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# *Puccinia meyeri-albertii* in leaf and fruit of calafate (*Berberis microphylla*) in La Araucanía Region, Chile

## *Puccinia meyeri-albertii* en hojas y frutos de calafate (*Berberis microphylla*) en la región de La Araucanía, Chile

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**Abstract**

Calafate (*Berberis microphylla* G. Forst) is an endemic species of the Patagonian Andes of Chile and Argentina, with potential use for agroindustry. One aspect that could be restrictive for the production is the Calafate rust (*Puccinia meyeri-albertii* P. Magn), which the last reference in Chile dated from 1945. The objective of this study was to identify and updated report about to the prevalence of Calafate rust in La Araucanía Region, Chile. The rust identification was consistent as that reported for *P. meyeri-albertii,* aspects also confirmed by Centre for Agricultural Bioscience International (CABI) under number IMI-50016. The sequence amplified of the 28S rDNA (accession number KY555071) had an identity of 97% with others six *Puccinia* species, likewise, the genetic relationship analysis established that *P. meyeri-albertii* had higher divergences in the nucleotide substitutions respect to the others rusts; it consign that no nucleotide sequences available in NCBI database for *P. meyeri-albertii*. Prospecting realized the 2011-2012 seasons in an experimental Calafate orchard, the incidence and severity were respectively: 24.70% (range 4.72-36.90%) and severity 2.97 (2.15-3.30, range affected area) in leaves; and in fruits 13.78 % (range 1.13-23.84%) and 2.40 (2.01-2.78, range affected area). Hence, it was suggested that it is necessary to develop molecular, epidemiological and control research, as also to determine the infection level of this pathogen in others Calafate ecotypes and *Berberis* species growing in different edaphoclimatic conditions.

**Keywords**
*Berberis* • Calafate • *Puccinia meyeri-albertii* • rust

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**Resumen**

El Calafate (*Berberis microphylla* G. Forst.), es una especie endémica de Los Andes Patagónicos de Chile y Argentina, con potencial de cultivo para la agroindustria. Un aspecto que pudiere ser restrictivo para su establecimiento y producción es la roya del Calafate, cuya última referencia en Chile data del 1945. El objetivo de este estudio fue identificar y actualizar la prevalencia de esta roya en la Región de La Araucanía, Chile. La identificación morfológica fue consistente con *Puccinia meyeri-albertii* P. Magn, determinación corroborada por el *Centre for Agricultural Bioscience International* (CABI) y registrada con el número IMI-50016. La secuencia amplificada desde el 28S del ADN ribosomal (accession KY555071), tuvo una identidad de 97% con otras seis especies de *Puccinia*. El análisis de relaciones genéticas de *P. meyeri-albertii*, representadas en un árbol filogenético, generó amplias divergencias sobre las substituciones de nucleótidos respecto de las otras royas comparadas en este estudio; es de consignar que en la base de datos del NCBI no se dispone secuencias nucleotídicas para este hongo. A través de prospecciones realizadas durante la temporada 2011-2012 en un huerto experimental de calafate, la incidencia y severidad de *P. meyeri-albertii* fueron respectivamente: 24,70% (rango 4,72%-36,90%) y 2,97 (2,15 - 3,30, rango de área afectada) en hojas; mientras que en frutos fue de 13,78 % (1,13-23,84%) y 2,40 (2,01-2,78, rango de área afectada). Al respecto, sería conveniente generar información en aspectos moleculares, epidemiológicos y de control de este patógeno, como también evaluar el nivel de infección en ecotipos de calafate y especies de *Berberis* que crecen en diversas condiciones edafoclimáticas.

**Palabras clave**

*Berberis* • Calafate • *Puccinia meyeri-albertii* • roya

## Introduction

Calafate (*Berberis microphylla* G. Forst.) belongs to the Berberidaceae family, which contains around of 15 genus and 650 species distributed worldwide. In Chile, this genera it is represented by 20 species (10, 19). It is a perennial spiny shrub, growing to a height of two metres or more (16). Calafate grows in the different altitudes on pre-cordillera, in moist steppe areas, along the river and stream banks from Del Maule to Magallanes and Chilean Antarctica regions (6, 9).

