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Standard Paper

The lichen genus *Pseudopyrenula* (*Trypetheliaceae*) in India

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Abstract

A survey of the lichen genus *Pseudopyrenula* in India is presented, with morphotaxonomic accounts of all six accepted species. Two species, *P. himalayana* and *P. megaspora*, are new to science. Both species resemble *P. staphyleae* but have a lichenized thallus and eccentric ostiole. Furthermore, *P. himalayana* differs from *P. staphyleae* in having immersed perithecia and narrower ascospores, while *P. megaspora* differs in the larger ascospores. Detailed descriptions of the new species are presented, together with notes on their chemistry, distribution, ecology and taxonomy. A key to all known species of *Pseudopyrenula* from India is also presented.

Keywords: Asia; biodiversity; lichenized fungi; pyrenocarpous; revision; taxonomy

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Introduction

The lichen genus *Pseudopyrenula* was established by Müller Argoviensis in 1883 for the species of *Pyrenula* Ach. with dispersed unilocular ascomata and colourless ascospores. Harris (1998) revised the genus *Pseudopyrenula* and recognized four species and one variety, reducing 18 names to synonymy with *P. diluta* (Fée) Müll. Arg. Harris (1998) further proposed 13 recombinations for 70 names formerly assigned to *Pseudopyrenula*. The genus *Pseudopyrenula* can be differentiated from the similar genera *Bogoriella*, *Constrictolumina*, *Dictyomeridium*, *Novomicrothelia* and *Polymeridium* by its unique combination of an ecorticate or indistinct thallus with hyaline, transversely septate, astrothelioid ascospores that have diamond-shaped lumina. Many species within the genus contain yellow oil in the ascospore lumina and/or the hamathecium (Aptroot & Lücking 2016). According to molecular phylogenetic analyses, the genus *Pseudopyrenula* belongs to the family *Trypetheliaceae* (Del Prado *et al.* 2006; Nelsen *et al.* 2009, 2011, 2014; Sweetwood *et al.* 2012; Lücking *et al.* 2016*a*, *b*). The genus is distributed in all tropical regions of the world and is currently represented by 24 species (Aptroot & Lücking 2016; Aptroot *et al.* 2019; Aptroot 2021). Six species of *Pseudopyrenula* have been reported from India: *P. diluta* (Fée) Müll. Arg., *P. media* Aptroot & Diederich, *P. pupula* (Ach.) Müll. Arg., *P. subgregaria* Müll. Arg., *P. subnudata* Müll. Arg. and *P. subvelata* (Nyl.) Müll. Arg. (Awasthi 1991; Jagadeesh Ram *et al.* 2005; Singh & Sinha 2010; Aptroot & Lücking 2016; Jagadeesh Ram 2022). Of these, *Pseudopyrenula pupula* was transferred to *Astrothelium* as *A. pupula* (Ach.) Aptroot & Lücking, while *P. subvelata* was transferred to *Arthopyrenia* as *A. subvelata* (Aptroot & Lücking 2016). The aim of the present study is to update the status of the genus *Pseudopyrenula* in India. In the process, two new species are described and a key to all *Pseudopyrenula* species known so far from India is provided.

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Materials and Methods

The present study is based on specimens preserved in the herbarium LWG and the consultation of protologues. A Leica S8APO stereozoom microscope was used to study the external morphology of the specimens, while anatomical characters of ascomata were observed under a Leica DM500 compound microscope. Thin, hand-cut sections of the ascomata were initially mounted in water to record the colour and measurements of various structures. The sections were then observed after applying aqueous KOH and Lugol's solution (CDH, New Delhi) was used for iodine reactions. Colour tests were performed using routine reagents; aqueous KOH (10%; K), calcium hypochlorite (C) and *para*-phenylene-diamine (Pd). TLC was performed in solvent system C following Orange *et al.* (2001).

Taxonomy

Pseudopyrenula diluta (Fée) Müll. Arg.

Flora, *Regensburg* **66**(16), 249 (1883).

