

# New record of *Psathyrella squarrosa* (Psathyrellaceae) from India with additional notes

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## Abstract

A remarkable *Psathyrella* was collected on several occasions during our diversity study on the agaricoid fungi of Kerala State, India. The macro- and micromorphological features in conjunction with nrITS-based phylogenetic analysis identified the collections as *Psathyrella squarrosa*, a recently described species from China. A reassessment of the species is provided based on collections made in Kerala State, India. This forms the first world report of the species after the type.

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## INTRODUCTION

Within the cosmopolitan fungal genus *Psathyrella* (Fr.) Quél., about 550 species have been recognised world wide<sup>[1–10]</sup>. The genus is characterised by mostly delicate, fragile basidiomata, small to large hygrophanous pileus, central, exannulate stipe, basidiospores brownish typically with a truncated germ pore with smooth to complex wall, fading to greyish in concentrated sulphuric acid and hyphae with clamp connections<sup>[1,11]</sup>.

As in most other cases of agaricoid genera, knowledge on the genus *Psathyrella* in India is inadequate, irregular and uneven. Only 60 taxa were reported from this vast country<sup>[12–13]</sup> and only nine species from Kerala State<sup>[14]</sup>. In an investigation on the diversity of the genus *Psathyrella* of Kerala State, India, basidiomes of an apparently psathyrelloid fungus were spotted on several occasions on a dead angiosperm tree trunk in Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) campus. Detailed morphological and molecular studies confirmed it as *Psathyrella squarrosa* T. Bau & J.Q. Yan, a recently described species from China and so far known only from the type locality<sup>[15]</sup>. An updated account of the species is provided based on detailed morpho-anatomical and molecular studies.

## MATERIALS AND METHODS

### Morphological studies

Freshly collected *Psathyrella* specimens from Kerala State, India were exclusively used for the macromorphological descriptions. Color codes follow Kornerup & Wanscher<sup>[16]</sup>. Microscopic characters were studied on sections of basidiomata revived in 3% solution of KOH and stained with 1% Congo red, and were examined under an Olympus CX43 optical microscope (Olympus, Japan). Twenty basidiospores from each collection were measured for spore dimensions, range of spore quotient (Q) and its mean value. All collections studied were

preserved in the Mycological Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram [TBGT (M)], India.

### DNA sequencing and phylogenetic analysis

DNA extraction from the specimens of *Psathyrella* was carried out using the methods as mentioned in Izumitsu et al.<sup>[17]</sup>. The Internal Transcribed Spacer region (nrITS) was amplified and sequenced from the newly collected specimens. The ITS region was amplified using primer pairs ITS1 and ITS4<sup>[18]</sup>. The protocols for PCR amplification and sequencing followed Kumar et al.<sup>[19]</sup> and the sequences generated from TBGT(M) 18695 are deposited in GenBank [OM301743 (nrITS)]. nrITS sequences generated from the Kerala collection and additional GenBank sequences retrieved were used for the phylogenetic analyses (Table 1). Sequences were aligned using MAFFT ([www.ebi.ac.uk/Tools/msa/mafft](http://www.ebi.ac.uk/Tools/msa/mafft)) with default settings. The final aligned data matrix (584 bp) had 26 taxa, including *Coprinellus christianopolitanus* Örstadius & E. Larss., as an outgroup<sup>[15]</sup> which were then imported into BioEdit v7.2.6.1<sup>[20]</sup> for manual adjustment. Maximum likelihood (ML) analysis was conducted in MEGA X<sup>[21]</sup> using Tamura-Nei mode<sup>[22]</sup> which was selected automatically as the best fit substitution model as per BIC score in the same web platform.

