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Puccinia anaphalidis-virgatae, a new species, and a new variety of rust fungi from Fairy Meadows, Northern Pakistan

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Abstract — *Puccinia anaphalidis-virgatae* on *Anaphalis virgata* is described as a new species from Pakistan. Previous records of rusts on genus *Anaphalis* have been species of *Miyagia*, *Phakopsora*, and *Uromyces*; this is the first *Puccinia* species recorded on this host genus. A new variety *P. helictotrichi* var. *pakistanica* is described based on its resemblance to *P. helictotrichi*; however, it differs in size and number of germ pores of urediniospores and apical thickness of teliospores.

Key words — *Miyagia*, Nanga Parbat, *Phakopsora anaphalidis-adnatae, Pucciniales*, rust mycobiota

Introduction

This paper is a continuation of our publications describing the rust fungi of Pakistan. The taxa presented and described in this paper were collected from Fairy Meadows, Northern Pakistan. Out of all rust fungi previously recorded from Pakistan, 68 species of rust fungi have been reported from northern areas of Pakistan with only 12 taxa from Fairy Meadows, including one species each of *Aecidium*, *Chrysomyxa*, *Cronartium*, *Hyalopsora*, *Melampsora,* and *Pucciniastrum* and six species of *Puccinia* (Afshan et al. 2009, Iqbal et al. 2009).

Numerous new records and new species can still be expected as a result of ongoing fieldwork in these areas of Pakistan because of the high diversity of vascular plants i.e. 3000 species (Iqbal et al. 2009). During recent rust surveys in northern areas of Pakistan, one specimen was determined to be new to science, i.e., *Puccinia anaphalidis-virgatae* on *Anaphalis virgata*. *Puccinia helictotrichi*

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var. *pakistanica* on *Helictotrichon virescens* is also being proposed as new to science. The present paper contributes to the knowledge of the rust mycobiota of Fairy Meadows, Northern Pakistan.

Materials and methods

Specimens were collected from Fairy Meadows, Pakistan. Freehand sections of infected tissues and spores were mounted in lactophenol and gently heated to boiling. The preparations were observed under a NIKON YS 100 microscope and photographed with JSM5910 Scanning Electron Microscope. For SEM, dried plant material was hand-sectioned with a razor blade and mounted on SEM stubs. The samples were coated with gold in a sputter-coater and examined with a JSM5910 Scanning Electron Microscope. Spores and paraphyses were drawn using a Camera Lucida (Ernst Leitz Wetzlar, Germany). Spores were measured with an ocular micrometer. At least 25 spores were measured for each spore state. The specimens were deposited in the Herbarium of the Botany Department, University of the Punjab, Lahore (LAH).

Enumeration of taxa

Puccinia anaphalidis-virgatae Khalid, Afshan & S.H. Iqbal, **sp. nov.** Figs. A–H

