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Distribution of rust fungi (*Puccinia* and *Phragmidium*) and host plants in Pakistan

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ABSTRACT

During 2014, survey of rust fungi and their associated hosts was attempted in Swat and Murree regions of Pakistan. In this paper we provided descriptions and illustrations of five rust species *viz. Puccinia nepalensis, P. melasmioides, Phragmidium bulbosum, Ph. butleri*, and *Ph. barclayi*. *Rubus fruticosus* host of *Phragmidium bulbosum* collected as a new host record from Pakistan. The rust fungus, *Puccinia nepalensis* was sequenced using LSU and ITS DNA markers and consequently the sequences were deposited to GenBank as future barcode reference.

Key words: Northern regions, Malakand Division, Pucciniales, Taxonomy, Urediniales.

INTRODUCTION

Rust fungi (*Pucciniales*) constitute one of the largest and diverse groups of plant pathogens that can affect almost all the plants in the biosphere (Cummins & Hiratsuka 2003). *Puccinia*, the largest genus in *Pucciniales*, comprised of more than 4,000 species which are characterized by developing pedicillate and two-celled teliospores (Mahadevakumar *et al.* 2016). The genus *Phragmidium* contains over 66 species of which seventeen *Phragmidium* species have been reported from Pakistan (Cummins & Hiratsuka 2003, Ali *et al.* 2017).

Swat, district of Khyber Pakhtunkhwa (KP), lies in the north of Pakistan represent one of the growing regions in the context of rust fungi and hitherto, over 66 species have been described in addition with new hosts and new local distribution records (Ali *et al.* 2016). 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 (Ali *et al.* 2016). In 2014, 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.

MATERIALS AND METHODS

Collections of rust affected plants were carried out and then infected plants were preserved and submitted to the herbarium of Plant Sciences Department, Quaid-i-Azam University, Islamabad, Pakistan (ISL), 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 comparing new collections with flora of Pakistan and botanical specimens held in herb. ISL. For Scanning Electron Microscopy (SEM) the spores were scraped from the leaves, mounted on a stub, coated with gold in a sputter-coater and examined with a JSM-5910 SEM.

RESULTS AND DISCUSSION

Puccinia nepalensis Barclay & Dietel, Hedwigia 29: 265 (1890) **Plat. 1**

SPERMOGONIA and AECIA not found. UREDINIA amphigenous, minute, round, chestnut brown, scattered or loosely aggregate, 0.1–0.5 mm in diam, pulverulent; UREDINIOSPORES globose, subglobose, obovoid or ellipsoid, cinnamon brown, 17–30 × 20–22.5 µm, wall 1–2 µm, sparsely echinulate, germ pores 2, not always placed on helium; TELIA dark brown to blackish brown; TELIOSPORES ellipsoid, rounded at both ends, slightly constricted at the septum, chestnut brown, 30–45 × 17.5–25 µm, sometimes attenuated at apex, rounded at base, not constricted at the septum, wall chestnut brown, 1–2 µm thick, uniform in thickness, smooth, pore of the upper cell apical, covered with hyaline papilla, of the lower cell at the septum, 2–2.5 µm high or sometimes not distinct, pedicels hyaline, short, 5–12.5 × 5–10 µm, deciduous.

Material examined: On *Rumex nepalensis* (*Polygonaceae*) with II + III stages, Pakistan, Punjab Province, Murree, Sunny bank, at 2000 m. a. s. l, February, 2014 and 2016, Coll., Barkat Ali BA65 and BA66 (ISL-26648), ITS sequences (KU886228, KX225481) and LSU sequences (KX014746, KX225482).

Comments: In Pakistan, *Puccinia nepalensis* has been described from *Rumex nepalensis* in Changa Gali, Hazara, Kaghan Valley, Murree, Muzaffarabad (Azad Jammu & Kashmir), Naran, Saiful Maluk, Swat, Hunza Valley, and Fairy Meadows (Iqbal & Khalid 1996, Ahmad *et al.* 1997, Afshan *et al.* 2009).

