

New record of rust disease caused by *Puccinia oxalidis* on *Oxalis latifolia* from India

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Abstract

A severe rust infection was observed on the plantations of *Oxalis corniculata* (*Oxalidaceae*), commonly known as procumbent yellow sorrel, in Himachal Pradesh and Chandigarh, and on *O. latifolia*, known as garden pink-sorrel, in Uttarakhand in northern India. Detailed morphological examination of the diseased leaf samples was conducted, which confirmed the identity of the pathogen as *Puccinia oxalidis*. Rust symptoms on the host plants, along with taxonomic account of the phytopathogen are detailed in this paper. A taxonomic key of *Puccinia* species reported from *Oxalis* species is provided to facilitate its identity. In addition to understand its global host range, a worldwide host distribution of *P. oxalidis* is provided. The present study is the first detailed taxonomic account of *P. oxalidis* on *Oxalis corniculata* from Himachal Pradesh and Chandigarh in northern India. To the best of our knowledge, this is a new record of *P. oxalidis* from *O. latifolia* from India.

Keywords: host range, morphology, rust fungi, systemic account, taxonomy

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Introduction

Rust fungi are one of the largest groups of plant pathogenic fungi. All the members of this group are highly specialized obligate biotrophic parasites, reflecting distinct systematic characteristics on infected hosts (Duplessis et al. 2011). They generally appear as orange, red, brown or black powdery pustules, known as rust; on leaves (both adaxial and abaxial surfaces), young shoots and fruits of variety of host plants. These pathogenic fungi affect the growth and productivity of many economically important plants. Because of the worldwide distribution of these fungi, they constitute an element of global biodiversity and firmly influence vegetation composition and plant community structure when they occur in epidemics (Dobson and Crawley 1994; Helfer 2014). The complicated life cycles with unique strategies of infection allow the rust fungi to adapt well as biotrophic pathogens. They are perhaps one of the most speciose and the most multifaceted groups of plant pathogens. Besides having unique and specific systematic characteristics, rust fungi are adapted to specific climatic conditions. The average temperature of up to 35 °C along with 50–60

% relative humidity is suitable for their growth and development. Under favorable conditions, these fungi produce pale chlorotic leaf spots, which eventually develop into spore-producing structures called pustules or sorus of orange, yellow, brown, black or white color on mainly leaves, but also on leaf stalks (petioles), stems and rarely on flowers and fruits. The rust infection often reduces the vigor of the plants and in extreme cases can be fatal. Currently, around 120 genera and 6,000 species of rust fungi are reported (Peterson 1974, Cummins and Hiratsuka 2003, Mohanan 2010, Duplessis et al. 2011, Helfer 2014).

In northern India, Himachal Pradesh (H.P.), Union Territory of Chandigarh (U.T.), Uttarakhand (U.K.), sub-mountainous parts of Punjab and Haryana states comprise a floristic region. Geographically, this region lies between 29–35 °N latitude and 74–86 °E longitude and is about 800 km long and about 200–400 km wide. The type of vegetation and variable climatic conditions play a considerable role in the diversity and distribution of rust fungi (Helfer 2014) in this region. Previously, a checklist of rust fungi in the genus *Puccinia* was published by Gautam and Avasthi (2016) from Himachal Pradesh, which included a total of 80 species of *Puccinia* on 91 host plant species spreading over 33 families of monocots and dicots. This study aimed to revisit the taxonomy of phytopathogens causing heavy rust infection on plantations of *Oxalis corniculata* in Himachal Pradesh and Chandigarh, and on *O. latifolia* in Uttarakhand, India.

Material and methods

Sample collection

Plant samples with distinct disease symptoms were collected in well-labelled paper bags during field excursions, taken to the laboratory and processed by following standard procedures (Hawksworth 1974, Savile 1962). The field diary was maintained in order to record the information on infection in natural conditions and their relation with climatic conditions. A map of study area was made by using the geographical co-ordinates documented at the site from where the samples were collected to depict the distribution of species with DIVA-GIS v.7.5.0 software (Hijmans et al. 2011), as shown in Figure 1.

