



First Report of *Panaeolus sphinctrinus* and *Panaeolus foenisecii* (Psathyrellaceae, Agaricales) on Elephant Dung from Sri Lanka

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Abstract: *P. sphinctrinus* (Fr) Quél. and *P. foenisecii* (Pers.) J. Schröt. are described from Sri Lanka for the first time. Both were collected from elephant dung in dry zone forest reserves of Sri Lanka. Identity was confirmed by sequencing the internal transcribed spacer (ITS) region in the nuclear ribosomal repeat unit, using the primers ITS1F and ITS4B. The morphological studies and phylogenetic analysis were carried out to characterize the fungus. Accordingly, *P. sphinctrinus* consists of a grey brown pileus with whitish margin and citriform, bluish brown basidiospores with a distinct germ spore. Whereas *P. foenisecii* has a characteristic white to smoke grey, hygrophane pileus and various shaped cheliocystidia. Further in the phylogenetic analysis, the two species clustered with their respective groups.

Keywords: Basidiospores, Elephant Dung, *Panaeolus*, Phylogenetic Analysis

1. Introduction

Macrofungi occur in different types of habitats such as grasslands, forests, mangroves and coastal sand dunes utilizing various substrates like soil, leaf litter, woody litter and dung [1]. Out of them dung fungi, technically known as coprophilous fungi is a specific group exhibiting unique adaptations to their peculiar lifestyle [2].

Coprophilous fungi within an ecological system are very important in demonstrating the diversity and morphology of that particular system [3]. Dung is an ephemeral substrate which often does not support fungi with long life cycles and large basidiomes. However elephant dung is exceptional in that case favouring colonization of diverse agarics because the droppings take almost a year for full disintegration while they are comparatively large and consist of only lignocellulosic materials. Other than *Panaeolus* sp., agarics belonging to genera *Agrocybe*, *Bolbitius*, *Conocybe*, *Entoloma*, *Macrocybe*, *Panaeolina*, *Stropharia* and *Volvoriella* were observed growing on elephant dung [4].

Species belonging to the genus *Panaeolus* (Fr) Quél. are known to be coprophilous fungi characterized by small to

medium sized fruiting bodies often bluing when bruised or with age and spores which do not fade in concentrated sulphuric acid [5,6,7]. According to reference [8], this particular genus consists of 15 species and is widespread. They are saprotrophic in habitat and grow solitary, scattered or in groups.

In Sri Lanka 3 species were previously recorded which includes *P. campanulatus* (Fr) Quél., *P. cyanescens* (B. & Br.) Sacc. and *P. rubricaulis* Petch. [9,10]. During a macrofungal survey in the dry zone of Sri Lanka two *Panaeolus* species were encountered from Sigirya wilderness and Kaudulla national park. These two species: *P. sphinctrinus* (Fr) Quél. and *P. foenisecii* (Pers.) J. Schröt. which do not have any previous records from Sri Lanka are described in this paper along with a phylogenetic analysis.

2. Methodology

2.1. Morphological Study

The studied specimens were collected from Sigirya wilderness and Kaudulla National Park, Sri Lanka. The

specimens are deposited at the herbarium of the Department of Plant Sciences, University of Colombo, Sri Lanka (UOC: SIGWI: S47 and UOC: KAUNP: MK62). Sections were studied at magnifications up to $\times 1000$ using an Olympus CX21FS1 microscope and phase contrast illumination. Size dimensions were determined for 30 basidiospores, 10 basidia and 10 cystidia from each basidiomata. Photographs of microscopic observations were taken from Canon, Power Shot A2600 camera. Special colour terms follow reference [11].

2.2. DNA Extraction and Sequencing

DNA was extracted from the fruit body according to CTAB based method [12]. PCR products were obtained using $5\times$ HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne) according to the manufacturer's instructions on a Veriti 96 well Thermal Cycler (Applied biosystems). ITS region was amplified using primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and ITS4B (CAG GAG ACT TGT ACA CGG TCC AG) (Gardes and Bruns 1993). The PCR procedure was as follows: initial denaturation at 95°C for 12 minutes, followed by 13 amplification cycles of denaturation (95°C for 35 s), annealing (55°C for 55 s) and extension (72°C for 45 s). The extension step was lengthened to up to 120 s from 14th to 26th cycle and 180 s from 27th to 35th cycle. Then the final extension was performed for 10 minutes at 72°C [13]. PCR products were sequenced by Macrogen, Inc. (Korea).

