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ORIGINAL RESEARCH PAPER

Fungi associated with *Pistacia integerrima* with a description of a new species and one new record from India

Ajay Kumar Gautam^{1*}, Shubhi Avasthi²¹ School of Agriculture, Faculty of Science, Abhilashi University, Mandi 175028, Himachal Pradesh, India² Department of Botany, Abhilashi Post Graduate Institute of Sciences, Ner Chowk, Mandi 175008, Himachal Pradesh, India* Corresponding author. Email: a2gautam2006@gmail.com**Abstract**

Pistacia integerrima is a deciduous tree species belonging to the family Anacardiaceae. The plant possesses numerous phytochemicals of ethno-medicinal importance. In a routine mycological survey carried out from July 2013 to June 2014, leaves of *P. integerrima* were found infected with fungi causing rust and blight diseases. The morphological and microscopic observations revealed three fungi, namely *Skierka himalayensis*, *Pestalotiopsis* sp., and *Pileolaria pistaciae*, which were found to cause rust and blight diseases. One new species of rust fungi, namely *Skierka himalayensis* sp. nov., and *Pestalotiopsis* sp. are reported for the first time from India. The detailed descriptions and illustrations of these three phytopathogenic fungi are provided in this paper.

KeywordsIndia; leaf blight; new report; *Pistacia integerrima*; rust; taxonomy**Introduction**

Pistacia integerrima J. L. Stewart ex Brandis is a tree species native to Asia. The plant belongs to family Anacardiaceae. The common names for this species in Hindi are kakar singhi, kakra, and kakring. It is a large deciduous tree having numerous ethno-medicinal properties. Traditionally, the plant parts are used to treat respiratory distress, all types of coughs, jaundice, chronic wounds, dysentery, and also have the antiseptic properties [1–5].

Pistacia integerrima is reported to suffer from a number of fungal diseases. During a routine mycological survey in India carried out from July 2013 to June 2014, two rust and a blight disease on *P. integerrima* caused by different fungal pathogens were noticed. All rust and blight samples were examined in detail for morphological characteristics. In the course of identification of these fungi, two were reported as new records from India.

Material and methods**Specimen sampling**

The specimens infecting leaves of *Pistacia integerrima* were collected in different seasons, specifically: rust samples during the pre-rainy (May–July) and winter (October–December) season, while leaf blight samples in post-rainy (August–October) season. The collection site – Mandi, is located in Himachal Pradesh. The area is a well-known region

of the state and popular for its fertile land as well as plain and hilly regions. Specimens collected were studied in a laboratory and deposited at School of Agriculture, Faculty of Science, Abhilashi University, Mandi, Himachal Pradesh, India.

Fungal isolation and identification

Standard methods for collection, preservation, and description of fungi have been followed. For examination of morphological features of rusts, the specimens were mounted in lactophenol and examined with an Olympus BH-2 microscope. For generic assignment of rust species, the manual of Cummins and Hiratsuka [6] was consulted.

For examination of morphological features of *Pestalotiopsis*, the material was cultured. For this purpose, twigs and young leaves showing blight symptoms were randomly collected, put in polythene bags, and transported to the laboratory for further investigation. Small dissected infected leaf tissues were surface sterilized in 95% ethanol (20 s) followed by 0.525% NaOCl (60 s) and then washed three times with sterilized distilled water. The leaf pieces were incubated aseptically on potato dextrose agar (PDA; Hi Media, India). The cultures were maintained at $25 \pm 2^\circ\text{C}$ for 12 hours. Fungi that grew from lesions were subcultured on PDA containing 50 mg/L streptomycin sulfate to maintain single species cultures. Subsequently, microscopic and cultural examinations were carried out for pathogen identification.

Results – taxonomy

Skierka himalayensis A. K. Gautam & S. Avasthi, sp. nov. (Fig. 1a,b)

Mycobank: MB 821919.

