

# A New Report of *Uropyxis daleae* in the Andes Range Mountains of Atacama Desert, Chile

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## Abstract

A rust disease caused by *Uropyxis daleae* (Dietel & Holw.) Magnus was registered on the natural population of *Dalea pennelli* (Mac Bride) Mac Bride var. *chilensis* Barneby in the highland near the Andes in the northernmost of Chile. This pathogen shows uredinia with the presence of paraphyses and features, larger urediniospores and teliospores than *U. daleae* (Dietel & Holway) Magn. var. *daleae*. Based on morphological combined with 28S sequence analysis, we propose this pathogen as a new fungal variety: *U. daleae* var. *socromensis*. This is the first report of *Uropyxis daleae* var. *socromensis* attacking *D. pennelli* var. *chilensis* in Northern Chile.

## Keywords

Uredinales, Neotropic Fungi, Endemic Fungi, Atacama Desert

## 1. Introduction

*Dalea pennellii* (J. F. Macbr.) J. F. Macbr. var. *chilensis* Barneby (Fabaceae), commonly known as “retamilla,” is a small endemic shrub distributed in the highlands in northern Chile and southern Peru, above 3500 m above sea level (asl) [1]. It is a typical plant component of the native flora of arid landscapes [2]. In Chile, there is no information regarding the fungal species associated with this host plant. However, a rust fungus has been found attacking *D. pennellii* var. *chilensis* developing dusty black sori on the leaflets and stems. The rust fungi are a monophyletic, highly specialized group of obligate parasites in the phylum Basidiomycota, with a complex life cycle. Most of the rust fungi exhibit a high degree of host specificity [3].

Nowadays, the identification of rust fungi includes morphological and molecular studies [3] [4] [5]. The cell wall thickness, ornamentation, color, size, and shape of the telia and teliospores, and the pedicel characteristics and number and position of the germinative pores in the teliospores are important taxonomical features in the identification of rust fungi.

The Uredinales associated with native plants have been scarcely studied in Northern Chile but, some rust fungi have been described in coastal range and coastal valleys [6]. This study aims to report and describe a rust fungus associated with the canopy of *D. pennellii* var. *chilensis*, in Socoroma, Chile.

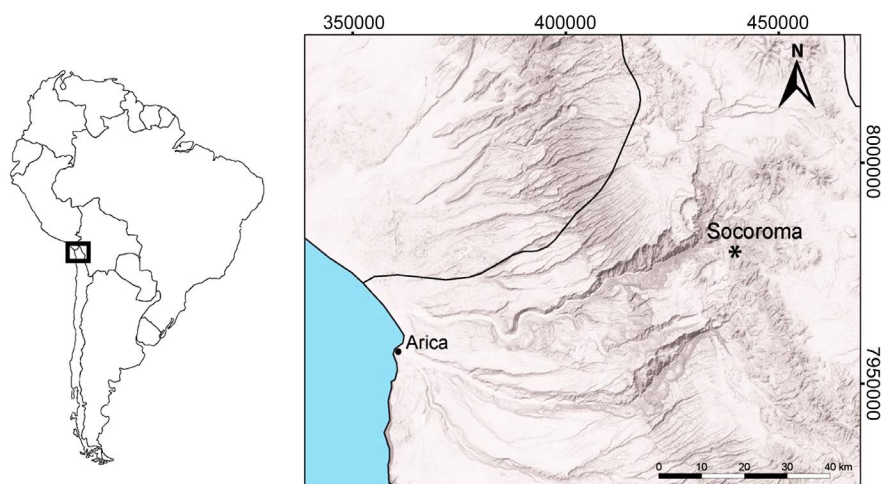
## 2. Materials and Methods

### 2.1. Sample Collection

Samples of *D. pennellii* var. *chilensis* were collected in Socoroma (18°16'38"S, 69°35'28"W) in the highlands of the Andes mountains at 3200 m asl. *D. pennellii* var. *chilensis* were growing in a slope with a northern exposure, 125 km northeast of Arica city and 3 km from Socoroma, in the north of Chile (Figure 1). A total of 10 entire plants of *D. pennellii* var. *chilensis* showing rust, symptoms, and signs on the leaflets were collected in April and June 2017. Each sample was identified, labeled, and taken to the Plant Pathology Laboratory at Universidad de Tarapacá, where morphological and molecular studies were conducted. Besides, ten plant samples were desiccated at room temperature (20°C - 22°C) and kept in a herbarium.

