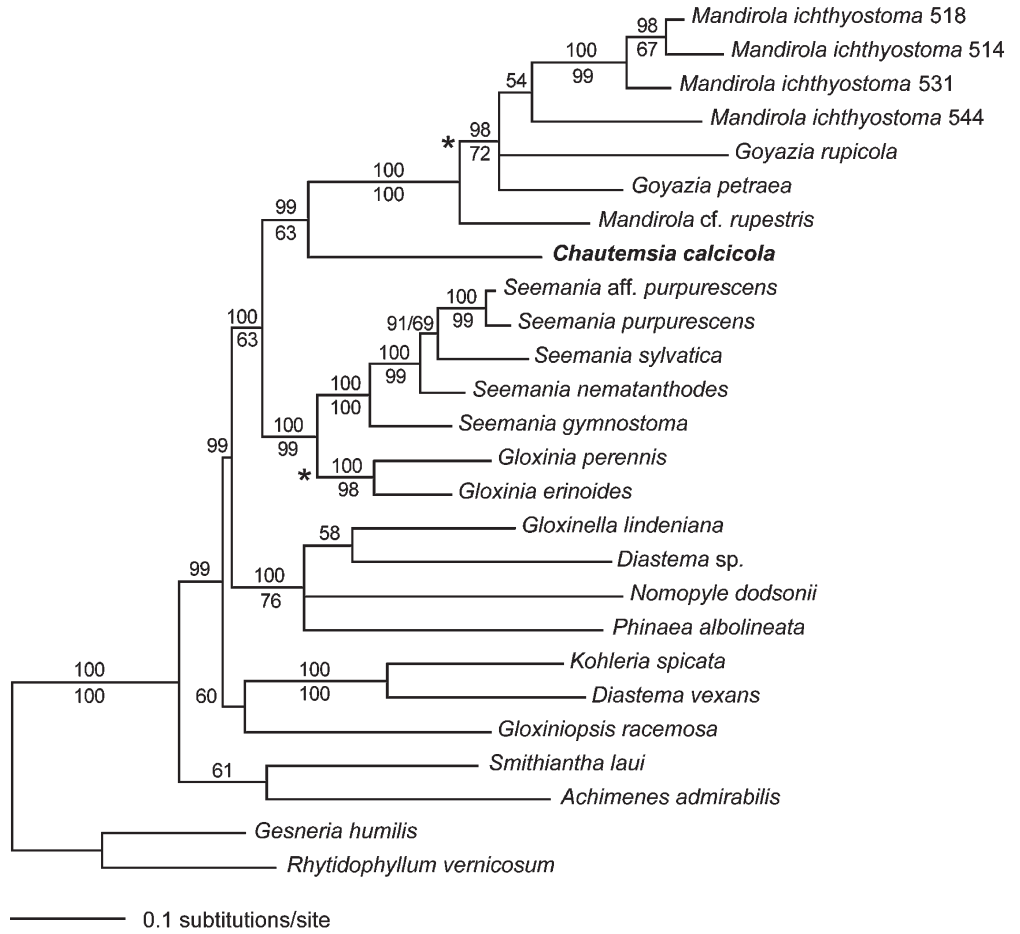
## ■ RESULTS

**Phylogenetic analyses.** — The combined matrix of all DNA regions comprised a total of 4124 characters with 294 variable sites and 96 parsimony-informative characters. Of these, 2273 (294 variable, 96 parsimony-informative) were derived from plastidic sequences, 701 (185 variable, 68 parsimony-informative) from *ncpGS*, and 650 (244 variable, 141 parsimony-informative) from ITS.

