

Research Article

Diversity of Rust Fungi Causing Plant Diseases in Abbottabad, Khyber Pakhtunkhwa (KP), Pakistan

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Abstract

In 2018, a survey of rust fungi and their associated hosts was attempted in the thandiani regions of Pakistan. In this paper, we provided descriptions and illustrations of six rust species viz. *Melampsora euphorbiae*, *Phragmidium barclayi*, *Puccinia ambegua*, *Puccinia calcitraipae*, *Puccinia mentha* and *Puccinia ustalis*. The *Melampsora euphorbiae*, *Puccinia ambegua*, and *Puccinia ustalis* were collected as a new host record from Abbottabad. Most of the rust fungi were collected from herbaceous wild plants. This study suggests further exploration of the area of rust disease in order to assess the diversity of these fungi. This research work is an addition to available data related to the Uredinales of Pakistan having special reference to Abbottabad District, Khyber Pakhtunkhwa, Pakistan.

Introduction

Rust fungi (Pucciniales) constitute one of the largest and most diverse groups of plant pathogens that can affect almost all the plants in the biosphere [1]. *Puccinia* is the largest genus in Pucciniales, comprised of more than 4,000 species that are characterized by developing pedicellate and two-celled teliospores [2]. The genus *Phragmidium* contains over 66 species of which seventeen *Phragmidium* species have been reported from Pakistan [3]. Abbottabad, a district of Khyber Pakhtunkhwa (KP), lies in the north of Pakistan and represents one of the growing regions in the context of rust fungi hitherto, over 66 species have been described in addition to new hosts and new local distribution records Murree, situated in the northwest of Pakistan, a diverse floristic region as well as one of the attractive sites of rust fungi is being explored for investigation of rust fungi, Swat and Murree regions were explored for the observation of rust fungi and as a result we found five rust species on five host plants, among them *Rubus fruticosus* host of *Phragmidium bulbosum* presented here as new host record from Pakistan [4]. The rust fungi belong to the order Uredinales of class Basidiomycete and comprise a large group of obligate biotrophic parasites. They cause rusty

pustular outgrowths on different plant parts especially leaves and stems of a wide range of host plants including ferns, conifers, and wild and cultivated flowering plants [5]. Plants and rust biotrophs are known to have specific relationships. The host-parasite association between plants and rust is an outcome of a long evolutionary process of continuous mutual adaptation [6]. Rust fungi are among the most economically important pathogens of many native and cultivated plants. However, they typically cause only minor damage to a population of wild host plants in an area belonging to their natural range of distribution [7] (Image 1).

Materials and methods

Collections of rust-affected plants were carried out and then infected plants were preserved and submitted to the herbarium of the Department of Botany Hazara University, Mansehra, Pakistan, and then the preserved rust spores were mounted in glycerin jelly and fixed as semi-permanent slides. The spores were measured and photographed under a Leitz HM-LU compound light microscope. At least 30 spores were measured for each spore stage, including the smallest and the largest spores found. Host plants were identified by

