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Research Article

STROBILOMYCES POLYPYRAMIS — REDISCOVERY OF A WILD MUSHROOM FROM SIKKIM, INDIA

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ABSTRACT
Strobilomyces polypyramis which was reported earlier from India in 1851 is rediscovered from North district of Sikkim and described here with its macro- and micromorphological details supported with illustrations. Its relations with the allied taxa are also discussed.

Keywords: *Macrofungi, Boletaceae, Strobilomyces, Sikkim, India, Taxonomy*

INTRODUCTION
The taxonomic study of the genus *Strobilomyces* Berk. (Boletaceae) dates back to Berkeley (1851) who rendered an invaluable service by working on a number of macrofungi collected by Dr. Hooker from different parts of India including Sikkim Himalaya. This genus is one of the easily identifiable genera amongst the boletes. It is characterized as: pileus coarsely fibrillose-squamulose or scaly; scales grey to black; pore surface white when young, becoming black with maturity; stipe dry, wooly to shaggy or floccose to villose (completely or partly), partly reticulate or smooth, with or without an annulus, mostly concolorous to pileus; spore print blackish brown to black; spores globose to broadly ellipsoid with partial to complete reticulum. At present, this genus is represented by seven species from India, namely, *Strobilomyces montosus* Berk. (from Jilla-pahar/Jalapahar of W.B.), *S. annulatus* Corner (from Kulu, Manali, H.P.), *S. polypyramis* Hook. f. (from Jilla-pahar/Jalapahar of W.B.), *S. kalimpongensis* Bose (from Kalimpong, W.B.), *S. mollis* Corner (from Summer-Hill, Kharapahar, Kalatop of H.P.), *S. nigricans* Berk. (from Kala-panee/Kalapani, Assam) and *S. strobilaceus* (Scop.) Berk. (from Sonmarg, J & K). Morphological characters of *Strobilomyces indica* Lloyd, reported earlier (Lloyd, 1924) from this subcontinent does not show sufficient deviation from *S. strobilaceus* (Murrill, 1924). Hence, in the present paper it is not considered as a separate taxon and kept under *S. strobilaceus*. Another species: *S. floccopus* (Vahl) P. Karst., reported earlier from this subcontinent (Lakhanpal, 1996) is now considered as a synonym of *S. strobilaceus*.
During a recent macrofungal survey to North district of Sikkim (a small Himalayan state in India), the first author came across a number of ectomycorrhizal macrofungi including specimens of *Strobilomyces* which after thorough examination appeared as *S. polypyramis* which has not been reported after 1851 from this country and hence is a rediscovery after a gap of over 160 years. As the micromorphological study form holotype deposited in K was not so fruitful because of the bad condition of the exsiccatum (Horak, 1980) the description after the protologue (Berkeley, 1851) and that of Boedijn (1951) and Horak (1980) were considered as authentic. Detailed macro- and micromorphological characters coupled with field and microscopic illustrations are given for the first time from India as well.

MATERIALS AND METHODS
Macromorphological characterization was made from the fresh basidiomata. Field photographs of the fresh basidiomata were taken with the aid of Nikon D300s. Colour codes and terms (mostly) are as in the Colour identification chart of British Fungus Flora (Henderson *et al.*, 1969) and Methuen Handbook of Colour (Kornerup and Wanscher, 1978) which are referred in the description as ‘a’ and ‘b’ successively. After recording the macromorphological characters, basidiomata were dried in the base camp with a field drier. Herbarium names are after Holmgren *et al.*, 1990.

In the laboratory, macromorphological characters were again observed from the dry samples with the help of a stereo zoom dissecting microscope Nikon SMZ 1500 and photographs were taken through the attached dedicated camera. Micromorphological characters were noted with the aid of a light microscope: Olympus CX 41 based on the dry samples stained in a mixture of 5 % KOH, Phloxin and mounted in 30% Glycerol. Amyloidy test were done in Melzer's reagent. Drawings of all the micromorphological structures were made with the help of a drawing tube at an original magnification of 1000×. Basidium length excludes the length of sterigmata. Spore measurements are recorded based on that of twenty basidiospores. Spores are measured in side view. Spore-measurement and Quotient indicating length-width ratio (Q = L/W) are presented as minimum–**mean**–maximum. Scanning Electron Microscope (SEM) illustrations of basidiospores were obtained from dry spores from spore print that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with gold coating at different magnifications in high vacuum mode to observe patterns of spore-ornamentation. SEM work was carried out with a FEI's Quanta FEG 250 model imported from The Netherlands and installed at the S.N. Bose National Centre for basic Sciences, Kolkata, India.