The Bayesian 50% majority rule consensus tree of the total combined dataset is shown in Fig. 1. This topology is identical to the one obtained using parsimony analyses. Values of posterior probability and maximum parsimony bootstrap are indicated at each node. The comparison of this tree with results derived from separate analyses of the nuclear and plastid datasets indicated two supported topological incongruences (BS > 70%). Both are in the plastid-based topology: one is related with the placement of *Mandirola cf. rupestris* (Gardner) Roalson & Boggan within the *Mandirola/Goyazia* clade and the other concerns the monophyly of the two *Gloxinia* species with the *Gloxinia/Seemannia* clade. Because these topological incongruences do not involve the newly described genus, only results obtained from the complete dataset will be discussed. *Chautemsia* appears sister of a clade including the Brazilian genera *Goyazia* Taub. and *Mandirola* (Fig. 1). This relationship has a posterior probability of 99% but a bootstrap support of only 63%. This lineage is in turn sister with the *Seemannia/Gloxinia* clade (PP = 100%, BS = 63%). The other relationships within Gloxinieae agree with the topology presented in Roalson & al. (2005b: fig. 6) and Roalson & al. (2008: fig. 2). Unfortunately, the low supports for the basal relationships within the Gloxinieae observed by Roalson & al. (2005b) were little improved in our analyses despite the larger number of molecular characters used. The SH-tests of alternative topologies forcing *Chautemsia* to be monophyletic with other Gloxinieae genera, indicate significant differences in the likelihood scores for all genera ( $P < 0.05$ ) except *Gloxiniopsis* ( $P \geq 0.06$ ). In further Bayesian analysis of the extended ITS/*trnL-F* matrix of Roalson & al. (2005b), *Chautemsia* appears in an unresolved position, as a single branch at the base of a large polytomy that groups all the major lineages of South American Gloxinieae (result not shown; see Roalson & al., 2005b).

**Morphological traits.** — The new taxon displays the following floral characters: frondose florescence, corolla infundibular-cylindrical, erect in the calyx, floral anthocyanin absent (yellow only in the throat), corolla tube internally glabrous except at the throat with glandular trichomes, nectary divided into five finger-like glands. The fruit structure found

**Fig. 1.** Bayesian 50% majority rule consensus tree resulting from the combined analysis of the *rpl16* intron, *rps16* intron, *trnL-F* region, *ncpGS* and ITS sequenced for 24 accessions in Gloxinieae and two outgroups in Gesnerieae. Numbers above the branches are PP values; those below are bootstrap values when  $\geq 50\%$ . Asterisks indicate conflicting nodes with BS > 70% between this tree and the plastid-derived tree.



**Table 1.** Morphological comparison of *Chautemsia* with other genera in Gloxinieae.

Character	<i>Chautemsia</i>	<i>Gloxinia</i>	<i>Gloxiniopsis</i>	<i>Mandirola</i>	<i>Seemania</i>	<i>Goyazia</i>	<i>Diastema</i>
Florescence	Frondose	Bracteose, rarely frondo-bracteose	Bracteose	Frondose	Fondose, rarely frondo-bracteose	Frondose	Bracteose
Corolla color	White with yellow throat	White, purple, pinkish or partly maroon	White with purple stripes in throat	White and partly purple, violet or pinkish	Red, orange or dark pink	White or white with violet stripes, pinkish, purple	White with purple markings on lobes or reddish
Corolla shape	Infundibular-cylindrical	Campanulate, campanulate-infundibular or cylindrical-bilabiate	Campanulate	Campanulate-infundibular or hypocrateriform	Cylindrical-ventricose or infundibular-ventricose	Infundibular-cylindrical	Infundibular-cylindrical
Nectary	5 finger-like glands	Absent or annular with 5 lobes	Absent or vestigial	5 lobed glands, or annular with 5 lobes or unlobed	Annular unlobed	5 lobed glands or annular unlobed	5 finger-like glands
Capsule consistence	Fleshy	Dry	Fleshy	Dry	Dry	Dry	Fleshy
Capsule dehiscence	Dorsal; splitting the hypanthium to the base	Apical	Dorsal; splitting the hypanthium to the base	Apical	Apical	Apical	Dorsal; splitting the hypanthium to the base

in this species matches the definition of horizontal follicular fruits (Weber, 2004). This kind of fruit is fleshy at maturity, horizontally held with the hypanthium splitting dorsally and completely to the base, exposing the seeds (Fig. 2). None of these characters is exclusive of this taxon, but this set of characters is not found in any other genus of Gloxinieae (Table 1).

