



## *Prillieuxina citricola* sp. nov. from Himachal Pradesh, India

Gautam AK<sup>1</sup> and Avasthi S<sup>2</sup>

<sup>1</sup> Faculty of Science, School of Agriculture, Abhilashi University, Mandi, Himachal Pradesh, India

<sup>2</sup> Department of Botany, Abhilashi Institute of Life Sciences, Mandi, Himachal Pradesh, India

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### Abstract

The present paper deals with a new black mildew collected on living leaves of lemon (*Citrus aurantifolia*, Rutaceae), from Bilaspur, Himachal Pradesh, India. The fungus was identified as the species of *Prillieuxina* as it contains substraight, branched hyphae without appressoria and setae; orbicular thyriothecia and brown uniseptate ascospores. A critical comparison of current taxon was carried out with species of genus *Prillieuxina* reported earlier on plants of the family Rutaceae and other closely similar species and found that there are no earlier reports on *Citrus aurantifolia*. Therefore, new species *Prillieuxina citricola* is described and illustrated in the present paper based on morphology and specificity of host association.

**Key words** – Asterinales – Black mildew – *Citrus* – foliicolous asexual fungi – taxonomy

### Introduction

Black mildews are fungi that produce black colonies on the host surface. These fungi are thought to be host-specific, and infect mostly leaves, often soft stems and petioles. They are mostly epiphyllous, but also hypophyllous, occurring commonly in the tropics and subtropics. Black mildews require humid environments for infection initiation and development and produce various structures like mycelium, appressoria, setae, thyriothecia, pycnothyria, asci, ascospores, pycnothyriospores, perithecial appendages and consistently, brown septate ascospores (Hansford 1961, Hosagoudar & Agarwal 2008, Hosagoudar 2012, Hongsanan et al. 2014).

*Prillieuxina* was established by Arnaud (1918) based on *P. winteriana* ( $\equiv$  *Asterina winteriana*) as the type species. It is characterized by substraight, branched mycelium devoid of appressoria and setae; orbicular thyriothecia and brown uniseptate ascospores (Hongsanan 2014). This genus has been reported on a wide range of angiosperms from tropical to subtropical regions. About 78 epithets have been reported worldwide till date (Index Fungorum 2016); of which about 12 are reported from India on the members of Aquifoliaceae (*P. aquifoliacearum*), Clusiaceae (*P. garciniae*), Bheemamyces (*P. argyreiae*), Ebenaceae (*P. diospyri*), Elaeagnaceae (*P. elaeagni*), Gangagamyces (*P. polyalthiae*), Menispermaceae (*P. anamirtae*), Myrsinaceae (*P. ardisiae*), Oleaceae (*P. jasmine*), Rubiaceae (*P. ixorigena*), Rutaceae (*P. aeglicola*) and Sterculiaceae (*P. pterigota*) (Hosagoudar 2012, Gautam 2015). *Aegle marmelos*, *Anamirta cocculus*, *Ardisia solanacea*, *Argyrea* sp., *Diospyros malabaricus*, *Elaeagnus kologa*, *Garcinia imberti*, *Ilex denticulate*, *Ixora coccinea*, *Jasminum flexile*, *Polyalthia longifolia* and *Pterygota alata* are reported to be infected with black mildew disease caused by different species of *Prillieuxina* (Hosagoudar 2012, Gautam 2014, Hongsanan et al. 2014, Gautam 2015).

Although *Prillieuxina* is an important genus that lacks appressoria, it is still included in the family Asterinaceae based on characters such as colonies spread on host surface, superficial