## RESULTS

### Molecular phylogeny

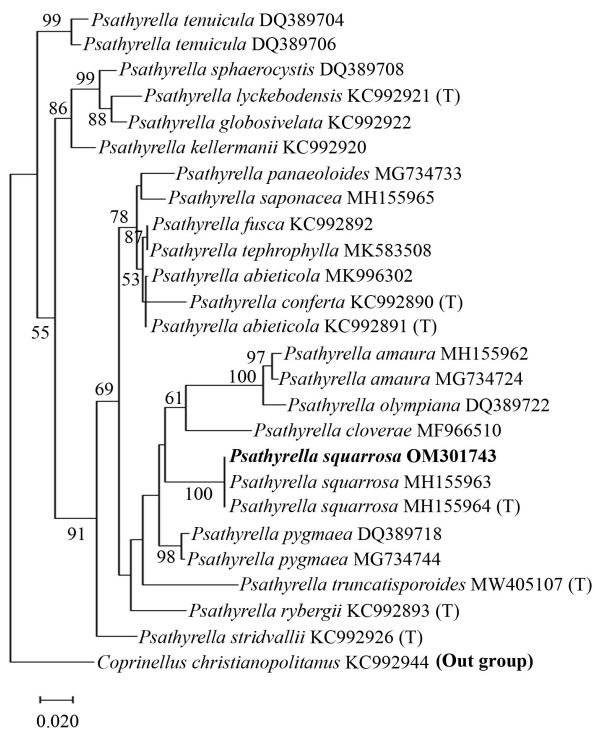
Molecular phylogenetic analysis data of the nrITS sequences (Fig. 1) of the Kerala collections strongly supports the identity of the present collection as *Psathyrella squarrosa* T. Bau & J.Q. Yan<sup>[15]</sup> and cluster with the holotype from China (MH155964) with 100% ML Bootstrap support.

### Taxonomy

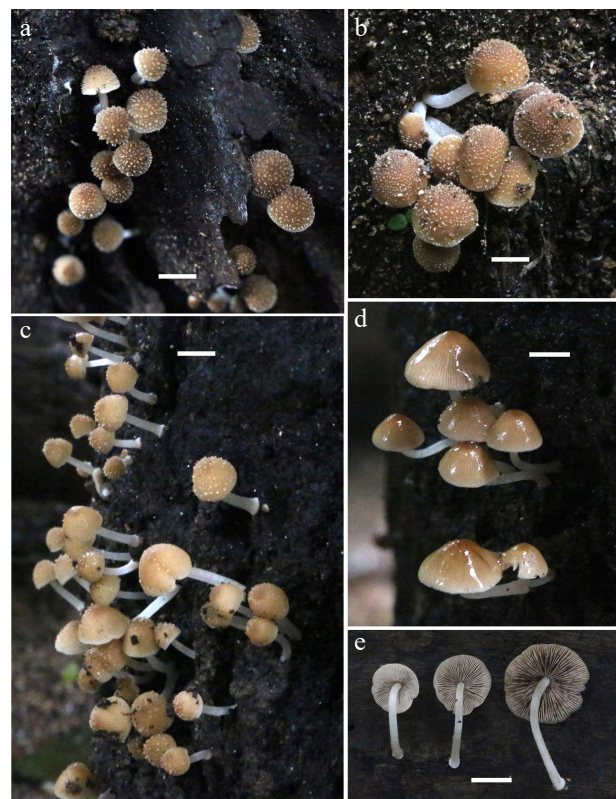
*Psathyrella squarrosa* T. Bau & J.Q. Yan, *Mycosystema* 40(3): 466 (2021)<sup>[15]</sup> (Figs 2 & 3)

**Table 1.** List of species, source and GenBank accession numbers of ITS sequences used in the molecular analysis. Type specimens are identified with a 'T'.