## DISCUSSION

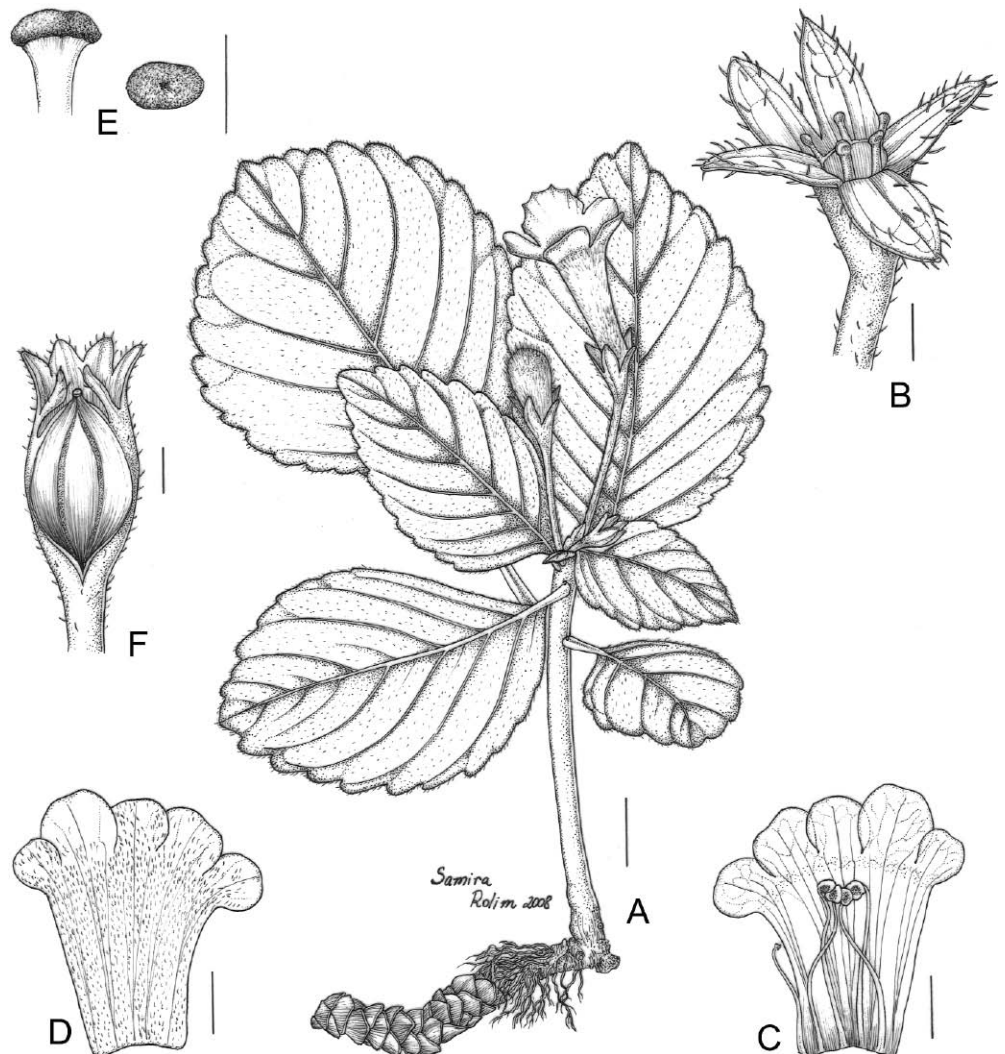
According to the classification of Wiehler (1976) and his identification key to tribes of Gesnerioideae (Wiehler, 1983), *Chautemsia* fits in the tribe Gloxinieae. Indeed, the new taxon displays all the diagnostic traits of Gloxinieae such as rhizomes with fleshy leaf scales, opposite leaves and inferior ovary. Moreover, our phylogenetic result clearly confirmed that *Chautemsia* is nested within Gloxinieae (Fig. 1).

Combined analyses of five DNA regions support *Chautemsia* as the sister lineage of a clade comprising the genera *Goyazia* and *Mandirola* (Fig. 1). This grouping is geographically consistent since species in this clade are endemic to central or

southeastern Brazil, outside of the range of other Gloxinieae species. *Chautemsia* can be easily distinguished from other Brazilian taxa based on features such as its white corolla, horizontal follicular fruit and nectary composed of five long finger-like and separate glands (Table 1; Fig. 2). The phylogenetic affinities of *Chautemsia* with Brazilian taxa should be considered with caution. Indeed, the SH-tests indicated that our data do not allow to reject the alternative relationship that places *Chautemsia* and the monotypic genus *Gloxiniopsis* in a same clade ( $P = 0.06$ ). Analysis of the phylogenetic relationship of *Chautemsia* based on the taxonomically more exhaustive ITS/*trnL-F* dataset of Roalson & al. (2005b) did not provide further information about the generic affinities of *Chautemsia*. These appeared as unresolved at the base of a large polytomy including the main lineages of South American Gloxinieae (Roalson & al., 2005b, 2008). Therefore, a more confident placement of *Chautemsia* in Gloxinieae will need additional data to help improve the supports of the basal relationships among the main Gloxinieae lineages.