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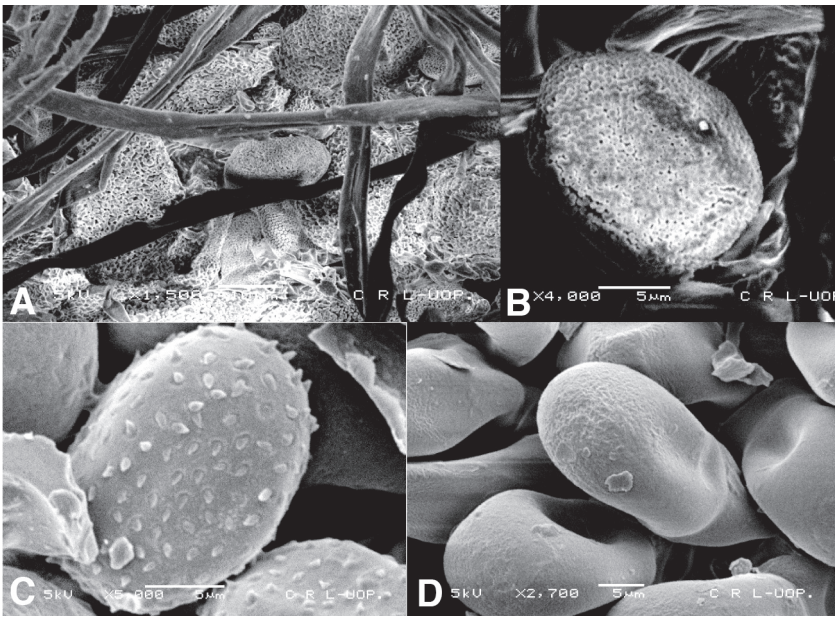
Aeciosporae ovoideae, obovoideae vel ellipsoideae, hyalinae vel dilute flavidae, densiter verrucosae vel coronatae, 19–22 × 21–29 µm. Cellulis peridii irregularibus, 47–65 × 20–29 µm, hyalinis vel dilute flavidis. Uredinia in pagina abaxiali foliorum, 0.7–2.0 × 4–7 mm, aureo-brunnea. Urediniosporae globosae vel subglobosae, 17–24 × 20–28 µm; pariete 1.5–2 µm crasso, echinulato, pallide flavido vel dilute brunneo; poris germationis 5–6, dispersis; paraphysibus clavatis, hyalinis vel dilute flavidis, 5–7 × 55–72 µm; pedicellis 4–8 × 10–25 µm. Telia atra, 0.9–2 × 2–7 mm. Teliosporae 2-cellulares, raro unicellulares, aureo-brunneae vel castaneo-brunneae, oblongae, fusiformes, ellipsoideae vel late ellipsoideae, 20–24(–26) × 50–64(–70) µm; apicaliter castaneo-brunneae, basaliter pallidae, apice obtuso, conico vel oblique conico, 5–10 µm crasso, pariete 1.5–2 µm crasso, levi, pedicellis persistentibus, hyalinis vel dilute brunneis, 8–13 × 32–50 µm.

Holotype: On *Anaphalis virgata* Thomson ex C.B. Clarke (*Asteraceae*), I + II + III, Pakistan, Northern Areas, Fairy Meadows, 3036 m a.s.l., 12 Aug 2007. NSA # G01 (LAH - NSA 1004).

Etymology: Named after the host plant *Anaphalis virgata.*

Spermogonia not found. Aecia on stems, orange, 0.1–0.2 × 0.2–0.3 mm, cupulate. Aeciospores ovoid to obovoid or ellipsoid, hyaline to pale yellow, finely verrucose to coronate, 19–22 × 21–29 µm. Peridial cells irregular to fusiform in shape, moderately rugose, 47–65 × 20–29 µm, hyaline to pale yellow. Uredinia on leaves and stems, abaxial, 0.7–2 × 4–7 mm, golden brown. Urediniospores globose to subglobose, 17–24 × 20–28 µm; wall 1.5–2 µm