The following description is based on observations made by Aptroot & Lücking (2016). The original specimen has not been examined.

Thallus ecorticate, white, smooth to uneven lacking chemical substances.

Ascomata solitary, 0.3–0.5 mm, prominent, hemispherical, exposed and black with apical ostioles; *hamathecium* inspersed with hyaline oil droplets; *asci* 8-spored; *ascospores* hyaline often partly with yellow oil in the lumina, 3-septate, fusiform-ellipsoid, IKI−, not ornamented, 24–32 × 7–10 µm.

Chemistry. Thallus UV−, K−. TLC: no secondary substances detected.

Distribution. The species is reported from Arunachal Pradesh, Uttarakhand and the West Bengal plains (Singh & Sinha 2010).

Discussion. *Pseudopyrenula diluta* shows a close resemblance to *P. subnudata* in its exposed, black ascomata and inspersed hamathecium but differs in having longer ascospores. Harris (1998) previously treated *P. diluta* and *P. subnudata* as varieties but subsequently both taxa were accepted as distinct species by Aptroot & Lücking (2016).

Pseudopyrenula himalayana Ingle, Nayaka & Upreti sp. nov.

MycoBank No.: MB 850274

Differs from *Pseudopyrenula staphyleae* by a lichenized thallus, immersed perithecia, eccentric ostiole and narrower ascospores measuring 40–53 × 9–11 µm.

Type: India, Himachal Pradesh, Hamirpur district, Jean nala, 500 m, on bark of *Phoenix sylvestris*, 6 April 2003, *D. K. Upreti* et al. 03-001471 (LWG—holotype).

(Fig. 1A–F)

Thallus corticolous, crustose, greyish white, smooth, ecorticate, up to 5 cm, UV−; *photobiont Trentepohlia*.

Ascomata perithecia, immersed, solitary, conical, 0.2–0.5 mm diam. *Ostiole* eccentric, immersed. *Involucrellum* entire, carbonized, 30–52 µm thick. *Hamathecium* hyaline, not inspersed with oil droplets, pseudoparaphyses branched and anastomosing. *Asci* clavate, 8-spored, mostly uniseriate, 135–220 × 18–23 µm. *Ascospores* transversely 3-septate, colourless, fusiform, with angular, diamond-shaped lumina, 40–53 × 9–11 µm.

Chemistry. Thallus UV−, K−. TLC: no secondary substances detected.