Morphologically, *Chautemsia* most closely resembles *Diastema* Benth., which also includes weak herbs with an

**Fig. 2.** *Chautemsia calcicola*. **A**, habit; **B**, calyx and finger-like nectary glands (ovary and corolla removed); **C**, longitudinal section of inner corolla, showing stamens and stamino-  
node; **D**, longitudinal section of outer corolla; **E**, lateral and superior views of stigma; **F**, mature dehiscent fruit (seeds removed). Scales: A = 1 cm; B = 0.5 mm; C, D = 5 mm; E = 1 mm; F = 2 mm. (Illustrator Samira Rolim).



infundibular-cylindrical corolla, a nectary of five long, finger-like, separate glands, and horizontal follicular fruit, dehiscent dorsally and splitting the hypanthium to the base (Weber, 2004). *Chautemsia* can be distinguished from *Diastema* by the following characters: frondose florescence with solitary flowers (vs. bracteose), corolla white with unspotted lobes, and stigma stomatomorphic (vs. bilobed; Table 1). Furthermore, *Chautemsia* occurs only in southeastern Brazil whereas *Diastema* is found from Bolivia to Mexico and rarely occurs in Brazil (only in Acre near the border of Peru). Our result confirms the finding of Roalson & al. (2005b) that *D. vexans* H.E. Moore is segregated from the rest of the genus (Fig. 1). However, neither of these two lineages was found related with *Chautemsia* indicating that their morphological similarities could be due to convergences. Beside *Diastema*, other taxa share with *Chautemsia* a fleshy fruit with a splitting hypanthium, e.g., *Gloxiniopsis*, *Kohleria* Regel, and the monophyletic group comprising *Gloxinella*, *Monopyle* Moritz ex Benth., *Nomopyle* and *Phinaea* Benth. (Kvist & Skog, 1992; Roalson & al., 2008). None of these taxa have a nectary similar to that of the new genus, however.

In their revision of the genus *Kohleria*, Kvist & Skog (1992) stated: “As is often the case in genera of Neotropical Gesneriaceae, no single feature distinguishes the genus *Kohleria* from related and similar genera in the tribe Gloxinieae, but the genus is set apart by a suite of characters.” In a similar way, the unique combination of characters that define this new taxon and its lack of close relatives in the tribe, leads us to recognize this taxon as a new genus. With this addition, tribe Gloxinieae now comprises 21 genera.

## ■ TAXONOMICAL TREATMENT

*Chautemsia calcicola* A.O. Araújo & V.C. Souza, **gen. & sp. nov.** – Type: Brazil. Minas Gerais, Mun. Iguatama, Fazenda Faroeste, próximo ao município de Arcos, 20°15'45.1" S, 45°40'10.3" W, 690 m alt., 13 Jan. 2005, A.O. Araújo, V.R. Scalon, A. Chautems, G. Barriera & R. Tsuji 500 (holotype: ESA!; isotypes: G!, SPF!, SPFR!). – Fig. 2.

Affinis *Diastemati* Benth. nectario quinque glandulis digitiformibus et capsula carnosae dorsaliter omnino secedente, sed floribus axillaribus solitariis, lobis corollae plene candidis et stigmatibus stomatoformi differt.

Perennial herbs, 4.0–8.5 cm high, with scaly rhizomes. Stems terete, erect, unbranched, usually reduced to 3–4 nodes. Internodes 0.2–3.2 cm long. Leaves opposite, anisophyllous, sometimes strongly unequal in a pair, petiole 0.3–3.2 cm long, blades 0.5–5.5 × 0.4–3.3 cm, elliptic, the basal ones frequently broadly elliptic or circular, apex acute or obtuse, base symmetrical, cuneate or attenuate, margin serrate, sometimes irregularly, adaxial and abaxial surface pubescent, discolor green, 4–5 pairs of secondary veins. Florescence frondose, epedunculate, flowers solitary, 1 or 2 flowers in the upper node, sometimes another flower in the lower node, subtending leaves 1.3–3.1 × 0.3–1.7 cm. Flowers without bracteoles, with pedicel erect-ascending, 0.9–1.5 cm long, glabrescent, zygomorphic, floral tube conic, ca. 2 mm; calyx aestivation open, sepals free,