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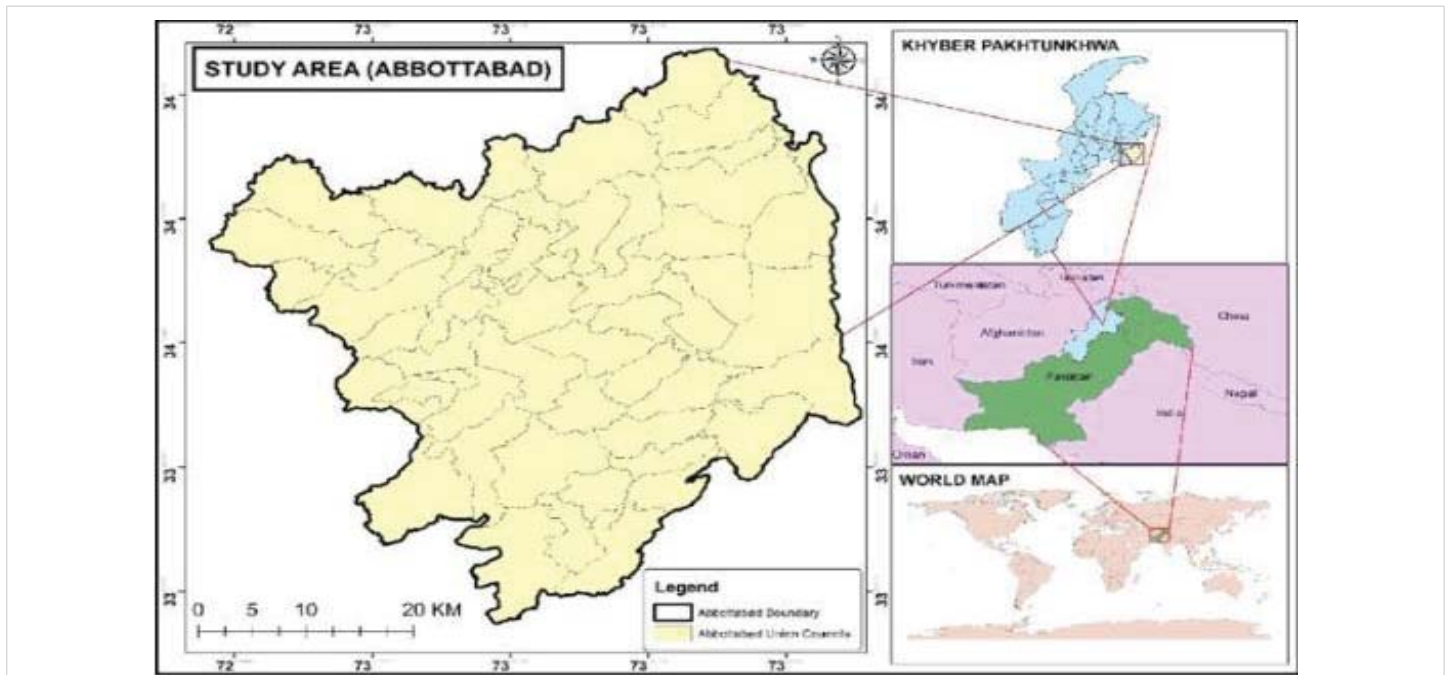


Image 1: Map of study area.

comparing new collections with the flora of Pakistan and botanical specimens held in the herb. An alcoholic solution of Mercuric Chloride ((Hg Cl_2) was used to poison these specimens and it prevented fungal attack and insect damage. With the help of an expert the host of the plants was identified [8].

Survey and collection

The study was conducted on rust fungi in different seasons in 2018. From Thandhiani, District Abbottabad, from selected sites, the plants were collected which were under attack by rust fungi. It was brought under the detailed and closed study according to the instructions of the supervisor. The plants that were healthy and green with flowers or inflorescence and fruits were given special attention during the identification of the exact host. During collection three pictures of every infected plant were taken. Samples were carefully and artistically pressed and then these collected samples were kept among the blotted papers and newspaper. With the passage of time, every day the specimens were exposed to air under strict surveillance, and the newspapers and blotted papers were changed to make it dry. Under the guidance of the supervisor, the above-mentioned process was repeated again and again to make these plants completely dry. For taking clear and high-megapixel pictures, the modern digital camera was used and pictures were taken. An alcoholic solution of Mercuric Chloride ((Hg Cl_2) was used to poison these specimens and it prevented the fungal attack and insect damage [8]. With the help of an expert the host of the plants was identified. According to the flora of Pakistan, botanical names, family names, and authors' name was corrected. To the herbarium of the Botany department, Hazara University, Mansehra, further study was carried out on these infected plants [9].