Description. Leaf spots hypophyllous, initiated as small brown to blackish, rounded rust sori, surrounded by a reddish yellow or chlorotic zone. The pustules initially scattered, later coalesced to form a hard dry crust on the leaf surface. Telia subepidermal; teliospores one-celled, sessile, $35.7\text{--}48.3$ (mean \pm SD, 41.26 ± 3.99) \times $10.5\text{--}18.9$ (mean \pm SD, 14.28 ± 3.1) μm in size, walls $2.5\text{--}3.5$ μm thick; teliospores produced in irregular succession, strongly adherent and extruded in long, hair-like columns, germ pore not observed.

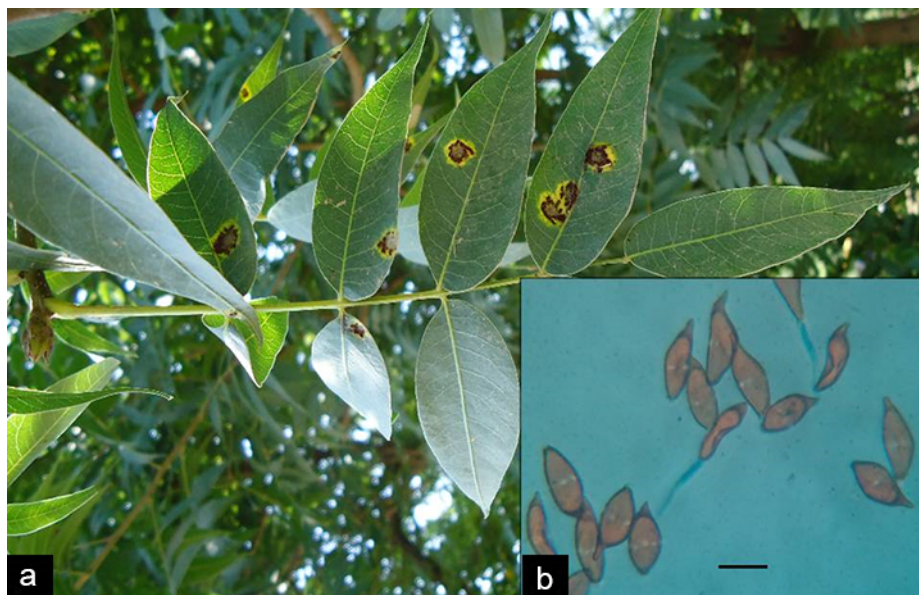


Fig. 1 *Skierka himalayensis*. **a** Leaf showing rust infection. **b** Teliospores seen in LM. Scale bar: 10 μm .

Type. India, Himachal Pradesh: Mandi, elev. ca. 760 m a.s.l. (2495 ft), on leaves of *Pistacia integerrima* (Anacardiaceae), November 24, 2014, leg. A. K. Gautam (holotype: AUMH 1097).

Host and distribution. On *Pistacia integerrima* (Anacardiaceae), India. The rust symptoms were noticed on mature leaves during May 2014. The disease symptoms were observed only on mature leaves.

Notes. There are 13 *Skierka* species reported on different hosts across the world (Index Fungorum, <http://www.indexfungorum.org>, accessed on April 1, 2017). The species newly described here, *Skierka himalayensis*, is easily distinguishable from morphologically similar species, namely *S. canarii* Racib. [7] and *S. petchii* (Syd.) Mains [8]. *Skierka canarii* has smaller teliospores (64–110 µm long) with thinner wall (1.5–2.5 µm). Similarly, broader length range of teliospores, greater wall thickness at sides and apex, and shorter beak size has been observed in *S. petchii* (Syd.). The morphological characters of all reported species of *Skierka* are presented in Tab. 1.

Tab. 1 The morphological characters of *Skierka* species.