### 2.2. Morphological Studies

Morphometric studies were conducted on water mount urediniospores and teliospores collected from rust sori developed on leaflet samples of *D. pennellii* var. *chilensis*.



**Figure 1.** South American map showing northern Chile, the geographical location of Socoroma and the sample collection point (asterisk) of “retamilla” rust (*Dalea pennellii* var. *chilensis*).

The identification included studies of wall thickness, ornamentation, color, size, and shape of the 30 urediniopores and 30 teliospores. The characteristics of the teliospore pedicels and the number and position of the germinative pores and spore size were determined. An aqueous solution of chloral hydrate 450 g/L was used to facilitate seeing the germinal pores of the teliospores [7]. For the observation of morphological details of the spores, the Bruzesse and Hassan dye [8] was used. The preparations were sealed with nail polish, labeled, and stored at room temperature until used. Measurements and digital photographs were made with the aid of an Olympus BX51 microscope and the Q Capture-Pro version 6 Software (Surrey, BC, Canada).

For scanning electron microscope (SEM) EVO LS 10 (Carl Zeiss, Bonn, Germany) observations, where sections of leaflets of (0.2 cm<sup>2</sup>) with telia were obtained. The specimens were mounted on aluminum sample holders with double-contact carbon adhesives and analyzed in a low vacuum and in variable pressure mode, where the chamber pressure was 150 Pa (under vacuum) and in the column  $2 \times 10^{-5}$  Torr (high vacuum). The working distance used (WD) varied depending on the type of sample, and what was needed to observe, the acceleration voltage used was 15 KV and a tilt (tilt) from 0° to 90°, while the images were taken with a resolution of 3024 × 2304 pixels at a scanning speed of 12 minutes with 54 seconds.

The specimens were deposited in the mycological collection of Universidad de Concepción Herbarium (CONC-F 1894).

### 2.3. Molecular and Phylogenetic Analysis

Due to rust cannot be grown in culture media, teliospores were used for molecular analysis, which was removed and collected from telia on leaves with a sterile scalpel. DNA from three independent infected plants was extracted using EZNA® Insect DNA Kit (Omega Bio-Tek Inc, Norcross, GA, USA) according to the manufacturer's instructions. Subsequently, the D1/D2 domain of the large subunit ribosomal DNA 28S (LSU rDNA) was amplified using primers LR6 (5'-CGCCAGTTCTGCTTACC-3') and Rust2inv (5'-GATGAAGAACACAGTGAAA-3') [9]. Polymerase chain reactions of LSU rDNA were conducted in an Amplitronyx®6 thermal cycler (Nyx-Technik, San Diego, California, USA) using a final volume of 20 µl. Each reaction contained 1 µl of DNA extract, 5 pmoles of each primer, 2.5 mM of each dNTP, 2 mM MgCl<sub>2</sub>, 1X PCR buffer (KCl), 1 unit of Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA), and sterile distilled water. Cycling conditions were: 5 min at 94°C; 35 cycles of 1 min at 94°C; 1 min at 55°C; 1 min at 72°C and a final elongation step of 2 min at 72°C. PCR blank reaction control without template DNA was always incorporated. The PCR products were visualized on 1.0% agarose gel and stained with GelRed (Biotium Inc., Hayward, CA, USA). The amplified PCR products were purified and sequenced by Macrogen (Macrogen, Geumcheon-gu, South Korea). The nucleotide sequences were visualized and edited using the 4 Peaks software (<http://nucleobytes.com>). A BLASTn analysis

was used to compare the DNA sequence obtained of the rust fungus isolates from Socoroma with rDNA D1/D2 data sequences available in GenBank (<http://www.ncbi.nlm.nih.gov/>).

