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Independent evolution of pouched flowers in the Amazon is supported by the discovery of a new species of *Lesia* (Gesneriaceae) from Serra do Aracá tepui in Brazil

Gabriel E. Ferreira¹ · Alain Chautems² · Michael J. G. Hopkins¹ · Mathieu Perret²

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Abstract We describe and illustrate *Lesia tepuiensis*, a new species of subshrub from rock outcrops of the Serra do Aracá tepui in Amazonas, Brazil. Phylogenetic analyses based on 7219-aligned base pairs of the plastid and nuclear DNA sequences recovered the new species as sister to *Lesia savannarum*, the type species of *Lesia*, a genus recently described as monotypic. The new species is readily distinguished from *L. savannarum* by having a red, pouched corolla tube, lanceolate calyx lobes, a hirsute to tomentose leaf indument and a rupicolous habit. Phylogenetic analyses of the relationships of *Lesia* with other genera in the subtribe Columneinae indicated that *Lesia* and *Codonanthopsis* form a monophyletic group sister to a clade endemic to the Brazilian Atlantic forest formed by *Nematanthus* and *Codonanthe*. The pouched corolla tube found in the genera *Lesia* and *Nematanthus* appears to have evolved independently in these two lineages. Ecological and distributional data, as well as a key to the species of *Lesia* and related genera, are also provided.

Keywords Amazonas · Columneinae · Convergence · Endemism · Pouched corolla · Taxonomy

Introduction

Plant biodiversity in the Brazilian Amazon is probably underestimated due to the low sampling effort (0.1 specimens per km², Hopkins 2007) and the limited number of floristic surveys in this region (Sousa-Baena et al. 2014; BFG 2015). This is particularly true for the Serra do Aracá, a Brazilian tepui that constitutes the southernmost extension of the Roraima Formation. This table mountain is delimited by vertical escarpments up to 1000 m high resulting from gradual erosion of softer sandstone rocks. As revealed by the pioneer works of Prance and Johnson (1992), this conspicuous formation has a specialized flora with a strong affinity with the Guyana Highland tepuis and many endemic taxa that could have evolved from old vicariance (Maguire 1970) or from dispersal, by successive connection and disconnection of summit floras under the effect of Pleistocene climatic changes (Huber 1988; Rull 2005). Berry and Riina (2005) estimated that 65 % of vascular plant species of the Guiana Shield are endemic and that around 25 % are restricted to the summit of a single tepui. However, most of the investigations took place in the mountains of Venezuela and Guyana, leaving the Brazilian tepuis and their floristic diversity particularly poorly known despite the recent effort to fill this knowledge gap (Silva et al. 2013).

During a survey of Gesneriaceae collections held at the *Instituto Nacional de Pesquisas da Amazonia* (INPA) in Manaus, Brazil, we found unidentified specimens from the top of the rock escarpment of the Serra do Aracá that could not be readily assigned to a known taxa occurring in the

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All samples were collected by researchers following current Brazilian regulations, ICMBio SISBIO License no. 43226-4.

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region. They were characterized by an unusual suite of characters including a rupicolous subshrub habit and red–orange corollas with constricted opening and a large expanded pouch on the lower surface (i.e. hypocyrtoid flowers; [Clark et al. 2015](#)). So far, such features were only known for some species belonging to the genus *Nematanthus* that is endemic to the Brazilian Atlantic forest ([Araújo et al. 2015](#); [Chautems et al. 2005](#)). When compared to Gesneriaceae species occurring in the Amazon and Guyana shield, these specimens from Serra do Aracá share some similarities in habit and flower morphology with the genus *Lesia* J.L.Clark & J.F.Sm., which has been recently segregated from *Nematanthus*, based on phylogenetic evidence ([Smith and Clark 2013](#)). As currently defined *Lesia* comprises a single species, *Lesia savannarum* that occurs in rain forests at low and medium elevation along a peri-Amazonian arc from the Andes foothills in eastern Peru to Colombia, northern Brazil (Amazonas and Roraima) and the Guyana Shield ([Skog and Feuillet 2008](#); [Smith and Clark 2013](#)). Phylogenetic analyses based on a large number of taxa representative of all genera in the subtribe Columneinae (former Episcieae, [Weber et al. 2013](#)) indicated that both *Lesia* and *Nematanthus* form a clade together with *Codonanthe* (Mart.) Hanst. and *Codonanthopsis* Mansf. despite their contrasting flower morphology with white funnel-shaped corollas ([Clark et al. 2006, 2012](#); [Chautems and Perret 2013](#)). A better resolution of the relationships among these morphologically distinct taxa is, however, needed to further explore how flower and biogeographic distribution have evolved in this clade.

To assess the placement of this unknown taxon, we generated DNA sequences from samples collected during a field trip made by the first author to the top of the Serra do Aracá in 2014. New sequences of seven loci from the plastid and the nuclear genome were analysed together with a large data set available for most Columneinae species occurring in Amazonia, Guyana shield and Brazilian Atlantic forest ([Serrano-Serrano et al. 2015](#)). Based on our phylogenetic results and morphological observations, we recognize and describe herein a new species of *Lesia* and clarify the phylogenetic position of the genus in the Columneinae. Our results indicate that despite their close morphological similarity, *Lesia* and *Nematanthus* are not related supporting the independent evolution of pouched flower in the Amazon and the Brazilian Atlantic forest biomes.

Materials and methods

Specimens of the described species were collected during fieldwork carried out at the Serra do Aracá, Barcelos, Amazonas, Brazil, in August of 2014. All pressed material is deposited in the INPA and G herbaria.