The scientific information in Chile about *Puccinia meyeri-albertii* associated to Calafate is scarce. The first reference is comprise in "*Flora Fungosa Chilena*" (13) associated to the Calafate (*B. micropylla*) and also other species belonging to Berberidaceae family (*i.e. B. blaurina* Bellb., *B. chilensis* Gill, *B. darwinii* Hook, *B. linearifolia* Ph. and *B. congestiflora* Gay). Other historic and relevant reference is that mentioned in "The Repertoire of Literature Cryptogamic" (1892) associated with *Berberis* genus growing in South America (14).

The pathogenic and epidemiological information regarding *P. meyeri-albertii* is scarcely and principally associated with *Berberis* genus (5, 13, 21).

Recently, there has been increasing interest for commercial use of its fruits, which can be consumed fresh or in processed foods (jams, jellies, and beer) (4, 20) due to its high level of antioxidants as well as alkaloids of the berberine group (3, 12, 18).

The quality and condition of Calafate fruit for the industrial process could decrease due to the severity of the attack of *P. meyeri-albertii*. In this sense, as firstly required identify causal agent of the Calafate rust, and quantify the prevalence in ecotypes, seasons and different environment conditions, to design control strategies for this pathogen in endemic plant species from Chile and Argentina, which last reference in Chile dated from 1945. Hence, the objective of this study was to identify and updated report about the prevalence of Calafate rust in La Araucanía Region, Chile.

## Material and methods

### Sample collection and geographical location

Symptomatic leaves and fruits were collected every 15 days in 2011-2012 growing seasons from Calafate plants of 10 years localized in the Maquehue Experimental Station (38°50' S, 72°41' W), belonging to Universidad de La Frontera.

The symptoms in leaves corresponding to a yellow discolouration, reddish brown patches, and dehydration of the fruits and signs are orange uredosoric pustules and brown teleutosoric pustules. The samples collected from different twigs (n=10) homogeneously distributed in the plant were maintained under refrigeration at 4°C for approximately seven days until their analysis in the plant pathology laboratory of Instituto de Agroindustria, Universidad de La Frontera.

### Morphometric and molecular features

The teliospores (n = 30) were measured according to described by Zuluaga *et al.* (22, 23).

The shape, colour, dimensions, apical and lateral wall size, ornamentation, pedicel length and the viability of the teliospores, and also basidiospores were morphometrically characterized using a Carl Zeiss stereoscopic microscope (model Stemi DRC) and a Nikon optical microscope (model Alphaphot-2 YS2-Hse).

For the germination of teliospores, a suspension using 10 ml with sterile distilled water was homogenised in test tubes for five-minutes, and placed on Petri dishes with water agar culture medium (2%). The dishes were incubated (20 ± 2°C; RH 90%) and evaluated at 24-hour intervals during three days (data not shown). To confirm the morphological identification, leaves and fruit samples infected were sent to the Centre for Agricultural Bioscience International (CABI), United Kingdom.

Aeciospore masses removed from sorus growth on Calafate fruits were kept into a mortar followed by frozen using liquid nitrogen and crushed with a pestle.

The powder was transferred to a 1.5 mL Eppendorf tube continuing with the manufacturer's protocol for DNA extraction with the Qiagen Plant Mini kit (ref. 69104). PCR reactions were processed in My Cycler™ thermal cycler (BioRad) including 25 µL reaction volume, where: 1x GoTaq Flexi Buffer (Promega, cod. M890A), 2 mM $MgCl_2$, 0.4 mM dNTPs, 1 µM for each primer, 1 U GoTaq® Flexi G2 DNA polymerase (Promega, cod. M780B) and 2 µL DNA template. Large-subunit ribosomal RNA gene (LSU rDNA) was amplified by both NL1 (5' -GCATATCAATAAGCGGAGGAAAAG- 3')

and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers. The thermal profile consisted in initial denaturation step (94°C, 2 min) followed by 31 cycles at 94°C for 1 min, 57°C for 30 s, 72°C for 30 s, and a final extension step for 7 min at 72°C.

The PCR product was observed by 1% agarose electrophoresis gel stained with ethidium bromide in 1x TAE buffer and visualized under UV light. The amplicon was purified using QIAquick® PCR Purification Kit (Qiagen, cat. 28104), and sequenced at Unidad de Secuenciamiento Automático at Pontificia Universidad Católica de Chile. Nucleotide sequence was edited using Lasergene software (DNASTAR, Inc. Madison, WI U.S.) and compared with homologous sequences of *Puccinia* species by Basic Local Alignment tool (BLAST) (1) in National Center for Biotechnology Information website (NCBI, https://www.ncbi.nlm.nih.gov/).