Phylogenetic reconstruction was performed using the maximum likelihood algorithm, using Molecular Evolutionary Genetics Analysis (MEGA version 6.0) software [10], and the robustness of the branches was assessed by bootstrap analysis [11] of 1000 replicates. The sequence of *Encyclometra* sp. (KY781819) was used as an outgroup.

### 3. Results

#### 3.1. Taxonomy

*Uropyxis daleae* (Dietel & Holw.) Magnus var. nova *socoromensis*.

**Holotype.** *Dalea pennelli* (Mac Bride) Mac Bride var. *chilensis* Barneby, Socoroma, Arica and Parinacota, Chile, Sep. 2017, leg. G. Sepúlveda 101 Col-Mic-UTA 183. The specimen was deposited as a dried specimen in the the mycological collection of Universidad de Concepción Herbarium (CONC-F 1894).

**Etymology.** From the Andean village of Socoroma, in reference to the collection site (**Figure 1**).

#### 3.2. Description

Uredinial stage with amphiphigenous subepidermal uredinia with inconspicuous rupture of the epidermis, reddish to tan-brown, pulverulent, solitary or in small groups, round to oblong. Paraphyses not observed. Ellipsoid to obovoid urediniospores, hyaline to pale yellow, of 22 - 28 × 20 - 23 µm, finely echinulate wall of 2 - 2.5 µm of thickness, golden brown with 5 to 8 scattered germinative pores (**Table 1**).

Telia were anfigenous, subepidermal in origin, with inconspicuous epidermal rupture, dark brown to black, predominantly hypophyllous, frequently coming from uredinium, pulvinate, paraphyses were absent-Teliopores were bicellular, ellipsoid to oblong, 32 - 50 × 25 - 30 µm. Uniform laminate wall, with scattered warts, 2.5 µm thick and hyaline, two germinating pores per cell; fragile pedicel, 30 - 35 µm long, hygroscopic at the point of union with the spore, up to 23 µm wide, hyaline in color. Spermogonia (pycnial) and aecia were absent.

#### 3.3. Specimens Studied

Fresh leaves of *Dalea pennelli* (Mac Bride) Mac Bride var. *chilensis* Barneby, Socoroma, Arica and Parinacota, Chile. 2017, Province of Parinacota, Socoroma, S lat. 18° 16'38", W 69° 35'28", 3200 m asl. Leg. et det. W. Huanca-Mamani & G. Sepúlveda, (CONC-F 1894).

Spermogonia (pycnia) and aecia were not detected. Sori containing teliospores, hypophyllous subepidermic, erumpents, small, dark brown, 1 - 2.1 × 1 - 1.8 mm, powdery, non-paraphysated (**Figure 2**). Teliospores elipsoides to oblong 32 - 50 × 25 - 30 µm, echinulated, dark brown, two germinative pores per



**Figure 2.** Signs and symptoms of “retamilla” rust (*Daleae pennellii* var. *chilensis*). (A) Telia on “retamilla” stems. (B) and (C) Slight chlorosis and dark brown to dusty black telia on “retamilla” leaf. Bar = 2 mm.

**Table 1.** Morphological characterization of the telia and teliospores of *Uropyxis daleae* var *socoromensis* isolates from *Dalea pennellii* var. *chilensis* collected in Socoroma, an Andean village located at 3500 m asl near Arica in Northern Chile.