This new taxon and *L. savannarum* were sequenced for five plastid DNA regions (*atpB-rbcL*, *matK*, *rps16*, *rpl16* and *trnL-trnF*), the nuclear ribosomal internal transcribed spacer (ITS) and a portion of the plastid-expressed glutamine synthetase gene (*ncpGS*). These sequences were added to available sequence data generated by [Serrano-Serrano et al. \(2015\)](#) and [Perret et al. \(2013\)](#). A total of 59 accessions were analysed, representing 19 genera from the subtribe Columneinae and three outgroups. Most species in the genera *Lesia* (1 out of 1), *Nematanthus* (20 out of 39), *Codonanthe* (8 out of 8) and *Codonanthopsis* (13 out of 13) were sampled. The species *Gesneria humilis*, *Kohleria spicata* and *Sinningia schiffneri* representing the main other subtribes in the tribe Gesneriaceae were selected as outgroups ([Perret et al. 2013](#)).

DNA was isolated from silica-gel-dried leaf tissue using the NucleoSpin Plant II (Macherey–Nagel, Düren, Germany) following the manufacturer’s protocol. Both polymerase chain reaction (PCR) amplifications and sequencing reactions followed the procedures described in [Perret et al. \(2013\)](#). The primers used to amplify the *atpB-rbcL*, *matK*, *rpl16*, *rps16*, *trnL-trnF* intron-spacer and the *ncpGS* are given in [Perret et al. \(2003, 2013\)](#). ITS was amplified and sequenced with primers ITS 5P and ITS 8P ([Möller and Cronk 1997](#)). The program Sequencher 4.7 (Gene Code Corp., Ann Arbor, MI, USA) was used to edit and assemble complementary strands. Newly acquired sequences have been deposited in GenBank. Voucher information and GenBank numbers for each sequence used in this study are given in Online Resource 1.

All sequences were first aligned using MAFFT ([Kato and Toh 2010](#)) and then imported to Mesquite 3.03 ([Maddison and Maddison 2015](#)) for verification and manual adjustment (Online Resource 2). For each DNA region, the optimal substitution models were assessed using jModelTest2 ([Darriba et al. 2012](#)) according to the Akaike information criterion (AIC). The best-fit model was HKY + Γ for *ncpGS*, and GTR + Γ for all other genes.

Phylogenetic analyses were performed on the CIPRES portal (www.phylo.org; [Miller et al. 2010](#)) using maximum likelihood (ML) and Bayesian Inference (BI). Since prior analyses of each separate DNA region did not reveal any topological conflicts concerning the phylogenetic position of *Lesia*, we conducted phylogenetic analyses on the concatenated matrix. Maximum likelihood analyses were conducted using the software RAXML ([Stamatakis 2014](#)). We performed 100 runs using the preferred model for each gene partition as described above. The robustness of the tree was calculated with nonparametric standard bootstrap resampling and 1000 pseudo-replicates ([Felsenstein 1985](#)). Bayesian inference analyses were performed using MrBayes 3.2.3 ([Ronquist et al. 2012](#)) following the procedure described in [Serrano-Serrano et al. \(2015\)](#). All the

parameters values were unlinked across gene partitions and estimated during the Markov chain Monte Carlo (MCMC) runs. Two independent analyses were run from different random trees. The chains were run for 20,000,000 generations, with trees sampled every 1000th generation. We determined chain convergence and burn-in length (20 % of the sampled generations) by examining trace plots of each parameter in Tracer v.1.6 (Drummond and Rambaut 2007). A consensus tree was calculated by removing the burn-in period and combining the two runs.

The evolution of pouched flower was reconstructed on the Bayesian phylogram using maximum likelihood as implemented in Mesquite 3.03 (Maddison and Maddison 2015). To test the hypothesis that clades with pouched flowers (i.e. *Lesia* and *Nematanthus*) are monophyletic, we performed a Bayes factor comparison (Kass and Raftery 1995) between this topological hypothesis and the alternative hypothesis that clades occurring in the Amazon (i.e. *Lesia* and *Codonanthopsis*) are monophyletic independently of their flower morphology. The marginal likelihood for each topology model was estimated through the stepping-stone method (Xie et al. 2011) in MrBayes 3.2 using 50 steps. The estimation was performed with a run of 10,000,000 generations, with a diagnostic frequency of 2500 generations, for each topology model. Partitions and other parameters were set as described above.

Results

The combined matrix of all DNA regions comprised a total of 7219 characters with 1418 variable sites and 694 parsimony-informative characters. Of these, 5655 (857 variable, 351 parsimony-informative) were derived from plastidic sequences, 748 (223 variable, 113 parsimony-informative) from *ncpGS*, and 816 (338 variable, 230 parsimony-informative) from ITS. The ML and BI analyses resulted in congruent topologies, and only the BI consensus tree is shown in Fig. 1. Our results support the placement of the new species as sister to *L. savannarum* (PP 1, BS 90). *Lesia* appears to be sister, to *Codonanthopsis* (PP 1, BS 64; Fig. 1), which form a monophyletic group (PP 1, BS 78), agreeing with the recent revision of *Codonanthopsis* (Chautems and Perret 2013). The clade formed by *Lesia* and *Codonanthopsis* occurs in the Amazonia, Guyana and Central America, whereas its sister clade composed of the genera *Nematanthus* and *Codonanthe* is endemic to the Brazilian Atlantic forest (Fig. 2).

Our reconstruction of ancestral states indicates that pouched flowers evolved independently in *Lesia* and *Nematanthus* (Fig. 1). The topological test showed that the hypothesis of monophyly between clades including

pouched flowers was significantly less probable than the alternative hypothesis of monophyly between clades that co-occur in the Amazon biome but exhibit distinct flower morphology (i.e. *Lesia* and *Codonanthopsis*), (marginal log likelihoods -27958.76 and -27926.53 , respectively). The large difference in log likelihoods (>5) (Kass and Raftery 1995) constitutes strong contradictory evidence against the monophyly of *Lesia* and *Nematanthus* supporting the independent evolution of pouched flowers in the Amazon and the Brazilian Atlantic forest.