In this paper *Rumex nepalensis* is re-described with new illustrations, electron micrographs and DNA sequences for future analysis.

Puccinia melasmioides Tranzschel (1904) **Plat. 2**

SPERMOGONIA and AECIA not found. UREDINIA hypophyllous, small, reddish brown, rounded, spread, rarely in aggregation, 0.3–0.6 × 0.2–0.3 mm in diam, covered by epidermis; UREDINIOSPORES globose to subglobose or ellipsoidal, yellow to brown, 15–22.5 × 10–17.5 µm, wall 3–5 µm, verrucose; PARAPHYSES numerous, incurved, hyaline; TELIA hypophyllous, black, rounded, swelled, compact, spread, 0.3–0.6 × 0.2–0.3 mm in diam, covered by epidermis; TELIOSPORES light brown to light yellowish, ellipsoidal v. oblong-ellipsoid, 2-celled, often apical cell is small and wider as compared with basal cell, mostly rounded at the apex, slightly or not constricted at the septum, 47.5–62.5 × 10–12.5 µm (with pedicel 55-70 µm), wall thin at sides while thickened at the apex, light brown to deep brown at the apex, 1.8–3.4 µm, apex 5–7.5 × 7.5–15 µm, pedicel hyaline to subhyaline, very short, 7.5–12.5 × 5–7.5 µm, fragile.

Material examined: On *Aquilegia pubiflora* (*Rununculaceae*) with II + III stages, Pakistan, Khyber Pakhtunkhwa, Swat District, Malam Jabba, 2376 m. a. s. 1., October 2014, Coll., Barkat Ali BA70.

Comments: *Puccinia melasmioides* has been reported previously on *Aquilegia pubiflora* from Swat District, KP, Pakistan (Ahmad & Lodhi 1953). However, Sultan Ahmad (1953) only mentioned the species with it is host plant while no description was provided. Here we provided the description along with illustrations.

Phragmidium bulbosum (Str.) Schlect., Fl. Berol. 2: 156, (1824) **Plat. 3**

SPERMOGONIA and AECIA not found. UREDINIA hypophyllous, mixed with telia, irregularly scattered without spots or with small yellowish or reddish spots, erumpent, powdery, surrounded by paraphyses; UREDINIOSPORES globose to ovate or ellipsoidal, yellow to pale yellow, wall 2–3.5 µm, hyaline, echinulate, indistinct germ pores, 15–20 × 13–18 µm; TELIA hypophyllous, black, scattered, small, rounded or irregular, pulverulent; TELIOSPORES cylindrical, not constricted at septa, sienna to dark amber brown, 65–100 × 25–30 um, 2–8 celled, mostly 6 or 7 celled, wall 4–6 µm, dark brown, verrucose, germ pores 2-4 in each cell, apex rounded with hyaline, acute or conical apiculus, 7.5–12.5 × 7.5–9.5 µm, pedicels 40–112.5 × 5–14.5 µm, hyaline, usually longer than the spores, swollen at the base, persistent.

Material examined: On *Rubus fruticosus* (*Rosaceae*) with II + III stages, Pakistan, Punjab Province, Murree, Bansra Gali, at 1767 m. a. s. l., December, 2014, Coll., Barkat Ali BA54.

Comments: *Phragmidium bulbosum* has been reported already from Pakistan: on *Rubus pungens* (Ahmad 1956b), *R. hoffmeisteianus* (Ono 1992), *R. ulmifolius* (Kaneko 1993, Khalid *et al.* 1995, Sultan *et al.* 2006). *Rubus fruticosus* presented here as new host record for *Ph. bulbosum* in Pakistan.

