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***Nicotiana rupicola* sp. nov and *Nicotiana knightiana* (*Paniculatae*, Solanaceae), a new endemic and a new record for the flora of Chile**

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Nicotiana rupicola sp. nov and *Nicotiana knightiana* (*Paniculatae*, Solanaceae), a new endemic and a new record for the flora of Chile

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Abstract. *Nicotiana knightiana* is recorded for the first time for the flora of Chile. A new species of *Nicotiana*, endemic to the coast of the Coquimbo region is described and illustrated. Molecular analysis placed the new species within the section *Paniculatae*, as sister to *Nicotiana cordifolia*, endemic to Juan Fernandez islands. The new species can be considered critically endangered (CR) according to the IUCN categories for its restricted and fragmented distribution, small population number, and the threat that urbanization and mining activities represent for the conservation of the biodiversity of the area.

Keywords. Coquimbo, endemism, exotic species, Nicotianoideae, systematics, taxonomy.

Introduction

Nicotiana L. is one of the largest genus in the Solanaceae with 75 recognised species (Clarkson et al. 2017) including the important crop plant *Nicotiana tabacum* L. *Nicotiana* is naturally distributed in America and Australia, and one species from south-west Africa (Hunziker 2001). Evidence of homoploidy and polyploidy showed that hybridization has been a major driver of speciation in the genus (Clarkson et al. 2010).

Since Reiche's (1903) treatment, no modern revision of *Nicotiana* in Chile has been published. According to the last catalogue of the flora of Chile (Rodríguez et al. 2018), the country is home to 12 native accepted species of *Nicotiana* distributed from Arica y Parinacota region to Magallanes region and Juan Fernandez Archipelago, and two introduced species, *Nicotiana tabacum* L., in Juan Fernandez and Easter Island, and *Nicotiana glauca* Graham in North and Central Chile. In his monograph of the genus, Goodspeed (1954) provided a taxonomical classification in which he recognised three subgenera further divided into 14 sections. Most of his sections have been confirmed as monophyletic by molecular analyses (Aoki & Ito 2000; Chase & al. 2003; Clarkson et al. 2004; Mehmood et al. 2020). Two Chilean species are included in the section *Paniculatae*, the endemics *Nicotiana cordifolia* found in Juan Fernandez Archipelago and *Nicotiana solanifolia* Walp., that grows between Tarapacá and Coquimbo regions and has been traditionally used in the economic and cultural life of the Atacama coastal communities (Ballester and Carrasco 2016). According to Goodspeed (1956), *Nicotiana* sect. *Paniculatae* share cordate-ovate leaves, evenly spaced along the stem, a long, narrow cylindrical thyse with thickened central axis, a calyx with regular, broadly triangular teeth, a short and distinct tube proper with a several times longer throat, a greenish to yellowish corolla, stamens of almost equal length, filaments curving

immediately above their insertion (except *N. cordifolia* and *N. raimondii*) and anthers bending towards the stigma.

The aim of the present work is to record *Nicotiana knightiana* for the first time for the flora of Chile and describe a new species of *Nicotiana* endemic to Chile, determine its phylogenetic position and conservation status.

Methods

Herbarium and fieldwork

Fieldwork was carried out during November 2020 in Fuerte Lambert and during March 2020 in the proximity of the rivermouth of Elqui river, within the city of La Serena, Coquimbo region (**Fig.1**). Specimens were collected and deposited in SGO. Physical and digital specimens of *Nicotiana*, including types, were revised at SGO, EIF, CORD, L, E, SI, F, NYBG, UC and US to reach a confident identification and check for possible previous collections of the species found in the field. Additionally, the citizen science platform iNaturalist (www.inaturalist.org) was consulted to search for possible observations of any of the two species. Terminology of the descriptions followed Goodspeed (1954).

Taxon sampling for phylogenetic analysis

DNA sequences for cpDNA intergenic spacers *trnF-trnL*, *trnS-trnG*, and genes *ndhF* and *matK* were obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank) for all species of *Nicotiana* used in Clarkson et al. (2004). Sequences for the new species were generated in the present study. As outgroups we used *Symonanthus aromaticus* (C.A.Gardner) Haegi, and *Mandragora officinarum* L.