A final multiple alignment performed by Jotun Hein algoritm (1990) in MegAlign program, it compared 28S rDNA sequences for 12 *Puccinia* species; this analysis considered segments of 510-nucleotides accurately chosen for matched. Likewise, based in the alignment precedent were established the genetic relationships among the diverse taxons building a phylogenetic tree with parameters of pairwise sequence divergence. *Phragmidium violaceum* (JN790619) was added as an outgroup specie.

**Incidence and severity**

The incidence of Calafate rust was evaluated in three plants quantifying 100 leaves and 100 fruits for each plant. The frequency of uredosoric pustules were expressed as a percentage using the following formula: Incidence (%) = affected organs/total organs (healthy + diseased) x 100**.**

To determine the magnitude or severity, the area affected in the same leaf and fruit was estimated by a scoring system to indicate the degree of infection, where: 1 (apparently healthy), 2 (1-25%), 3 (26-50%), 4 (51-75%) and 5 (76-100%). Incidence and severity were expressed as monthly average (figure 1, page 335).

## Results

**Morphometric and molecular features**

The teliospores were bicellular, cylindraceous or ellipsoidal, ochre colour, pointed at the distal end, semi-contracted in the area of the division, with slightly pigmented hyaline walls and germination pores in the apical and basal cells semi-contracted in the area of the division from the basal or septal cell, broadening in the centre (figure 2, page 335). The characteristics recorded for the teliospores, with a length greater than 80 µm, thickened wall in the apex, and pedicel over 100 µm (table 1, page 336).

Occasionally, were observed basidia (6 - 10 µm diameter; average length 8.12 µm (n = 20)) with spherical-ovoid and hyaline basidiospores (figure 2, page 335).

The morphometric analysis of the uredospores is included in table 2 (page 336) and figure 3 (page 335).

The uredosoric phase in Calafate leaves presented exponential growth, declining at the end of summer when they turned brown and hard. Uredospores presented different degrees of dehydration with thick and uneven walls. These morphometric characteristics corresponded to that mentioned by Hennen *et al.* (2005) and Lindquist (1978) for *Puccinia meyeri-albertii* P. Magn.

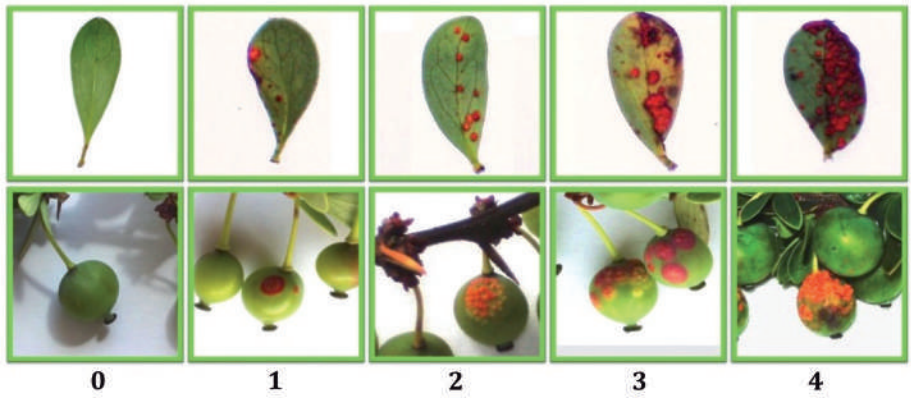


**Figure 1.** Average severity of *P. meyeri-albertii* in leaf and fruits of Calafate based on the note scale from 0 to 4.

**Figura 1.** Escala de notas (0 a 4) para estimar severidad de *P. meyeri-albertii* en hoja y fruto de Calafate.

**Figure 2.** Teliospores of *P. meyeri-albertii*: Mature and germinated teliospores, basidia and basidiospores in germinated teliospores. 100x. (yellow bar = 10µm).

**Figura 2.** Teliosporas de *P. meyeri-albertii*: teliosporas maduras germinadas, basidios y basidiospora en teliosporas germinadas. 100x. (Barra amarilla = 10µm).