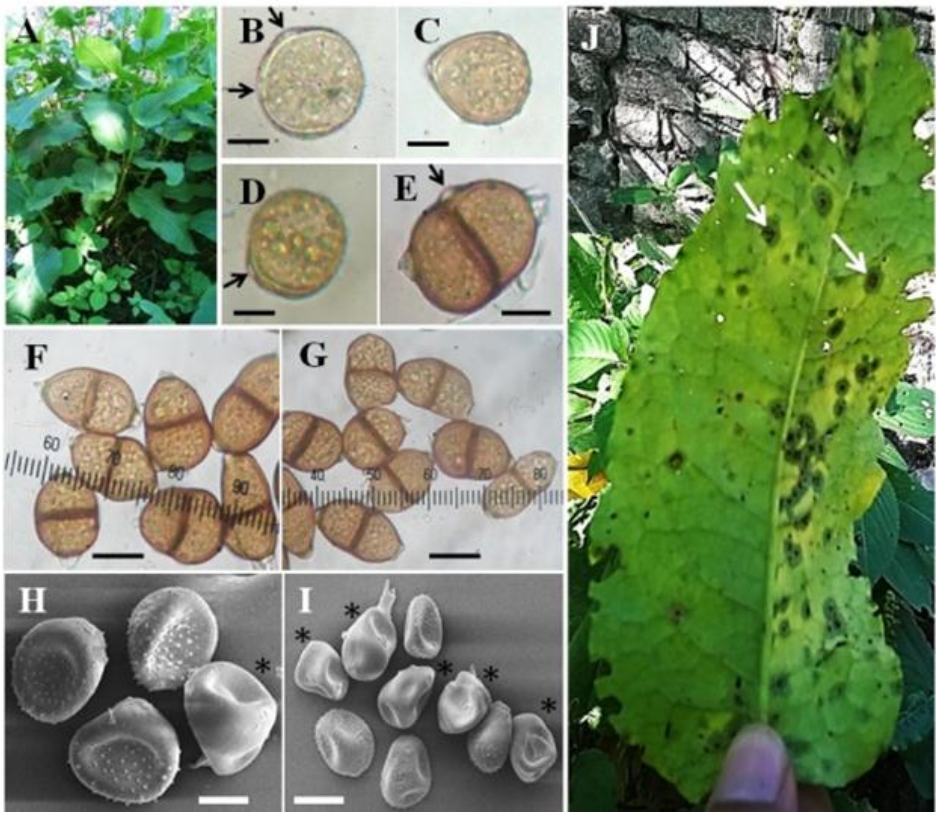

PLATE 1. ***Puccinia nepalensis.*** (A) *Rumex nepalensis* plant growing under natural conditions. (B–D) Urediniospores of different shapes, showing germ pores (black arrows), (E) Teliospore showing a germ pore (black arrow). (F–G) Teliospores of different shapes, some showing a pedicel, (H) SEM of urediniospores showing sparse echinulation and a teliospore (marked with asterisk). (I) SEM of sparsely echinulated urediniospores and smooth-walled teliospores (marked with asterisk). (J) Infected leaf showing abaxial brown sori (infected regions illustrated with white arrows). Scale bars = B–D. 10 µm; E–G. 12 µm; H–I. 10 µm.

Phragmidium barclayi Dietel, Hedwigia 29: 264, 1890 **Plat. 4**

SPERMOGONIA and AECIA not found. UREDINIA hypophyllous, scattered, small, orange; UREDINIOSPORES globose to subglobose, 17–26 × 15–18 µm, wall 2–4 µm thick, TELIA hypophyllous, scattered, minute, 0.2–2 mm in diam, black, pulverulent; TELIOSPORES cylindrical, oblong, 5–7 celled, mostly 6 celled, 70–130 × 35–45 µm, not to slightly constricted at the septum, wall 4–6 µm thick, smooth, yellowish brown, rounded, papilla lacking, Pedicels cylindrical, hyaline, rough, elongated, 55–160 × 16–23 µm, yellow contents at the base.

Material examined: On *Rubus pedunculosus* (*Rosaceae*) with II + III stages, Pakistan, Khyber Pakhtunkhwa (KP), Swat District, Mallam Jabba, at 2300 m. a. s. l., October, 2014, Coll., Barkat Ali BAR11

Comments: *Phragmidium barclayi* has been reported from *Rubus lasiocarpus* in KP and Changla Gali by Ahmad (Ahmad 1956a, b), on *R. aff. hoffmeisteianus* from Kaghan valley by Ono & Kakishima (1992), and on *R. pedunculosus* from Mingora (Swat) by (Khalid et al. 1993b). *Phragmidium barclayi* on *Rubus pedunculosus* is again described from swat with latest illustrations.