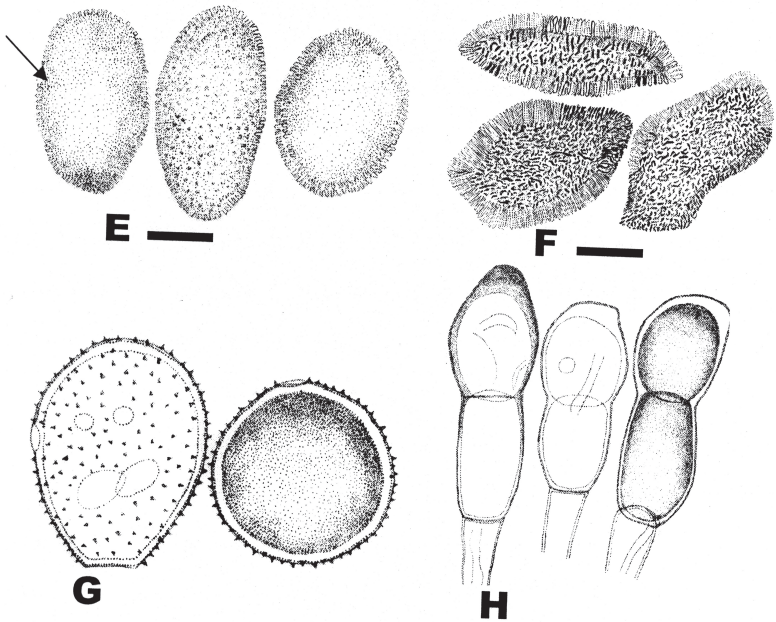


Figs. A–D: *Puccinia anaphalidis-virgatae* (type) (A) SEM photograph of peridial cells and aeciospores. (B) An aeciospore showing verrucose wall ornamentation. (C) Echinulate urediniospores. (D) SEM photograph of smooth walled teliospores.

thick, pale yellow to pale brown, sparsely echinulate; germ pores 5–6, scattered; paraphyses clavate, hyaline to pale yellow, 5–7 × 55–72 µm; pedicel fragile, 4–8 × 10–25 µm. Telia on stems, black, 0.9–2 × 2–7 mm. Teliospores mostly two-celled, few one-celled, golden brown to chestnut brown, oblong to fusiform or ellipsoid to broadly ellipsoid, 20–24(–26) × 50–64(–70) µm; apex chestnut brown, paler basally, apex mostly rounded but sometimes conical or obliquely conical, 5–10 µm thick; wall 1.5–2 µm thick, smooth; pedicel persistent, hyaline to pale brown, 8–13 × 32–50 µm.

Comments: Previously, *Phakopsora anaphalidis-adnatae* Khalid & S.H. Iqbal was reported on *Anaphalis adnata* DC. from Pakistan (Khalid & Iqbal 1996b).

Other rust fungi reported on *Anaphalis* spp. include *Miyagia anaphalidis* Miyabe on *A. acutifolia, A. aureopunctata, A. brevifolia*, *A. hancockii, A. margaritacea* subsp. *angustior, A. margaritacea* subsp. *japonica, A. margaritacea* subsp. *yedoensis, A. morrisonicola, A. sinica, A. subdecurrens, A. yedoensis,* and *A. zeylanica* from Japan, China, Sri Lanka, and Taiwan; *Miyagia macrospora* Hirats. f. on *A. aureopunctata, A. contorta, A. morrisonicola, A. nepalensis,* and *A. xylorhiza* from China, Nepal, and Taiwan; *Phakopsora artemisiae* Hirats. on



Figs. E–H: *Puccinia anaphalidis-virgatae* (E). Aeciospores showing germ pores (F). Peridial cells of the aecidia (G). Urediniospores showing germ pores (H). Teliospores Scale bar for E & G = 10 µm, F = 5 µm, H = 15 µm.

A. margaritacea, and *A. sinica* from China and Nepal; *Phakopsora compositarum* T. Miyake on *A. sinica* from China; *Phakopsora elephantopi* Hirats. on *A. sinica* from China; *Uromyces amoenus* Syd. & P. Syd. on *A. alpicola*, *A. busua, A. contorta,* and *A. margaritacea* from China, Japan and Nepal; and *Uromyces langtangensis* Durrieu on *A. nepalensis* from Nepal (Sawada 1943, Ito 1950, Hiratsuka 1969, Hiratsuka 1973, Tai 1979, Azbukina 1984, Durrieu 1987, Guo 1989, Ono et al. 1990, Hiratsuka & Chen 1991, Hiratsuka et al. 1992, Zhuang 1993, Zhuang & Wei 1994, Gjaerum 1995, Khalid & Iqbal 1996a,b, Cao et al. 2000, Zhuang 2005).

Puccinia anaphalidis-virgatae is characterized by the absence of peridia in uredinia and telia and up to 6 scattered germ pores in urediniospores. Another characteristic feature is the presence of thickened, rounded, or conical apices of the teliospores with persistent pedicels.

Species in the genus *Miyagia* have peridiate uredinia and telia while the absence of peridial uredinia and telia is characteristic of the genus *Puccinia* (Cummins & Hiratsuka 2003). The uredinia and telia of *Miyagia anaphalidis* are peridiate with a peridium of laterally adherent, palisade-like paraphyses.