Lab work

The Sori part or infected section of the plants was brought under a stereo microscope for study which had a magnification power of 25 to 50. We took at least three pictures with the help of a high megapixel digital camera which was attached to the microscope. All the compulsory equipment and apparatuses were ready in the next step: spirit, spirit lamp, slide, nail lacquer, lectophenole, cover slip, syringe, needle, and tissue paper. In the coming step, we were ready and prepared the slide. We used a needle to take spores for preparing the slide (in the rust fungi case) and mounted it in the lacto phenol. For the semi-permanent slides, we prepared cementing coverslips with the help of nail lacquer, and slight heat due to heating the spore was dispersed. In the coming step, the prepared slide was transferred to the biological microscope and we took from it at least 20 pictures with the help of a digital camera which was attached to the microscope (Nikon YS 100). Lucida camera (Ernst Leitz Wetzlar Germany) was taken and used for the exact drawing of various types of spores and Paraphyses. The spore dimensions were taken by an ocular micrometer (Zeiss Eye Piece Screw Micrometer). For the recording dimension of the spore, extra care was given and it was checked at least twenty pictures of the smallest and biggest spore were taken. After this all the significant data was written in copy and the specimen was described one by one theoretically and then its description was contrasted to the accessible literature for the correct recognition of rust fungi. Then all the infected species were taken and the study was deposited in the Herbarium of the Department of Botany, Hazara University Mansehra, Pakistan [10].

Results

Melampsora euphorbiae (Ficinius and C. Schuib.) (Figure 1, A-H)

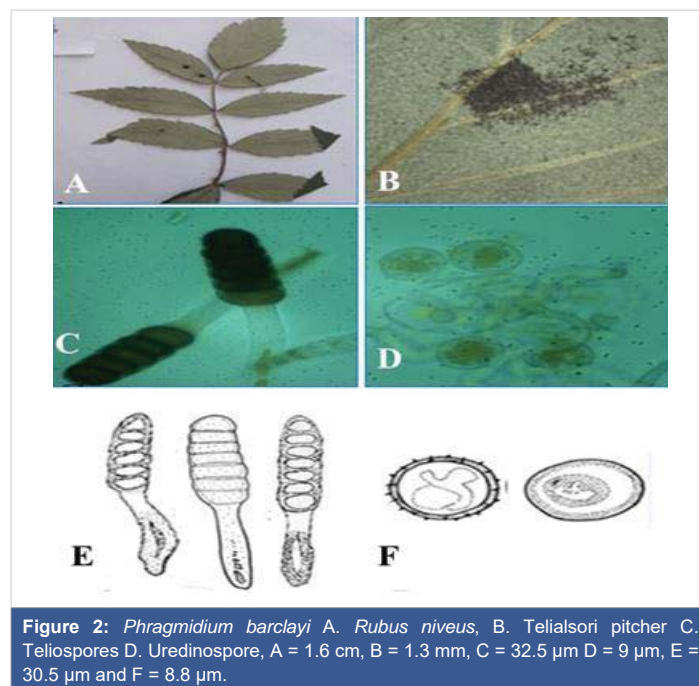
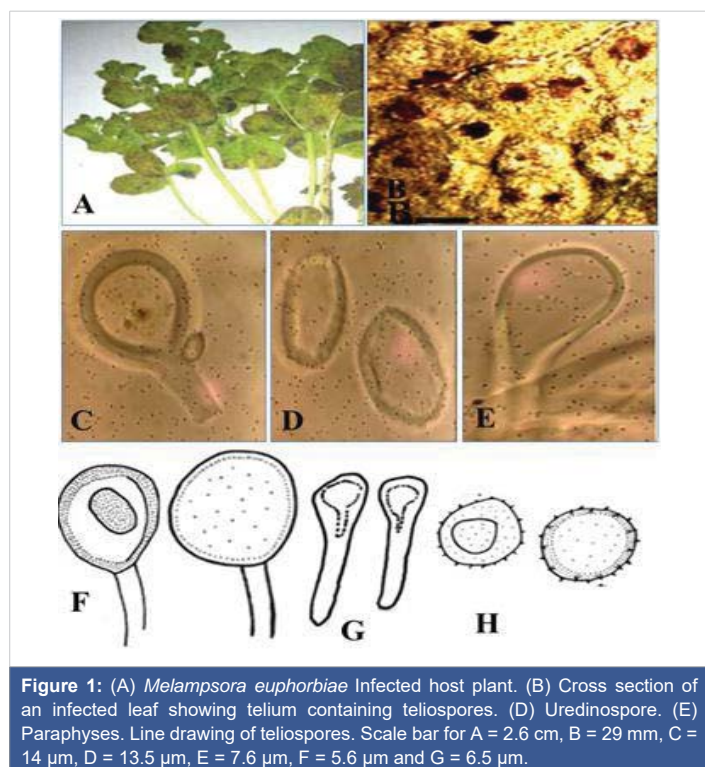
In the aeciospores as well as Spermogonia are totally absent or unknown. Uredia, amphigenous, spread or circinate, yellowish, Uredinospore globosely, sub globosely or ellipsoid, $17.5 - 20.5 \times 13 - 18 \mu\text{m}$. Paraphyses capitate, thick-walled $14.3 - 30 \mu\text{m}$. Telia sub-epidermal, amphigenous, brownish. Teliospores are cylindrical oblong, yellowish brown, $36 - 66 \times 9 - 14 \mu\text{m}$.