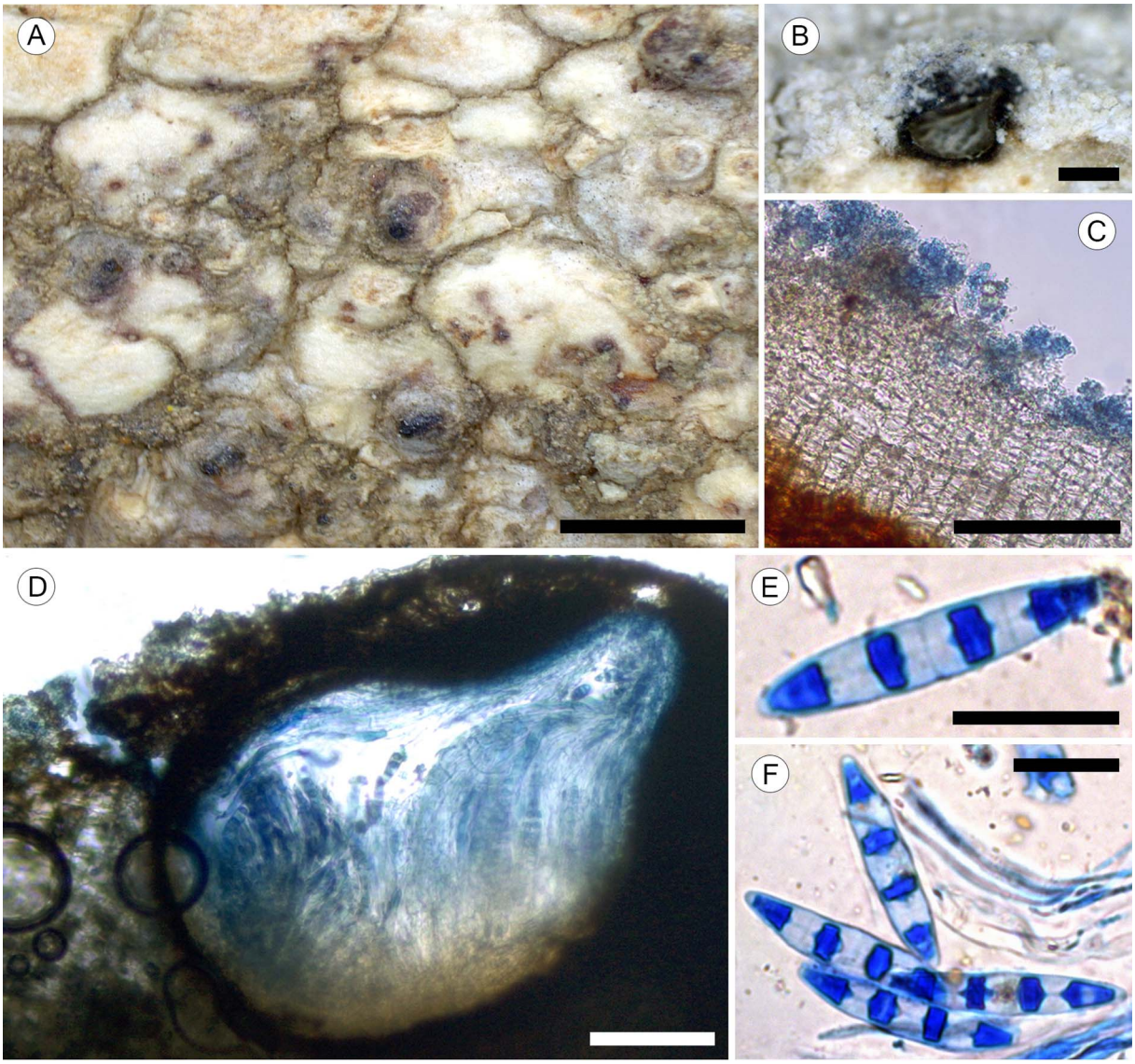

Figure 1. *Pseudopyrenula himalayana* (holotype) A, thallus. B, transverse section of perithecium. C, transverse section of thallus. D, detail of perithecium. E & F, ascospores. Note: C, D, E & F treated with lactophenol cotton blue. Scales: A = 5 mm; B = 0.2 mm; C = 200 µm; D = 100 µm; E & F = 25 µm. In colour online.

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Etymology. The species epithet refers to the type locality within the Himalayan region.

Distribution and ecology. Currently the new species is known only from its type locality, Himachal Pradesh in the foothills of Western Himalaya. It was found on the bark of *Phoenix sylvestris* in deciduous forest with a subtropical climate at an elevation of 500 m.

Notes. The new taxon is characterized by its greyish white, smooth, ecorticate thallus, immersed ascomata with eccentric ostioles, a clear hamathecium and large ascospores (40–53 × 9–11 µm). The thallus is mostly smooth, but occasionally forms granular patches. *Pseudopyrenula himalayana* closely resembles *P. staphyleae* (Petr.) Aptroot in having a clear hamathecium and similar ascospore length but *P. staphyleae* has a non-lichenized thallus, apical ostioles and wider ascospores (12–15 µm) (Table 1). *Pseudopyrenula himalayana* is also close to the other new species, *P. megaspora* Ingle, Nayaka & Upreti, in having eccentric ostioles and a clear hamathecium but *P. megaspora* has a non-carbonized involucrellum and longer ascospores (40–60 µm).

Pseudopyrenula media Aptroot & Diederich

Herzogia **30**(1), 213 (2017).