Moreover, aecia of *Miyagia* are erumpent and uredinioid with aeciospores borne singly on pedicels. The aecia of *P. anaphalidis-virgatae* are of the aecidium type with a peridium. *Miyagia anaphalidis* is somewhat comparable to the *P. anaphalidis-virgatae* in the size and wall ornamentation of the urediniospores and teliospores. *Puccinia anaphalidis-virgatae* with urediniospores having 5–6 scattered germ pores differs from *M. anaphalidis* with 2 equatorial germ pores.

Puccinia anaphalidis-virgatae differs from *P. horti-kirstenboschi* Berndt & E. Uhlmann reported on *Helichrysum* sp. by the size and shape of teliospores. *P. anaphalidis-virgatae* has larger (20–26 × 50–64 (–70) µm vs. 17–23 × 40–55 µm) teliospores with thicker (5–10 µm vs. 0.5–1.5 µm) apices than in *P. horti-kirstenboschi*.

P. anaphalidis-virgatae is similar to *P. subindumentana* Berndt reported on *Helichrysum chrysophorum* by the shape and apical thickness of teliospores. However, aeciospores are smaller (19–22 × 21–29 µm vs. 25–30 × 27–33 µm) and teliospores are wider (20–26 µm vs. 16–22.5 µm) with a persistent pedicel. *P. anaphalidis-virgatae* has smaller aeciospores (19–22 × 21–29 µm vs. 23–31 × 29–41 µm) and urediniospores (17–24 × 20–28 µm vs. 24.5–29.5 × 28–34.5 µm) than in *P. cornurediata* Berndt reported on *Helichrysum petiolatum* D. Don. Moreover, *P. anaphalidis-virgatae* lacks peridia in uredinia while *P. cornurediata* possesses slightly tapering, orange-yellow peridium in uredinia.

Puccinia helictotrichi var. ***pakistanica*** Afshan & Khalid, **var. nov.** Figs. I–J

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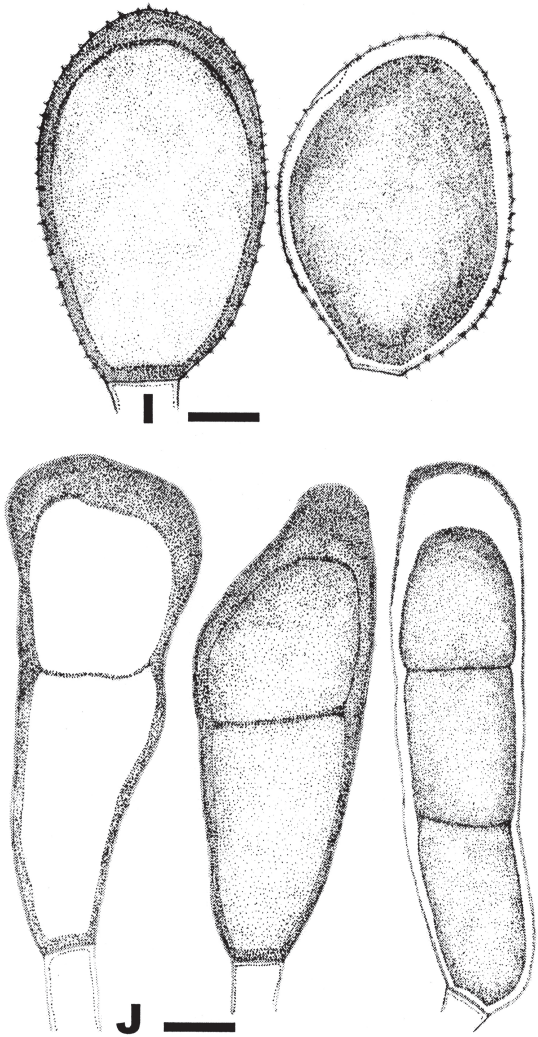
Spermogonia et aecia ignota. Uredinia amphigena, subepidermalia. Urediniosporae globosae vel subglobosae, ovoidae vel ellipsoideae, 19–25 × 23–29(–32) µm; pariete 1–1.5 µm crasso, pallide brunneo vel brunnea, echinulato; poris germinationis 2–4, aequatorialibus vel supra-aequatorialibus. Telia amphigena, subepidermalia, atra. Teliosporae 1–3-cellulares, oblongae vel clavatae, 14–21 × (36–)44–56 µm, pariete levi, 1.5–2 µm crassa, ad apicem 4–7 µm crasso, cinnamomea vel atro-brunneo, basaliter pallidae; apice truncato vel obtuso; pedicellis hyalino vel dilute brunneis, 5–8 × 11–14 µm.