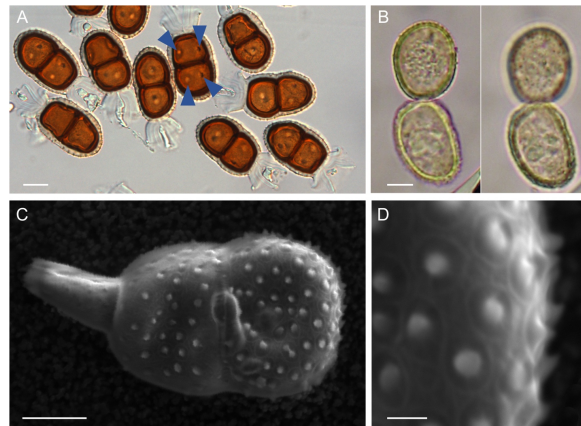
Criteria	<i>U. daleae</i> <sup>1</sup>	<i>U. daleae</i> var. <i>socoromensis</i>
<b>Teliospores</b>		
<b>Size</b>	35 - 50 × 24 - 30 μm	32 - 50 × 25 - 30 μm
<b>Shape</b>	Ellipsoid	Ellipsoid to oblong
<b>Spore surface</b>	Verrucose, echinulate	Verrucose, echinulate
<b>Spore ends</b>	Rounded	Rounded
<b>Wall</b>	Laminate	Laminate
<b>Wall thickness</b>	4 - 6 μm	1.3 - 3.3 mm
<b>Wall inner layer</b>	Dark brown	Dark brown
<b>Wall outer layer</b>	Yellowish	Hyaline to yellowish
<b>Pedicel attachment</b>	Swelling	Swelling
<b>Pedicel wideness</b>	15 - 20 μm	23 μm
<b>Pedicel length</b>	35 μm	30 - 35 μm
<b>Germinative pores per cell</b>	2	2
<b>Septum constriction</b>	Slightly or absent	Slightly or absent
<b>Telia</b>		
<b>Size</b>	0.5 - 1.0 mm in diameter	1 - 2.1 × 1 - 1.8 mm
<b>Color</b>	Brown to black	Dark brown to black
<b>Aspect</b>	Pulverulent	Pulverulent
<b>Origin</b>		Subepidermic
<b>Paraphyses</b>	Peripheral	Non observed

<sup>1</sup>Based on Baxter’s description (1959) [12].

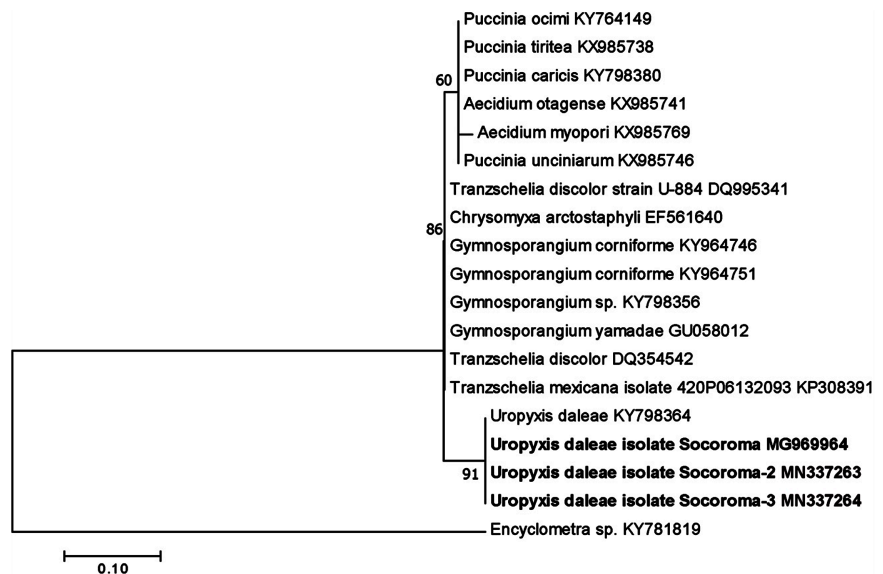
cell, walls of uniform thickness 1.3 - 3.3 mm, the majority 2.5 mm thick, pedicellated, hyaline pedicels, campanulated lateral wall of 14.3 - 10 mm of thickness, the majority of 12.5 mm thick (Figure 3).