**Figure 3.** Uredospores of *P. meyeri-albertii* (yellow bar = 10 µm). 100x.

**Figura 3.** Uredosporas de *P. meyeri-albertii* (Barra amarilla = 10 µm). 100x.

**Table 1.** Measurement (µm) of length, width, apex, pedicel and full length of teliospores of *P. meyeri-albertii*.

**Tabla 1.** Medidas (µm) de largo, ancho, ápice, pedicelo y largo total de teliosporas de *P. meyeri-albertii*.

| | Teliospores (µm) | | | | |
|---|---|---|---|---|---|
| | **Length** | **Width** | **Apex** | **Pedicel** | **Full length** |
| Minimum | 58.0 | 10.0 | 4.0 | 70.0 | 134.0 |
| Maximum | 90.0 | 16.0 | 14.0 | 180.0 | 260.0 |
| Average | 74.2 | 13.6 | 8.9 | 127.4 | 201.6 |
| SD* | 7.64 | 1.8 | 2.6 | 26.9 | 28.2 |

* Standard deviation; n = 30.

* Desviación estándar; n = 30.

**Table 2.** Measures (µm) uredospores of *P. meyeri-albertii* P. Magn.

**Tabla 2.** Medidas (µm) de uredosporas de *P. meyeri-albertii* P. Magn.

| | Uredospores (µm) | |
|---|---|---|
| Parameter | Diameter | wall |
| Minimum | 20.0 | 2.0 |
| Maximum | 32.0 | 4.0 |
| Average | 25.9 | 2.5 |
| S.D* | 3.44 | 0.9 |

* Standard deviation; n = 30

* Desviación estándar; n = 30.

The preliminary identification corroborated by International Centre for Agricultural Bioscience International (CABI-UK) was registered under the number IMI-500169.

The sequence amplified of the 28S rDNA of 655 bp (accession number KY555071) -where, no sequences available in NCBI database- it had an identity of 97% with *Puccinia andropogonis* (GU057993.1), *P. recondita* (KX036373.1), *P. striiformis* (DQ417407.1), *P. triticina* (DQ664194.1), *P. windsoriae* (GU057995.1) and *P. graminicola* (KX190872.1). The phylogenetic tree (figure 4, page 337) analysis permitted to establish 13 lineages showing a genetic relationship closest among the *Puccinia* species; at the same time, it was evidenced that *P. meyeri-albertii* had higher divergences (nucleotide substitutions) respect to the others rusts.

The percentage identity of *P. meyeri-albertii* with the others *Puccinia* spp. compared in this study, ranged from 96.9% to 98.6%, wherever the score divergences 1.4 to 3.2 (figure 5, page 338).

**Incidence and severity in leaves and fruits**

The mean annual incidence in leaves was 25.43 %, with minimum 4.72% and maximum 36.90 % recorded in September and December respectively.

The incidence was higher in younger than mature leaves. At the end of summer, the uredosori in senescent leaves ceased production of uredospores and entered a stage of latency in the form of sub-epidermal mycelia characterized by brown patches (table 3, page 339).

At the end of winter, signs of teleutosore were growing on the abaxial side of senescent Calafate leaves. They were round to irregular in shape, scattered or confluent, exposed, compact, and brown in colour turning greyish with teliospore germination.

The symptoms were yellowness and necrotic patches on the leaf. In leaves, the average notes of severity were between 2.15 and 3.30 (25%-50% leaf area affected); the lowest score recorded in September and the highest in November and December.

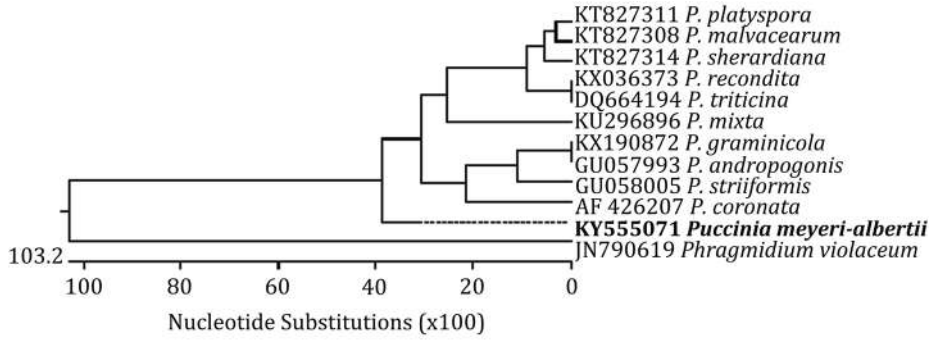


**Figure 4.** Phylogenetic tree of homologous species to *P. meyeri-albertii* based in the partial sequences of the 28S rDNA (fragment of 510 nucleotides). The relative length of the branches denotes the distance between pair sequences. The units at the bottom of the tree indicate the number of substitution events.