DNA extraction, amplification, sequencing, and phylogenetic analyses

Total genomic DNA was extracted from silica-dried material collected in the field using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Santiago, Chile) following the manufacturer's instructions. Genomic DNA was used to amplify by PCR the following chloroplast regions: *trnL-trnF* using the primers c and f (Taberlet et al. 1991), *trnS-trnG* using the primers *trnS*(GCU) and 3'*trnG* (UUC) (Hamilton 1999; Shaw et al. 2005), *ndhF* using the primers 972F and 2110R (Olmstead and Sweere 1994), *matK* using the primers pairs *matK*-TR and *matK*_4F, *trnK*-710F and *matK*-1848R, and *matK*_1F and *matK*_4R (Johnson and Soltis 1995, Aoki and Ito 2000, Bremer et al. 2002). We amplified all regions in 25 µl PCR reactions using the following thermocycling conditions: initial denaturation of 95°C for 5 min; 35 cycles at 95°C for 1 min, a specific annealing temperature for 1 min at 50°C (60°C for *trnS-trnG*), 72°C for 1 min; and a final elongation period of 72° for 15 min. Sanger sequencing was performed using the same primers used during amplification in the case of regions *matK* and *trnL-trnF*, the same primers used during amplification plus the primers *ndhF*_1318 and *ndhF*_1603R (Olmstead and Sweere 1994) in the case of region *ndhF*, and the primers *trnS*(GCU) and *trnG*(UUC) (Hamilton 1999) for the *trnS-trnG* region. Sequencing was performed in the Plataforma de Secuenciación y Tecnologías Ómicas, Pontificia Universidad Católica de Chile, using the ABI PRISM 3500 xl Genetic Analyzer (Applied Biosystems). GenBank accession numbers for all DNA sequences are given in Suppl. material 1.

The assembled sequences were aligned using the MAFFT v7.450 algorithm (Katoh et al. 2002; Katoh and Standley 2013) in Geneious Prime 2021.1.1 (<https://www.geneious.com>).

Phylogenetic analyses were run for both Maximum-likelihood (ML, Felsenstein 1981) and Bayesian inference (BI, Huelsenbeck & Ronquist 2001), using RAxML-AVX3 version (Stamatakis 2014) included in RAxMLGUI v.2.0 (Silvestro and Michalak 2012, Edler et al. 2020) and MrBayes x64 v3.2.7 (Ronquist et al. 2012), respectively. The best-supported model of nucleotide sequence evolution for each partition was determined based on the Akaike Information Criterion (AIC) using MrModeltest v2 (Nylander 2004). For the BI analysis, four partitions were used corresponding to each region, in which evolutionary models for each one were: GTR+G for *ndhF*, *trnL-trnF* and *trnS-trnG*, and GTR+I+G for *matK*. Maximum likelihood analyses were run using the GTRGAMMA approximation. The analysis included 1000 ML slow bootstrap replicates with 500 runs. Bayesian analyses were conducted under the respective best fit models for each partition, with two independent runs for 4 million generations, sampling every 1000 generations. Time series plots and effective sample size (ESS) were analysed using TRACER v.1.7 (Rambaut et al. 2018) in order to check convergence for each run. The first 1 million generations were discarded as burn-in.

Conservation assessment

The assessment of the conservation status of the new species was made using the International Union for Conservation of Nature (IUCN 2017) criteria. The extent of occurrence (EOO) and area of occupancy (AOO) were calculated using GeoCat (Bachman et al. 2011).

Results

We could not find any described species of *Nicotiana* for Chile that matched the morphology of the plants from Elqui river and Fuerte Lambert and neither could we find any collected specimen ascribable to the former. A specimen matching the morphology of the species from Fuerte Lambert was found at EIF, collected in 2006 in the whereabouts of Chungungo, Coquimbo, aprox 50 km north of Fuerte Lambert. Plants from Elqui river are a 2 m, somewhat ineffectively rooted, branching short perennial shrubs with a pale yellow-green corolla, a dark green limb and sericeous indumentum (**Fig. 2, Fig. 3C-D**). This species was identified as *Nicotiana knightiana*, a member of *Nicotiana* sect. *Paniculatae* known from coastal southern Peru. Two georeferenced and misidentified observations of *Nicotiana knightiana* in Chile are available on iNaturalist, one from November 2018 in the proximity of our collection site (<https://www.inaturalist.org/observations/19777218>) and one from May 2021 at approx. 6 km southwards (<https://www.inaturalist.org/observations/80390445>) (**Fig. 1**). Plants from Fuerte Lambert are perennial, rupicolous shrubs, with glandular indumentum, a characteristic compressed inflorescence, and small, yellow flowers with an almost glabrous corolla (**Fig. 3A-B, Fig. 4**). Both species have capsules that produce a large amount of seeds but the ones of *N. knightiana* are more rounded in shape compared to those of the new species (**Fig. 5**).