Morphological characterization and microscopy

The rust sori were photographed using a trinocular stereomicroscope (VL-Z60). For microscopic observations, specimens were prepared by hand-sections along with rust symptoms and scrapings. Microscopic observations based on freehand sections from the infected area were made in clear glycerin, while microscopic slides were prepared from scrapings of air-dried specimens in hydrous lactophenol cotton blue stain. All microscopic observations were made using a light microscope at 40× and 100×. A research transmission microscope Matrix (VRS-2f) connected with camera was used for photography. All measurements were taken with the help of ProMED software.

Specimens were also examined by scanning electron microscopy (SEM) at the Department of USIC, HNB Garhwal University, Uttarakhand, India. For SEM, samples obtained from pre-collected specimens were dried in hot air oven under 50 °C for 2 days. Dried samples were then attached to specimen holders by double-sided adhesive carbon tape, coated with Gold-palladium using a Quorum SC7620 Ion Sputter Coater and examined with a MA15/EVO 18 SEM (Carl Zeiss) operated at 20 kV. About 25 measurements were made for each characteristic, with the extremes given in parentheses.

Taxonomy

The systematics of the taxon is provided here by following Cannon and Kirk (2007) and Kirk et al. (2008). While describing the fungus, key distinguishing characteristics such as: 0, pycnia (pycniospores); I, aecia (aeciospores); II, uredinia (urediniospores); III, telia (teliospores); IV basidiospores were recorded. The specimens were deposited in the herbarium of the Department of Botany, Panjab University, Chandigarh (PAN), India.

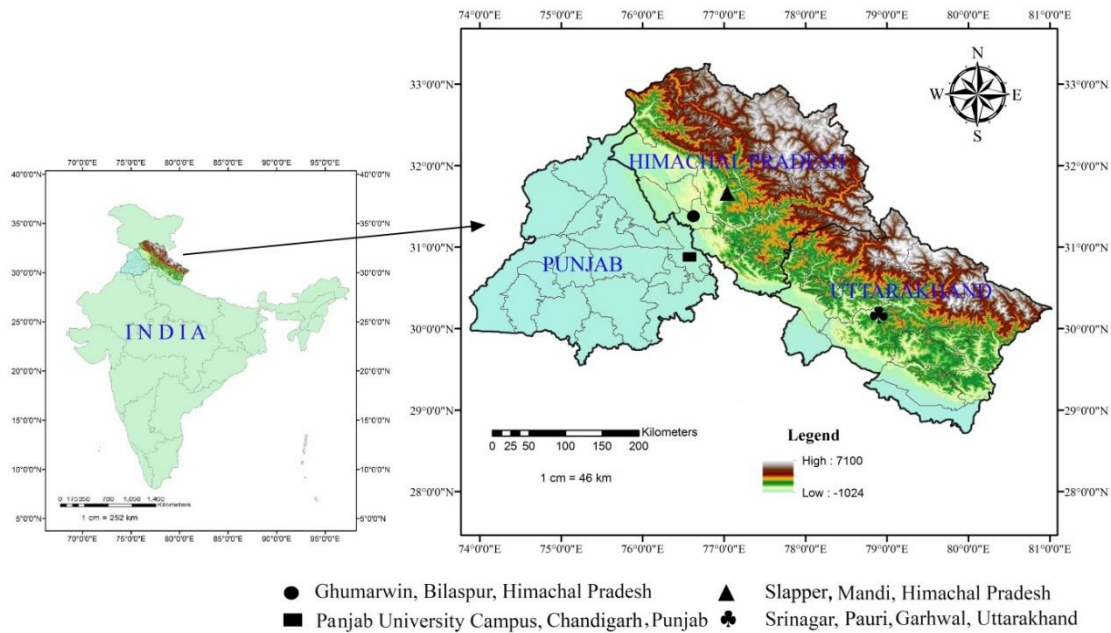


Figure 1. Map showing collection sites of *Oxalis corniculata* and *O. latifolia* infected with *Puccinia oxalidis* from Chandigarh, Himachal Pradesh, and Uttarakhand in northern India.