The following description is based on observations made by Jagadeesh Ram (2022). The original specimen has not been examined.

Thallus ecorticate, white, suborbicular to irregular, smooth, with an anthraquinone as a chemical substance.

Ascomata solitary, sometimes confluent, subglobose to hemispherical, black with apical ostioles; *hamathecium* inspersed with yellow oil droplets; *asci* 8-spored; *ascospores* 3-septate, oblong-fusiform, IKI−, not ornamented, 25–30 × 7.5–10 µm.

Chemistry. Thallus UV−, K−, hamathecium K+ deep yellow to reddish. TLC: anthraquinone.

Distribution. In India, *P. media* is reported only from the Andaman Islands (Jagadeesh Ram 2022).

Discussion. *Pseudopyrenula media* is similar to *P. subgregaria* in its yellow hamathecium inspersion. However, the latter differs in having smaller ascospores (18–22 × 5–7 µm) (Jagadeesh Ram 2022).

Pseudopyrenula megaspora Ingle, Nayaka & Upreti sp. nov.

MycoBank No.: MB 850275

Differs from *Pseudopyrenula staphyleae* by a lichenized thallus, eccentric ostiole and longer and narrower ascospores, measuring 40–60 × 9–11 µm.

Type: India, Uttar Pradesh, Sonbhadra district, Hathinala, 284 m, on bark of tree, 23 June 2008, *Santosh Joshi* 08-015764/B (LWG—holotype).

(Fig. 2A–H)

Thallus corticolous, crustose, smooth to uneven, visible as pale green discoloration of the bark, up to 7 cm, ecorticate, UV−. *Photobiont Trentepohlia,* sparse.

Ascomata perithecia, immersed, solitary, subglobose, 0.2–0.6 mm diam. *Ostiole* eccentric, immersed to erumpent. *Involucrellum* entire, not carbonized. *Hamathecium* hyaline, not inspersed with oil droplets, pseudoparaphyses branched and anastomosing. *Asci* cylindrical, 8-spored, mostly uniseriate, 170–235 × 16–18 µm. *Ascospores* transversely 3-septate, colourless, fusiform, with angular, diamond-shaped lumina, 40–60 × 9–11 µm.

Chemistry. Thallus UV−, K−. TLC: no secondary substances detected.

Etymology. The epithet refers to the large ascospores of the species.

Distribution and ecology. The new species is currently known only from the type locality in the Gangetic plains of Uttar Pradesh. It was growing on the bark of a tree in deciduous forest at an elevation of 284 m.

Notes. *Pseudopyrenula megaspora* is characterized by its ecorticate, pale greenish thallus, eccentric, ascomata with immersed to erumpent ostioles, clear hamathecium and large ascospores (40–60 × 9–11 µm). The species has the largest ascospores of all *Pseudopyrenula* species. Another species, *P. staphyleae*, shows a

Table 1. Comparison of two new species of *Pseudopyrenula* with similar species.

	P. cubana	*P. himalayana*	*P. megaspora*	*P. staphyleae*
Thallus colour	white to pale brownish	greyish white	pale green	brownish
Thallus texture	smooth to uneven	smooth	smooth to uneven	smooth to uneven
Lichenization	lichenized	lichenized	lichenized	non-lichenized
Ascomata	solitary	solitary	solitary	solitary
Ostiole	eccentric, immersed	eccentric, erumpent	eccentric, immersed to erumpent	apical, erumpent to prominent
Hamathecium	inspersed	clear	clear	clear
Ascospore size	21–25 × 6–9 µm	40–53 × 9–11 µm	40–60 × 9–11 µm	45–55 × 12–15 µm
Chemistry	K−, no secondary substances	K−, no secondary substances	K−, no secondary substances	K−, no secondary substances
References	Aptroot & Lücking (2016)			Aptroot (1998), Aptroot & Lücking (2016)