Phragmidium butleri Syd., Ann. Mycol. 5: 501, 1907 **Plat. 5**

SPERMOGONIA, AECIA and UREDINIA not found. TELIA hypophyllous, black, minute, scattered, pulverulent; TELIOSPORES cylindrical, 95–123.5 × 27.5–37.5 µm, 8–10 celled (mostly 9 celled), apical papilla long, hyaline, 6-12 µm, wall

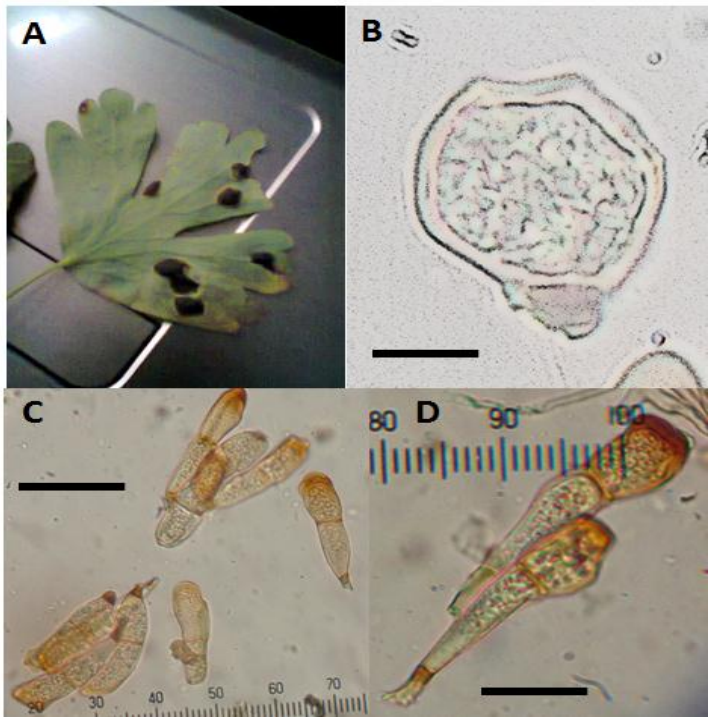

PLATE 2. *Puccinia melasmioides*. (A) Infected leaf of *Aquilegia pubiflora* showing black spots on the abaxial surface. (B) Urediniospore showing thick wall. (C) Teliospores with short pedicels. (D) Apically thickened teliospores. Scale bars for B and C= 20 µm, D= 12 µm.