thyriothechia, globose asci and 1–3-multi-septate ascospores. The genus mainly adopts obligate biotrophic lifestyle on living leaves and develops different heterogeneous infection strategies like expanded hypostromata, intercellular hyphae or penetration of the host stomata. There are number of thyriothechia forming fungi such as *Halbania*, *Schenckiella* and *Uleothyrium* which also contained mycelium devoid of appressoria. These genera differ from each other on the basis of thyriothechia, asci and ascospores. The asci were observed without an ocular chamber with 3-septate, fusiform, inequilateral and brown ascospores in genus *Halbania*, while elongated clavate asci with brown pseudoparaphyses containing 4–5-septate brown ascospores in *Schenckiella* and clavate to ellipsoidal, apedicellate with opaque thickening at apical region having straight or slightly curved, elongate fusoid, hyaline, 1-septate ascospores, parallel inside asci in *Uleothyrium* (Hofmann 2009, Hosagoudar 2012, Hongsanan et al. 2014). The asci formed in *Schenckiella* are rarely observed in the Dothideomycetes. Different dothideomycetes like *Elsinoe fawcettii* (Jayawardena et al. 2014), *Polychaeton citri* and *Pseudocercospora angolensi* (Schoch et al. 2009) have been reported to cause scab and anthracnose on various *Citrus* spp.

During November 2014, a black mildew infection was observed on leaves of *Citrus aurantiolia* (Christm.) Swingle. The plant contains numerous phytochemicals which exhibit anti-inflammatory, antimicrobial, anticancer and anti-oxidative activities. It is used intensively in the preparation of various juices and pickles (Tanaka 1959, Penjor et al. 2014). The detailed microscopic observations revealed structures like orbicular and astomatous thyriothechia with radiating cells and dehisce stellately at the center. The presence of mycelium devoid of appressoria and setae pointed out the relatedness of the fungus to genus *Prillieuxina* G. Arnaud. This fungal genus exhibits a wide diversity and host range and believed to be host specific; however, no earlier reports on *C. aurantifolia* are available. The aim of this study is the description of new species, based on morphology and host-specificity. The detail morphological description, colour photographs, line drawings and discussions are provided here.

## Materials & Methods

The infected leaf samples of the *C. aurantifolia* were collected in district Bilaspur of Himachal Pradesh during the course of a field survey in winter of 2014. All the collected samples were taken to laboratory and analyzed for morphological and microscopic characteristics. Field notes were prepared regarding the disease symptoms, nature of colonies, nature of infection, locality and altitude, etc. The morphological examination of diseased spots was carried out with the help of hand lenses for colour and texture. Nail polish technique was used to study the micromorphological characters of the fungi. Surface scrapings of black colonies were taken directly from infected host, mounted in 5% KOH solution and then replaced by lactophenol to make the septa visible (Hosagoudar & Kapoor 1984). Surface scrapings and sections were taken through infection spots and mounted in cotton-blue and lactophenol mount mixture for microscopic examination. Morphotaxonomical details were observed and photomicrographs were taken under CH2 Olympus light microscope equipped with a SONY DSC WX200 digital camera. Drawings of microscopic structures like mycelium, thyriothechia, asci, ascospores, pycnothyria and pycnothyriospores were made with the help of a camera-lucida under oil immersion and measurements were carried out with the help of micrometry at 1000× magnification. All measurements were given in the form: min–max (mean ± standard deviation) and extreme sizes are omitted. Facesoffungi and MycoBank numbers are added (Jayasiri et al. 2015).