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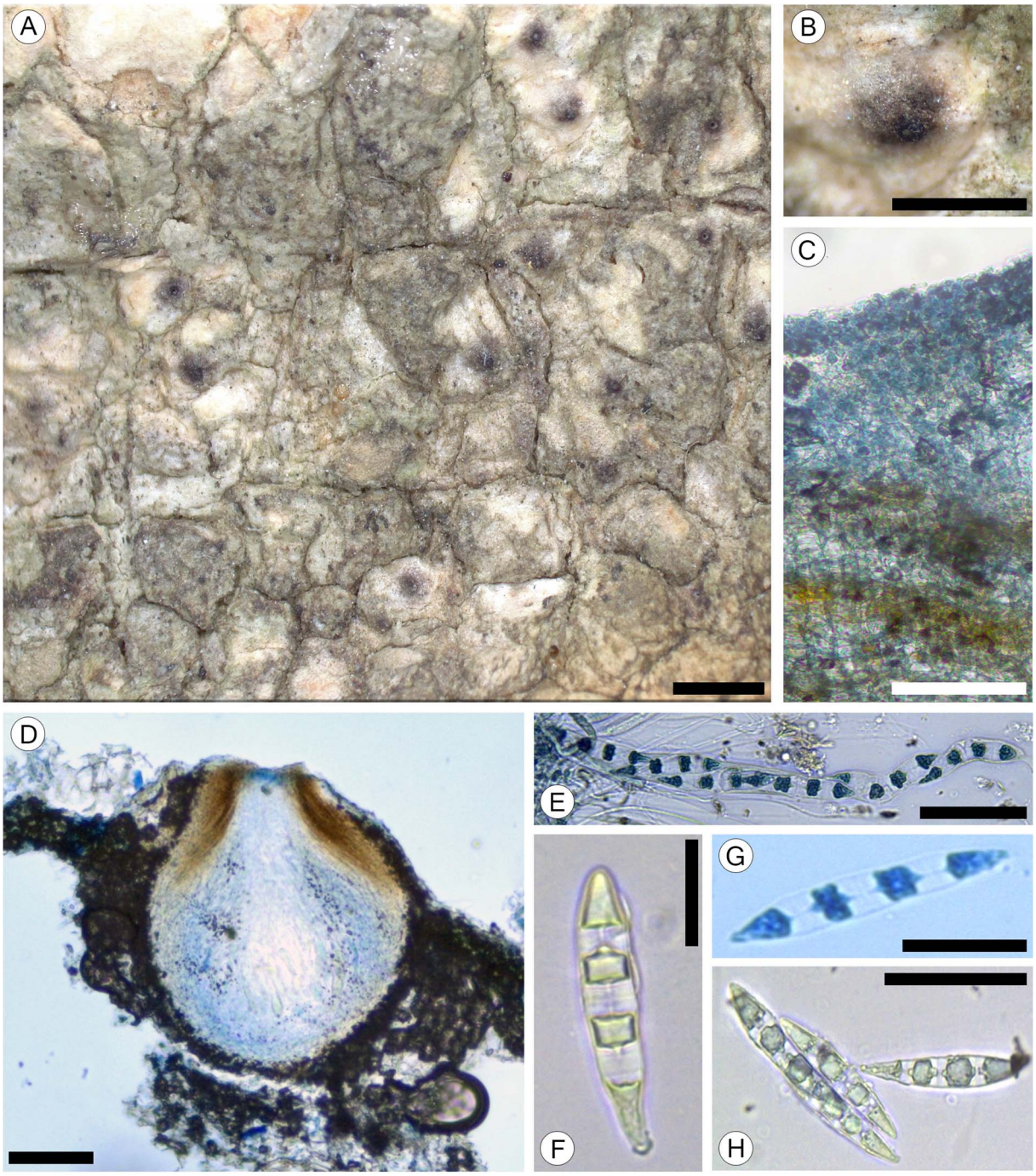