Results

Initial symptoms of rust disease were observed during March–May 2019, as light-green spots in the centre of the upper surface (adaxial) of leaves which later spread as sporadic pustules. Golden-yellow to orange-yellow powdery pustules were noticed on corresponding lower (abaxial) surface of leaves. The powdery pustules of rust fungi often arranged in circles and were found to be suppressing the growth of plants. The average size of the uredosori ranged between 0.2 to 0.5 mm in diameter. Severely infected leaves soon withered and died (Figures 2–4). The description and illustration of the fungus, along with a discussion on its taxonomy and distribution are detailed below.



Figure 2. Rust infections on *Oxalis latifolia*. **A.** Plant without infection. **B & C.** Plants with heavy infection from Srinagar, Pauri Garhwal, Uttarakhand.

Puccinia oxalidis Dietel & Ellis, Hedwigia 34: 291 (1895)

Figures 2–7

= *Dicaeoma oxalidis* (Dietel & Ellis) Kuntze, Revis. gen. pl. (Leipzig) 3(3): 469 (1898)

= *Trichobasis oxalidis* (Lév.) Lév., in Orbigny, Dict. Univ. Hist. Nat. 12: 785 (1848)

= *Uredo oxalidis* Lév., Anns Sci. Nat., Bot., sér. 2 16: 240 (1841)

= *Uromyces oxalidis* (Lév.) Lév., Anns Sci. Nat., Bot., sér. 3 8: 371 (1847)

0, I: Not seen. (For description see Long and Harsch 1918)

II Uredinia abaxial, erumpent, subepidermal, round, up to 500 μm in diam., scattered or confluent and often covering the entire leaf surface, yellow to yellowish brown; paraphyses intrasoral, abundant, hyaline, cylindrical with truncate apex, up to 40 μm long and up to 5 μm wide at apex. (Figures 2, 3, 4 and 5A)

Urediniospores globose, subglobose 17–24.5 μm in diameter or broadly ellipsoidal 16.5–20 \times 10.5–16 μm , yellow; wall 0.5–1.5 μm thick, minutely echinulate. (Figures 5, 6B and 7)

III Telia on leaves, subepidermal, abaxial, erumpent, yellow to yellowish brown, up to 0.5 mm diam., aggregating into larger sori, paraphyses same as that of uredinial paraphyses.

Teliospores ellipsoidal or occasionally diorchidioid, with rounded apex, yellow, 16–22 \times 10–12 μm ; wall 0.5 μm thick, smooth, 2-celled, slightly constricted at septum, pedicel persistent 8–30 μm . (Figure 6A)

Known distribution: This rust has been reported from many countries/ continents, including America, Africa, New Zealand, Nepal Argentina, Brazil, Colombia, Hawaii, Uruguay, etc. It is a heteroecious rust (requiring two different host plants to complete life cycle) with uredinia and telia

on different species of *Oxalis*, and its pycnia and aecia are produced on an alternate host viz. species of *Berberis* (*B. repens*, *B. aquifolium* and *B. trifoliata*). This rust was introduced to Japan from North America occurring on *Oxybaphus corymbosus* DC., a plant introduced from North America and also to Nepal (Ono et al. 1988). Probably, this rust might have been introduced in India from North America via Japan & Nepal (Patil et al. 2004). This species has already been reported from various states of India viz. Arunachal Pradesh, Assam, Rajasthan, Karnataka, Tamil Nadu, Maharashtra, Kerala, Uttar Pradesh, Andhra Pradesh (Bilgrami et al. 1991; Ahmed 1986, 1990; Saikia and Ashok 1994, De 1997).

Materials examined – India, Chandigarh, Panjab University campus, RK Verma and IB Prasher, 321 m, 11 March 2019 (PAN 32815); Himachal Pradesh, Bilaspur, RK Verma and Harpreet Singh, 673 m, 5 April 2019 (PAN 32816); Mandi, AK Gautam and S Avasthi, 760 m, 7 May 2019 (PAN 32817); Pauri Garhwal, Uttarakhand, A Singh, 1814 m, 15 March 2019 (PAN 32818). The materials are available for further research, including molecular identification of the fungus.