lobes equal, 0.1–0.3 × 0.1–0.2 cm, green, inside glabrous near base and sparsely pubescent toward apex, outside pubescent to densely pubescent, elliptic, sometimes obovate, with margin entire; corolla infundibular-cylindrical, aestivation imbricate, tube 0.9–1.5 cm long, inside glabrous, sparsely pubescent only in the throat, with glandular, uniseriate, capitate, short or long trichomes, outside sericeous, near base glabrous; tube white to white-yellowish; throat not constricted, yellow, 0.3–0.5 cm diameter; lobes white, broadly depressed-ovate with entire or slightly sinuate margins, glabrous inside, outside glabrous to sparsely sericeous, 0.3–0.5 cm long; stamens 4, included in the corolla, 0.9–1.3 cm long, filaments glabrous, anthers all coherent opening by longitudinal slits, staminode ca. 0.8 cm long.; nectary of 5 long, finger-like, separate glands; ovary inferior; style 1.2–1.4 cm long, sparsely pubescent, glabrous toward apex; stigma stomatomorphic. Capsule 0.8–1.2 × 0.2–0.6 cm, pubescent, ellipsoid, apex erect, fleshy, dehiscent dorsally and splitting the hypanthium to the base, capsule without prominent costae. Seeds without enlarged funicle.

*Etymology.* – The name of this genus (*Chautemsia*) honors Dr. Alain Chautems, curator at the Conservatoire & Jardin Botaniques de la Ville de Genève, who investigated Brazilian Gesneriaceae for over 20 years. He deserves this homage in recognition to his important contribution to the knowledge of this family and to his deep involvement in training new researchers.

*Distribution and ecology.* – Despite extensive investigations of the limestone flora in central and southeastern Brazil made by the first collector of this taxon (Melo, 2008), the species has been so far only found in the localities Arcos, Pains and Iguatama in the region of Formiga, in the western part of Minas Gerais. The species is restricted to deciduous forest remnants on limestone outcrops between 600 and 800 m. Weber (2004) stated that horizontal follicular fruits are probably associated with a rain-splash dispersal mechanism: when seeds are exposed, they are washed away by water drops. In view of the occurrence of the new species on dripping rocks, this kind of dispersal strategy seems quite appropriate.

*Conservation status.* – Endangered, based on the occurrence in an area smaller than 5000 km<sup>2</sup> and known by fewer than five localities (IUCN, 2001).

*Phenology.* – The peak flowering period was observed in January and fruiting specimens were found in February and March, matching the rainy season. During the dry season from May to September, plants are dormant and reduced to scaly rhizomes hidden in rock crevices.

*Paratypes.* – Brazil. Minas Gerais, Mun. Arcos, Fazenda Faroeste, margem direita do Rio São Miguel, 1 Jan. 2003, P.H.A. Melo & J.A. Lombardi 408 (BHCB, G); Mun. Pains, Fazenda Amargoso, MG 439 Km 16, 20°15'05.7" S, 45°39'46.7" W, 6 Mar. 2003, P.H.A. Melo 446 (BHCB, G); Mun. Pains, fronteira entre os municípios de Pains e Formiga, região da nascente do Rio São Miguel, 20°27'22" S, 45°39'15" W, 700–800 m, 25 Jan. 2005, P.H.A. Melo & N.L. Assunção 1257 (BHCB, G); Mun. Iguatama, Fazenda Faroeste, margem esquerda do Rio São Miguel, 20°15'45" S, 45°40'10" W, 700–800 m, 25 Mar. 2005, P.H.A. Melo & J.F. Martins 1315 (BHCB, G).

## ■ ACKNOWLEDGEMENTS

We are grateful to Pablo Hendrigo Melo for communicating the discovery of this plant, sending pictures as well as dried and pickled material and for guiding us to one of its localities. John Boggan kindly provided material of *Seemannia purpurascens* grown at the Smithsonian Institution. We also thank Eric H. Roalson for providing his alignment of DNA sequences and Fadi Avdiya for his help in the laboratory work. The first author thanks Fundação de Amparo a Pesquisa do Estado de São Paulo, International Association for Plant Taxonomy and the Elvin McDonald Research Endowment Fund of the Gesneriad Society, Inc. for supporting this research.