Discussion

Our phylogenetic results clearly demonstrate that the new species described here is best placed in *Lesia*, a genus previously recognized as monotypic (Smith and Clark 2013). This species differs from its sister species *L. savannarum* by having a red–orange pouched corolla tube, lanceolate calyx lobes with nearly entire margin, hirsute to tomentose leaf indument and a rupicolous habit (Table 1; Fig. 3b). The contrast between the widespread distribution of *L. savannarum* in the lowland rainforest along a large peri-Amazonian arc and the narrowly distributed *L. tepuiensis* on the top of a single tepui (Fig. 3f) suggests that their divergence could have resulted from either peripatric or parapatric speciation along an altitudinal gradient. Further calibration of this divergence in time would help to identify whether long term isolation or Pleistocene climatic changes best explain the divergence of these sister species (Rull 2005).

Our phylogenetic analysis, based on the most complete taxonomic sampling used so far in the group, did not support previous results showing *Lesia* as sister to *Nematanthus* or sister to a clade composed of *Codonanthopsis*, *Codonanthe* and *Nematanthus* (Clark et al. 2006; Smith and Clark 2013). Instead, *Lesia* is here found sister to *Codonanthopsis* (Fig. 1). This grouping is geographically consistent since species in both clades co-occur in a wide area comprising the Amazon basin and the Guayana highlands (Fig. 2; Chautems and Perret 2013). By contrast, the sister clade composed of *Codonanthe* and *Nematanthus* is restricted to Brazilian Atlantic Forest (Fig. 2). According to Perret et al. (2013), such divergence of lineages between Amazonia and the Brazilian Atlantic forest biomes could date back to the Oligocene/Miocene boundary (ca. 23 Mya) and suggest, together with other evidences from various groups of plants and animals, that the occurrence of a more open vegetation along a dry diagonal (i.e. Chaco, Cerrado, Caatinga) has been an efficient barrier to dispersal of rainforest taxa during the Miocene (Pellegrino et al. 2011; Fouquet et al. 2012; Werneck et al. 2012; Terra-Araujo et al. 2015).

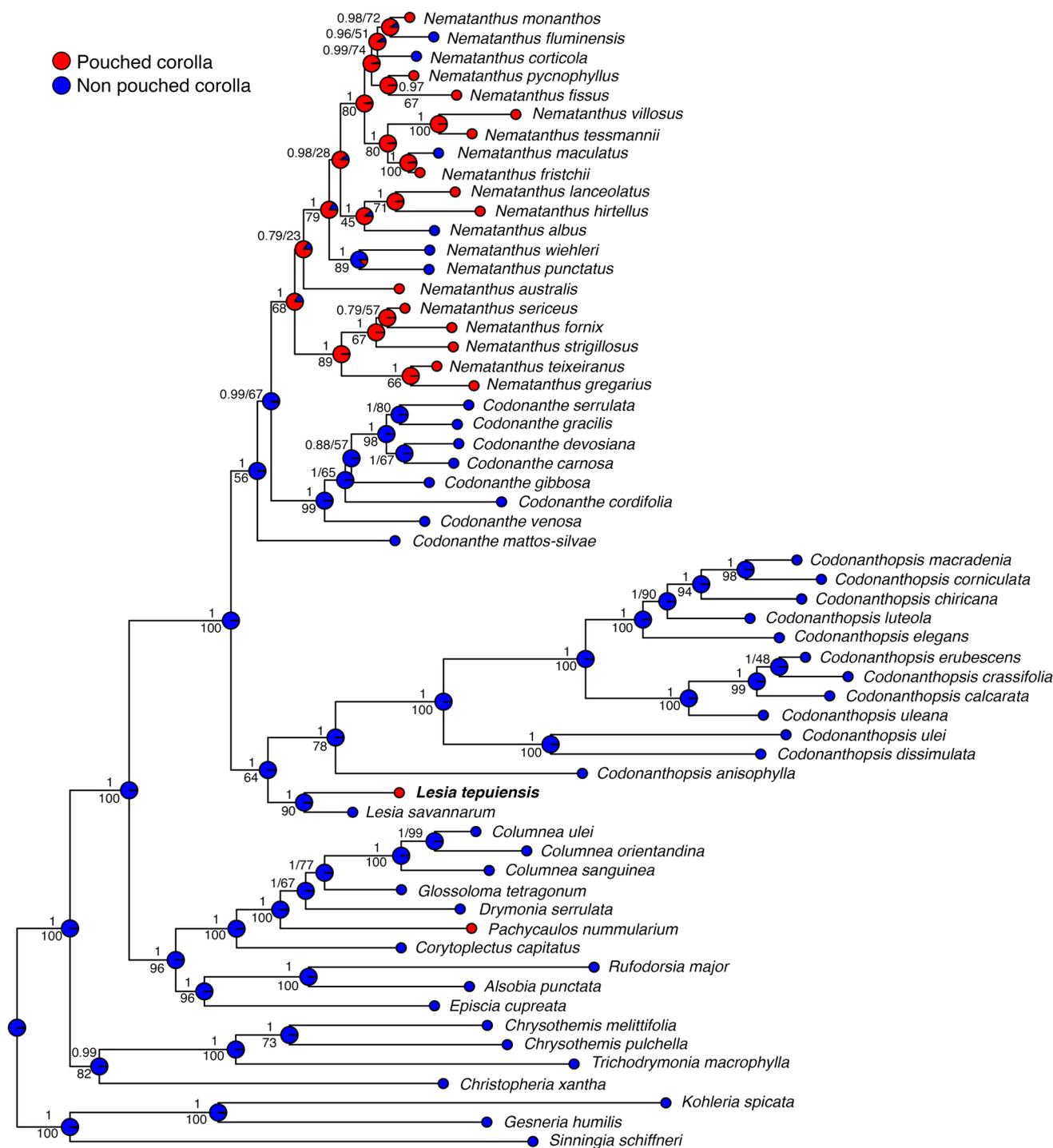


Fig. 1 Bayesian 50 % majority rule consensus tree of Columneinae resulting from the combined analysis of plastid loci *atpB-rbcL*, *matK*, *rps16*, *rpl16*, *trnL-trnF* and the nuclear regions *ncpGS* and ITS.