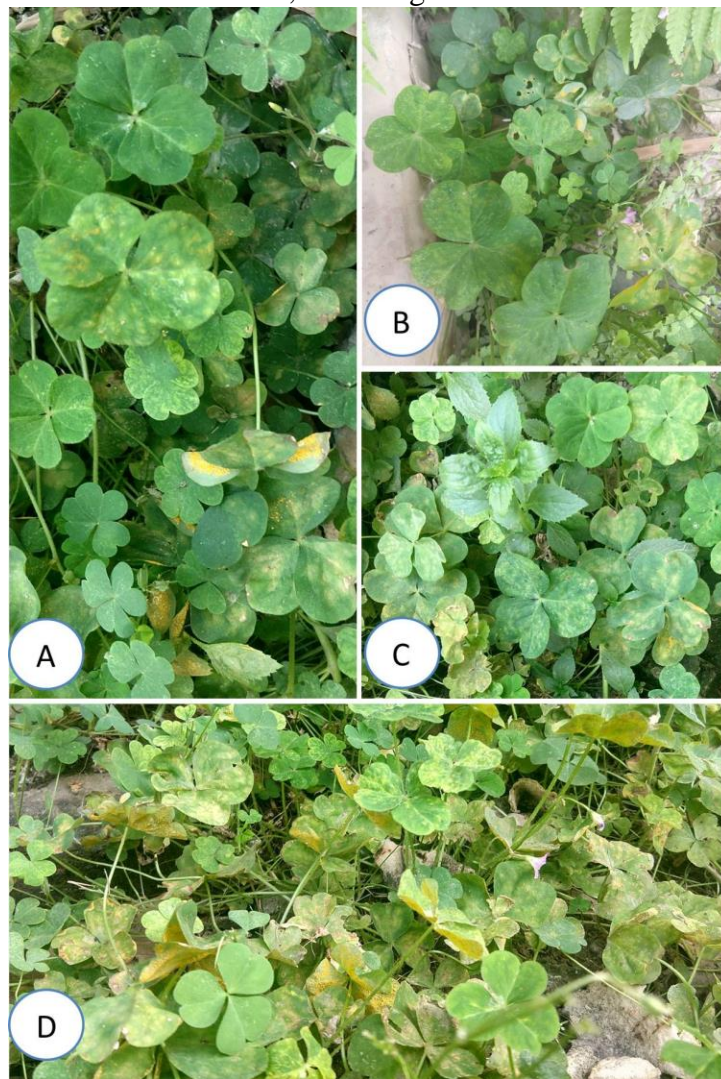


Figure 3. Rust infection on *Oxalis corniculata* from different localities. **A & B.** Bilaspur (Himachal Pradesh) **C.** Mandi (Himachal Pradesh) **D.** Panjab University, Chandigarh (U.T.).