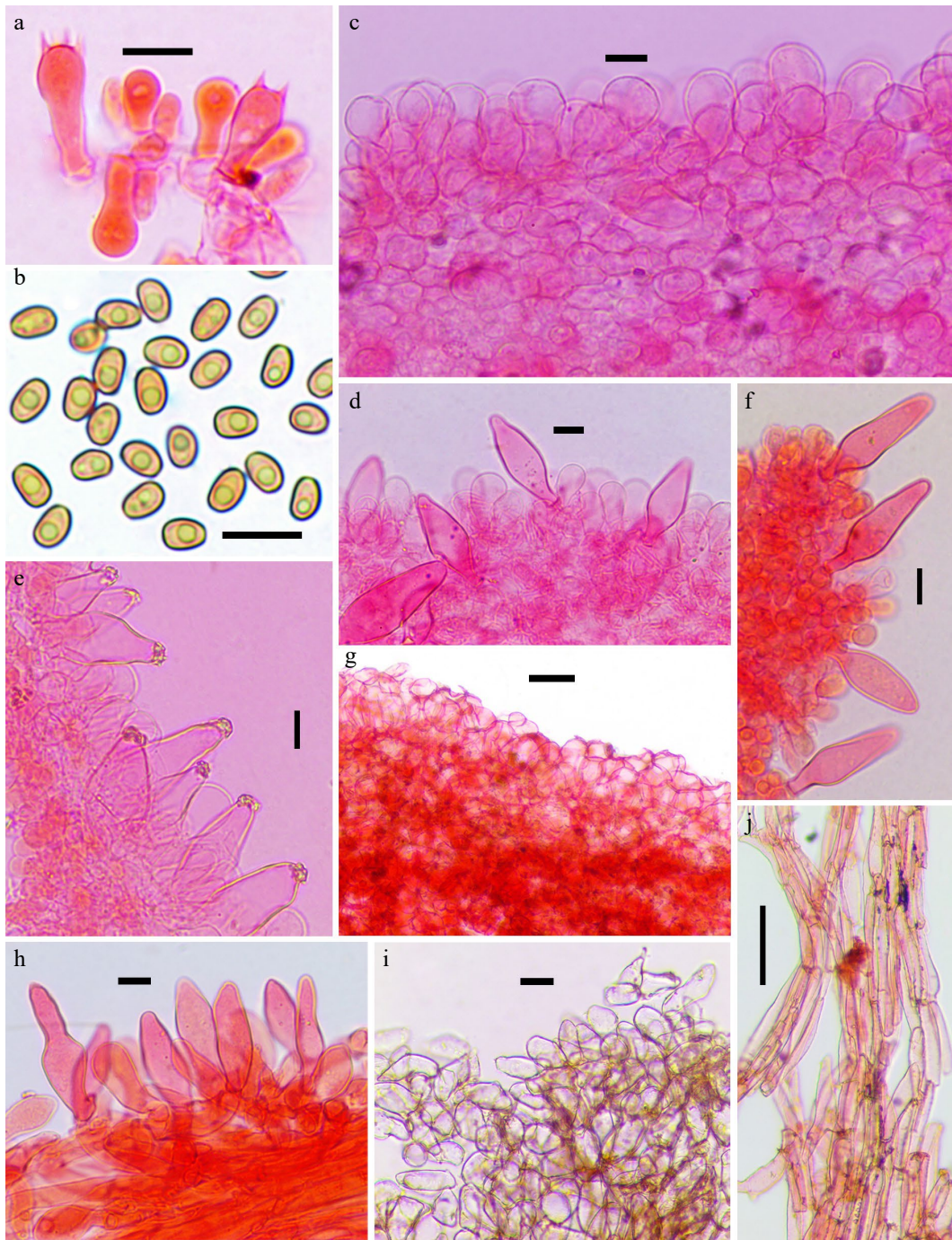
Taxon	Specimen	GenBank acc. no.	Geographic origin	Reference
<i>Psathyrella abieticola</i>	Smith58673 T	KC992891	America	Non referenced
<i>P. abieticola</i>	JLF6233	MK996302	America	Non referenced
<i>P. amaura</i>	HMJAU37810	MG734724	China	Yan & Bau <sup>[23]</sup>
<i>P. amaura</i>	HMJAU37907	MH155962	China	Yan & Bau <sup>[23]</sup>
<i>P. cloverae</i>	INB-3718172	MF966510	–	Non referenced
<i>P. conferta</i>	GE2.007 T	KC992890	France	Non referenced
<i>P. fusca</i>	LÖ287-04	KC992892	Sweden	Non referenced
<i>P. globosivelata</i>	Schumacher035	KC992922	Germany	Non referenced
<i>P. kellermanii</i>	de Meulder11242	KC992920	Belgium	Non referenced
<i>P. lyckebodensis</i>	LÖ301-11 T	KC992921	Sweden	Örstadius et al. <sup>[8]</sup>
<i>P. olympiana</i>	LÖ32-02	DQ389722	Sweden	Larsson & Örstadius <sup>[24]</sup>
<i>P. panaeoloides</i>	HMJAU 23696	MG734733	China	Yan & Bau <sup>[23]</sup>
<i>P. pygmaea</i>	LÖ97-04	DQ389718	Sweden	Larsson & Örstadius <sup>[24]</sup>
<i>P. pygmaea</i>	HMJAU 37850	MG734744	China	Yan & Bau <sup>[23]</sup>
<i>P. rybergii</i>	LÖ373-06 T	KC992893	Sweden	Örstadius et al. <sup>[8]</sup>
<i>P. saponacea</i>	HMJUA 37935	MH155965	China	Non referenced
<i>P. sphaerocystis</i>	TROMSivertsen65-89	DQ389708	Sweden	Larsson & Örstadius <sup>[24]</sup>
<i>P. squarrosa</i>	TBGT18695	<b>OM301743</b>	India	<b>This paper</b>
<i>P. squarrosa</i>	HMJAU 37828	MH155963	China	Yan & Bau <sup>[15]</sup>
<i>P. squarrosa</i>	HMJAU37827 T	MH155964	China	Yan & Bau <sup>[15]</sup>
<i>P. stridvallii</i>	LÖ104-98 T	KC992926	Sweden	Örstadius et al. <sup>[8]</sup>
<i>P. tenuicula</i>	LÖ37-04	DQ389704	Sweden	Larsson & Örstadius <sup>[24]</sup>
<i>P. tenuicula</i>	LÖ58-03	DQ389706	Sweden	Larsson & Örstadius <sup>[24]</sup>
<i>P. tephrophylla</i>	DD-INDET	MK583508	Belgium	Non referenced
<i>P. truncatisporoides</i>	HMJAU37947 T	MW405107	China	Bau & Yan <sup>[10]</sup>
<b>Outgroup</b>				
<i>Coprinellus christianopolitanus</i>	LO141-08 T	KC992944	Sweden	Örstadius et al. <sup>[8]</sup>