Holotype: On *Helictotrichon virescens* (Nees ex Steud.) Henrard (*Poaceae*), II + III stages, Pakistan, Northern Areas, Fairy Meadows, 3036 m a.s.l., 12 Aug 2007. NSA # 69. (LAH - NSA 1075).

Etymology: Named after the country, Pakistan.

Spermogonia and aecia unknown. Uredinia amphigenous, subepidermal, yellowish brown to golden brown, 0.09–0.1 × 0.1–2.0 mm. Urediniospores globose to subglobose or ovoid to ellipsoid, 19–25 × 23–29(–32) µm; wall 1–1.5 µm thick, pale brown to cinnamon brown, echinulate; germ pores 2–4, equatorial to supraequatorial, pedicel hyaline, 4–6 µm wide and up to 16 µm long. Telia amphigenous, covered by epidermis, dark brown to blackish brown, loculate with paraphyses, 0.09–0.5 × 0.2–0.8 mm. Teliospores 1–3-



Figs. I–J: *Puccinia helictotrichi* var. *pakistanica*
(I) Echinulate urediniospores. (J) Teliospores. Scale bar = 10 μm.

celled, oblong to clavate, septa usually horizontal, but sometimes oblique in three-celled spores, 14–21 × (36–)44–56 μm (mean 17 × 44 μm); wall 1.5–2 μm thick, cinnamon brown to golden brown but paler basally, smooth; apex mostly truncate, sometimes rounded, 4–7 μm thick; germ pores obscure; pedicel hyaline to pale brown, 5–8 × 11–14 μm.

Comments: *Puccinia helictotrichi* var. *pakistanica* is characterized by the presence of 1–3-celled teliospores with sometimes oblique septa in three-celled spores. The presence of 2–4 equatorial germ pores in urediniospores and the absence of uredinial paraphyses also make it different from other *Puccinia* species reported on hosts in the same tribe.

Puccinia helictotrichi var. *pakistanica* closely resembles *P. helictotrichi* Jørst. by the shape and wall ornamentation of urediniospores and size of teliospores. These varieties can be separated by the size of urediniospores. *P. helictotrichi* var. *pakistanica* has smaller urediniospores (19–25 × 23–29 (–32) µm vs. 18–26 × 24–48 µm) than *P. helictotrichi*. Another characteristic difference is the presence of 1–3-celled teliospores with thicker apices (4–7 µm vs. 2–4 µm) and 2–4 equatorial germ pores of urediniospores in the *P. helictotrichi* var. *pakistanica* than in *P. helictotrichi* that possesses 1–2 celled teliospores with 6–12 scattered, obscure germ pores.

Puccinia helictotrichi var. *pakistanica* is similar to *P. brachypodii* var. *poae-nemoralis* (G.H. Otth) Cummins & H.C. Greene in the shape, wall ornamentation, and size of urediniospores. These species differ in the size of teliospores, which are smaller in *P. helictotrichi* var. *pakistanica* (14–21 × (36–) 44–56 µm vs. 12–27 × 30–80 µm). The presence of 1–3-celled teliospores, sometimes with a vertical septum in three-celled teliospores and absence of uredinial paraphyses in *P. helictotrichi* var. *pakistanica* make it different from *P. brachypodii* var. *poae-nemoralis*.

On the basis of close resemblance with *P. helictotrichi*, this species is described as a new variety of *P. helictotrichi* i.e. *P. helictotrichi* var. *pakistanica*.

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