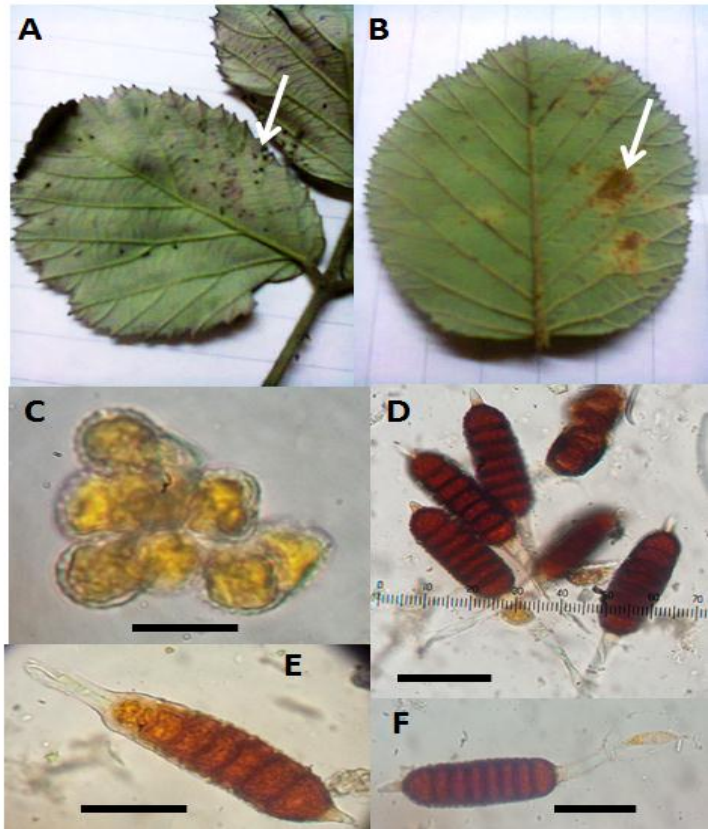

PLATE 3. *Phragmidium bulbosum*. (A) Infected leaf showing telia (indicted by white arrow). (B) Infected leaf showing yellowish to reddish brown uredinia (indicted by white arrow). (C) Urediniospores showing echinulations with yellowish contents. (D) Teliospores showing different number of cells and apical hyaline papillae. (E-F) Teliospores showing pedicels with yellowish contents at the base. Scale bars for C,D,F = 20 µm, E = 12 µm.

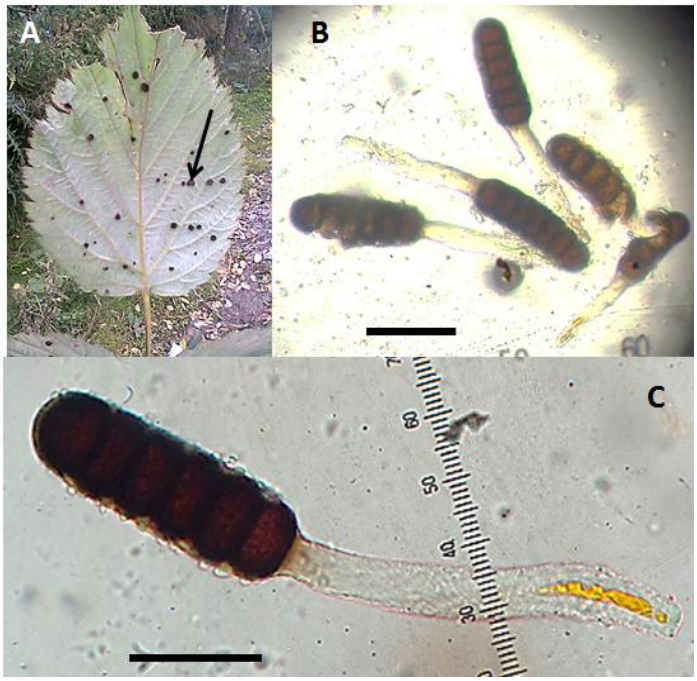

PLATE 4. *Phragmidium barclayi*. (A) Infected leaf of *Rubus pedunculosus* showing black spots of telia (indicated by black arrow). (B) Teliospores showing different number of cells and hyaline pedicels. (C) Teliospore showing pedicel with yellowish contents at the base. Scale bars for B = 20 µm, C= 12µm.

PLATE 5. *Phragmidium butleri*. (A) Infected leaves of *Rosa brunonii* showing adaxial brownish telia (Indicted by white arrows). (B-C) Teliospores showing number of cells and pedicels with yellowish contents at the base. Scale bars B = 20 µm, C = 12 µm.

5-7 µm thick, dark chocolate brown, verrucose, not constricted at the septa, pores 2-3 in each cell, pedicels hyaline, palely colored next the spore, up to 113 µm long, swelled at the lower part with yellowish contents, up to 25 µm wide at the base.

Material examined: On *Rosa brunonii* (*Rosaceae*) with III stage, Pakistan, Punjab Province, Murree, Ghora Gali, at 1767 m. a. s. l., November, 2014, Coll., Barkat Ali BA51.

Comments: *Phragmidium butleri* on *Rosa brunonii* has been reported previously from Pakistan (Ahmad 1956, Iqbal *et al.* 1996, Ahmad *et al.* 1997). In this paper, *Phragmidium butleri* is described from Murree region of Pakistan.

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