**Fig. 1** Maximum likelihood tree generated from ITS sequence data. Bootstrap values > 50% are indicated above/below branches. Collection from Kerala, India is shown in bold. GenBank accession numbers are given after each taxon name.



**Fig. 2** *Psathyrella squarrosa*. (a–d) Habit in situ. (e) Lamella view. Scale bars = 10 mm.



**Fig. 3** *Psathyrella squarrosa*. (a) Basidia. (b) Basidiospores. (c) Lamella edge with paracystidia. (d) Cheilocystidia. (e) Pleurocystidia with crystals. (f) Pleurocystidia. (g) Pileipellis. (h) Caulocystidia. (i) Veil cells. (j) Veil hyphae. Scale bars: a–f, h, i = 10 μm; g, j = 50 μm.

Index Fungorum number: IF824883; Facesoffungi number: FoF10835.

Basidiomata small, up to 52 mm high, fragile. Pileus 3–21 mm diam., parabolic in buds, becoming conical, convex to broadly convex with or without a small obtuse umbo at centre; surface brown (6E5) to dark brown (6F5) in buds with off white to yellowish white (4A2) upright to recurved velar squamules throughout, vanishing when mature or on handling; later become brownish orange (5C4/5D5) to light brown (5D6) in mature basidiomata, pellucid striate up to the disc, with or without velar remnants, moist to dry, hygrophanous; margin

straight, entire to incised. Lamellae adnexed, yellowish white (4A2) in buds, later become greyish orange (5B3/5C3) to brown (6D3–6E4) when mature, up to 2.5mm wide, crowded with lamellulae of 3–4 lengths; edge white, minutely fimbriate. Stipe 8–50 × 1–2.5 mm, central, cylindric, hollow, curved, equal with a slightly broad base, brittle; surface white, turning brownish orange (5C4) on keeping, floccose throughout in buds, vanishing on handling; base with white strigose hairs and mycelial mat. Context thin, white up to 1 mm thick at the disc, soft. Odor mild, not distinctive. Spore print dark brown (7F5).

Basidiospores 6.0–6.4 × 3.6–4.0 μm (avL = 6.12 μm, avW = 3.72 μm), Q 1.5–1.87 μm (Qm = 1.55 μm), ellipsoid to elongate, subphaseoliform, brownish orange in water, greyish brown in 3% aqueous KOH, fading in concentrated H<sub>2</sub>SO<sub>4</sub>, thick-walled, smooth, germ pore indistinct. Basidia 16.4–19.2 × 6.0–7.2 μm, clavate, 2, 4-spored, thin-walled, hyaline. Gill edge sterile with both cheilocystidia and paracystidia. Cheilocystidia scattered, scarce, 20.0–34.6 × 11.2–15.2 μm, utriform to broadly utriform with obtuse to subobtuse apex, slightly thick-walled, hyaline, apex with crystals, dissolving in 3% aqueous KOH. Paracystidia crowded, 14–32 × 10–22 μm, clavate, broadly clavate to vesiculose, thin-walled, hyaline. Pleurocystidia abundant, scattered, 33–46 × 9.6–15 μm, similar to cheilocystidia, narrowly utriform, to very broadly utriform, with broadly obtuse apex, rarely with subcapitate to bifurcate apex, thick-walled with abundant crystals at apex, dissolving in 3% aqueous KOH. Hymenophoral trama regular, hyphae 3.2–18.0 μm broad, inflated thin-walled, hyaline. Subhymenium pseudoparenchymatous. Pileal trama composed of thin-walled, hyaline, branched, inflated, 4–36 μm broad hyphae constricted at septa. Pileipellis is an epithelium composed of globose to subglobose cells, 26.0–65.6 × 25.6–41.5 μm, thin-walled, hyaline. Stipitipellis composed of thin-walled, hyaline, 4–14 μm broad hyphae with clamp connections. Stipe contains both caulocystidia and paracystidia throughout. Caulocystidia in clusters, crowded at the stipe apex, abundant, 22.4–52.0 × 8.0–12.8 μm, versiform, lageniform to narrowly utriform, utriform, flexuous with acute, obtuse, broadly obtuse, mucronate to rostrate apex, slightly thick-walled, hyaline, apex with crystals, dissolving in 3% aqueous KOH. Paracystidia in clusters, crowded at the apex, abundant, 20–28 × 10.0–13.6 μm, clavate, thin-walled, hyaline. Oleiferous hyphae present in all tissues. Velar squamules consists of both hyphae and subglobose to ellipsoid cells; 43.6–19.2 × 4–22.8 μm, thin-walled, hyaline; hyphae, 3.2–20.0 μm wide, hyaline, thin-walled with clamp connections.