To confirm the morphological identification, a PCR fragments of LSU rDNA of isolates from Socoroma were successfully amplified and sequenced, obtaining fragments of LSU of 1446, 1456 and 1443 bp, which were deposited in the Gen-

Bank (Accession number MG969964, MN337264 and MN337263, respectively). A BLAST search showed 91.20% similarity of the isolates Socoroma with *Uropyxis daleae* (KY798364) and 87.85%, 87.78%, and 87.76% with *Puccinia ocimi*, *P. uncinarum*, and *P. caricis*, respectively (KY764149, KX985746, and KX798380). The phylogenetic analysis using sequences of the LSU rDNA grouped the isolates Socoroma in a different cluster from other rust fungi, and it was phylogenetically close to the reference isolate of *U. daleae* (KY798364) (Figure 4).



**Figure 3.** *Uropyxis daleae* var. *socoromensis*. (A) Dark bicellular teliospores, verrucose to echinulate, with a brown inner layer a hyaline outer layer, two germinative pores (blue arrowhead), and a campanulate pedicel. (B) Ellipsoid to obovoid unicellular urediniospores with gray to yellow walls. (C) and (D) Teliospores under scanning electron microscopy. Echinulate surface and detail of teliospore pedicel (C). Detail of teliospore surface showing scars and spines. Bars: (A)-(C) = 10  $\mu$ m, (D) = 1  $\mu$ m.



**Figure 4.** Phylogenetic tree based on LSU analysis of *Uropyxis daleae* var. *socoromensis* and close Basidiomycota species using the Kimura two-parameter model and maximum likelihood algorithm with 1000 bootstrap replicates. The new *U. daleae* sequences generated for this study are indicated in bold type. Numbers at nodes represent bootstrap support values. *Encyclometra* sp. was used as an outgroup for rooting purposes.

## 4. Discussion

In this study, a rust fungus attacking *D. pennelli* (“retamilla”) was characterized and identified as *Uropyxis daleae*, and based on morphological and molecular differences, a new variety, *U. daleae* var. *socoromensis*, is proposed. Rust fungi are very specific obligate parasites. To our knowledge, *U. daleae* var. *socoromensis* was only found on “retamilla” growing above 3500 m asl in the Andes Mountains in Northern Chile, where a high frequency of endemic plant species, animals, and fungi have been described [13]. Therefore, it is possible that this new variety of *U. daleae* may have coevolved *in situ* with its plant host. At present, “retamilla” grows as a wild shrub; however, it has a high potential as an ornamental plant.

The genus *Uropyxis* J. Schröt. (Basidiomycota, Pucciniales, Uropyxidaceae), was first distinguished from *Puccinia* spp. in 1875 by the presence of teliospores with two germination pores in each cell [11]. Nowadays, 30 species have been identified in the genus *Uropyxis*, two var. in *U. steudneri* and three in *U. daleae*. The recognized varieties of *U. daleae* are *africana*, *daleae*, and *eysenhardtiae* (Index Fungorum 2019, <http://www.indexfungorum.org/>, Mycobank 2019, <http://www.mycobank.org/>).

Based on morphological and molecular results obtained in this study, *U. daleae* var. *socoromensis* is described as a new fungal variety. The main distinguishing characters of *U. daleae* var. *socoromensis* are the bigger urediniospores with uniform walls up to 2.5 µm thick, uredinia with scattered paraphyses, and teliospores up to 50 µm long. It differs from *U. daleae* (Dietel & Holway) Magn. var. *daleae* by the size of the urediniospores and the wall, longer teliospores with smaller wall size, and telia lack of paraphysis. It differs from *U. daleae* (Dietel & Holway) J.W. Baxter var. *eysenhardtiae* by the absence of spermogonia, aecia uredinoid, and the urediniospore size and wall thickness.

The presence of uredinia distinguishes *U. daleae* var. *socoromensis* from *U. farlowii* (Arthur) J.W. Baxter, a rust fungus that is also parasite *Dalia* spp., but apparently lacks of the uredinial stage.

Therefore, *Uropyxis daleae* var. *socoromensis* is described for the first time attacking *D. pennelli* var. *chilensis* in Northern Chile. Previously, only *U. naumanniana* has been reported in southern Chile [14].

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## Conflicts of Interest

The authors declare no competing interests.

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