Taxonomy

A Provisional Key to the Species of Strobilomyces in India

1. Spores dacryoid, up to 7 µm long……..*S. kalimpongensis*

1a. Spores globose to broadly ellipsoid, more than 7 µm in length…..……..……..2

2. Spores with a complete/uninterrupted reticulum ……..……..3

2a. Spores with isolated spinoid warts and/or confluent cylindrical to conic warts and/or ridges to form echinulate pattern or broken to incomplete reticulum ……..……..5

3. Pore surface yellow; context brown ……..*S. montosus*

3a. Pore surface white or reddish, finally black after bruising; context white becoming reddish brown to black on exposure ……..……..4

4. Annulus present ……..……..…..*S. strobilaceus*

4a. Annulus absent ……....……...*S. mollis*

5. Annulus present ……..…….*S. annulatus*

5a. Annulus absent ……..6

6. Spores large, 9.5 – 12 µm, with crest like ornamentation to form mostly incomplete reticulum ……..…*S. nigricans*

6a. Spores comparatively smaller, 8.0 – 10.8 × 7.0 – 10.0 µm, echinulate with isolated or confluent cylindrical to conic warts ……..……..……...*S. polypyramis*

Strobilomyces polypyramis Hook. f., in Berkeley, *Hooker's J. Bot. Kew Gard. Misc.* **3**: 78 (1851).

Figure 1 & 2

Pileus 55 – 105 mm diam., convex when young, becoming broadly convex with maturity (never becoming flat), surface dry, unpolished, whitish, squamulose, covered with numerous mostly erect, small conical to pyramidal, pointed or rarely spinoid black scales (10 – 20 mm high); margin mostly incurved to decurved with irregularly torn cottony flaps of partial veil. *Pore surface* white (b: 1A1) when young, slowly becoming grey to black, turning orange to pastel red (b: 7A5), then light brown (b: 7D 4 – 5), brown (b: 7E5) to fuscous black (a: 36) when bruised; pore angular, 1-2 mm wide (0.4-1mm wide after drying). *Tubes* up to 13 mm long, decurrent to sinuate, white, quickly turning light orange (b: 6A5), then gradually black. *Stipe* 70 – 80 x 10 – 18 mm, central, nearly equal but distinctly enlarged to bulbous at base, surface dry, finely reticulate and somewhat floccose at apex, rest is almost smooth except the base which is villose or wooly (in stereo-zoom microscope), cigar brown (a: 16) at apex, gradually fuscous black (a: 36); veils and annulus absent. *Context* solid in stipe, white, quickly becoming light orange (b: 6A5), then sahara (b: 6C5) and finally fuscous black, turning cadmium orange (b: 5A8) to saffron (a: 49) with KOH, bluish grey with $FeSO_4$. *Odour* not distinctive. *Taste* mild. *Spore print* blackish brown.