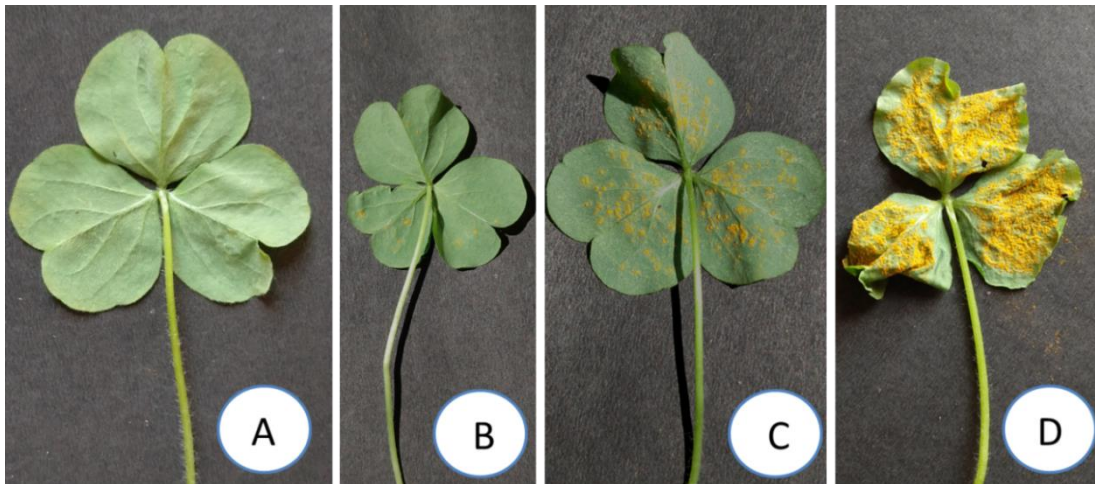


Figure 4. Different stages of rust infection on *Oxalis corniculata*. **A.** Healthy leaf with no rust infection, **B.** Initiation of rust infection, **C.** Rust infection on whole abaxial leaf surface, **D.** Severely infected leaf with drying symptoms.

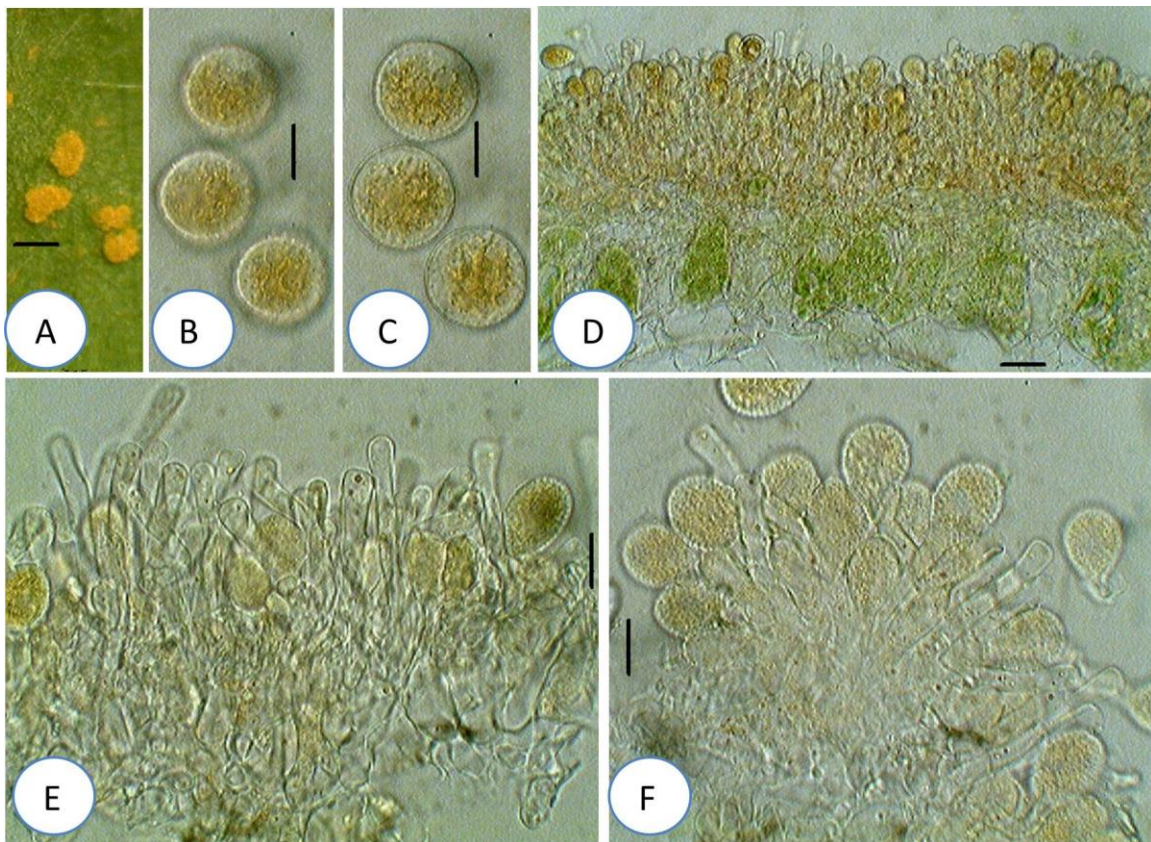


Figure 5. **A.** Uredinia on lower surface of *Oxalis* leaf, **B & C.** Uredinospores (globose), **D.** Transverse section of rust infected leaf showing paraphysis intermingled with uredinospores, **E & F.** Paraphysis and Uredinospores (elliptical). Scale bars: A= 500 mm B, C, E and F = 10 μ m, D = 20 μ m.