Molecular phylogenetic analyses

The DNA matrix contained 4427 nucleotide characters (1554 *matK*, 1074 *ndhF*, 932 *trnL-trnF* and 867 *trnS-trnG*), representing 60 ingroup and 2 outgroup accessions. Both BI and ML analyses yielded congruent topologies. The topology of the phylogenetic tree constructed in this study is congruent with the clades found by Clarkson (2004) (**Fig.6**). Overall, the support given by Bayesian posterior probabilities are higher than bootstrap values given by ML analyses. *Nicotiana* sections *Tomentosae* (PP=1, BS=88), *Undulatae* (PP=1, BS=95), *Paniculatae* (PP=1, BS=87), *Trigonophyllae* (PP=1, BS=100), *Petunioides* (PP=1, BS=100),

Alatae (PP=1, BS=96), Repandae (PP=1, BS=100), *Noctiflorae* (PP=1, BS=100), and *Suaveolentes* (PP=0.94, BS=60) all form monophyletic groups with moderate to high supporting values.

Section *Paniculatae*, including *Nicotiana rustica*, forms a well-supported clade (PP=1.0, BS=87). Relationships among the clades largely reflect Clarkson's (2004), including the position of the section *Paniculatae* as sister to section *Undulatae* and together as sister to the rest of the genus excluding section *Tomentosae*. The new species falls within section *Paniculatae* as sister to *N. cordifolia* (PP=0.99, BS=89), and together they form a clade with *N. solanifolia* (PP=1, BS=100). This clade is sister to a clade including the rest of section *Paniculatae* (PP=0.92, BS=66). *Nicotiana knightiana* and *Nicotiana paniculata* are closely related species (PP=0.98, BS=63) and form a clade with *Nicotiana rustica* (PP=1, BS=100). The species collected in the mouth of Río Elqui, and identified as *Nicotiana knightiana*, falls as sister to *Nicotiana knightiana* sequenced in Clarkson (2004) with moderate support (PP<0.5, BS=62). A base-by-base comparison of the sequences of *Nicotiana knightiana* from Clarkson (2004) and *Nicotiana knightiana* from this study, show that they are identical, while they present differences at 6 nucleotide positions (3 from *trnS-trnG* and 3 from *matK* regions) with *Nicotiana paniculata*.

Taxonomic treatment

Nicotiana knightiana Goodsp., Univ. Calif. Publ. Bot. 18: 139, pls. 11, 12b (1938).
Figure 2, Fig. 3C–D, Fig. 5A–C, Fig. 7.

Description. Robust annual or short-lived shrub up to 3 m with many new stems at different stages of development arising from a lignified horizontal stem poorly anchored to the soil. **Stems** herbaceous, green, tomentose. **Leaves** ovate, undulate, base rounded to subcordate, apex obtuse to acute; bigger leaves 13 × 10 cm, indumentum similar to the stem but much denser on the abaxial side which confers a whitish colour, hairs simple, pluricellular, brochidodromous venation, petioles a third or half as long as the leaves. **Inflorescence** a broad thyrse or loose panicle, 40 cm. Pedicels 0.5–1 cm in mature fruits, covered in glandular hairs. **Calyx** up to 7 mm, cylindrical, tomentose, teeth short, triangular. **Corolla** 20–23 mm excluding the limb (tubular part), tube proper 4 mm, throat 16 mm, pale yellow-green, covered in short, sericeous, hairs, limb bottom 3 mm wide, dark green, same indumentum as tube proper, notched into 5 small lobes. **Stamens** extending below the limb, 19 mm except one slightly shorter, filaments adnate to the tube proper, then free, pubescent approx. in the proximal 6 mm, then glabrous and slightly curved, with stamens bending toward the stigma. **Capsule** 6–8 mm, ovoid. **Seeds** mainly subrotund, 0.5–0.7 mm, brown, surface reticulate. **Embryo** straight.

Distribution and habitat. *Nicotiana knightiana* grows naturally in the coast of southern Perú in roadsides, pastures and rocky ravines bottoms and it is found in Chile in the proximity of the rivermouth of Río Elqui, Coquimbo region (**Fig. 1**). It grows in a dense *Tessaria absinthioides* (Hook. & Arn.) DC. scrub, associated with *Myoporum laetum* G. Forst., *Phyla nodiflora* (L.) Greene, *Schoenoplectus californicus* (C.A. Mey.) Soják, *Solanum pinnatum* Cav., *Lycium chilense* Miers ex Bertero, *Sarcocornia neei* (Lag.) M.A. Alonso & M.B. Crespo, *Distichlis spicata* (L.) Greene, *Thypha angustifolia* L., *Ambrosia chamissonis* (Less.) Greene, *Nicotiana glauca* Graham and *Stemodia durantifolia* (L.) Sw. var. *chilensis* (Benth.) C.C. Cowan. It thrives in sandy soils with a phreatic layer located very close to the surface.

