Description and molecular diagnosis of a new species of *Brunfelsia* (Solanaceae) from the Bolivian and Argentinean Andes

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Abstract
*Brunfelsia plowmaniana* N.Filipowicz & M.Nee sp. nov., a species from humid and cloud forests of the Bolivian and Argentinean Andes, is described and provided with a molecular diagnosis, using provisions available in the recently approved *International Code of Nomenclature for algae, fungi and plants*. Specimens belonging to the new species were previously placed in the polymorphic *B. uniflora* (Pohl) D.Don, which a molecular phylogeny revealed as polyphyletic. Revision of numerous collections revealed clear morphological differences between the new species and *B. uniflora*, the type locality of which is in the state of São Paulo, Brazil.

Keywords
Brunfelsia, Argentina, Bolivia, Brazil, molecular species diagnosis, morphological description

Introduction

The genus *Brunfelsia* L. (Solanaceae) comprises ca. 50 species of shrubs and small trees in the Greater and Lesser Antilles, the eastern slopes of the Andes, and the Brazilian Shield. More than half of the species are narrowly endemic and may be adapted to special habitats. The first species of *Brunfelsia* was described in 1703 (Plumier 1703), and the first and only comprehensive treatment of the genus was carried out in the 1970s and 80s...
One of the earliest species named is *Brunfelsia uniflora* (Pohl) D. Don, a relatively common and frequently collected species described from the vicinity of Rio de Janeiro (as *Franciscea uniflora*; Poh, 1826). When revising the abundant and heterogeneous material, Plowman (1979, 1998) chose to retain a broad concept of this species although he noted that the species had a disjunct range, occurring in rain forests of southeastern Brazil, the relatively dry coast of Venezuela, and high altitude cloud forests of the Bolivian and Argentinian Andes, and that there seemed to be morphological differences that coincided with geography (Plowman 1979, 1998). However, there was insufficient fertile material for Plowman to find a satisfactory solution before his untimely death in 1989, when material from Bolivia and northwestern Argentina was just beginning to flood into herbaria. The numbers of collections in F, MO, and NY of the Andean *Brunfelsia* species described here show this explosive increase: 1920s – 1 collection, 1970s – 4, 1980s – 9, 1990s – 15, and 2000s – 14 collections. Because of his uncertainty as to the Andean populations, (Plowman 1979, 1998) annotated *Steinbach 8345* (GH) as *B. uniflora* in 1974, *Beck 7439* (NY) as “*Brunfelsia sp. aff. B. uniflora*” in 1982, and *Vervoorst-Legname 4564* (NY) as “*Brunfelsia sp. nov. aff B. uniflora***” in 1985. “All three in fact represent the new species described here.

Study of all collections from Bolivia and Argentina (plus numerous collections from Brazil) and insights from a molecular phylogeny for *Brunfelsia* (Filipowicz and Renner 2012), revealed that the Andean element is a separate species, distinct from *B. uniflora*. We here describe the new species, named to honor Timothy Plowman, and provide information about its range, habitats, and conservation status. In addition to several morphological features that distinguish the new species from *B. uniflora, a molecular diagnosis based on plastid and nuclear sequences clearly differentiates the new species from all its relatives.

**Taxonomic treatment**

*Brunfelsia plowmaniana* N. Filipowicz & M. Nee, sp. nov.

http://species-id.net/wiki/Brunfelsia_plowmaniana

**Molecular diagnosis.** The new species differs from all other species of *Brunfelsia* at the following nucleotide positions in the plastid *ndhF* gene, position 237: Guanine not Thymine; 270: Cytosine not Guanine; and 887: Thymine not Cytosine; and in the
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Figure 1. Isotype specimen of *Brunfelsia plowmaniana* N. Filipowicz & M. Nee: *M. Nee 37571* (NY).
nuclear ITS region (ITS1 spacer, 5.8S rRNA gene, ITS2 spacer) at position 52: Guanine not Adenine; 80: Cytosine not Guanine; 215 Guanine not Cytosine; and 232: Cytosine not Guanine (Coordinates from Nicotiana tabacum complete chloroplast genome, GenBank accession Z00044, ndhF gene from 12072 to 114294, and Nicotiana tabacum GenBank AJ300215, ITS region) (compare Table 1).