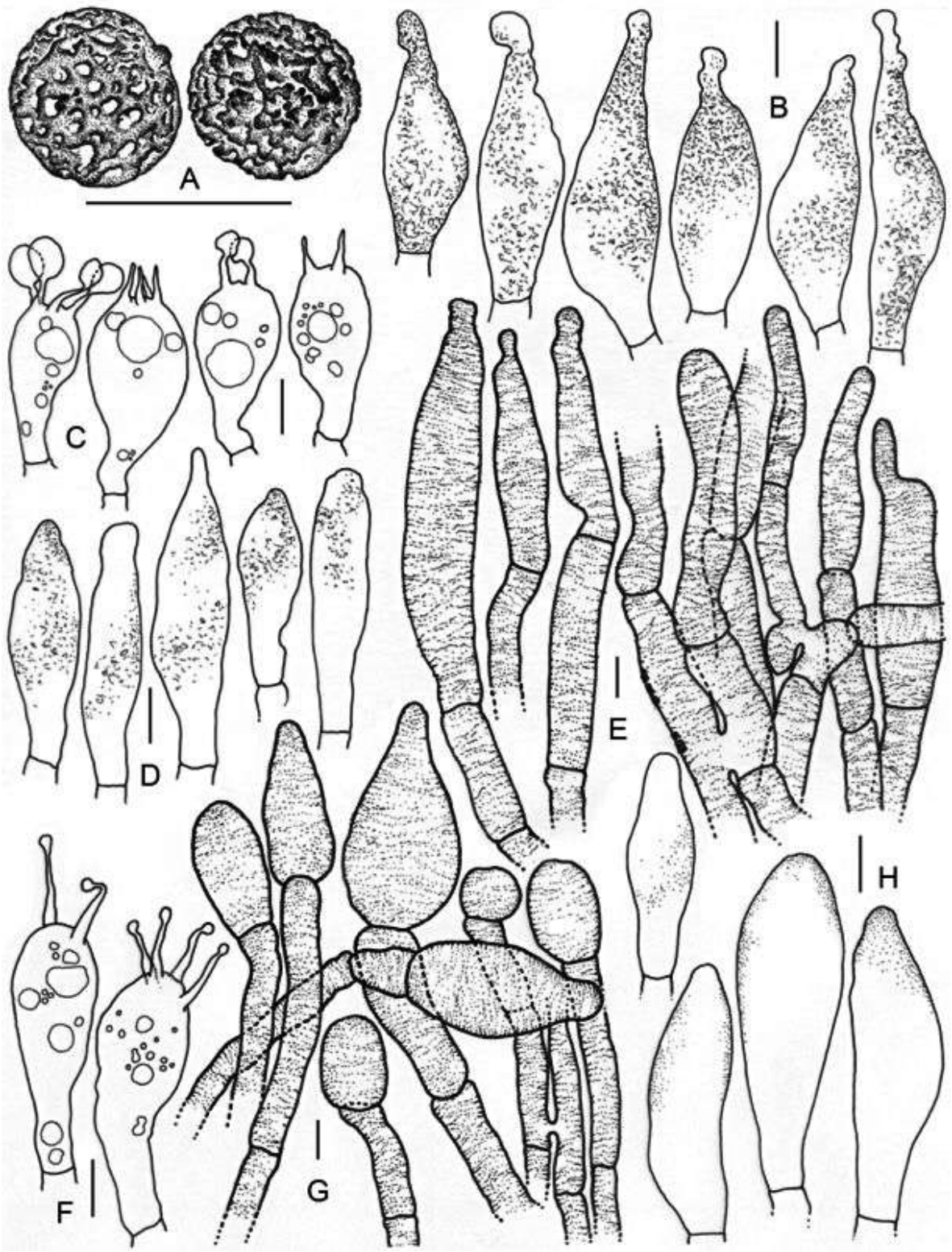

Figure 1 (A-H): *Strobilomyces polypyramis*. A- Basidiospores. B - Pleurocystidia. C- Basidia. D - Cheilocystidia. E - Terminal cells of hyphal elements showing incrustations in zebroid pattern. F - Caulobasidia. G - Hyphal elements of stipe covering flocci showing incrustations in zebroid pattern. H - Caulocystidia. Scale bars: A – H = 10 µm