Numbers above branches are Bayesian posterior probabilities. Numbers below branches are maximum likelihood bootstrap. Evolution of pouched flowers is reconstructed using maximum likelihood

Morphologically, *Lesia* can be easily distinguished from *Codonanthopsis* by its yellow or red tubular corolla and the lack of corolla spur (Fig. 3; Chautems and Perret 2013). On the other hand, *Lesia* lacks unambiguous synapomorphies that could be used to distinguish it from *Nematanthus*. In

particular, *Lesia tepuiensis* shares the same flower morphology, characterized by a constricted flower opening and a throat with an expanded pouch on the lower surface (hypocrytoid), with some species of *Nematanthus* such as *N. strigillosus* (Mart.) H.E.Moore (Fig. 1). Pouched flowers

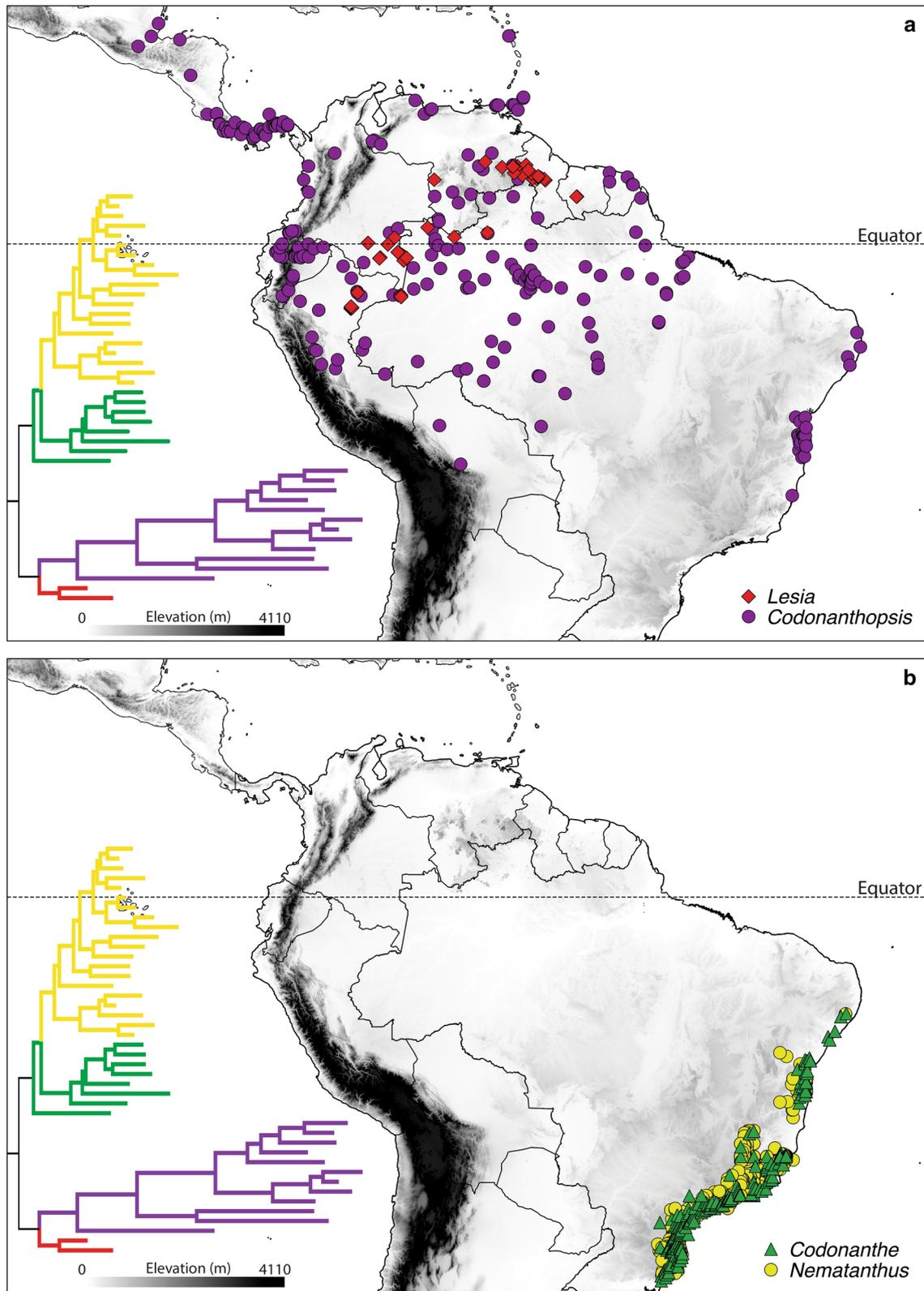


Fig. 2 Distribution of *Codonanthe*, *Codonanthopsis*, *Lesia* and *Nematanthus* in South and Central America. Clades is identified with colours on the Bayesian phylogram shown on the left. **a** Occurrences of *Lesia* and *Codonanthopsis*, **b** occurrences of *Codonanthe* and *Nematanthus*

Table 1 Comparison of *Lesia tepuiensis* with related genera and species

Traits	Species			
	<i>Codonanthopsis calcarata</i>	<i>Lesia savannarum</i>	<i>Lesia tepuiensis</i>	<i>Nematanthus strigillosus</i>
Petiole length (cm)	1	2.0–5.5	0.3–0.7	0.1–0.9
Leaf blade indumentum abaxial surface	Glabrous	Hirsute to tomentose	Densely lanate	Pubescent to tomentose
Leaf blades length (cm)	2.0–6.8	7.5–16.0	3.0–6.0	1.5–3.5
Pedicel indument	Puberulent	Hirsute to tomentose	Lanate	Sericeous
Pedicel length (cm)	0.7–1.7	0.9–1.2	0.9–1.4	0.2–1.4
Calyx colour	Green	Red	Green	Green
Calyx lobes (cm)	0.3	2–3	1.2–1.4	0.6–1.3
Calyx lobes—margin	Entire	Dentate at base and entire near apex	Entire to sparsely serrate	Entire
Corolla	Funnel-shaped, oblique in calyx	Slightly ventricose, erect in calyx	Strongly pouched, erect in calyx	Pouched, erect in calyx
Corolla colour	White internally pinkish or light purple	Yellow	Red–orange	Orange to red
Corolla length (cm)	1.6–2.5	2.0–2.8	2.8–3.0	1.3–2.5
Corolla lobes—length (mm)	0.3–0.8	2	2–3	2
Corolla lobes—colour	White	Yellow	Red	Yellow
Corolla spur	Present	Absent	Absent	Absent
Fruit	Fleshy capsules	Fleshy capsules	Fleshy capsules	Berries
Extrafloral nectaries	Present	Absent	Absent	Absent
Habit	Epiphytic, growing in ant-gardens	Epiphytic or terrestrial	Rupicolous	Rupicolous
Geographic distribution	Northern South America	Amazonian basin	Amazonian basin	Brazilian Atlantic forest