Figure 2. *Pseudopyrenula megaspora.* (holotype) A, thallus. B, perithecium. C, transverse section of thallus. D, transverse section of perithecium. E, ascus. F–H, ascospores. Note: C, D, E & G treated with lactophenol cotton blue. Scales: A = 1 mm; B = 0.5 mm; C = 200 μm; D = 100 μm; E & H = 50 μm; F & G = 25 μm. In colour online.

close resemblance to the new species in having a similar thallus coloration and a clear hamathecium, but it differs in having a non-lichenized thallus, apical ostioles and wider ascospores (45–55 × 12–15 μm). The other two species of *Pseudopyrenula* with eccentric ostioles are *P. himalayana* and *P. cubana* (Müll. Arg.) Aptroot & Lücking (Table 1). However, *P. himalayana* differs in having a carbonized involucrellum and slightly shorter ascospores (40–53 μm), and *P. cubana* differs in having erumpent ostioles, an inspersed hamathecium and smaller ascospores (21–25 × 6–9 μm).

Pseudopyrenula subgregaria Müll. Arg.

Bot. Jb. **6**, 408 (1885).

The following description is based on observations made by Jagadeesh Ram (2022). The original specimen has not been examined.

Thallus ecorticate, white, smooth to uneven, with an anthraquinone as a chemical substance.

Ascomata solitary, 0.3–0.4 mm diam., prominent, hemispherical, exposed and black, with apical ostioles; *hamathecium*

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inspersed, with yellow oil droplets; *asci* 8-spored; *ascospores* hyaline, 3-septate, fusiform-ellipsoid, IKI−, not ornamented, 18–22 × 5–7 µm.

Chemistry. Thallus UV−, K−, hamathecium K+ deep yellow to reddish. TLC: anthraquinone.

Distribution. This species is reported from West Bengal and the Andaman Islands (Jagadeesh Ram 2022).

Discussion. The species shows a close resemblance to *Pseudopyrenula subnudata* in having an ecorticate thallus and ascospores of a similar size, but *P. subnudata* differs in having an inspersed hamathecium with hyaline oil droplets (Aptroot & Lucking 2016).

Pseudopyrenula subnudata Müll. Arg.

Flora, Regensburg **66**(16), 249, 272 (1883).

The following description is based on observations made by Jagadeesh Ram *et al.* (2005). The original specimen has not been examined.

Thallus ecorticate, white, smooth to uneven, with no chemical substance.

Ascomata solitary, 0.2–0.4 mm diam., prominent, hemispherical, exposed and black with apical ostioles; *hamathecium* hyaline, inspersed with hyaline oil droplets; *asci* 8-spored; *ascospores* hyaline, sometimes yellow oil in the lumina, 3-septate, fusiform-ellipsoid, IKI−, not ornamented, 21–25 × 6–9 µm.

Chemistry. Thallus UV−, K−. TLC: no secondary substances detected.

Distribution. This species is reported from Arunachal Pradesh, West Bengal and Odisha (Singh & Sinha 2010; Singh *et al.* 2015; Nayak *et al.* 2016).

Discussion. *Pseudopyrenula diluta* and *P. subgregaria* resemble *P. subnudata* morphologically and anatomically, but *P. diluta* differs in having longer ascospores (24–32 µm), and *P. subgregaria* contains anthraquinones and has a yellow hamathecium inspersion.

Key to the species of *Pseudopyrenula* in India

1	Ostiole eccentric	2
	Ostiole apical	3
2(1)	Involucrellum carbonized, ascospores 40–53 × 9–11 µm	**P. himalayana**
	Involucrellum non-carbonized, ascospores 40–60 × 9–11 µm	**P. megaspora**
3(1)	Hamathecium inspersed with hyaline oil droplets	4
	Hamathecium inspersed with yellow oil droplets	5
4(3)	Ascospores 21–25 × 6–9 µm	**P. subnudata**
	Ascospores 24–32 × 7–10 µm	**P. diluta**
5(3)	Ascospores 25–30 × 7.5–10 µm	**P. media**
	Ascospores 18–22 × 5–7 µm	**P. subgregaria**

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