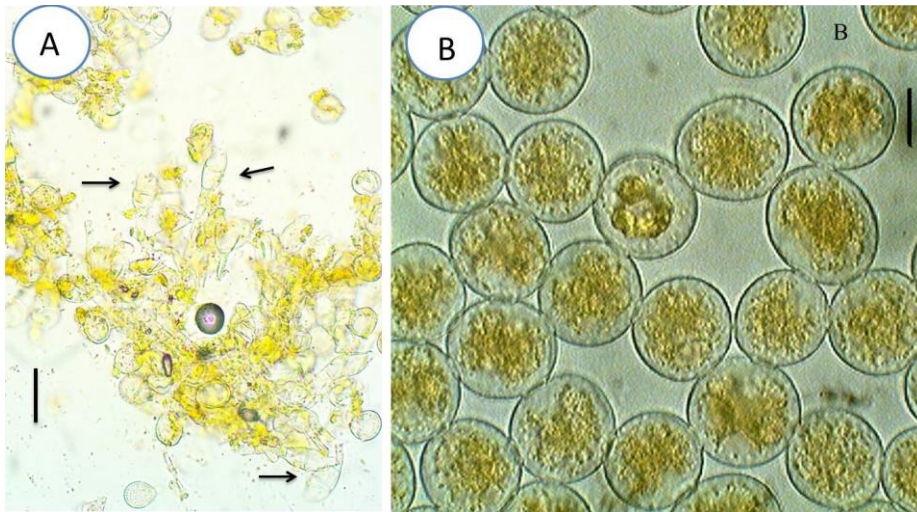


Figure 6. A. Bicelled teleutospore (marked with an arrow head) with Urediospores B. Urediospores. Scale bars: A= 20 μm, B=10 μm.

Key to species of *Puccinia* on *Oxalis* spp.

Pycnia & aecia on *Berberis* spp. and uredinia and telia on *Oxalis* spp., urediniospores 12–15 μm in diam. (globose) verrucose, 15–20 × 11–14 μm (elliptical) and teleutospores 2–celled, hyaline to faint yellow and 16–22 × 9–12 μm..... *P. oxalidis*
 Pycnia & aecia on *Oxalis* spp. and uredinia and telia on *Zea mays*, urediniospores verrucose, 23–29 × 26–32 μm and teleutospores 2–celled, hyaline to light yellow and 16–23 × 29–55 μm..... *P. sorghi*