have been shown to be associated with hummingbird pollination in *Nematanthus* (Buzato et al. 2000, Wolowski et al. 2013) and in *Drymonia* where it has evolved independently at least three times (Clark et al. 2015). Such constricted flowers may have evolved to better guide hummingbird bills and increase precision and consistency of pollen placement. Alternatively, the narrow openings of these flowers could promote specialization on hummingbird by excluding visitation by less efficient bees (i.e. anti-bee trait; Clark et al. 2015). Convergent evolution of pouched flowers also occurs in several other genera of Gesnerioideae such as *Besleria* Plum. ex L., *Columnnea* L., *Corytoplectus* Oerst., *Gasteranthus* Benth., *Pearcea* Regel, *Pachycaulos* J.L.Clark & J.F.Sm. and *Paradrymonia* Hanst. (Wiehler 1978; Clark et al. 2015). Our results indicate that the evolution of pouched flowers in *Lesia* constitutes an additional origin of this flower type in the Neotropical Gesneriaceae.

Taxonomic treatment

Lesia tepuiensis G.E.Ferreira & Chautems, **sp. nov.**—
TYPE: Brasil, Amazonas, Barcelos, Serra do Aracá,

rupícola nas margens do igarapé Preto, próximo a cachoeira Eldorado, 23 Aug 2014, G.E. Ferreira et al. 262 (INPA! holotype; G! isotype) (Figs. 3, 4).

Lesia tepuiensis can be distinguished from *L. savannarum* by its smaller leaf blades (3–6 vs. 7.5–16 cm), densely lanate abaxially (vs. hirsute to tomentose), petiole 0.3–0.7 cm (vs. 2–5.5 cm), green calyx (vs. dark red) with margin entire at base (vs. dentate), corolla orange–red and strongly pouched (vs. yellow and ventricose towards apex).

Rupicolous subshrub. Stems erect, sometimes branched, terete, 0.4–1.0 m tall, up to 0.5 cm diam., subwoody, internodes 0.3–1.0 cm long, sericeous, whitish. Leaves opposite, deciduous at base, subequal in a pair; petiole green–whitish, sericeous, 0.3–0.7 cm long; blade ovate to elliptic, 3–6 × 0.9–2.2 cm, coriaceous, apex acute, base rounded to subcordate, margin sparsely denticulate, adaxial surface sericeous, abaxial surface densely lanate, lateral veins 5–7 per side. Inflorescences axillary, reduced to one flower; pedicels 0.9–1.4 cm long, lanate. Calyx lobes fused at base, erect, both sides sericeous, subequal, lanceolate, 1.2–1.4 × 0.4–0.6 cm, apex attenuate, margin nearly entire to sparsely serrate. Corolla erect in relation to calyx, orange–red, 2.8–3.0 cm long, tube 0.4 cm diam and