Phenology. *Nicotiana knightiana* is found flowering and fruiting between November and May.

Additional specimens examined. PERÚ. Arequipa: Prov. Islay, Quebrada Canyon, 5-6 km north of Mollendo, 300 m, 29 September 1938, *C.R. Worth & J. L. Morrison 15742* (US); 12 km southeast of Islay, 250-300 m, 28 September 1938 *C.R. Worth & J. L. Morrison 15724* (US). CHILE. Coquimbo: Prov. del Elqui, La Serena, ribera sur del Río Elqui a ca. 200 m de la desembocadura, 2 m, 23 March 2021, *L. Santilli 210323* (SGO)

Nicotiana rupicola L.Santilli, C.De Schrevel, N.Lavandero & P.Dandois **sp. nov.**

Type. Chile. Región de Coquimbo: Prov. Elqui, Comuna de Coquimbo, Fuerte Lambert, 29° 56' 2.52" S 71° 20' 16.46" W, 29 m, 12 November 2021, *N. Lavandero 1011* (holotype: SGO!; isotypes: EIF!, CONC!).

Diagnosis. *N. rupicola* is most similar to *Nicotiana solanifolia*, from which it differs by its compressed panicle (vs. loose panicle), its short corolla up to 18 mm (vs. corolla of 35-50 mm), glabrous corolla (vs. pubescent), non-retroflexed limb (vs. retroflexed limb), capsule small and included or slightly exerted from calyx, 6-10 mm (vs. large and more than half the length excluded from calyx, 12-18 mm).

Description. Perennial shrub up to 2 m with many stems arising from a lignified horizontal stem. **Stems** lignified, light brown, glabrous. **Leaves** orbicular to ovate, flat to slightly undulate, margins slightly revolute, base rounded to cordate, apex retuse to obtuse; bigger leaves 10 × 8.5 cm, reducing their size towards the apex, densely covered in 2 type of hairs in both sides: simple, straight, pluricellular, up to 1 mm long, and glandular (capitate), straight, pluricellular, 50–600 µm long, brochidodromous venation, petioles a quarter to half as long. **Inflorescence** a compact panicle, up to 35 cm; pedicels up to 0.5 cm, same indumentum as the leaves. **Calyx** up to 10 mm, cylindric, same indumentum as the leaves, teeth short, triangular. **Corolla** 17–18 mm excluding the limb (tubular part), tube proper up 5.5–6 mm, throat up to 12 mm, yellow, glabrous, limb 4 mm wide, yellow, glabrous or with scattered hairs, notched into 5 lobes. **Stamens** extending below the limb, similar length; filaments adnate for the first 5 mm to the tube proper and pubescent approx. in the proximal 7 mm, then glabrous and slightly curved with stamens bending toward the stigma. **Capsule** 6–10 mm long, ovoid. **Seeds** mainly angular, laterally compressed, 0.4–0.6 mm long, dark brown, surface reticulate. **Embryo** unknown. **Chromosome number** unknown. (**Fig. 3; Fig. 4B-D; Fig. 8**)

Distribution and habitat. *Nicotiana rupicola* is endemic to Chile where it is currently known from two locations, one being Fuerte Lambert, and the other being Chungungo, both in the region of Coquimbo (**Fig. 1**). In Fuerte Lambert it grows between the rocks in a cliff near the ocean together with *Myrcianthes coquimbensis* (Barnéoud) Landrum & Grifo, *Loasa elongata* Hook. & Arn., *Alstroemeria magnifica* Herb., *Diplolepis boerhaviifolia* (Hook. & Arn.) Liede & Rapini, *Eulychnia breviflora* Phil., *Nolana sedifolia* Poepp., *Nolana rupicola* Gaudich., *Nolana acuminata* (Miers) Miers ex Dunal, *Stachys pannosa* Phil., *Plumbago caerulea* Kunth, *Sicyos baderoa* Hook. & Arn. var. *baderoa*, *Solanum pinnatum* Cav., *Ophryosporus triangularis* Meyen, *Polyachyrus poeppigii* Kuntze ex Less. and *Cistanthe grandiflora* (Lindl.) Schtdl. In Chungungo it grows on a rocky cliff facing the ocean, together with *Nolana sedifolia* Poepp and *Nolana crassulifolia* Poepp., and surrounded by a

scrub of *Heliotropium stenophyllum* Hook. & Arn., *Balbisia peduncularis* (Lindl.) D. Don and *Oxalis virgosa* Molina.