Material examined: On *Euphorbia helioscopia*, with stages II and III, Pakistan, Khyber Pakhtunkhwa province, district Abbottabad, Thandiani region, at 2790 m. a. s. l., 15 september, 2018.

Comments: This rust fungus has previously been reported on *Euphorbia hypericifolia* L. and *E. helioscopia* L., from Gilgit, Karachi, and Muzaffarabad [11]. It is a new record for the Thandiani region.

Phragmidium barclayi Dietel. (Figure 2, A-F)

In this case, Barclay shows the uredinal stage as well as Teliospores. But aecia and Spermogonia are absent. Uredinia, light, brown, spread, biaxial, 2 – 3.9 mm. and uredinal spore are mostly ellipsoid and their color is yellow $9.3 - 14.12 \times 13 - 21 \mu\text{m}$. germ pores obscured, wall thickness $1.24 - 1.44 \mu\text{m}$. telia brown dark, hypophyllous, and scattered about 4mm in diameter, and spores are slightly cylindrical at the base and composed of 3 to 7 cells and the apex is $4.4 - 5.7 \mu\text{m}$. In the case of Teliospores about $25 - 34 \times 73 - 117 \mu\text{m}$, and wall



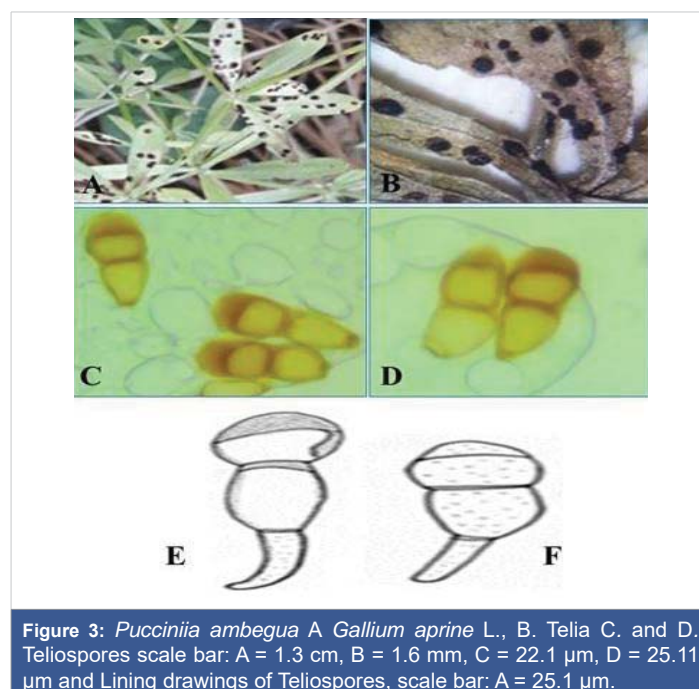
length 2.3 – 3.2 μm . Pedicels are hyaline, $16.6 - 19.3 \mu\text{m}$, broad, up to 137 μm long.

Material examined: Pakistan, Khyber Pakhtunkhwa, Abbottabad District, at 2770 m a.s.l., On *Rubus niveus* II and III stages, 30 September, 2018.

Comment: *Phragmidium barclayi* has been reported on *Rubus lasiocarpus* in KP and ChangaGali by Ahmad [10], *Phragmidium barclayi* on *Rubus spediunculatus* is again described from swat. It is a new record for Abbottabad district.

Puccinia ambegua (Alb.) (Figure 3, A-D)

Spermogonia and aecia, not found. Uredinia hypophyllous,



large may be dense, darkly, brownly 1.3 to 1.5 mm. Telia clavate ellipsoidal, apexil, conical, thickened 1.3 to 1.5 mm, teliospores darkly brawny hyaline to pale yellowish, 38. 24 - 57.77 $\mu\text{m} \times$ 13. 35 - 20.66 μm . Pedicel 2.35 - 6.35 \times 7.88 - 27.97 μm , and hyline.