Species	Teliospores	References
<i>Skierka himalayensis</i> A. K. Gautam & S. Avasthi	10.5–18.9 (mean ±SD, 14.28 ±3.1) × 35.7–48.3 (mean ±SD, 41.26 ±3.99) µm; wall 2.5–3.5 µm thick	Present study
<i>Skierka congensis</i> Henn.	7–10 × 60–80 µm	[14]
<i>Skierka philippinensis</i> Mains	11–19 × 64–110 µm; wall smooth, hyaline, 1.5–2.5 µm thick	[15]
<i>Skierka canarii</i> Racib.	11–19 × 64–110 µm; wall smooth, hyaline, 1.5–2.5 µm thick	[7]
<i>Skierka diploglottidis</i> (Cooke & Massee) Mains	15–18 × 70–90 µm; wall colorless, 1.5 µm thick	[15]
<i>Skierka petchii</i> (Syd.) Mains	12–18 × 38–44 µm, the apex long attenuate, 25–50 µm long; wall hyaline, inner wall 1 µm thick	[8,15]
<i>Skierka agallocha</i> Racib.	8–12 × 60–100 µm; wall smooth, thin-walled, the apex 18–25 µm	[16]
<i>Skierka robusta</i> Doidge	20–27 × 120–180 µm; acuminate, elongated into a long filiform process; wall 3.0–3.5(–5.0) µm thick	[17]

Pestalotiopsis sp. Steyaert, Bull. Jard. bot. État Brux. 19: 300 (1949) (Fig. 2a–c, Fig. 3a–c)

Description. Mycelial growth on potato dextrose agar (PDA) cottony white; conidia produced in ink-like fruiting bodies. Hyphal tips of the fungus were inoculated on PDA medium, where acervular conidiomata (22.8–29.1 × 5.3–8.9 µm) were developed after 2 weeks of inoculation. Conidia five-celled, smooth, with hyaline cylindrical to conical apical and basal cells; middle conidial cells dark brown with two upper cells sometimes darker; conidial size variable, 18.7–28.6 (mean ±SD, 24.82 ±3.26) × 5.3–9 (mean ±SD, 6.05 ±1.1) µm; both apical and basal cells bear appendages: three apical appendages, 6.6–17.6 (mean ±SD, 9.35 ±3.52) µm long and single basal appendage, 3.85–7.7 (mean ±SD, 5.33 ±1.07) µm long. Based on morphological characters described above, the fungus was identified as *Pestalotiopsis* sp. [9].

Material investigated. India, Himachal Pradesh, Mandi, ca. 760 m a.s.l. (2495 ft), on leaves of *Pistacia integerrima* (Anacardiaceae), September 9, 2013, leg. A. K. Gautam (AUMH 1015).

Hosts and distribution. On *Pistacia integerrima*, *Corylus avellana* L. (Betulaceae), *Juglans regia* L. (Juglandaceae); India, Turkey. In India, the first symptoms of the



Fig. 2 *Pestalotiopsis* sp. a,b Leaves showing blight symptoms. c Mycelial colony grown on potato dextrose agar for 10 days.