Table 1. Brunfelsia plowmaniana-specific substitutions in chloroplast and nuclear DNA regions as compared to 59 other accessions representing 39 species of Brunfelsia.

<table>
<thead>
<tr>
<th>Position</th>
<th>Specific substitution</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plastid ndhF gene</strong></td>
<td></td>
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</tr>
<tr>
<td>237</td>
<td>T → G</td>
<td>B. plowmaniana-specific</td>
</tr>
<tr>
<td>270</td>
<td>G → C</td>
<td>B. plowmaniana-specific</td>
</tr>
<tr>
<td>887</td>
<td>C → T</td>
<td>B. plowmaniana-specific</td>
</tr>
<tr>
<td><strong>Nuclear ITS1 spacer, 5.8S rRNA gene, ITS2 spacer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>A → G</td>
<td>B. plowmaniana-specific</td>
</tr>
<tr>
<td>215</td>
<td>C → G</td>
<td>B. plowmaniana-specific</td>
</tr>
</tbody>
</table>

1 coordinates from Nicotiana tabacum complete chloroplast genome (GenBank accession no. Z00044), ndhF gene from 12072 to 114294
2 coordinates from Nicotiana tabacum AJ300215

**Type.** Bolivia. La Paz: Prov. Inquisivi, between Yamora and Iguasani, 5 km (by air) SE of Inquisivi, 16°57'S, 67°06'W, 3100 m, 13 Jan 1989 (fl), M. Nee 37571 (holotype: LPB; isotypes NY [01418954], AD [AD99103316], CAS [26326], CORD [00006706], F [V0093209F], G [00340058], jbsc (informal acronym for the herbarium of the Jardín Botanico de Santa Cruz, Bolivia), K [K000787830], MADw 46246, MG, MO [5752063], MY, P [00478848], SP, TEX, US [01050455], USZ [27345], WIS [v0262652WIS]).

**Description.** Shrubs or small trees, mostly with a single stem at the base and branched only above the base, 1–4 (–10) m high, to 14 cm in diameter; bark peeling or flaking, light gray or yellow-brown. Branches with the new twigs densely pubescent with hairs to 0.3 mm long, the older branches with bark smooth, glabrous, light tan, drying in irregular longitudinal ridges or almost winged, glabrous. Internodes 4–12 mm long. Leaves scattered along the branchlets, simple, alternate, exstipulate, shiny and dark green above, chartaceous to subcoriaceous; lamina (2–) 4–9 × 1.3–3.8 cm, broadly lanceolate to obovate, narrowing abruptly to the acute to attenuate apex, the base somewhat asymmetrically cuneate to attenuate, the margin entire, slightly revolute, appearing glabrous at maturity, but often pubescent when young and then glabrescent, usually remaining sparsely pubescent on the midrib below with weak hairs to 0.3 (–0.4) mm long on the midrib and with shorter and fewer hairs on the midrib above, more rarely sparsely pubescent on the surface below and with fewer and shorter hairs above; lateral nerves 5–8 pairs, spreading, arching, adaxially often impressed, the tertiary veins raised and forming a fine reticulum when dry to nearly invisible, abaxially the lateral veins slightly raised and forming a looping interconnecting vein 2/3–3/4 of
the way to the margin, the tertiary veins often not apparent; petiole 0.4–0.6 cm, pubescent with hairs to 0.3 mm long, Stomata paracytic. Flower solitary, terminal, often nodding, fragrant during the day. Pedicel (4–) 5–10 (–15) mm long in anthesis, not lengthening in fruit, articulate at the base, very sparsely glandular-pubescent, less so than the subtending stem and usually only with the shortest hairs. Calyx gamosepalous, tubular to narrowly campanulate, the tube 6–10.5 mm long, gradually narrowing to the pedicel and not sharply delimited from it, 0.9 cm diameter at summit, the lobes 5, more or less equal, (2–) 2.5–3 mm long, triangular or triangular-ovate, the margin cartilaginous, especially in fruit, striately veined, with very sparse glandular hairs on the outer surface, fewer than on the pedicel, the tube accrescent, not lengthening in fruit, but broadened and closely investing the proximal ½ of the fruit, the lobes stretched and broadened, and sometimes lengthened to 3.5–4 mm long. Corolla gamopetalous, with five subequal, broadly ovate lobes; tube 1.5–2.5 cm long, twice as long to a little more than twice as long as the calyx, more rarely only slightly longer than the calyx, erect, the limb spreading and 2–2.5 cm wide in anthesis, violet (lilac) with a pale yellowish-green throat, this raised and forming a ring, abruptly changing to violet on the lobes, with glandular hairs present on the mouth of the corolla tube, the lobes overlapping at the margins in bud, ca. 9 × 11 mm in anthesis; flower color fading to white with age. Stamens 4, didynamous, epipetalous; free part of filaments of the upper pair 2.5–4.5 mm long, those of the lower pair 0–2 mm long; anthers 4, dorsifixed, semicircular, 1 mm long, the dehiscence around the perimeter; stigma and upper two anthers visible at the mouth of the tube. Ovary bicaul pupellate, syncarpous, superior, ovoid, 2 mm long, glabrous, with oblique septa, ovules several per locule; style 1.5 cm long, slender, promptly deciduous; stigma clavate, bifid, 1.5 mm long. Fruit obovoid, coriaceous, capsular, 1.3–1.5 × 1.2–1.3 cm, probably green, and perhaps turning dark purple or black when ripe. Seeds ca. 9, brown, irregularly ovoid or oblong and subangular, 5.5–7 mm long, 3–3.5 mm wide and thick, the surface very minutely foveolate.