Material examined: Pakistan, Khyber Pakhtunkhwa, Pakistan, Khyber Pakhtunkhwa, Abbottabad, at 2740 m a.s.l., On *Gallium aprine* L., II and III stages, October 2018.

Comment: In *Gallium aprine* plants first of all only the uredinal stage was discovered but here telio stage was also discovered previously it is reported from Murree Ghoregalii by [12] new records for Abbottabad.

Puccinia calcitraipae DC. (Figure 4, A-F)

In this case, aecia and spermogonia are totally absent. Hypophyllous, Uredinia spread curved, brawny to palely radish. Uredinia - spores globose to ellipsoids. 19 -24 \times 23.22 - 27 μm . Telia hypophyllous infrequently, spotted; into groups of various sizes and dark brown color. Teliospores are generally ellipsoidal or fuse-form 15 - 27 \times 18 - 40 μm . High and inferior end round, rare case at the septum, also present. Yellow to brown, germ pore apically are sub-apical cells. Pedicle hyaline.

Material examined: *Puccinia calcitraipae* on *Cirsium vulgare* with II and III, stages, Pakistan, Khyber Pakhtunkhwa Province, District, Abbottabad, Thandiani region, 2600 m. a. s. l., 10 september, 2018.

Comment: *P. calcitraipae* before report on *Centaurea bruguieriana* (DC.) From Peshawar; on leaves of *Cardus edelbergii* Rech. f., from Batakundi, Shogran (Kaghan valley), and Kalam (Swat), on leaves of *Cirsium arggracanthum* Dc,c.

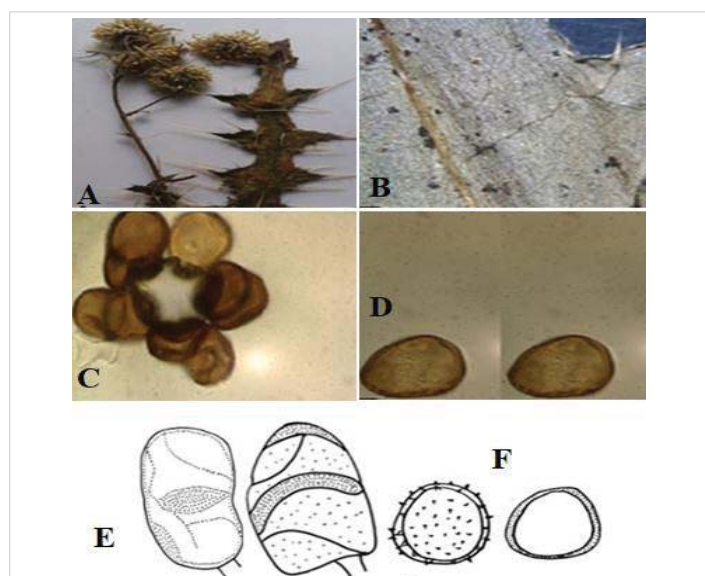


Figure 4: *Cirsium vulgare*. A. Host Plant. B. Infected leaf showing telium. C. Urediniospores D. Telio-spores scale bar A = 3 cm, B = 1 mm, C = 7 μm , D = 4 μm , A - B Line drawing of A Teliospores B. Uredinia spores scale bars A. = 4 μm B = 2 μm .

Walichii Dc and *Cnidus* from Maindam, Kalam, Changla Gali, and Shogran [13,14].

Puccinia mentha Pers. (Figure 5, A-D)

Spermogonia and aecia are unknown. Uredinia brown, abaxial, naked, uneven, and spread, Urediniospores hyaline, sub-globose, 18 - 20 \times 19 - 28 μm ; wall echinulate, 1.4 - 2.3 μm broad, Telia, brownie to black, wall 2- 3 μm thick, verruculose, pedicle little, typically broken.

Material examined: Pakistan, Khyber Pakhtunkhwa, Abbottabad District, Thandiani region, 2730 m a. s. l., On *Mentha longifolia*, with II, III stage, 8 Sep. 2018.

