



Nicotiana azambujae (Solanaceae): A wild tobacco presumably extinct rediscovered after 73 years

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ABSTRACT

Nicotiana azambujae is an endemic species from Santa Catarina state, southern Brazil, that was described in 1964 and has not been seen since then. During fieldwork, we found a population in Alto Matador, about 70 km from the presumable type collection, after 73 years of its last known collection. Thus, we bring the first in vivo pictures of this species, assess its conservation status, update the morphologic description and discuss its habitat preferences. Also, we discuss a possible mistake in the type of voucher label from the originally collected locality.

Keywords: Atlantic Forest, Extinct species, Nicotianeae.

Introduction

Nicotiana L. (Solanaceae) is composed about 82 species (Knapp 2020). The genus is most diverse in America, as well as in Australia, where it is represented by 26 species (Chase *et al.* 2018), one species in Namibia (Africa), and one in Melanesia (Goodspeed *et al.* 1954; Hunziker 1979; 2001; Knapp 2020). Perhaps, the most studied species is *Nicotiana tabacum* L., a globally cultivated species, widely investigated due to its insecticide and stimulant properties by the tobacco industry, and other areas such as pharmacy, physiology, virology, and transgenic plants (Goodspeed *et al.* 1954; Hawkes 1999; Hunziker 2001; Vieira *et al.* 2003).

The main literature about the other species focusing on South American and Brazilian species (i.e. Sendtner 1846; Dunal 1852; Bentham & Hooker 1876; Smith & Downs 1966; Vignoli-Silva & Mentz 2005; Vignoli-Silva & Stehmann 2020) are systematics and nomenclatural works. In Brazil, there are ten *Nicotiana* species, eight natives – most in the South and Southeast regions, inhabiting grasslands and rocky outcrops – and two exotic: *Nicotiana tabacum* and the ruderal *Nicotiana glauca* Graham (Vignoli-Silva & Stehmann 2020; Augsten *et al.* 2022).

Most species that occur in Brazil were described between 1830-1905, and all species have many samples in Brazilian herbaria. There are two recently described species, *Nicotiana mutabilis* Stehmann & Semir (Stehmann *et al.* 2002),

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an endemic and vulnerable species from Rio Grande do Sul state (Martinelli & Moraes 2013), and *Nicotiana gandarela* Augsten & Stehmann, another endemic species, but from the Quadrilátero Ferrífero, Minas Gerais. The latest species is classified by the authors as Critically Endangered (Augsten *et al.* 2022).

From the Brazilian species, there is one known only from the type collection *Nicotiana azambujae* L.B. Sm. & Downs, described by Smith & Downs (1966). The species was collected in Azambuja, Brusque municipality, dated 1948. Since the type species was collected, besides some field expeditions by many botanists in the type locality, no other individuals have been seen (73 years), thus being considered extinct (CONSEMA 2014). Surprisingly, in August 2021, during a field expedition in Alto Matador, in the municipality of Rio do Sul, we found a small population of this species. So, to improve the knowledge of this rare species, we made a detailed description based on the isotype and the new samples, assessed its conservation status, and brought field photographs of the species as well an updated distribution map.

Material and methods

The morphological description of the species was made from field observations and studying specimens housed in the HBR Herbarium and images of specimens kept in the US Herbarium (acronyms according to Thiers 2021, continuously updated). The terminology of morphological description follows Goodspeed *et al.* (1954) and Vignoli-Silva & Stehmann (2020). The photographs were taken with a stereomicroscope. The maps were drawn via QGIS software (GIS Development Team 2021), and the AOO and EOO were calculated using GeoCat (Bachman *et al.* 2011), using the standard square suggested by IUCN (2012; 2019) of 2 × 2 km. The conservation status of the species was assessed following the IUCN (2012; 2019) criteria. All field photographs were taken by the authors.

Results

Taxonomy

Nicotiana azambujae L.B.Sm. & Downs Type: BRAZIL, Santa Catarina: Brusque, In thicket, Azambuja, alt. 50 m, 1 November 1948, Reitz 2245 (holotype US [photo!]; isotype HBR!) (Fig. 1).