**Figura 4.** Árbol filogenético de especies homólogas a *P. meyeri-albertii* basado en secuencia parcial 28S del ADN ribosomal (510 nucleótidos). La longitud de los brazos denota la distancia entre pares de secuencias. Las unidades bajo el árbol indican el número de eventos de substituciones.

Calafate rust infection predominate from September to the beginning of April, being most susceptible in October and November when the pathogen can infect active growing plant tissues (table 3, page 339).

The mean incidence of rust on fruit was 12.43%, from 1.13% recorded in January associated with fruit formation, whereas that highest incidence (23.84%) occurred in mid-spring (November) when the fruit is in the process of ripening (table 3, page 339).

In immature fruits infected was associated yellow-ochre discolouration and concentric ring surrounded for a reddish halo with radial growth.

In the other hand, in advanced stages of infection the symptoms corresponded to fruit deformation, mummification and abscission (berry and peduncle) (figure 6, page 339).

Pustules observed in the fruit from October, increased their number and size as resulted of the reinfection by uredospores.

Nevertheless, the severity did not influence the highest degree on the scale (score 4 = 76 - 100 %, affected area) due to the brief period of fruit formation (October - December). The severity average was the fruit 2.01 in January and 2.65 in December for the same year (table 3, page 339).

## Discussion

Species of genus *Puccinia* affecting a broad host range of the genus *Berberis* (2, 21, 24).

On these fungi, the life cycle corresponds to a hemiform rust evidencing only teliospores and uredospores stages (2, 17).

The findings of this study suggest that the uredosoric phase appears from October in younger leaves and immature fruits, after declining towards February giving way to the telial and basidial phases in mature leaves.

The symptoms on Calafate leaves were yellowness, reddish adaxial patches, orange abaxial pustules and defoliation. Whereas in fruits were observed orange pustules with different size, dehydration and abscission of fruits (figure 6, page 339). *P. meyeri-albertii* in Calafate had an average incidence of 24.7 % and 13.8 % on fruits in leaves, respectively. Meanwhile average severity scored of 2.9 and 2.4 on leaves and fruits. Spatial analysis studies are fundamental in order to plan strategies to manage crop health and improve sampling methods (15).

Considering the precedent results with implication of local weather conditions, the Calafate plants were most susceptible to *P. meyeri-albertii* infection from October until December, situation attributed to adaptation and colonization of the rust on younger tissues, situation eventually related with the thickening of the cuticle in mature tissues. We consider appropriated mentioning that *P. meyeri-albertii* was also identified on fruits of Michay plants (*Berberis darwinii* Hook) nearly localized to the study area (data not shown).

The morphometric identification of *Puccinia meyeri-albertii* P. Magn. affecting leaf and fruits of Calafate, it was registered by CABI under number IMI-500169 (2014), determination similar that those previously realized during the year 2010 (IMI-398821), updating the information referenced in Chile from 1945.