Comments: *P. mentha* has been reported on *M. Sylvester's* L., from Peshawar, Mingora, Kaghan Valley, Chalianwala, Quetta, Poonch, and Muree Hills; on *Origanum vulgare* L. from ChanglaGali and Kaghan Valley; by [15,16]. Recently it has been reported from Battagram (Fahad 2017). It is the first time reported for the study area.

Puccinia ustalis Bark. (Figures 6, A-D)

Spermogonia, aecia unknown. Uredinia hypophyllous, dark brown to blackish spots, scattered or aggregated in large groups, compact. Urediniospores are ovoid, 9.8 - 23 \times 11 - 22 μm , wall thickness 0.6 - 1.7 μm . Telia is usually hypophyllous as dark brown to blackish spots, scattered or aggregated in large groups, compact. Teliospores are mostly two-celled, occasionally 1 or 3 celled teliospores co-exist. ellipsoid, oblong to cylindrical or irregular, conical or rounded above and narrowed below, constricted at the septum, yellowish brown, pale brown basally, 2 celled spores 10-17.2 \times 31.6-62.2 μm , smooth, 1-1.4 μm thick at the side, epically 2-8 μm

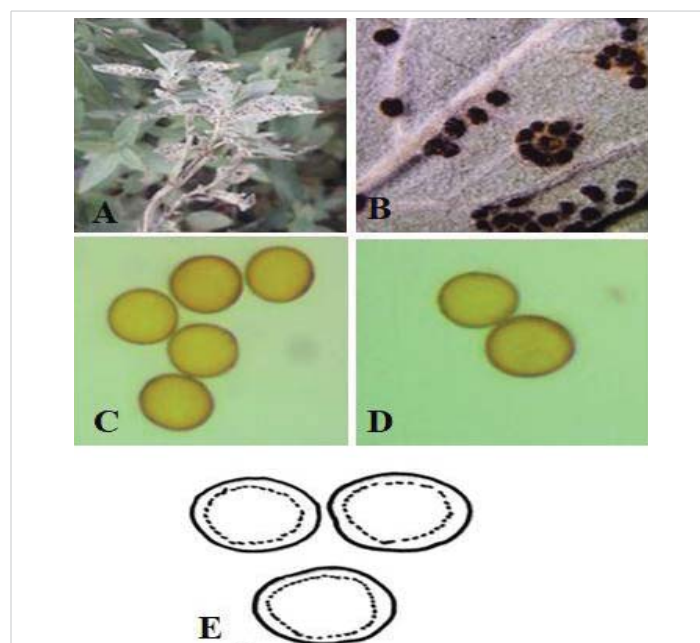
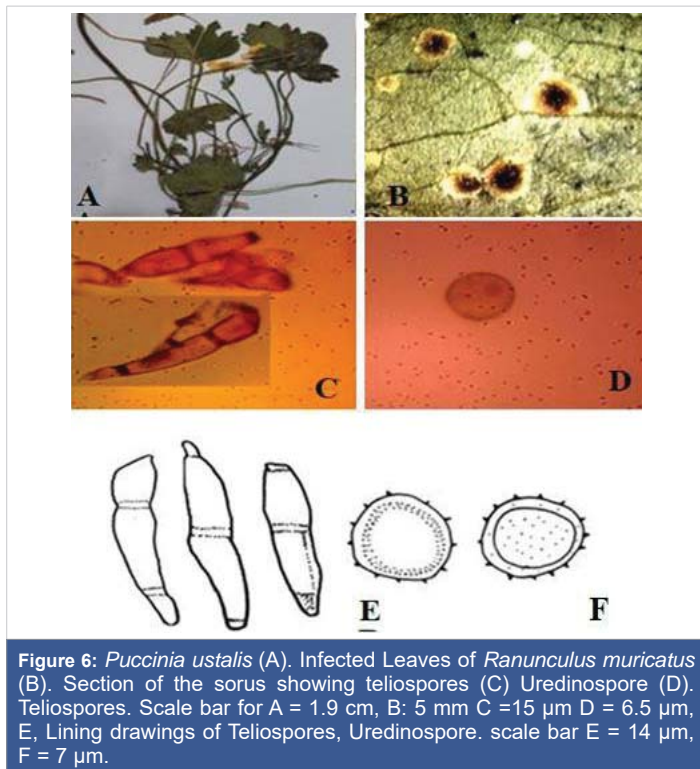