## ■ LITERATURE CITED

- Boggan, J.K., Skog, L.E. & Roalson, E.H. 2008. A review of the Neotropical genera *Amalophyllon*, *Niphaea*, and *Phinaea* (Gesneriaceae-Gloxiniaceae). *Selbyana* 29: 157–176.
- Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- Goldman, N., Anderson, J.P. & Rodrigo, A.G.. 2000. Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* 49: 652–670.
- IUCN. 2001. *IUCN Red List categories and criteria*, version 3.1. IUCN Species Survival Commission. Gland, Switzerland & Cambridge, U.K.: IUCN
- Kvist, L.P. & Skog, L.E. 1992. Revision of *Kohleria* (Gesneriaceae). *Smithsonian Contr. Bot.* 79: 1–83.
- Maddison, W.P. & Maddison, D.R.. 1992. *MacClade*, version 3.01. Sunderland, Massachusetts: Sinauer.
- Melo, P.H.A. 2008. *Flora vascular relacionada aos afloramentos de rocha carbonática no interior do Brasil*. Master thesis, Universidade Federal de Lavras, Minas Gerais, Brazil.
- Möller, M. & Cronk, Q.C.B. 1997. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *Amer. J. Bot.* 84: 956–965.
- Oxelman, B., Lidén, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410.
- Perret, M., Chautems, A., Spichiger, R., Kite, G. & Savolainen, V. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear *ncpGS*. *Amer. J. Bot.* 90: 445–460.
- Posada, D. & Crandall, K.A. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Roalson, E.H., Boggan, J.K. & Skog, L.E. 2005a. Reorganization of tribal and generic boundaries in the Gloxinieae (Gesneriaceae: Gesnerioideae) and the description of a new tribe in the Gesnerioideae, Sphaerorrhizeae. *Selbyana* 25: 225–238.
- Roalson, E.H., Boggan, J.K., Skog, L.E. & Zimmer, E.A. 2005b. Untangling Gloxinieae (Gesneriaceae). I. Phylogenetic patterns and generic boundaries inferred from nuclear, chloroplast, and morphological cladistic datasets. *Taxon* 54: 389–410.
- Roalson, E.H., Skog, L.E. & Zimmer, E.A. 2008. Untangling Gloxinieae (Gesneriaceae): II. Reconstructing biogeographic patterns and estimating divergence times among New World continental and island lineages. *Syst. Bot.* 33: 159–175.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with application to phylogenetic inference. *Molec. Biol. Evol.* 16: 1114–1116.
- Swofford, D. L. 1999. *PAUP\*: Phylogenetic analysis using parsimony (\*and other methods)*, version 4. Sunderland, Massachusetts: Sinauer.
- Swofford, D. 2002. *PAUP\*: Phylogenetic analysis using parsimony (\*and other methods)*, version 4.0b10. Sunderland, Massachusetts: Sinauer.
- Weber, A. 2004. Gesneriaceae. Pp. 63–158 in: Kubitzki, K. & Kadereit, J.W. (eds.), *The families and genera of vascular plants*, vol. 4. Berlin, Heidelberg: Springer.
- Wiehler, H. 1976. A report on the classification of *Achimenes*, *Eucodonia*, *Gloxinia*, *Goyazia* and *Anetanthus* (Gesneriaceae). *Selbyana* 1: 374–404.
- Wiehler, H. 1983. A synopsis of the neotropical Gesneriaceae. *Selbyana* 6: 1–219.
- Zimmer, E.A., Roalson, E.H., Skog, L.E., Boggan, J.K. & Idnurm, A. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences. *Amer. J. Bot.* 89: 296–311.

**Appendix.** Taxa sampled for the study. Samples taken from cultivated material with indicated accession numbers were collected in CJB (Conservatoire et Jardin botaniques de la Ville de Genève greenhouse), GRF (Gesneriad Research Foundation, Sarasota, U.S.A.), MP (Mauro Peixoto private collection, Brazil), or USBRG (Botany Research Greenhouses of the Smithsonian Institution). Voucher numbers are given when present. N.A., no data

Species, accession numbers and/or voucher specimen (herbarium), GenBank accessions (ITS, *ncpGS*, *trnL-F*, *rps16*, *rpl16*)