Habit, habitat and phenology—Scattered to gregarious on dead angiosperm wood in tropical evergreen forest, Kerala State, India. September - December.

Materials examined—India, Kerala State, Thiruvananthapuram district, Palode, JNTBGRI campus, 19 Sep 2021, TBGT(M)18694; *ibid.*, 20 Sep 2021, TBGT(M) 18695; *ibid.*, 04 Oct 2021, TBGT(M)18713; *ibid.*, 11 Oct 2021 TBGT(M)18718; *ibid.*, 29 Oct 2021, TBGT(M)18737; *ibid.*, 2 Nov 2021, TBGT(M)18738; *ibid.*, 03 Nov 2021, TBGT(M)18741; *ibid.*, 05 Nov 2021, TBGT(M)18743; 10 Nov 2021, TBGT(M)18745; *ibid.*, 11 Nov 2021, TBGT(M)18748; *ibid.*, 19 Nov 2021, TBGT(M)18751; *ibid.*, 30 Nov 2021, TBGT(M)18752; 2 Dec 2021, TBGT(M)18753.

## DISCUSSION

*Psathyrella squarrosa* is a recently described species from China and so far known only from the type locality<sup>[15]</sup>. It is characterized by small basidiomata, conico-convex hygrophanous pileus, adnexed lamellae, central, cylindrical, hollow, brittle stipe, base with white strigose hairs and white mycelial mat, ellipsoid, elongate subphaseoliform basidiospores with indistinct germ pore, sterile lamella edge with abundant paracystidia and scattered cheilocystidia, abundant thick-walled pleurocystidia with crystals at apex, versiform thick-walled caulocystidia and thin-walled paracystidia, velar

squamules with both hyphae and subglobose cells and presence of clamp connections. The Indian collections match with the type description both morphologically and molecularly<sup>[15]</sup>. Additional features observed in the Indian collections include: strigose stipe base and mycelial mat; abundant thin-walled clavate, vesiculose paracystidia in the lamella edge; abundant pleurocystidia with crystals at apex, but lacking yellow deposition at apex and versiform caulocystidia intermingled with thin-walled paracystidia.

The comparison of sequence data generated from *P. squarrosa* with the nucleotide sequences of taxa available in GenBank shows 100% identity (Fig. 1) with *P. squarrosa* from China (MH155964).

*Psathyrella squarrosa* is very closely related to *P. pygmaea* (Bull.:Fr.) Sing. in most macro- and micromorphological characters. However, *P. pygmaea* is distinct by its smaller basidiomata, slightly larger basidiospores (5.5–7 × 3.5–4.5 μm) with distinct and large germ pores<sup>[4]</sup>.

The discovery of *Psathyrella squarrosa* from India is significant as it is so far known only from the type collections in China. This report thus forms the first ever report of this species outside the type locality and is phytogeographically significant. In China, *P. squarrosa* was found in hardwood forest on rotten wood while in India the species was found in evergreen forest on dead angiosperm wood. Smaller basidiospores, thick-walled muricate pleurocystidia place *P. squarrosa* in subg. *Psathyra*, sect. *Spadiceae* (Morg.) Kits van Wav.<sup>[4]</sup>.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## Dates

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New record of *Psathyrella squarrosa*

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