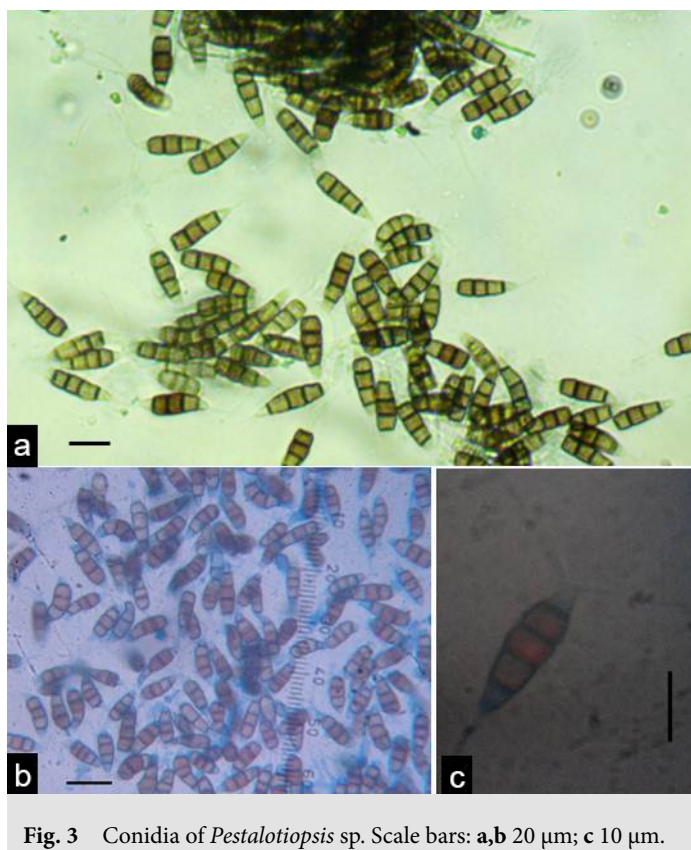


Fig. 3 Conidia of *Pestalotiopsis* sp. Scale bars: a,b 20 µm; c 10 µm.

disease on *Pistacia integerrima* were noticed during August–September 2013. Interestingly, the disease symptoms were observed only on young twigs or leaves. The initiation of disease on leaves started from tips and progressed towards leaf axil.

Pileolaria pistaciae F. L. Tai & C. T. Wei,
Sinensia 4: 108 (1933) (Fig. 4a,b)

Description. Telia epiphyllous, subepidermal, dark-brown to black; teliospores dark brown, discoid or very broadly ellipsoid, one-celled with one germ pore, 18.9–27.3 (mean \pm SD, 22.79 \pm 2.55) \times 25.2–27.3 (mean \pm SD, 26.4 \pm 1.12) μ m in size, teliospore walls pigmented, 1.5–4.2 (mean \pm SD, 2.55 \pm 0.86) μ m thick, chestnut-brown, rugose or reticulated, pedicels hyaline, 52.5–77.7 (mean \pm SD, 61.16 \pm 8.12) μ m long.

Material investigated. India, Himachal Pradesh, Mandi, ca. 760 m a.s.l. (2495 ft), on leaves of *Pistacia integerrima* (Anacardiaceae), November 4, 2013, leg. A. K. Gautam (AUMH 1017).

Hosts and distribution. On *Pistacia* spp. (Anacardiaceae), *Pistacia integerrima*, *P. chinensis* Bunge, *P. weinmannifolia* J. Poiss. ex Franch; Asia (China,

India, Japan, Philippines, Taiwan). In India, the rust symptoms were noticed on mature leaves of *P. integerrima* during initiation of winter season in November 2013 and lasts up to leaf senescence. Symptoms appeared as small rust sori, which were round, hard, and brown to blackish in color. Drying and early dropping of premature leaves were observed when the infection was severe.



Fig. 4 *Pileolaria pistaciae*. **a** Leaves showing rust infection. **b** Teliospores. Scale bar: 10 μ m.

Discussion

Bhardwaj and Sharma [10] conducted a survey for the fungal diseases of *Pistacia integerrima* in district Solan, Himachal Pradesh, India during 1989–1990 and reported four foliar diseases, including leaf spot and yellow and brown leaf rust caused by *Cercospora megaspermae* L. H. Bhardwaj & R. C. Sharma, *Septoria pistaciae* Desm., *Uraecium* sp., and *Pileolaria pistaciae*. A blight disease caused by *Septoria pistaciarum* Caracc. on *Pistacia vera* L. in India was reported by Ahmad et al. [11]. On the other hand, *Pestalotiopsis guepinii* has been reported previously from Turkey on *Pistacia lentiscus* var. *chia* (Desf. ex Poiret) DC. [12] and *Corylus avellana* [13]. *Pestalotiopsis* sp. and *Skierka himalayensis* on *P. integerrima* were not reported previously from India. Additionally, *Skierka himalayensis* represents new species of rust fungi. Both fungi constitute also new records on *Pistacia integerrima*.

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