Examined specimens

Santa Catarina. Rio do Sul, Alto Matador, Cachoeira da Magia, -27.248245, -49.562573, 08 August 2021, L.A. Funez, M. Comin & F. Bittencourt 9876 (HBR, FURB);

Description

Herbs, probably annual or biannual, to 1 m tall. Stem short, glandular-pubescent, rarely branching from the base. Basal leaves forming a rosette, sessile, auriculate, oblong to oblanceolate, 11–21 × 4.5–11 cm, membranaceous, sparsely glandular-pubescent, with simple capitate trichomes, caulinar leaves oblanceolate, 4–18 × 0.8–6 cm, gradually reducing to the apical portion of the stems. Inflorescence paniculate 30–48 cm long, composed of monochasial cymes. Flowers with pedicels 3–4 mm long, densely glandular-pubescent. Calyx campanulate, glandular-pubescent, deeply lobate, fused 1.2–1.5 mm of the basal portion, the lobes linear-lanceolate 4.8–5.8 cm long. Corolla purple (according to Smith & Downs 1966) or pinkish-lilac (according to our field observations), hippocraterifom, sparsely glandular abaxially, pilose adaxially below the stamen insertion, 10–12 mm long, tube 6–8 mm long, swollen at the apex, corolla lobes 2–3 mm long, asymmetric-lobed, limb 8–10 mm diam. Stamens unequal 1+4, the shorter filaments 3 mm long, the longer 4 mm long; anther ellipsoid, 0.5–0.6 × 0.4–0.5 mm, pollen white in living plants. Ovary glabrous, ovoid, 2–2.5 × 1 mm, style 8 mm long, stigma discoid. Capsule ovoid 7–8 × 3 mm, apex acute, glabrous; seeds globose, 0.5 mm diam, brown, testa reticulate.

Distribution and ecology

Nicotiana azambujae was known only for the type locality, in Azambuja, Brusque municipality, in a regeneration fragment or edge of the Atlantic Rainforest, according to Smith & Downs (1966). The new population found inhabits an area of higher elevation (ca 800 m asl) and on the banks of a waterfall, in a rocky and open environment, similar to habitats occupied by other species of this genus (Fig. 2).

Flowering and Fruiting

November, according to Smith & Downs (1966). In our study, there was only a specimen flowering and fruiting in August, and some young individuals were in the vegetative stage. We did another visit to the area in November, where five mature individuals were detected blooming, some of them with mature fruits. Probably the blooming extends to December, and fruiting to January or February, but more studies need to be conducted to determine it more precisely.

Conservation status

Critically Endangered (CR-B2a,b[iii]). This species is only known from two locations: the type locality, where it has not been found there for more than 50 years, and the newly-discovered population in Alto Matador. Considering Azambuja as an extant population of *Nicotiana azambujae*, it has an area of occupancy (AOO) of only 4 km². Considering only the population of Alto Matador due to the long time-lapse of the plant being disappeared in Azambuja, its AOO is



estimated at 2 km², thus, both estimates assess *N. azambujae* as a critically endangered species. Additionally, the species does not occur in environmentally protected areas and is threatened by its habitat destruction, largely converted for diverse economic uses, such as urbanization, agriculture, livestock, and forestry (Vibrans *et al.* 2012).

A significant threat that we noticed is that the habitat of *Nicotiana azambujae* is heavily invaded by the butterfly

ginger (*Hedychium coronarium* J.Koenig), a highly competitive invasive exotic species in Brazil (Santos *et al.* 2005; Castro *et al.* 2021). The species seems to grow only in regions where *Hedychium* is absent, probably losing out in the competition for light with these plants. We strongly recommend that the butterfly ginger be managed and/or eradicated in the area, to carry out experiments to see if the species can recolonize previously affected areas.



Figure 1. Field photographs of *Nicotiana azambujae*. **A.** Flower in frontal view. **B.** Flower in lateral view, showing the short tube. **C.** Young specimen, showing the leaf blades and habit.