## Results

### Taxonomy

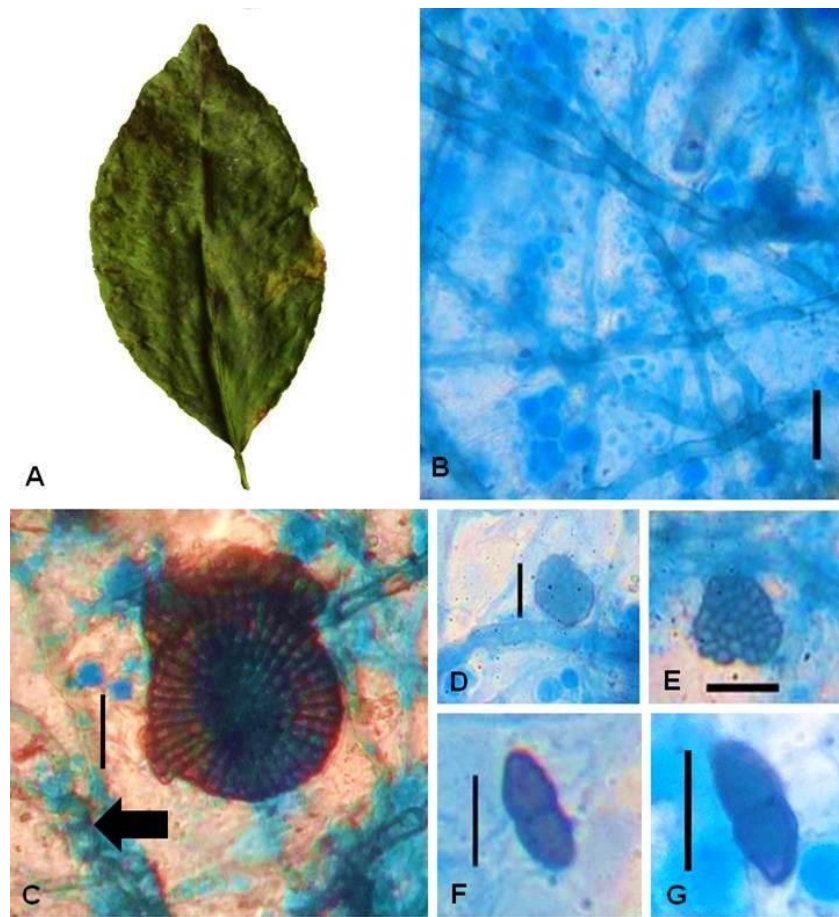
*Prillieuxina citricola* A. K. Gautam & S. Avasthi, **sp. nov.**

**Figs. 1-2**

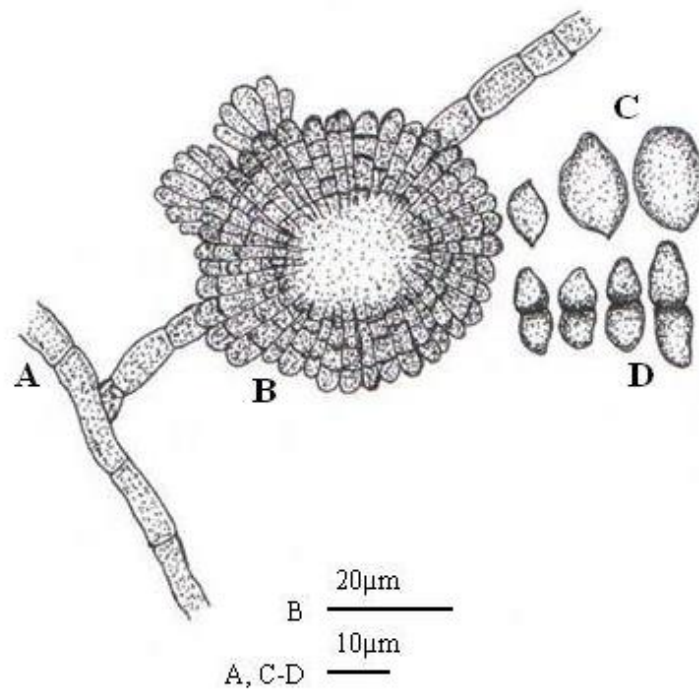
MycoBank: MB 816864

Facesoffungi Number: FoF 02663

Etymology –The specific epithet is named after the host plant species.



**Fig. 1** – *Prillieuxina citricola* sp. nov. A. Black mildew infection on leaf. B. Branched mycelium. C. Thyriothecium (germinating ascospore – arrowed). D,E. Developing thyriothecia. F,G. Ascospores. Scale bars = 10  $\mu$ m



**Fig. 2** – *Prillieuxina citricola* sp. nov. A. Branched mycelium. B. Thyriothecia with stellate dehiscence. C. Pycnothyriospores. D. Ascospores. Scale bars: A, C – D = 10  $\mu$ m, B= 20  $\mu$ m.

**Table 1** Comparative account of present taxon with closely resembled *Prillieuxina* species on Rutaceae and some other plant families.