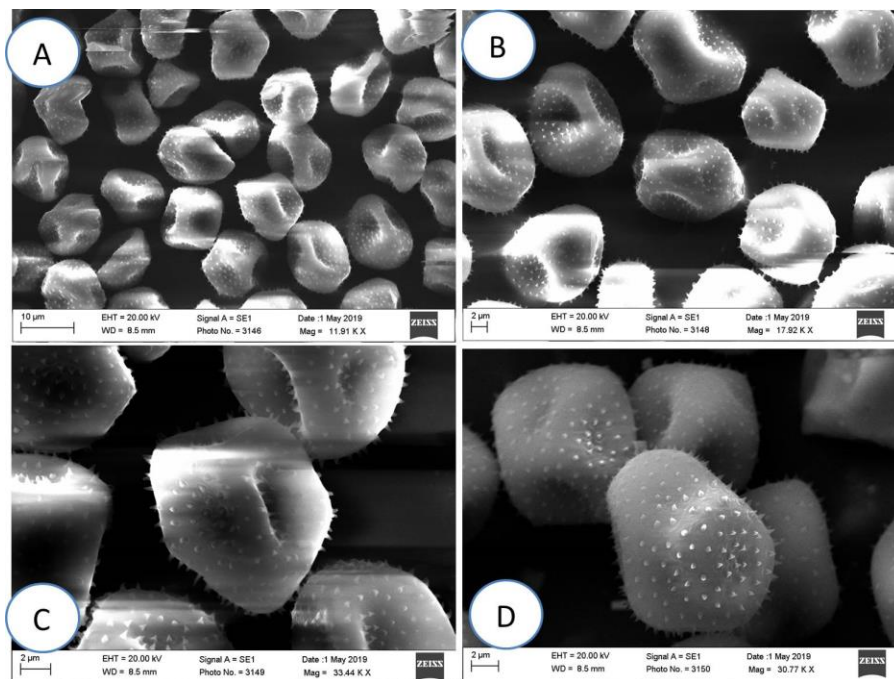


Figure 7. Scanning Electron Microphotographs (SEM). A-D. Urediniospores showing echinulation.

Table 1. Updated host distribution of *Puccinia oxalidis* worldwide (Farr & Rossman 2019).

S. no.	Host	Distribution	Reference
1.	<i>Oxalis adenophylla</i>	United Kingdom	Henderson (2000)
2.	<i>O. amplifolia</i>	Mexico	Gallegos and Cummins (1981)
3.	<i>O. articulate</i>	United Kingdom, Japan, New Zealand	Hiratsuka et al. (1992); McKenzie (1991); Henderson (2000)
4.	<i>O. bowiei</i>	New Zealand	McKenzie (1998)
5.	<i>O. brasiliensis</i>	New Zealand	McKenzie (1990); McKenzie (1998)
6.	<i>O. corniculata</i>	New Zealand, Puerto Rico, Virgin Islands, Chandigarh (Punjab), Bilaspur and Mandi (Himachal Pradesh) India	Patil et al. (2004); McKenzie (1992); Stevenson (1975); present study
7.	<i>O. corymbosa</i>	Australia, Azores, Brazil, Canada, China, England, India, Virgin Islands, Puerto Rico, Jamaica, United Kingdom, Japan, Madeira Island, Taiwan	Dale (1955); Stevenson (1975); Gjaerum and Dennis (1976); Gjaerum (1982); Zhuang (1983); Ginns (1986); Ahmed (1990); Hiratsuka and Chen (1991); Hiratsuka et al. (1992); Hennen et al. (2005); Langrell et al. (2008)
8.	<i>O. debilis</i>	New Zealand	Pennycook (1989)
9.	<i>O. debilis</i> var. <i>corymbosa</i>	New Zealand, Korea	McKenzie (1998); Lee et al. (2018)
10.	<i>O. dehradunensis</i>	India	Singh and Palni (2011); Palni and Pangtey (2002)
11.	<i>O. deppei</i>	New Zealand	McKenzie (1998)
12.	<i>O. divaricata</i>	Mexico	Gallegos and Cummins (1981)
13.	<i>O. griffithii</i>	China	Zhuang (2003)
14.	<i>O. hirta</i>	New Zealand	McKenzie (1998)
15.	<i>O. incarnata</i>	New Zealand	McKenzie 1998)
16.	<i>O. intermedia</i>	Puerto Rico	Stevenson (1975)
17.	<i>O. latifolia</i>	Azores, Brazil, Colombia, Costa Rica, Mexico, Nepal, New Zealand, Uganda, United Kingdom, Venezuela, Uttarakhand (India)	Chardon and Toro (1934); Stevenson (1975); Gallegos and Cummins (1981); Ono et al. (1988); Pardo-Cardona (1998); McKenzie (1998); Henderson (2000); Gjaerum and Namaganda (2003); Berndt (2004); Langrell et al. (2008); present study
18.	<i>O. lobata</i>	New Zealand	McKenzie (1998)
19.	<i>O. lotoides</i>	Colombia	Pardo-Cardona (1998)
20.	<i>O. martiana</i>	Argentina, Brazil, Colombia, Hawaii, Uruguay	Lindquist (1982); Hennen et al. (2005), Pardo Cardona (1998)
21.	<i>O. papilionacea</i>	Uruguay	Lindquist (1982)
22.	<i>O. pes-caprae</i>	New Zealand	McKenzie (1998)
23.	<i>O. pubescens</i>	Colombia	Kern et al. (1933)
24.	<i>O. purpurea</i>	Canary Islands	Gjaerum and Sunding (1986)
25.	<i>O. regnellii</i>	Hawaii	Gardner and Hodges (1989)
26.	<i>O. rubra</i>	Canada	Ginns (1986)
27.	<i>O. salva</i>	Brazil	Hennen et al. (2005)
28.	<i>O. scandens</i>	Bolivia	Jackson (1931)
29.	<i>O. spiralis</i>	Costa Rica	Berndt (2004)
30.	<i>O. triangularis</i>	Czech Republic	Šafránková (2014)
31.	<i>O. trinervis</i>	Mexico	Gallegos and Cummins (1981)
32.	<i>O. tuberosa</i>	Bolivia	Farr and Stevenson (1963)
33.	<i>O. tubiflora</i>	United Kingdom	Henderson (2000)