Figure 5: *Puccinia menthae* A. *Mentha longifolia* L., B. Telial. C D. Urediniospores. Scale bar: A = 1 cm B = 1 mm C = 10 μm D = 7 μm and E = Lining drawing of Urediniospores scale bar A = 4.2 μm .



thick. Germ pore one per cell, apical or subapical in the distal cell, in a basal cell near the septum, or obscure. Pedicel pale yellow to hyaline, deciduous, 6–9 μm long, deciduous.

Material examined: *P. ustalis* on *Ranunculus muricatus* with stage II & III, Pakistan, Khyber Pakhtunkhwa Province, District Abbottabad, Thandiani region, at 2600 m.a.s.l., 15 September 2018.

Comments: *P. ustalis* has been reported on *Anemone obtusiloba* D. Don. from Changla Gali and Nathia Gali by Ahmad (1956a, b); on *R. diffuses* DC. from Dunga Gali KP (NWFP) by [12] and from Kaghan Valley by [17]. *Ranunculus muricatus* is a new addition to the rust flora of Pakistan on the base of the uredinal stage and a new host.

Discussion

The goal of the current study project is to learn more about the rust fungi found in the Khyber Pakhtunkhwa Thandhiani region, specifically in District Abbottabad. September and October of 2018 saw the visitation of several carefully chosen locations and the collection of specimens needed for the study. Following a thorough morpho-anatomical search of a number of specimens, six genera—representing six rust species—on six different host vegetation species have been identified. With between 3000 and 4000 species, it is one of the largest groups of fungi. It targets all kinds of vegetation. Gymnosperm, bryophytes, Pteridophytes, and angiosperm may all be impacted. Puccinia is unique in that it can have two or three cells, smooth walls, and a well-developed pedicel. Each Puccinia spore has two or more germ pores and a wall with varying pigmentation [9]. The genus Puccinia is the largest

in the current examination, with four species; *Ph. Barclayi* on *Rubus niveus* is represented by one species; and *Melampsora euphorbiae* on *Euphorbia helioscopia* is represented by one species. Based on the morphology of the spores and the disease pattern, in the current research from Abbottabad, one Taxon *M. Euphorbiae*, was identified. Previous reports of the rust fungus *Euphorbiae hypericifolia* from Muzaffarabad, Karachi, and Gilgit [10]. From Charsadda on leaves, Nathiagali, Quetta, Peshawar, Muree, Lahore, Kaghan Valley, Swat, Wazirabad, Chalianwala [18]. It was determined that this was a new record for the study area. Ahmad has reported finding *Phragmidium barclayi* from *Rubus lasiocarpus* in KP and Changla Gali. Again from SWAT, *Phragmidium barclayi* on *Rubus pedunculatus* is described with the most recent illustrations. It's a first for the district of Abbottabad. *P. ambegua* has been documented on *Gallium aprine* L.; however, Asghar Ali (1955) only reported its uredinal stage in Muree Hills, Ghoregalii. The fungus's telial stage is a recent addition to Pakistan's rust flora. Additionally, it sets a new record for the Thandiani region's Abbottabad District. Prior research on *Centaurea bruguieriana* (DC.) Bornm was done by Calcitraipae. A recent addition to Pakistan's rust flora at the base of the uredinal stage is *Ranunculus muricatus*.

Conclusion

The results of our research indicate that District Abbottabad is extremely wealthy and has a wide variety of fungi, particularly rust. Puccinia, with four species, was found to be the largest genera among the reported genera, followed by Uromyces, with four species. The study's findings indicate that District Abbottabad, which is covered in 70% coniferous forest and is situated in a moist temperate climate, has a huge potential for fungal diversity. Future research in this area is necessary to look for extremely valuable species belonging to other groups.

Acknowledgment

The authors would like to have collection done in the study research area and lab work is Completed in the Mycology and Plants Pathology lab, Faculty of Health and Biological Science, Department of Botany Hazara University Mansehra Pakistan.

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