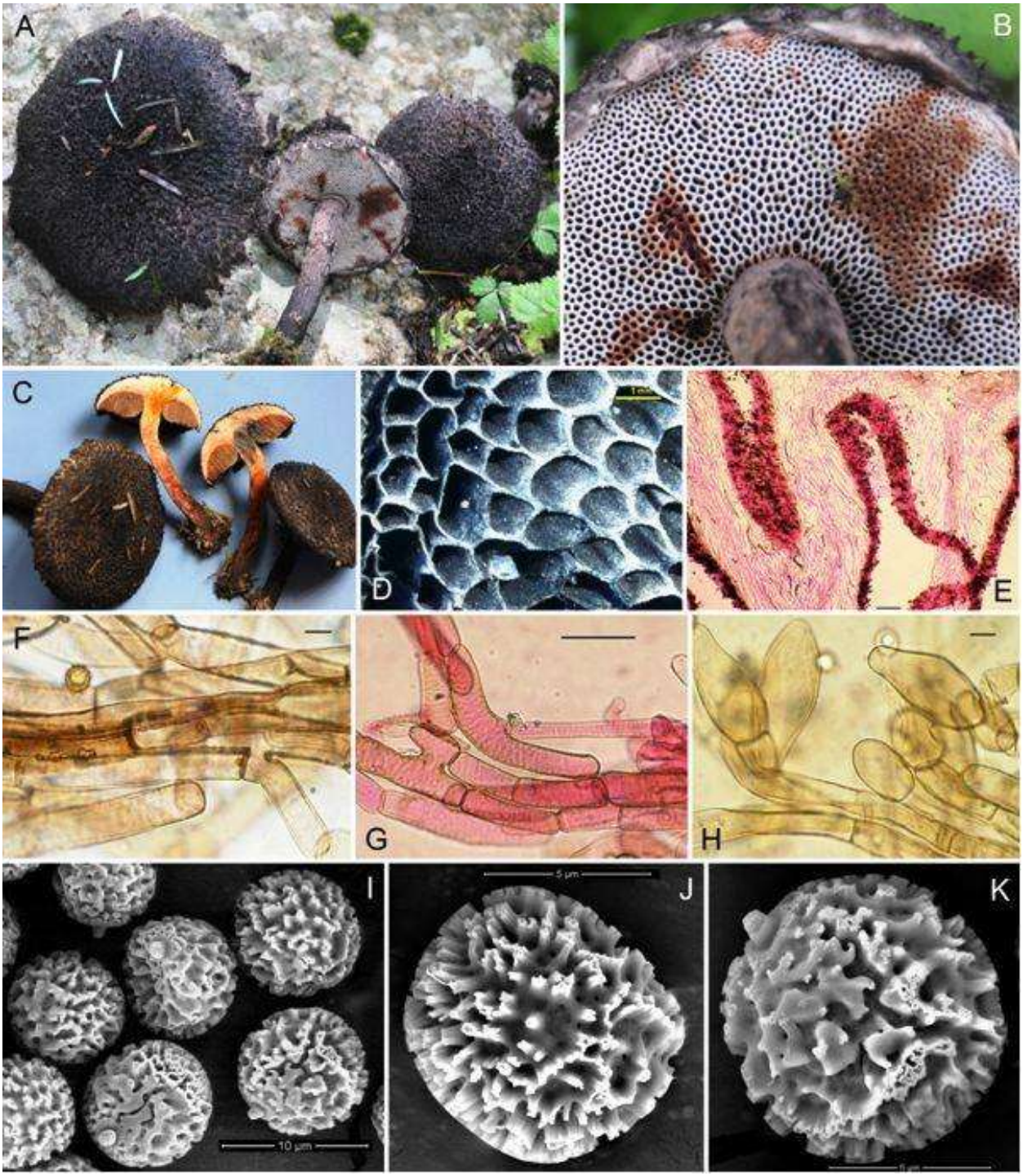

Figure 2: *Strobilomyces sikkimensis* (KD12-251). A - Dorsal and ventral view of fresh basidiomata in the field. B - Pore surface showing the change of colour (after bruising). C- Longitudinal section of basidiomata showing the exposed context. D - Pores from dry sample. E - Divergent hyphal arrangement in tube trama; F–G - Encrusted hyphal elements of pileipellis. H - Encrusted hyphal elements of stipitipellis (stipe covering flocci). I–K - SEM micrographs of basidiospores showing variable pattern of ornamentations. D Scale bars: D = 1 mm, E = 100 µm, F, H, I = 10 µm, G = 50 µm, J, K = 5 µm