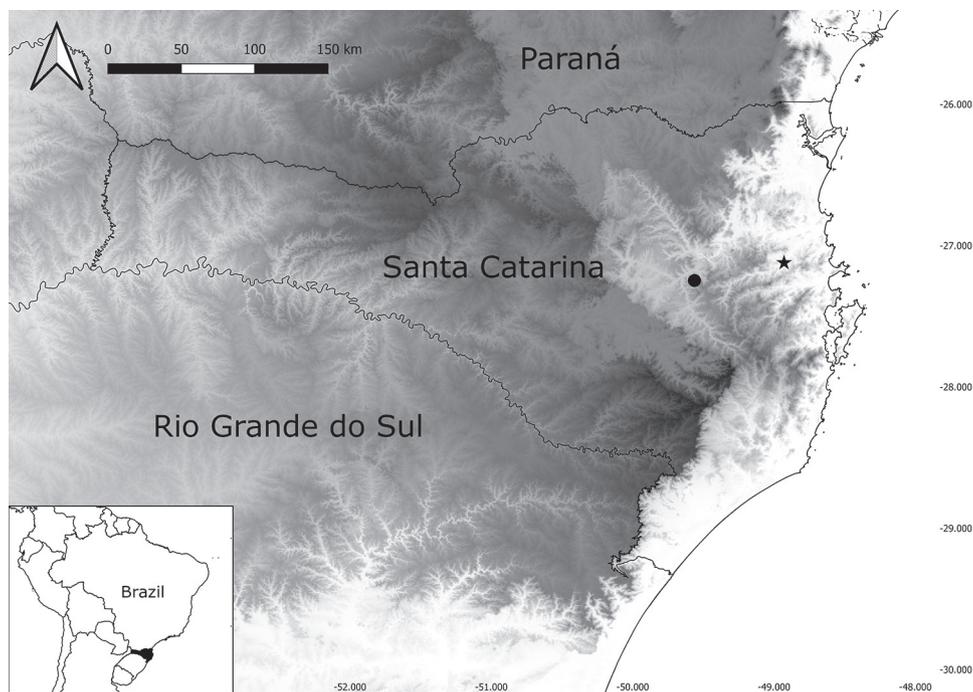


Figure 2. Distribution map of *Nicotiana azambujae*, showing the type locality (star) and the new population (dot).

Discussion

The discovered population has given us a better understanding of the type of habitat the species occupies and gives us again a chance to effectively conserve this species. There were some morphologic lacunes on the protologue and in Smith & Downs (1966), as the basal leaves, indumentum, and some floral characteristics that we fill with this updated description. Additionally, we take the first field photographs of this species.

According to the available morphologic information, *N. azambujae* belongs along with all indigenous species of *Nicotiana* from Brazil to *Nicotiana* section *Alatae*, characterized by its herbaceous habit, commonly rosulate basal leaves, flowers in false racemes or panicles, corolla salver-shaped, longer than broad, limb white, red, green or lavender, deeply lobed, stamens often unequal in length (Goodspeed *et al.* 1954). Although the same author points out that they have a haploid chromosome number of $n = 9$ (10, 12), we could not test this hypothesis (Goodspeed *et al.* 1954). With this new sample collected, the inclusion of *N. azambujae* in a phylogenetic work could clarify the relationship with other *Nicotiana* species.

About the history and distribution gap between Brusque and Rio do Sul municipalities (about 130 km), we discussed with Aloisius C. Lauth, biographer of the Raulino Reitz story, as to whether or not Reitz might have made a mistake about the species' locality. According to Lauth, "Reitz was transferred to the Parish of Itajaí in 1947, where he could not

find space to keep the collection of 600 exsiccatae, leaving it between Sombrio, in Araranguá municipality (south of Santa Catarina) and Azambuja, in Brusque municipality. His collection of living plants for studies was left with Sister Limbônia Bohn, responsible for the gardens and flowers of the Azambuja Sanctuary, and she set an area for the Reitz plants. However, in 1970, this area was destroyed to expand Azambuja hospital park. At that period, Reitz had no collection methodology for field excursions, barely recording it in field notebooks. This is the case of the *Nicotiana azambujae*, annotated anonymously only with number and municipality. Additionally, he was three times in the region of Ibirama and Rio do Sul during this period. In 1946, Reitz met Lyman Bradford Smith, at the Botanical Congress in Tucuman, Argentina, and started exchanging specimens with him. The material of *Nicotiana azambujae* was sent to Smith for studies, whose live specimen was planted in the garden of Azambuja. Thus, although the publication was in 1964, due to the loss of information on the origin of the specimens, this possible cultivated origin of the type material in the garden of the Sanctuary of Azambuja, was never associated with the samples used in the description (Lauth, personal communication).

The discovery of the population of a supposedly extinct species in Santa Catarina state reinforces the need for field expeditions more systematically, covering the great gaps in knowledge about biodiversity. Although Santa Catarina is a state with a relatively well-known flora, dozens of new species were described in the last years (Hassemmer *et al.* 2016; Funez *et al.* 2019; Sobral *et al.* 2019), even in areas where



historically there are already many studies (e.g. Florianópolis and Blumenau municipalities). Additionally, some portions of the state, even with the immense effort of the Herbário Barbosa Rodrigues and Inventário Florístico Florestal de Santa Catarina, remain practically unknown, such as the midwestern portion of the state, some mountains, and coast dunes. As the rates of original vegetation cover loss are growing in Santa Catarina, giving way to agriculture, silviculture, and urbanization (Fundação SOS & INPE 2009; Vibrans *et al.* 2012), it becomes essential to begin collecting in these areas since the forest cover is small and highly fragmented to prevent more species loss (Gasper *et al.* 2016).

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