Phenology. *Nicotiana rupicola* was found flowering and fruiting in November.

Etymology. The specific epithet derives from the latin *rupes*, *rupis* (rock), and *colere* (inhabiting), to refer to the rocky habitat where the species grows.

Additional specimens examined. Chile. Región de Coquimbo. Prov. Elqui, Comuna La Higuera, costa al Norte de Chungungo, 7 November 2006, *N. García* 3085 (EIF).

Conservation status. *N. rupicola* can be considered as Critically Endangered (CR) under the IUCN categories and criteria B1ab(iii); D. The criterion B1 was selected because its extent of occurrence is <100 km² (8 km²). The criterion “a” was selected because the distribution is highly fragmented. The criterion “b(iii)” was selected because there is a projected decline in the area, extent and quality of habitat. This area is constantly threatened by the expansion of urbanization that is affecting central-north coastal Chile. One of the locations is currently found at less than 300 m from the residential area of Coquimbo and the habitat is being altered by numerous and increasing amounts of formal and informal paths and human activity (camping, garbage, etc.). Moreover, mining activities within the extent of occurrence, especially Minera Dominga, which pretends to settle between the two known localities, will more likely affect possible unknown populations and the quality of the habitat. The criterion D was selected because we observed less than 50 individuals around the two known localities.

Key to the species of *Nicotiana* sect. *Paniculatae* found in Chile

1. Inflorescence a compact panicle, corolla tube glabrous except for sparse hairs on the limb..... *N. rupicola*
- Inflorescence a loose panicle, corolla tube entirely covered by hairs.....2
2. Corolla tube 3–5 cm, indumentum of corolla made of glandular hairs.....*N. solanifolia*
- Corolla tube 2–2.3 cm, corolla sericeous.....3
3. Corolla tube 2 cm, limb dark green, continental Chile.....*N. knightiana*
- Corolla tube 2–3 cm, limb yellow or purple, Juan Fernandez Islands.....4
4. Corolla tube purple, Alejandro Selkirk Island.....*N. cordifolia* subsp. *cordifolia*
- Corolla tube yellow, Santa Clara Island.....*N. cordifolia* subsp. *sanctaclarae*

Discussion

The characters that proved to be most useful to differentiate species of *Nicotiana* sect. *Paniculatae* are the type of inflorescence, the type of hairs and the pattern of distribution of the indumentum, the size and colour of the flowers. *N. knightiana* resembles most *N. paniculata* from which differs for its shorter flowers with dark green limbs (vs. yellow) (**Fig. 2E-F**). The large undulate leaves, the narrow panicles and the long throat of the flowers of *N. knightiana* also resemble *N. solanifolia* from which it can be distinguished for its smaller flowers, sericeous indumentum (vs. glandulous) and dark green limb (vs. yellow) (**Fig. 2E-F; Fig. 3C-D**). *Nicotiana rupicola* is morphologically similar to *Nicotiana solanifolia* from which it can be distinguished by its compact panicle (vs. loose), glabrous corolla tube (vs. hairy), its smaller flowers and no-retroflexed corolla (**Fig. 4**).

Molecular analyses showed that plants from Elqui river were correctly identified as *Nicotiana knightiana* and that our initial conjectures about the phylogenetic position of *Nicotiana rupicola* as part of the sect. *Paniculatae* were confirmed (**Fig. 6**). Our topology retrieves Clarkson's (2004) results of two separate clades within the sect. *Paniculatae* that reflect geography, one including species from Perú and one including the two endemics *N. solanifolia* from the North of Chile and *N. cordifolia* from Juan Fernández Archipelago (**Fig. 6**). Species of Chilean sect. *Paniculatae* share long tubular flowers and long-petiolate leaves. Surprisingly, *N. rupicola* results to be more closely related to the Juan Fernández species *N. cordifolia* than to the continental species, *Nicotiana solanifolia*, despite their morphological affinity.