| | | Percent Identity | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| Divergence | **1** | | 96.9 | 98.0 | 98.6 | 98.0 | 98.0 | 98.2 | 98.0 | 97.6 | 98.4 | 98.6 | 90.1 |
| | **2** | 3.2 | | 98.2 | 98.0 | 98.2 | 98.0 | 97.2 | 97.4 | 96.9 | 98.2 | 98.0 | 89.7 |
| | **3** | 2.0 | 1.8 | | 98.2 | 100.0 | 98.6 | 98.2 | 98.4 | 97.6 | 98.4 | 98.2 | 89.7 |
| | **4** | 1.4 | 2.0 | 1.8 | | 98.2 | 98.8 | 98.8 | 98.6 | 98.6 | 99.0 | 100.0 | 90.7 |
| | **5** | 2.0 | 1.8 | 0.0 | 1.8 | | 98.6 | 98.2 | 98.4 | 97.6 | 98.4 | 98.2 | 89.7 |
| | **6** | 2.0 | 2.0 | 1.4 | 1.2 | 1.4 | | 98.4 | 98.2 | 97.8 | 98.8 | 98.8 | 90.3 |
| | **7** | 1.8 | 2.8 | 1.8 | 1.2 | 1.8 | 1.6 | | 99.4 | 99.0 | 98.2 | 98.8 | 90.5 |
| | **8** | 2.0 | 2.6 | 1.6 | 1.4 | 1.6 | 1.8 | 0.6 | | 98.8 | 98.4 | 98.6 | 90.3 |
| | **9** | 2.4 | 3.2 | 2.4 | 1.4 | 2.4 | 2.2 | 1.0 | 1.2 | | 97.6 | 98.6 | 90.9 |
| | **10** | 1.6 | 1.8 | 1.6 | 1.0 | 1.6 | 1.2 | 1.8 | 1.6 | 2.4 | | 99.0 | 90.9 |
| | **11** | 1.4 | 2.0 | 1.8 | 0.0 | 1.8 | 1.2 | 1.2 | 1.4 | 1.4 | 1.0 | | 90.7 |
| | **12** | 10.7 | 11.1 | 11.1 | 10.0 | 11.1 | 10.4 | 10.2 | 10.4 | 9.8 | 9.8 | 10.0 | |
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |

**1: KY555071 *Puccinia meyeri-albertii*** / **2:** AF426207 *P. coronata* / **3:** KX190872 *P. graminicola*
**4:** DQ664194 *P. triticina* / **5:** GU057993 *P. andropogonis* / **6:** GU058005 *P. striiformis*
**7:** KT827308 *P. malvacearum* / **8:** KT827311 *P. platyspora* / **9:** KT827314 *P. sherardiana*
**10:** KU296896 *P. mixta* / **11:** KX036373 *P. recondita* / **12:** JN790619 *Phragmidium violaceum*

**Figure 5.** Divergence and identity values of each sequence pair based in the multiple alignments by Jotun Hein method. The divergences have been calculated comparing sequences pairs, wherever the percent identity relating directly the sequences.

**Figura 5.** Valores de divergencia e identidad de cada par de secuencias basado en alineamientos múltiples empleando el método de Jotun Hein. Las divergencias fueron calculadas comparando pares de secuencias, mientras que el porcentaje de identidad, relacionando directamente las secuencias.

**Table 3.** Month mean incidence (%) and severity (score) of rust *P. meyeri-albertii* in leaves and fruits of evaluated Calafate.

**Tabla 3.** Incidencia (%) y severidad (escala de notas) promedio mensual de *P. meyeri-albertii* en hojas y frutos de Calafate.

| | Incidence (%) | | Severity (score) | |
|---|---|---|---|---|
| **Month** | **Leaf** | **Fruit** | **Leaf** | **Fruit** |
| November | 18.59 | 12.95 | 2.78 | 2.78 |
| December | 28.23 | 6.49 | 3.08 | 2.21 |
| January | 29.52 | 1.13 | 3.06 | 2.01 |
| February | 34.61 | - | 3.09 | - |
| March | 26.34 | - | 3.00 | - |
| April | 19.35 | - | 2.95 | - |
| May | - | - | - | - |
| June | - | - | - | - |
| July | - | - | - | - |
| August | - | - | - | - |
| September | 4.72 | - | 2.15 | - |
| October | 24.25 | 7.76 | 2.94 | 2.40 |
| November | 31.82 | 23.84 | 3.30 | 2.40 |
| December | 36.90 | 22.40 | 3.30 | 2.65 |
| Average | 24.68 | 13.78 | 2.97 | 2.40 |

*(-) Asymptomatic tissue or absence of uredosore. n = 32.

*(-) Tejido asintomático o ausencia de uredosoros. n = 32.

**Figure 6.** Symptoms and signs of *P. meyeri-albertii* on Calafate fruits.

**Figura 6.** Síntomas y signos de *P. meyeri-albertii* en fruto de Calafate.

The nucleotide sequence determined in the 28S RNA gene showed a plausible preliminary identification complementing the morphometric characteristics. For all these, it is necessary to continue researching about the genetic features due to that there is yet scarce studies about *P. meyeri-albertii*.

The disease should imply a risk factor due to its high prevalence, causing significant economic losses for Calafate commercial production system.

In addition, it would be convenient to develop epidemiologically and control research, in order to determine the infection level of this pathogen in other species and ecotypes *Berberis* in different edaphoclimatic conditions.

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