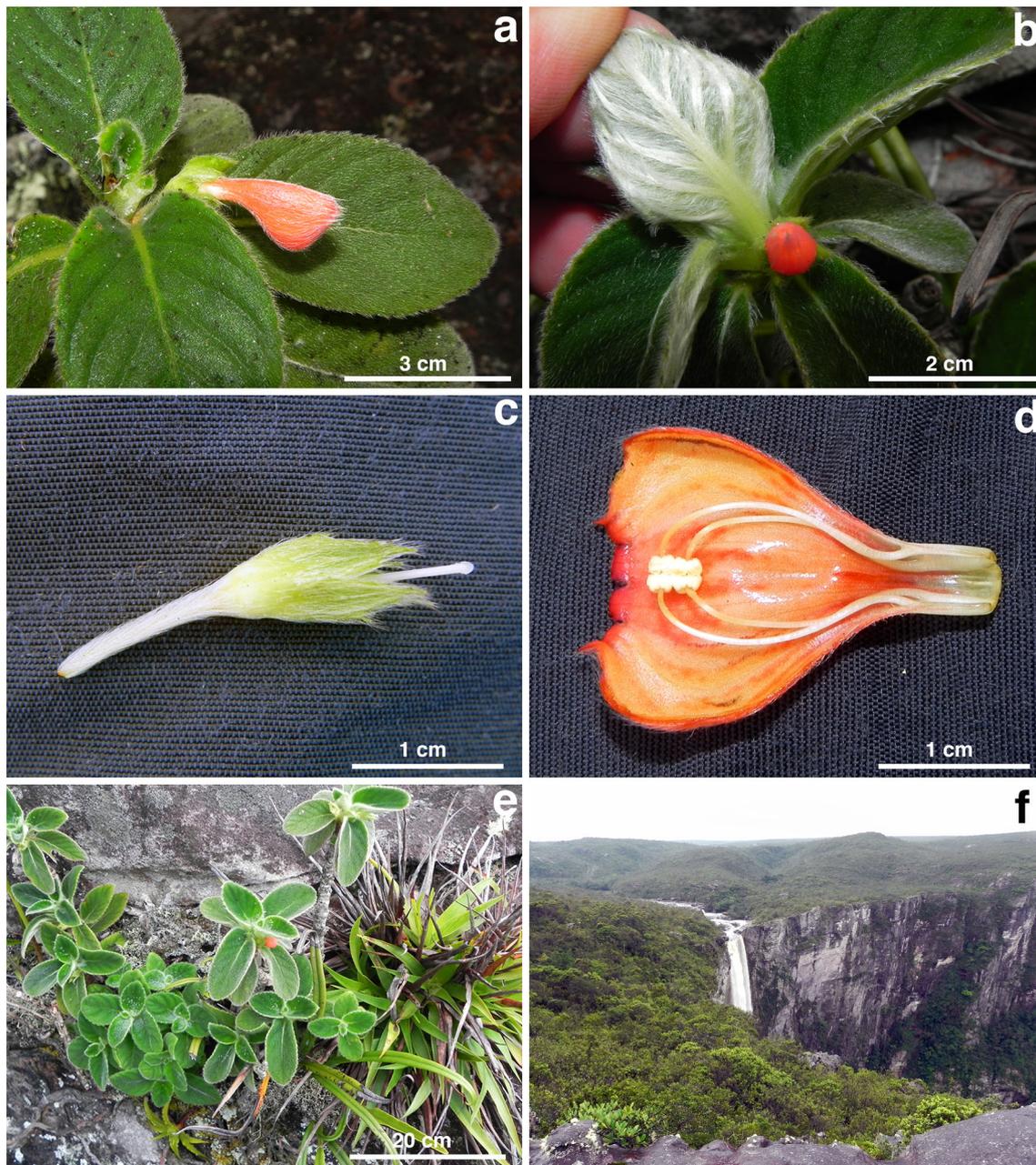


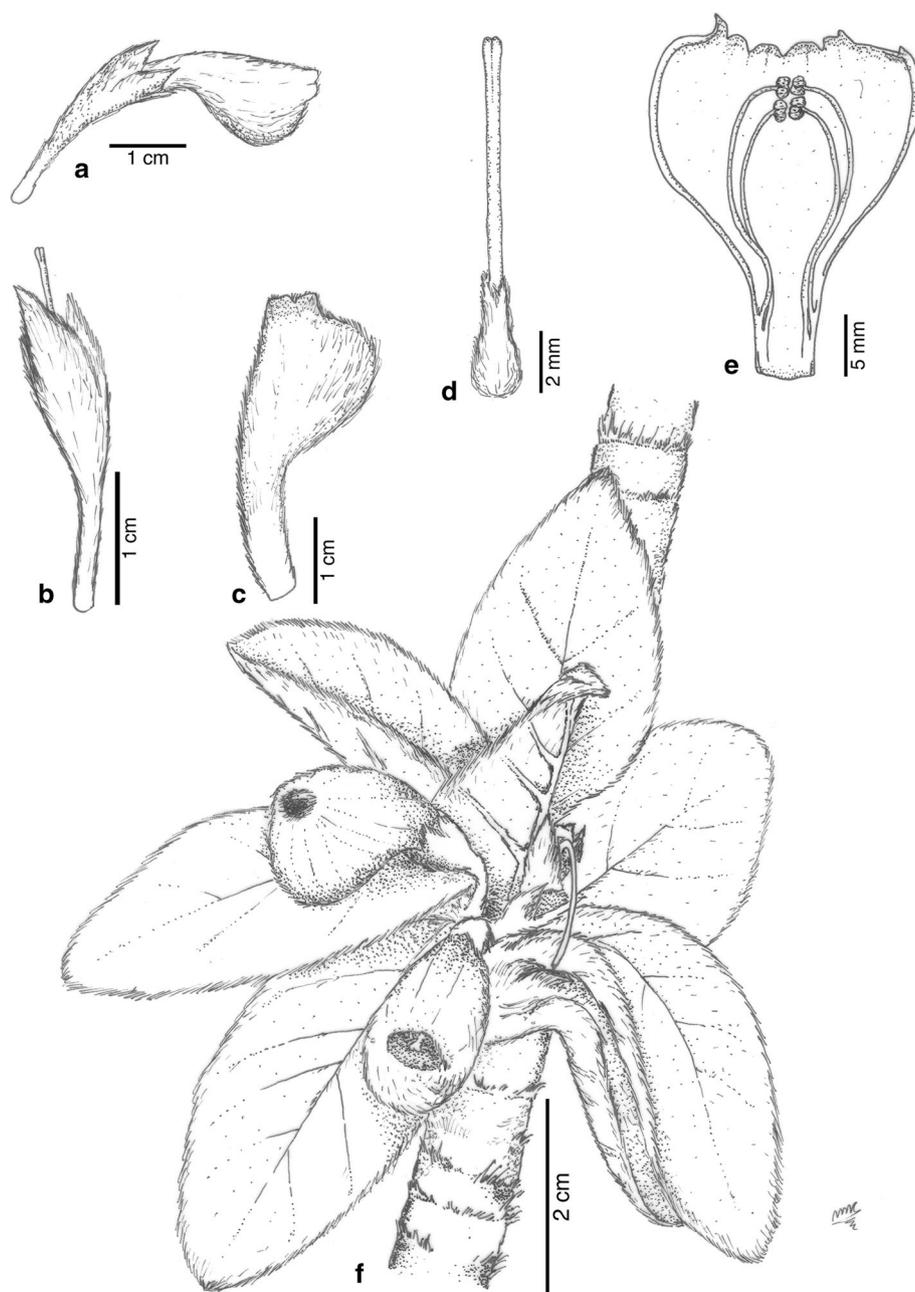
Fig. 3 *Lesia tepuiensis*. **a** Corolla, lateral view, **b** leaf venation on the lower surface and corolla, front view, **c** calyx, style and stigma, **d** laterally cut corolla showing the stamens and anthers, **e** habit, **f** general view of the species habitat (**a–e** from G.E. Ferreira et al. 262)

0.8–1.0 cm long at base, widened for most of its length in a ventral pouch (hypocyrtoïd), 1.6–1.9 cm long, constricted in a narrow mouth, ca. 3 mm diam, exterior glabrous at base, densely white-sericeous towards apex, interior glabrous, lobes subequal, erect, elliptic, 0.2–0.3 × 0.2 cm, margin erose to denticulate. Stamens 4, 2.0–2.8 cm long, included in the throat; filaments glabrous, adnate to the base of the corolla tube for 3–5 mm; anthers coherent in pairs, quadrangular, 1 × 1 mm, staminode 0.2 cm long. Ovary glabrous, ovoid, 3 mm long, style glabrous, 1.4 cm long, stigma 2-lobed, pubescent. Nectary a bilobed gland

on dorsal side of ovary. Fruit a fleshy display capsule, subglobose, ca. 1.0 × 0.8 cm, yellow, sericeous.