34.	<i>O. vallicola</i>	Mexico, New Zealand	Gallegos and Cummins (1981); McKenzie (1998)
35.	<i>O. versicolor</i>	New Zealand	McKenzie (1998)
36.	<i>O. violacea</i>	Georgia, Kansas, Louisiana, Mexico, Mississippi, Texas	Hanlin (1966); Rogerson (1958); Cooke (1980)
37.	<i>Ionoxalis martiana</i>	Brazil, Jamaica	Thurston (1940); Arthur (1916)
38.	<i>Mahonia repens</i>	New Mexico, Texas	Anonymous (1960)
39.	<i>Xanthoxalis</i> sp.	Venezuela	Kern et al. (1934)

Discussion

The genus *Puccinia* was introduced by Persoon (1801) with *P. graminis* Pers. ex. Pers. as the type. It is one of the largest and most commonly occurring genera of rust fungi with 3648 species (Index Fungorum, 2019). The genus is worldwide in distribution on both dicot and monocot plants, with autoecious (complete life cycle on single host) & heteroecious (complete life cycle on two different host plants) life cycle pattern with great variability (Kolmer et al. 2009). The species show a variety of forms of life cycles, from euform (macrocytic) to microform i.e. (microcytic) and show inclination of spore exclusion gradually and become very simple producing only teliospores, e.g. *P. thwaitesii*, *P. xanthii*, etc. There are around 400 species and nine varieties of *Puccinia* known from India (Bilgrami et al. 1991). *Puccinia oxalidis* has been reported on variety of host plants, including 35 different *Oxalis* species from about 29 countries of the world. Besides on *Oxalis* spp., *P. oxalidis* has also been recorded on *Ionoxalis martiana*, *Mahonia repens* and *Xanthoxalis* sp. (Table 1). This is the first detailed taxonomic account of *P. oxalidis* on *Oxalis corniculata* from Himachal Pradesh and Chandigarh U.T in northern India. The literature review suggests that *P. oxalidis* was reported on *Oxalis dehradunensis* from Uttarakhand (Sah et al. 2009; Singh and Palni 2011), and other parts of India viz. on *O. corymbosa* from Arunachal Pradesh, Tamil Nadu and Assam (Ahmed 1986, 1990; Bhowmick 1983; De 1997). Similarly, the infection of *P. oxalidis* on *O. corniculata* was reported previously from Assam and Karnataka (Bhowmick 1983; Patil et al. 2004). Additionally, infection of *Puccinia sorghi* was reported on *O. corniculata* in Himachal Pradesh (Gautam and Avasthi 2016, 2019). Similarly, occurrence of *P. oxalidis* on *O. latifolia* has been reported from various countries (Table 1), but there were no reports from India. However, an infection of *P. sorghi* was reported on *O. latifolia* in Himachal Pradesh (Gautam and Avasthi 2016, 2019). To the best of our knowledge, this is a new host record for *P. oxalidis* from *O. latifolia*, from India.

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Statement on conflict of interest

The authors declare no competing interests.

Author contribution

All the authors contributed equally in sample collection and further processing in the laboratory. The first and corresponding authors were equally involved in scientific writing and finalizing the manuscript.

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