Basidiospores 8.0 – 9.0 – 10.8 × 7.0 – 8.3 – 10.0 µm, globose to subglobose (Q = 1.02 – 1.08 – 1.14), ornamentations inamyloid, highly variable, composed of dense isolated cylindric spines or in combination of isolated and confluent spines/small ridges (series of fused spines) or irregularly arranged warts and ridges forming echinulate pattern or rarely with broken reticulum; warts and ridges up to 2 µm long; wall rather thick (0.7 – 0.9 µm thick). *Basidia* 29 – 36 × 12.5 – 18 µm, clavate, 2 – 4 spored; sterigmata up to 6.5 µm long. *Pleurocystidia* 44 – 62 × 12 – 17 µm, rather abundant, mostly lanceolate with subfusoid to appendiculate or lageniform apex; contents partially dense. *Cheilocystidia* 23 – 58 × 8 – 14 µm abundant, subcylindric to narrowly clavate or lanceolate with subfusoid to fusoid or rounded to slightly appendiculate apex, mostly hyaline or with slightly dense content. *Tube trama* bilateral, divergent; hyphae 6 – 10 µm wide, frequently septate, branched, some with strong brown-grey pigmentation and some wrinkled. *Pileipellis* a trichoderm in regular clusters; hyphal element erect to suberect composed of chain of elongated cells; terminal cell 49 – 110 × 5 – 20 µm, cylindric, subcylindric, subfusiform with rounded, subcapitate or apendiculate apex, thick-walled (up to 1 µm thick), pale grey-brown in KOH, pink in phloxin, with pale grey-brown incrustations forming zebroid pattern; subterminal cells also encrusted. *Stipitipellis* fertile at apex; stipe covering hairs composed of erect to suberect hyphal elements; terminal cells globose to ellipsoid, clavate to ampulliform or bullet shaped, with grey-brown incrustations in zebroid pattern. *Caulobasidia* (in fertile area) 35 – 45 × 12 – 16 µm, 2 – 4 spored, sterigmata very long (up to 11 µm). *Caulocystidia* 44 – 63 × 13 – 22 µm, subcylindric, subventricose to subclavate, mostly hyaline. *Stipe trama* composed of subparallel to interwoven hyphae.

Distribution: India, Indonesia, Java

Specimen examined**:** India. Sikkim, North district: Lachen top, 2846 m, N27°43′37.0″ E88°32′47.2″, 3 Sept. 2012, *K. Das*, KD12-251 (CAL 1135).

Habitat**:** Ground, under *Tsuga dumosa* (D. Don) Eichler, in subalpine mixed forest (broad-leaved and coniferous).

Notes**:** Distinct macromorphological features like squamulose pileus with black conic to pyramidal or pointed scales, pileus-margin with irregularly torn cottony flap of partial veil, white (turning black when bruised) pore surface, angular pores, partly floccose-reticulate stipe with bulbous base, absence of annulus, blackish brown spore print, globose to subglobose smaller (8.0 – 10.8 × 7.0 – 10.0 µm) spores with ornamentations of isolated spines or in combination of isolated spines, fused spines and small ridges forming at the most a broken reticulum place the present taxon under *Strobilomyces polypyramis*.

Other existing species of *Strobilomyces* reported from India appear to be close to *S. polypyramis*. But, both *S. annulatus*, *S. strobilaceus* (= *S. floccopus*) can be separated from the present species by the presence of annulus. Moreover, *S. annulatus* has completely floccose to squamulose stipe and larger [10 – 13.5(14) × 8.5 – 11.5 µm] spores (Lakhanpal, 1996) whereas, *S. strobilaceus* has spores with complete reticulum (Lakhanpal, 1996). *S. nigricans* probably the closest ally of *S. polypyramis* also has distinctly larger spores ("9.5 – 12 µm") with reticulate ornamentations (Berkeley, 1852; Horak, 1980). *S. montosus* has yellow pores, scaly dark brown stipe, spores with complete reticulum and is reported to grow on rotten wood (Horak, 1980). *S. mollis* shows spores with reticulum (never with spinoid warts) and ectomycorrhizal association with *Quercus* (Lakhanpal, 1996). *S. kalimpongensis*, can be separated from *S. polypyramis* by distinctly smaller spores (6 – 7 × 5 – 5.5 µm) and the habitat i.e. deadwood (Bose, 1946).

Strobilomyces confusus Singer reported from North America also resembles the present species. But, the earlier one is distinct by its annulus and larger spores (10.5 – 12.5 × 9.5 – 10.5 µm) (Bessette *et al.*, 2010).

Furthermore, mophological features of the collected materials from Sikkim agree completely with the description of *S. polypyramis* mentioned by earlier workers based on the collections made from the Asian countries (Berkley, 1851; Horak, 1980).

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