An important question regards whether *N. knightiana* has to be considered native or introduced to Chile. Either the species was never noticed or collected during the last 2 centuries of botanical expeditions, and that it presents a naturally disjunct population, being almost 1500 km apart from the closest population found in Peru, or it was recently introduced in Chile by anthropogenic means. The earliest evidence of its presence dates back to 2018 (iNaturalist) and it seems to have been established and possibly expanded to the surrounding area in sites with similar ecological conditions to the river mouth of Elqui river. The production of abundant and small seeds, together with the ability of some species to grow in a broad range of open and disturbed habitats, is considered as a common adaptation that ensures high probability of dispersal and establishment. Such is the case for *Nicotiana paniculata*, *Nicotiana glauca*, and *Nicotiana plumbaginifolia* Viv. that are considered invasive species (Gallo et al. 2008; Gairola et al. 2016; Rodríguez-Caballero et al. 2020; Alharthi et al. 2021)

Nicotiana rupicola presents a restricted distribution limited to a small portion of the coastal area of northern Chile, where it grows on two locations on easily accessible coastal rocky cliffs at less than 300 m from urbanization. The population from Fuerte Lambert is situated in an area where urban expansion has caused major damage to the vegetation. The coastal area between Tongoy and Coquimbo is catalogued as a site of interest for the conservation of woody and succulent species due to its high diversity and endemism (Squeo, 2000). The area is home to various threatened species such as: *Myrcianthes coquimbensis* (Barnéoud) Landrum & Grifo, *Porlieria chilensis* I.M. Johnst., *Carica chilensis* (Planch. ex A. DC.) Solms. Controversially, it is an area particularly affected by intense expanding urbanization that seriously threatens the conservation of the local biodiversity. Additionally, a new mining facility and a discharge port (Minera Dominga) will be constructed within its extent of occurrence, close to its northernmost known locality. It is likely that several more individuals of *Nicotiana rupicola* are present in the area, since the abiotic and biotic conditions are similar to the currently known localities. None of these individuals were considered during the environmental impact assessment of this controversial project. It is of great importance for the conservation of the species to search for more individuals and localities, and to raise the attention to much needed conservation measures for the species and the unique ecosystem where it is found.

Additional specimens examined

Nicotiana solanifolia. **CHILE. Antofagasta:** [Antofagasta Province]. Lomas de Taltal, near road from Taltal to the panamericana, 430 m, 25 October 2002, *Ackermann 500* (SGO); bei Hueso Parado, nahe von Taltal, 400 m, 9 July 1972, *O. Zöllner 5942* (L); Quebrada de Taltal,

410 m, 17 September 1992, *Teillier, Rundel & P. García* 2850 (F); Hueso Parado, *s.d., s.c.* (SGO); Ravine ca. 16 km north from Paposo, 207 m, 21 November 2008, *Baines et al.* 38. (E); Mirador above the ThermoElectric plant below Quebrada Paposo, 680 m, 1 December 2004, *Dillon & Finger* 8670 (SGO); Paposo, Peralito, 15 November 1959, *Torres s.n.* (SGO); Paposo, borde quebrada, 24 October 2009, *Moreira & Luebert* 1205 (SGO); Camino Paposo-Caleta Blanco Encalada, Queb. Miguel Diaz, 160 m, 15 November 1996, *Rodriguez* 3131 (SGO); El Rincón, al N de Paposo, 17 September 1941, *Muñoz & Johnson* 2877 (SGO); Quebrada el Despoblado, 25-26 August 1992, *Torres s.n.* (SGO); Taltal-Paposo, September 1909, *Reiche s.n.* (SGO); Paposo, entrada a la Q.da Los Peralitos, 30 September 2005, *Muñoz* 4607 (SGO); 10 km al sur de Caleta Blanco Encalado, 200-800 m, 11 December 1940, *Walter Biese* 3209 (SGO). Taltal, Quebrada de Taltal, 410 m, 17 September 1992, *Teiller et al.* 2837 (SGO); 6 Km east of Talta, 300-600 m, 14 October 1938, *C.R. Worth, J.L. Morrison* 16122 (US); Cerro Perales, ca. 5 km E of Taltal, 550-960 m, 27 September 1988, *M.O. Dillon, D. Dillon & V. Poblete* 5536 (F); Quebrada Rinconada, ca. 5 Km N of Caleta Paposo, 250 m, 25 October 1988, *M.O. Dillon, D. Dillon & B. Tay* 5741 (F);