**Distribution.** *Brunfelsia plowmaniana* is known from humid forests in the Provinces of Salta and Jujuy in northwestern Argentina, and the Departments of Santa Cruz, Cochabamba and La Paz (Prov. Inquisivi) in Bolivia. It grows at altitudes of 1500–3200 m on the predominantly N–S ridges separated by dry to arid scrub in the intervening valleys, perhaps mostly at the lower altitudinal range in the southern part of the distribution and the upper altitudinal range to the north. It has never been collected in the Provinces of Sud Yungas, Nor Yungas and Larecaja in the relatively well-explored central and northern parts of the Department of La Paz, so the northern limit of the range likely is in Prov. Inquisivi (Fig. 2).


**Etymology.** Named to honor the late Timothy Plowman (1944-1989), an American botanist fascinated with Neotropical plants of ethnobotanic importance, among them *Brunfelsia*.

**Common names.** Few common names have been recorded: “bella unión” (*Nee & Vargas 38253*); “hierba mala”, “mata burro” (*Arroyo et al. 4043*). “Bella unión” is commonly used for other species of *Brunfelsia* in Bolivia (*B. boliviana* and *B. grandiflora*), the “beautiful union” referring to the inflorescences with both violet and white flowers together. The terms “bad herb” and “burro killer” indicates that the foliage is poisonous to grazing animals.

**Figure 2.** Distribution of *Brunfelsia plowmaniana* N. Filipowicz & M. Nee based on the voucher specimens listed in Taxonomic treatment and in Appendix 1.
**Conservation status.** The species is often found in heavily grazed lower edges of the cloud forest where grazing, deforestation and fires are a threat to local populations. Even though the range is narrow, it is about 800 km long; and many populations are in undisturbed or protected areas.


**Discussion.** All of the specimens from Bolivia and Argentina cited above belong to a single, morphologically uniform species that differs from *B. uniflora*, the type of which is from eastern Brazil, in morphology as well as nuclear and plastid substitutions as specified in the molecular diagnosis and Table 1. The leaves of the Andean material are uniformly of a thicker texture, with distinct reticulate venation above; they look more like those of *B. latifolia* (Pohl) Benth. of eastern Brazil, rather than those of Brazilian material determined as *B. uniflora* by T. Plowman (mostly housed at F). The Brazilian material determined by Plowman is heterogeneous in indumentum; the petioles and pedicels (more particularly in the young stages) may be glabrous, very finely puberulent with tiny hairs, pubescent with weak hairs (the most like the Andean material), or pilosulose with straight hairs. The description given by Plowman (1998) has the inflorescence as “1-flowered, terminal, produced at tips of new shoots, sessile, rarely short pedunculate.” However, the protologues of *Franciscea uniflora* Pohl. and *Franciscea hopeana* Hook. both mention solitary flowers. Based on the vouchers from near the type locality, identified as *B. uniflora*, it appears that Plowman’s description is correct, however, and this species has highly reduced cymose inflorescences with (usually) single flowers and scars visible on it. The solitary flower thus seems to be a morphological feature restricted to the Bolivian/Argentinean entity that we describe here.