*Achimenes admirabilis* Wiehler, CJB AC-2510, *A. Chautems* & *M. Perret* 01-033 (G), GQ344504, AJ459689, AJ439827, N.A., AJ487784; *Chautemsia calcicola* A.O. Araujo & V.C. Souza, *A.O. Araujo* & al. 500 (ESA), GQ344506, GQ383515, GQ383543, GQ383581, GQ383631; *Diastema* sp., MP 0872, GQ344510, GQ383517, GQ383546, GQ383585, GQ383634; *D. vexans* H.E. Moore, GRF 840 306, GQ344511, GQ383518, GQ383547, GQ383586, GQ383635; *Gesneria humilis* L., CJB s.n., *A. Chautems* & *M. Perret* 97-020 (G), GQ344513, AJ459683, AJ439821, GQ383588, AJ487778; *Gloxinella lindeniana* (Regel) Roalson & Boggan, *A.O. Araujo* 604 (G), GQ344515, GQ383521, GQ383550, GQ383590, GQ383638; *Gloxinia erinoides* (DC.) Roalson & Boggan, CJB s.n., *A. Chautems* & *M. Perret* 01-034 (G), GQ344519, AJ459690, AJ439828, GQ383593, AJ487785; *G. perennis* (L.) Fritsch, *A.O. Araujo* & al. 536 (ESA), GQ344516, GQ383522, GQ383551, GQ383546, GQ383639; *Gloxiniopsis racemosa* (Benth.) Roalson & Boggan, *A.O. Araujo* 548 (ESA), GQ344514, GQ383520, GQ383549, GQ383589, GQ383637; *Goyazia petraea* (S.M. Phillips) Wiehler, *A.O. Araujo* & al. 457 (ESA), GQ344517, GQ383523, GQ383552, N.A., GQ383640; *G. rupicola* Taub., *A.O. Araujo* & al. 545 (ESA), GQ344518, GQ383524, GQ383553, GQ383592, GQ383641; *Kohleria spicata* (Kunth) Oerst., CJB s.n., *A. Chautems* & *M. Perret* 97-018 (G), GQ344520, AJ459682, AJ439820, GQ383594, AJ487777; *Mandirola ichtyostoma* (Gardner) Seem. ex Hanst., *A.O. Araujo* 514 (ESA), GQ344527, GQ383527, GQ383546, GQ383601, GQ383648; *A.O. Araujo* 518 (ESA), GQ344528, GQ383528, GQ383560, GQ383602, GQ383649; *A.O. Araujo* & al. 531 (ESA), GQ344522, GQ383526, GQ383555, GQ383596, GQ383643; *A.O. Araujo* 544 (ESA), GQ344521, GQ383525, GQ383554, GQ383595, GQ383642; *M. cf. rupestris* (Gardner) Roalson & Boggan, *A.O. Araujo* 530 (ESA), GQ344529, GQ383529, GQ383561, GQ383603, GQ383650; *Nomophyllum dodsonii* (Wiehler) Roalson & Boggan, GRF 9769, GQ344535, GQ383530, GQ383565, GQ383609, GQ383654; *Phinaea albolineata* (Hook.) Benth. ex Hemsl., *A.O. Araujo* & al. 538 (ESA), GQ344538, GQ383531, GQ383566, GQ383612, GQ383655; *Rhytidophyllum vernicosum* Urb. & Ekman, *A.O. Araujo* & al. 600 (G), GQ344539, GQ383532, GQ383567, GQ383613, GQ383656; *Seemannia gymnostoma* (Griseb.) Toursark., *A.O. Araujo* & al. 549 (ESA), GQ344542, GQ383534, GQ383569, GQ383616, GQ383658; *S. nematanthodes* (Kuntze) Schum., *A.O. Araujo* & al. 550 (ESA), GQ344541, GQ383533, GQ383568, GQ383615, GQ383657; *S. aff. purpurascens* Rusby, CJB AC-1481, *A. Chautems* & *M. Perret* 97-019 (G), GQ344540, AJ459684, AJ439822, GQ383614, AJ487779; *S. purpurascens* Rusby, USBRG 02-197, *Ibisch 960111* (US), GQ344544, GQ383535, GQ383571, N.A., GQ383659; *S. sylvatica* (Kunth) Hanst., CJB s.n., *A.O. Araujo* & al. 603 (G), GQ344546, GQ383537, GQ383573, GQ383618, GQ383661; *Smithiantha laui* Wiehler, GRF s.n., GQ344552, AJ459685, AJ439823, GQ383623, AJ487780.