Atacama: [Chañaral Province]. Parque Nacional Pan de Azúcar, Quebrada de Coquimbo, 10 November 1987, *Paez s.n.* (SGO); Chañaral, 24 October 1985, *Nieuwenhuizen* 132-27 (SGO); Camino Chañaral a Flamenco, 3.5 km al interior camino izquierdo desde Portofino, quebrada y cono de deyección, 14 October 1992, *Muñoz* 3095 (SGO). [Copiapó Province]. Caldera, Quebrada León, 20 m, October 1924, *Werdermann, E.* 437 (E, SI, F); Quebrada de los leones, Caldera, 1888, *W. Geisse s.n.* (Type of *Nicotiana cardiophylla* Phil.) (SGO); Caldera, September 1900, *Reiche s.n.* (SGO). [Huasco Province]. Camino Carrizal Bajo - Huasco, 30 m, 13 October 1991, *Teillier et al.* 2579 (SGO); sector Aguada Tongoy, road to Los Bronces near Corral El Sauce - road junction, 276 m, 6 December 2004, *Baxter et al.* 1877 (E, SGO); Carrizal Bajo, September 1885, *F. Philippi s.n.* (SGO); Camino Carrizal Bajo, km 50, 2 November 1991, *Muñoz et al.* 2944 (SGO); Camino de vuelta Carrizal a Canto de Agua en Qda. exposición sur, 23 September 1977, *Muñoz* 1119 (SGO); Carrizal, September 1885, *F. Philippi s.n.* (SGO).

Nicotiana cordifolia. **CHILE. Valparaíso:** [Valparaíso Province]. Archipiélago de Juan Fernandez, Isla Santa Clara, Bahía Matriz, 12 December 1998, *Ph. Danton s.n.* (SGO); Isla Masafuera, *s.d.*, *R. A. Philippi* 730 (F), Isla Masafuera, October 1854, *Germain s.n.* (SGO); Isla Masatierra, San Juan Bautista, Conaf Garden, 56 m, 13 December 2003, *Gardner et al.* 81 (E)

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References

Alharthi AS, Abd-ElGawad AM, Assaeeda AM (2021) Influence of the invasive shrub *Nicotiana glauca* Graham on the plant seed bank in various locations in Taif region, western

of Saudi Arabia. Saudi Journal of Biological Sciences 28(1): 360-370.
<https://doi.org/10.1016/j.sjbs.2020.10.014>

Aoki S, Ito M (2000) Molecular phylogeny of *Nicotiana* (Solanaceae) based on the nucleotide sequence of the matK gene. Plant Biology 2: 316–324. <https://doi.org/10.1055/s-2000-3710>

Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity science. ZooKeys 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>

Ballester B, Carrasco C, Del Desierto C (2016) *Nicotianas* litorales del desierto de Atacama: historia de registro y consumo de tabaco cimarrón (*Nicotiana solanifolia* Warp.). Taltalia (9): 69–87.

Bremer B, Bremer K, Heidari N, Erixon P, Anderberg AA, Olmstead RG, Källersjö M, Barkhordarian E. (2002) Phylogenetics of asterids based on three coding and three non-coding chloroplast DNA markers and the utility of noncoding DNA at higher taxonomic levels. Molecular Phylogenetics and Evolution 24: 274–301. [https://doi.org/10.1016/S1055-7903\(02\)00240-3](https://doi.org/10.1016/S1055-7903(02)00240-3)

Chase MW, Knapp S, Cox AV, Clarkson JJ, Butsko Y, Joseph J, Parokony AS (2003) Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). Annals of Botany 92(1): 107–127. <https://doi.org/10.1071/SB11006>

Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase, MW (2004) Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. Molecular phylogenetics and evolution 33(1): 75–90. <https://doi.org/10.1016/j.ympev.2004.05.002>

Clarkson JJ, Kelly LJ, Leitch AR, Knapp S, Chase MW (2010) Nuclear glutamine synthetase evolution in *Nicotiana*: phylogenetics and the origins of allotetraploid and homoploid (diploid) hybrids. Molecular phylogenetics and evolution 55(1): 99–112. <https://doi.org/10.1016/j.ympev.2009.10.003>

Clarkson JJ, Dodsworth S, Chase MW (2017) Time-calibrated phylogenetic trees establish a lag between polyploidisation and diversification in *Nicotiana* (Solanaceae). Plant Systematics and Evolution 303(8): 1001–1012. <https://doi.org/10.1007/s00606-017-1416-9>

Edler D, Klein J, Antonelli A, Silvestro D (2020) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12(2):1-5. <http://dx.doi.org/10.1111/2041-210X.13512>

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of molecular evolution 17(6): 368–376.

Gairola S, El-Keblawy A, Mahmoud T (2016) A note on the current distribution of *Nicotiana plumbaginifolia* (Solanaceae) in the United Arab Emirates. National Academy Science Letters 39(6): 461–464. <https://doi.org/10.1007/s40009-016-0490-9>

Gallo AG, de la Torre WW, Rodríguez VM (2008) Especies vegetales consideradas invasoras de hábitats, en la Historia Natural de Canarias. Lazaroa 29: 49.

Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Molecular ecology 8(3): 521–523.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>

IUCN (2017) Guidelines for using the IUCN red list categories and criteria, version 13. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>

Johnson LA, Soltis DE (1995) Phylogenetic inference in Saxifragaceae sensu stricto and Gilia (Polemoniaceae) using matK sequences. Annals of the Missouri Botanical Garden 149–175. <https://doi.org/10.2307/2399875>

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic acids research 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>

Mehmood F, Ubaid Z, Shahzadi I, Ahmed I, Waheed MT, Poczai P, Mirza B (2020) Plastid genomics of *Nicotiana* (Solanaceae): insights into molecular evolution, positive selection and the origin of the maternal genome of Aztec tobacco (*Nicotiana rustica*). PeerJ 8:e9552. <http://doi.org/10.7717/peerj.9552>

Nylander JAA (2004) MrModeltest v2. Program distributed by the author.

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology 43(4): 467–481. <https://doi.org/10.2307/2413546>

Philippi RA (1856) Bemerkungen über die Flora der Insel Juan Fernández. Botanische Zeitung, Berlin 14: 641–650.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic biology 67(5): 901. <https://doi.org/10.1093/sysbio/syy032>

Reiche K (1903) Estudio crítico sobre la Flora de Chile. Solanaceae. Anales de la Universidad de Chile 5: 378-390.

Rodríguez R, Marticorena C, Alarcón D, Baeza C, Cavieres L, Finot VL, Fuentes N, Kiessling A, Mihoc M, Pauchard A, Ruiz E (2018) Catálogo de las plantas vasculares de Chile. *Gayana Botánica* 75(1): 1-430. <http://dx.doi.org/10.4067/S0717-66432018000100001>

Rodríguez-Caballero G, Roldán A, Caravaca F (2020) Invasive *Nicotiana glauca* shifts the soil microbial community composition and functioning of harsh and disturbed semiarid Mediterranean environments. *Biological Invasions* 22:2923–2940. <https://doi.org/10.1007/s10530-020-02299-1>

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61:539-542. <https://doi.org/10.1093/sysbio/sys029>

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92:142–166. <https://doi.org/10.3732/ajb.92.1.142>

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12:335–337. <https://doi.org/10.1007/s13127-011-0056-0>

Squeo FA (2000) Libro rojo de la flora nativa y de los sitios prioritarios para su conservación: Región de Coquimbo. Ediciones de la Universidad de La Serena, La Serena, Chile.

Stamatakis A (2014) Raxml version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17(5): 1105–1109. <https://doi.org/10.1007/BF00037152>

Walpers WG (1844) Synopsis solanacearum, scrophularinarum, orobancheorum et labiatarum in *Repertorium botanices systematicae*. The New York Botanical Garden 3:12.

Captions:

Figure 1. Distribution map of known locations of *Nicotiana knightiana* (circles) and *Nicotiana rupicola* (triangles) in Chile. Service Layer Credits: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

Figure 2. *Nicotiana knightiana* (L. Santilli 210323). **A.** Habit; **B.** Inflorescence; **C.** detail of lignified horizontal stem; **D.** adaxial and abaxial side of a leaf; **E.** frontal view of a flower showing limb area **F.** lateral view of a flower. Scale bars = 1 cm.

Figure 3. Indumentum of leaves. **A-B.** *Nicotiana rupicola* (*N. Lavandero 1011*); **C-D.** *Nicotiana knightiana* (*L. Santilli 210323*).

Figure 4. *Nicotiana rupicola*. **A.** Habitat; **B.** Habit; **C.** Inflorescence; **D.** adaxial and abaxial side of a leaf; **E.** frontal view of a flower showing limb area; **F.** detail of indumentum; **G.** lateral view of a flower. Scale bars= 1 cm.

Figure 5. Fruits and seeds. **A,C.** *Nicotiana knightiana* (*L. Santilli 210323*); **B,D.** *Nicotiana rupicola* (*N. Lavandero 1011*).

Figure 6. Phylogeny of *Nicotiana* resulting from Maximum Likelihood analysis of the plastid regions *matK*, *rps16*, *trnS-trnG* and *trnL-trnF*. Numbers above and below the branches represent the Posterior probabilities from the BI analysis and bootstrap values from the ML analysis, respectively. The species whose sequences were obtained in the present study are highlighted in bold, while section *Paniculatae* including *N. rustica* is highlighted in grey.

Figure 7. Illustration of *Nicotiana knightiana*

Figure 8. Illustration of *Nicotiana rupicola*

