Distribution and Ecology: The species is so far known only from the Serra do Aracá, a tepui located in the municipality of Barcelos, in the northeastern part of Amazonas state (Fig. 5). The tepui is found in elevations from 1000 to 16,000 m and formed by Upper Precambrian quartzitic sandstone with an intrusion of granitic rock. It is inserted within the Amazon rainforest and has rainforest, cloud forest and rupicolous vegetation types (Prance and Johnson 1992). *Lesia tepuiensis* occurs predominantly on

Fig. 4 *Lesia tepuiensis* (from G.E. Ferreira et al. 262). **a** Corolla without calyx and trichomes. **b** corolla outline in lateral view, **c** ovary and style, **d** corolla in front view, **e** anthers in front view, **f** habit



rocky outcrops near the Eldorado waterfall (Fig. 3f) at ca. 1100 m elevation, but some specimens were found growing on rocks in the forest in the vicinity of the waterfall. This species is variable and ranges from very short internodes (0.3–0.5 cm long) and smaller leaf blades ($3\text{--}4 \times 0.9\text{--}1.2$ cm) in the open rock population (G.T. Prance 29032, G.E. Ferreira et al. 262, R.C. Forzza 8023) to longer internodes (0.7–1.0 cm long) and larger leaf blades ($4\text{--}6 \times 1.9\text{--}2.2$ cm) in shaded populations (A.S. Tavares 132). The population collected at the Eldorado waterfall included 30 individuals, but more individuals probably occur on nearby waterfalls that were not readily accessible.

Phenology: Flowers from February to September, fruits from May to November.

Etymology: The specific epithet refers to “tepuí” the indigenous word that is used locally to characterize the flat-topped table mountains where the species was found and means “house of the gods” (Huber 1987).

Diagnostic Characters and Relationships: *Lesia tepuiensis* is easily distinguished from *L. savannarum* by its smaller, leaf blades abaxially densely lanate, green calyx, 1.2–1.4 cm long, elliptic lobes, margin sparsely serrate and orange–red corolla (vs. hirsute to tomentose leaf blade, red calyx, ovate lobes, margin dentate at base and entire near apex, and yellow corolla; Fig. 6).

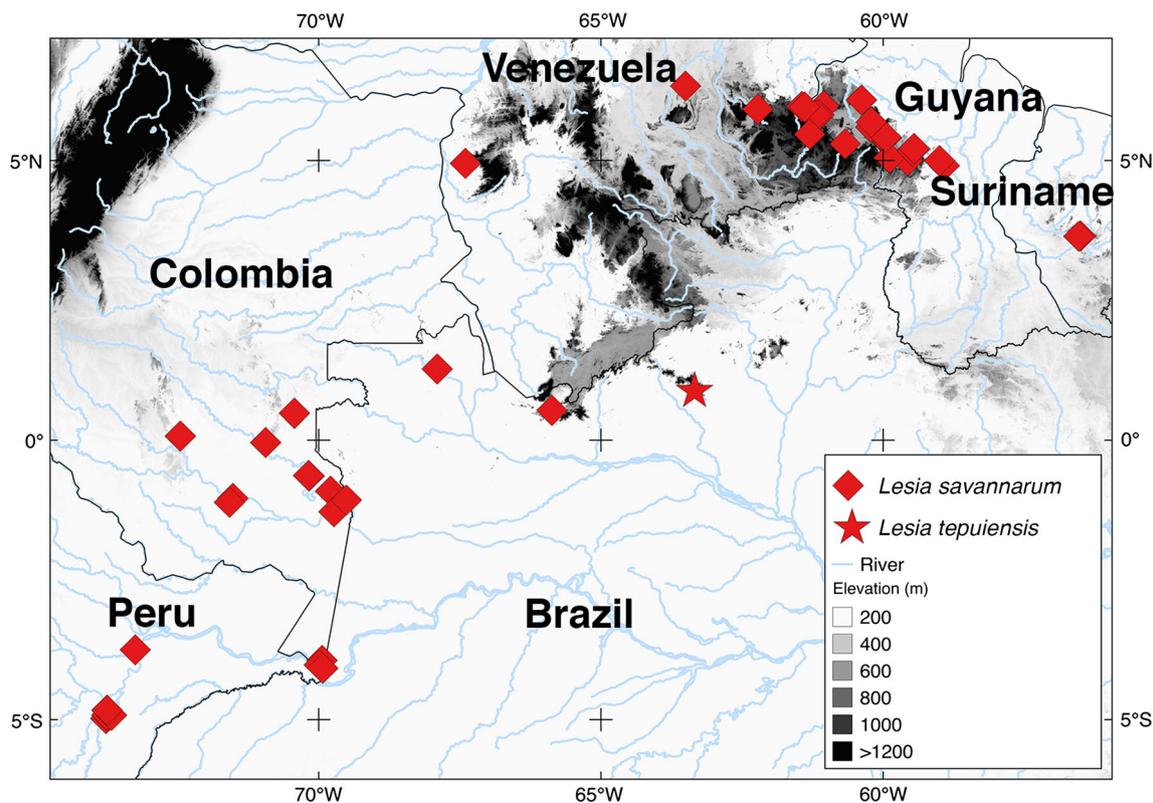


Fig. 5 Distribution of *Lesia* in South America

Furthermore, *L. tepuiensis* is rupicolous while *L. savannarum* is epiphytic (see Table 1).

When compared to the related genus *Codonanthis*, the differences are greater, including the white to cream, funnel-shaped, spurred corolla, which is oblique to the calyx and the presence of extrafloral nectaries (vs. yellow to red, ventricose to gibbous, not spurred corolla, which is erect to the calyx and the absence of extrafloral nectaries).