Species	Colonies	Thyriothecia (µm)	Asci (µm)	Ascospores (µm)	Host
<i>Prillieuxina citricola</i> sp. nov.	Epiphyllous	Up to 143	30.8	11- 16.5 × 4.4- 6.05	<i>Citrus aurantifolia</i>
<i>Prillieuxina aeglicola</i> A. K. Gautam	Epiphyllous to hypophyllous	Up to 114	Up to 22	7.7- 15.4 × 3.3-7	<i>Aegle marmelos</i>
<i>Prillieuxina aquifoliacearum</i> Hosag., Ravikumar & Archana	Hypophyllous	Up to 192	--	16-19 × 6- 10	<i>Ilex denticulata</i>
<i>Prillieuxina pterigotae</i> Hosag. & Abraham	Hypophyllous	Up to 140	Up to 55	26-29 × 12-14.5	<i>Pterigota alata</i>
<i>Prillieuxina polyalthiae</i> Hosag. & Abraham	Epiphyllous	Up to 350	Up to 45	20-23 × 9.5- 11	<i>Polyalthia longifolia</i>
<i>Prillieuxina elaeagni</i> Hosag. & C.K. Biju	Epiphyllous	Up to 235	Up to 30	17-23 × 9-11	<i>Elaeagnus kologa</i>

*Colonies* black, epiphyllous, dense, scattered, coalesced; hyphae superficial, substraight to crooked, branching irregular at acute angles, opposite to alternate, loosely to closely reticulate, 2.75–4.5 (3.42 ± 0.84) µm wide, cells 6–11 (8.58 ± 1.72) µm long; *appressoria* and setae absent; *thyriothecia* few, orbicular, scattered, up to 143 (73.07 ± 30.29) µm in diameter, stellately dehisced at the centre, margin fimbriate; asci globose, ovate, octosporous, up to 30.8 (23.95 ± 4.14) µm in diameter; *ascospores* oblong, brown, 1-septate, constricted at the septum, smooth walled, 11–16.5 (13.75 ± 2.33) × 4.4–6.05 (5.1 ± 0.82) µm; *pycnothyria* similar to thyriothecia, scattered; pycnothyriospores brown, unicellular, pyriform, 10–16.5 (13.62 ± 2.75) × 4.5–9 (6.68 ± 1.92) µm, wall smooth.

Material examined – India, Himachal Pradesh, Bilaspur, Berthin, alt. 686 m (2,495 ft), on leaves of *Citrus aurantifolia* (Christm.) Swingle (Rutaceae), collected A.K. Gautam, 24.11. 2014 (AUMH 1025, holotype).

## Discussion

Twelve species of *Prillieuxina* have been reported from India of which single species i.e. *P. aeglicola* on *A. marmelos* was reported from the family Rutaceae (Gautam 2015). Interestingly, the infection was found associated with the colonies of *Schiffnerula girijae* (Gautam 2014). The present species was therefore, compared with *P. aeglicola*. As comparison with single species is not adequate to propose a new taxon, therefore, the characteristics like colonies, thyriothecia, asci, ascospores and host association of some other species of *Prillieuxina* were also evaluated and compared with present taxon (Table 1). Comparative analysis revealed that this species have larger thyriothecia than *P. aeglicola*. Although, the size of thyriothecia showed similarity with *P. pterigotae* but observed small size of asci and ascospores. Present species showed similarity with *P. pterigotae* based upon size of thyriothecia, but variable in nature of colony (hypophyllous), size of asci (up to 55 µm), ascospores (26-29 × 12-14.5 µm) and plant host (*Pterigota alata*). Similar sized asci were observed with *P. elaeagni*, but here again found variability with reference to size of thyriothecia (up to 235 µm), ascospores (17–23 × 9–11µm) and plant host (*Elaeagnus kologa*). Similar type of comparison of present taxon with various *Prillieuxina* spp. is provided in Table 1. Moreover, species of genus *Prillieuxina* are thought to be host-specific and there are no earlier reports on *C. aurantifolia*. Therefore this should be new species of *Prillieuxina* based on morphology and host-specificity.

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