The leaves of *B. plowmaniana* are variable in size and shape, but usually are widest above the middle (ovate) with a rather abruptly narrowed apex (cuspidate) in the manner of many Myrtaceae. The raised and somewhat cartilaginous ring of the corolla throat is reminiscent of that of species of *Prestonia* (Apocynaceae) from the same geographic region; it probably reflects adaptation to pollinator foraging behaviour. Several collection labels mention that the corolla color changes from blue/violet to whitish while aging, which is common in the South American species of *Brunfelsia*. The notes from two vouchers (*M. Nee 37571; M. Nee 50606*) also mention diurnal anthesis, with a pleasant jasmine fragrance. Nothing is known directly of the pollinators of *B. plowmaniana*, but the floral features described above are shared by other South American brunfelsias for which butterfly pollinations has been observed (Plowman, 1998).

There are no observations on fully mature fruits or dispersal of the seeds. Herbarium specimens with ripe or nearly ripe fruits always show them splitting neatly from the top about 1/3 of the way to the base into two equal valves.

The species that is geographically closest to *B. plowmaniana* is *B. boliviana* Plowman from Depto. Santa Cruz, Prov. Vallegarande south to Depto. Chuquisaca. This species has broader, thinner leaves, a corymbiform inflorescence with up to 15 flow-
ers, a broader corolla limb, and is found in drier forest or Cháco vegetation, from a relatively narrow area in the foothills of Andes in southeastern Bolivia (up to 1200 m) (Plowman, 1981). The molecular phylogeny of Brunfelsia shows that B. boliviana and several Amazonian species are part of a larger eastern Brazilian and Amazonian clade (see Fig. 3; Filipowicz and Renner 2012).

Figure 3. Maximum likelihood phylogram (GTR + Gamma) for a reduced dataset consisting of 41 accessions of Brunfelsia and 8 outgroup genera and based on the analysis of combined plastid and nuclear sequences (3784 aligned nucleotides). Numbers above branches refer to ML bootstrap support ≥70%. Placements of B. plowmaniana N.Filipowicz & M.Nee and B. uniflora (Pohl) D.Don (both represented by two accessions) in the tree are marked in red. Main clades are marked in different colors. For the full tree and alignment see TreeBase under acc. no. 12245.
We initially became aware of B. plowmaniana during molecular-phylogenetic work. Sequencing of 59 accessions of Brunfelsia representing 39 species (plus relevant outgroups) for the nuclear ITS1-5.8S rRNA-ITS2 region, and the plastid ndhF gene and \textit{trnL} intron and \textit{trnL-F} spacer (together 3784 aligned nucleotides) revealed \textit{B. uniflora sensu} Plowman to be a polyphyletic entity (Fig. 3). Both \textit{B. uniflora} and \textit{B. plowmaniana} are placed in a South American group, however in distinct clades. Brunfelsia uniflora, represented by two accessions originating from Minas Gerais and São Paulo, belongs to a Southeastern South America and Amazon Basin clade (shaded green in Fig. 3), while \textit{B. plowmaniana}, represented by Argentinean and Bolivian specimens, falls in a clade from the Pacific coast and Andean region (shaded orange in Fig. 3). Molecular dating, using indirect calibration, suggests that these clades split from each other about 10 million years ago (Filipowicz and Renner 2012).

Acknowledgements

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References

Appendix 1

List of the voucher specimens included in the study. (doi: 10.3897/phytokeys.10.2558.app1) File format: MS Excel (XLS).

Explanation note: List of the voucher specimens used in the study with detailed locality, GPS coordinates where available and herbaria barcodes.

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Appendix 2

List of accessions included in the study. (doi: 10.3897/phytokeys.10.2558.app2) File format: MS Word document (DOC).

Explanation note: List of 49 accessions included in the study and used also in Filipowicz and Renner (2012) with voucher specimens, their geographic origin, and GenBank accession numbers. All the samples in the list are represented in Fig. 2. Herbarium acronyms follow Thiers (2011).

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