Morphologically, the new species is similar to the Atlantic Rainforest *Nematanthus strigillosus*, sharing a woody and deciduous stem at base with internodes of a few mm to 1 cm, and a strongly pouched, reddish corolla ca. 2.5 cm long, but *L. tepuiensis* has leaf blades measuring usually 3–6 cm long that are abaxially lanate whereas in *N. strigillosus* leaf blades reach only 1.5–3.5 cm and are pubescent to tomentose abaxially.

Additional Specimens Examined: Brasil, Amazonas, Barcelos, Serra do Aracá, Platô da Serra do Aracá, arbusto de 2 m de altura, botões florais vermelhos e cálice verde, bosque nublado, próximo ao paredão, 24 Feb 1984, A.S. Tavares 132 (INPA!); plateau of northern massif of Serra Aracá, southern extreme of northern part of Aracá, cloud forest on cliffs, 12 Feb 1984, G.T. Prance 29032 (INPA!); Serra do Aracá, igarapé Preto, acima da Cachoeira do Eldorado, formação campestre, 16 Aug 2011 G. Martinelli

17047 (RB!); margem do rio da cachoeira do Eldorado, rupícola, flores vermelhas, 20 Apr 2014, G. Barbosa-Silva 275 (RB!); margens da Cachoeira do Eldorado, arbusto entre rochas ou epífita, em mata nebulosa, ca. 40 cm alt., folhas discolores, argenteas na face abaxial, flores com cálice verde, corola vermelha, 21 Apr 2014, R.C. Forzza 8023 (RB!).

Key to distinguish *L. tepuiensis* from related genera and species

- 1a Corolla white to cream; oblique in calyx; corolla spur present *Codonanthis*
- 1b Corolla yellow to red; erect in calyx; corolla spur absent ... 2
- 2a Epiphytic herbaceous to suffrutescent plants from Atlantic rainforests *Nematanthus*
- 2b Epiphytic to terrestrial subshrubs from northern South America or Central America 3
- 3a Leaf blade hirsute to tomentose abaxially; calyx lobes 2–3 cm long; red; margin dentate at base and entire near apex; corolla yellow, ventricose towards apex; plants usually epiphytic *L. savannarum*
- 3b Leaf blade lanate abaxially, calyx lobes 1.2–1.4 cm long; green; margin entire to sparsely serrate; corolla

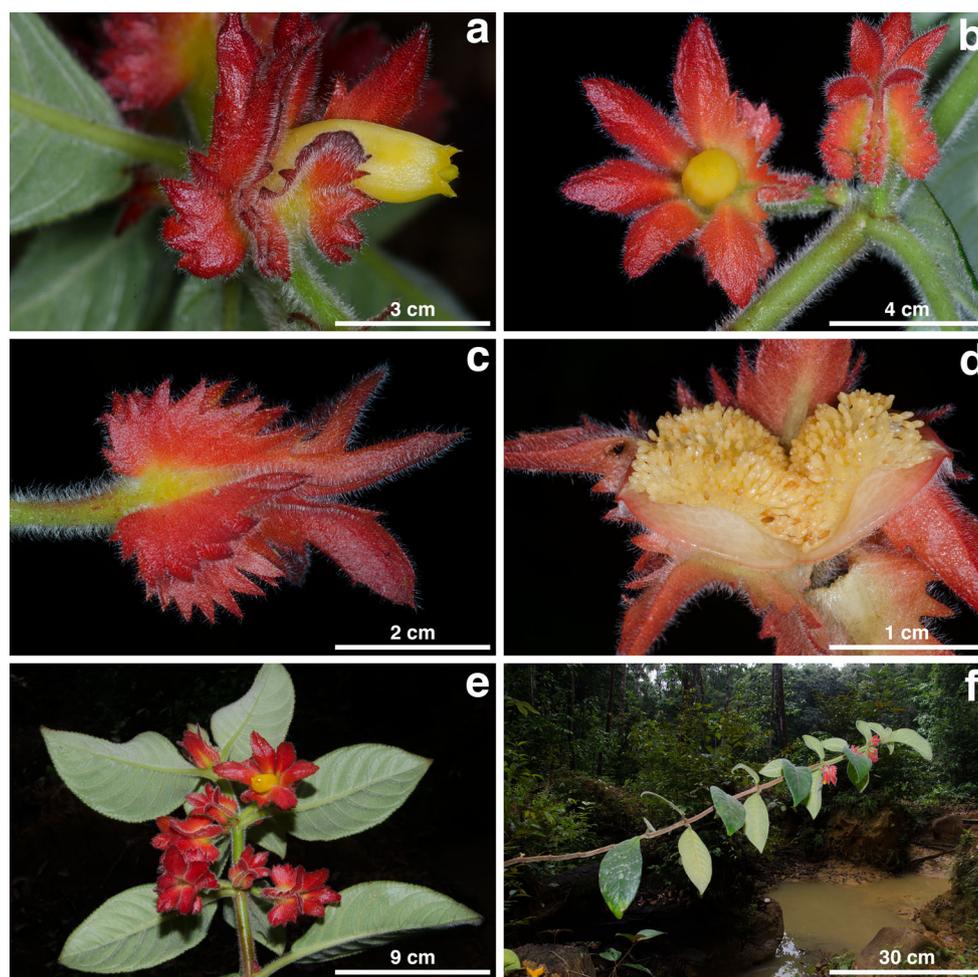


Fig. 6 *Lesia savannarum*. **a** Corolla and calyx, lateral view, **b** floral bud, front view, **c** calyx, lateral view, **d** fruit open, **e** leaf venation on the lower surface and corolla, front view, **f** habit. (photos provided by John L. Clark)

red–orange, strongly pouched; plants usually rupicolous *L. tepuiensis*

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic supplementary material

Online Resource 1. List of species sampled, voucher information and Genbank accession numbers.

Online Resource 2. Alignment sequence used for phylogenetic analyses in this study.

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