2.3. Sequence Alignment and Phylogenetic Analysis

Basic local alignment search tool (Blast) of USA database, National Center for Biotechnology Information (NCBI) was used for performing initial comparison and alignment of the sequence. Sequences of *P. sphinctrinus* and *P. foeniseccii* were submitted to NCBI database under accession numbers KP826787 and KP764810, respectively.

For phylogenetic analysis, closely related sequences were retrieved from NCBI database. The aligning of sequences and phylogenetic analysis was performed using MEGA 6 (Molecular Evolutionary Genetics Analysis) software [14,15]. Neighbour Joining method was based on the Jukes-Cantor model of nrITS sequences and phylogeny was tested by bootstrap value of 1000 replicates.

3. Results and Discussion

3.1. Taxonomic Descriptions

3.1.1. *Panaeolus sphinctrinus* (Fr) Quél. (Fig. 1)

Pileus 1-2 cm in height, 1-2 cm broad, conical or campulanate; surface brownish when immature, grayish brown when mature, smooth; margin crenulate, white, later becoming black. Gills adnexed, distant broad, mottled black and grey when young, later entire black; margin white. Stipe equal, hollow, 2.5-9 cm in height, 1-2 mm thick, snuff brown, sometimes whitish basal mycelium present. Spore print black.



Figure 1. *Panaeolus sphinctrinus*. A - Basidiomes growing in natural habitat; B - Lower side of the cap; C - View of gills under the stereomicroscope; D - View of stipe under the stereomicroscope; E - Spore print; F - Basidiospores; G - Parallel arrangement of hyphae in stipe; H - Caulocystidia; I - Basidia.

Basidiospores $10.4-11.3 \times 6.6-7.5 \mu\text{m}$ ($Q=1.53$), citriform, smooth, thick walled with a distinct protruding germ pore, blackish brown when mature. Basidia $11.0-14.7 \times 9.2-10.1 \mu\text{m}$, spherical to barrel shaped, 2-4 sterigmata, hyaline. Cystidia $11.0-16.5 \times 5.5-9.2 \mu\text{m}$.

Stipe context hypha longitudinal parallel, thin walled, clamp connections absent; caulocystidia $11.0-20.2 \times 5.5-9.17 \mu\text{m}$.

Material examined: Sri Lanka, Sigiriya wilderness, growing on elephant dung, 27th March 2013, specimen voucher (Holotype) – UOC:SIGWI:S47.

Remarks: *P. sphinctrinus* is characterized by pileus which is in mouse grey shades and smooth. Also the white margin of the pileus due to partial veil fragments. This particular species is widely distributed throughout the world including America, Europe, Iceland, South Africa, Canary Islands, Israel, Siberia, Japan, Kenya and India [6]. It also grows on cow and horse dung and is known to be poisonous [14].

3.1.2. *Panaeolus foeniseccii* (Pers.) J. Schröt. (Fig. 2)

Pileus 0.7-1.7 cm in height, 0.3-1.0 broad, hemispherical to conical, smooth, cracking in dry weather, hygrophanus, white to smoke grey, turning mouse grey, black and buff

when drying; margin slightly striate. Gills adnexed, distant, mottled with grey and black when young, later entire black; margin white. Stipe 1.2-5.8 cm in height, 1-1.5 mm thick, buff, late becoming dark brown, smooth.

Spore print dark brown.

Basidiospores $12.8-18.3 \times 10.4-11.3 \mu\text{m}$ ($Q=1.43$), ellipsoid to lemon shaped, thick walled, reddish brown in KOH. Basidia $20.2-25.1 \times 14.7-15.1 \mu\text{m}$, spherical to barrel shaped. Cheliocystidia $22.0-49.5 \times 8.3-22.0 \mu\text{m}$, in various shapes, flask shaped, clavate and lageniform.

Stipe context parallelly arranged hypae, septate, thick walled, $5.5-7.3 \mu\text{m}$ in diameter.

Pielus cuticle cellular, $22.5-31.2 \times 18.3-20.2 \mu\text{m}$.

Material examined: Sri Lanka, Kaudulla National Park, growing on elephant dung heap, 26th February 2014, specimen voucher (Holotype): UOC:KAUNP:MK62.

Remarks: *P. foeniseccii* is reported from North America and Europe and Known as lawn mower's mushroom. Hallucinogen chemical psilocybin was revealed from some collections from some parts of North America. *Panaeolina foeniseccii* is a synonym for this particular species [16].

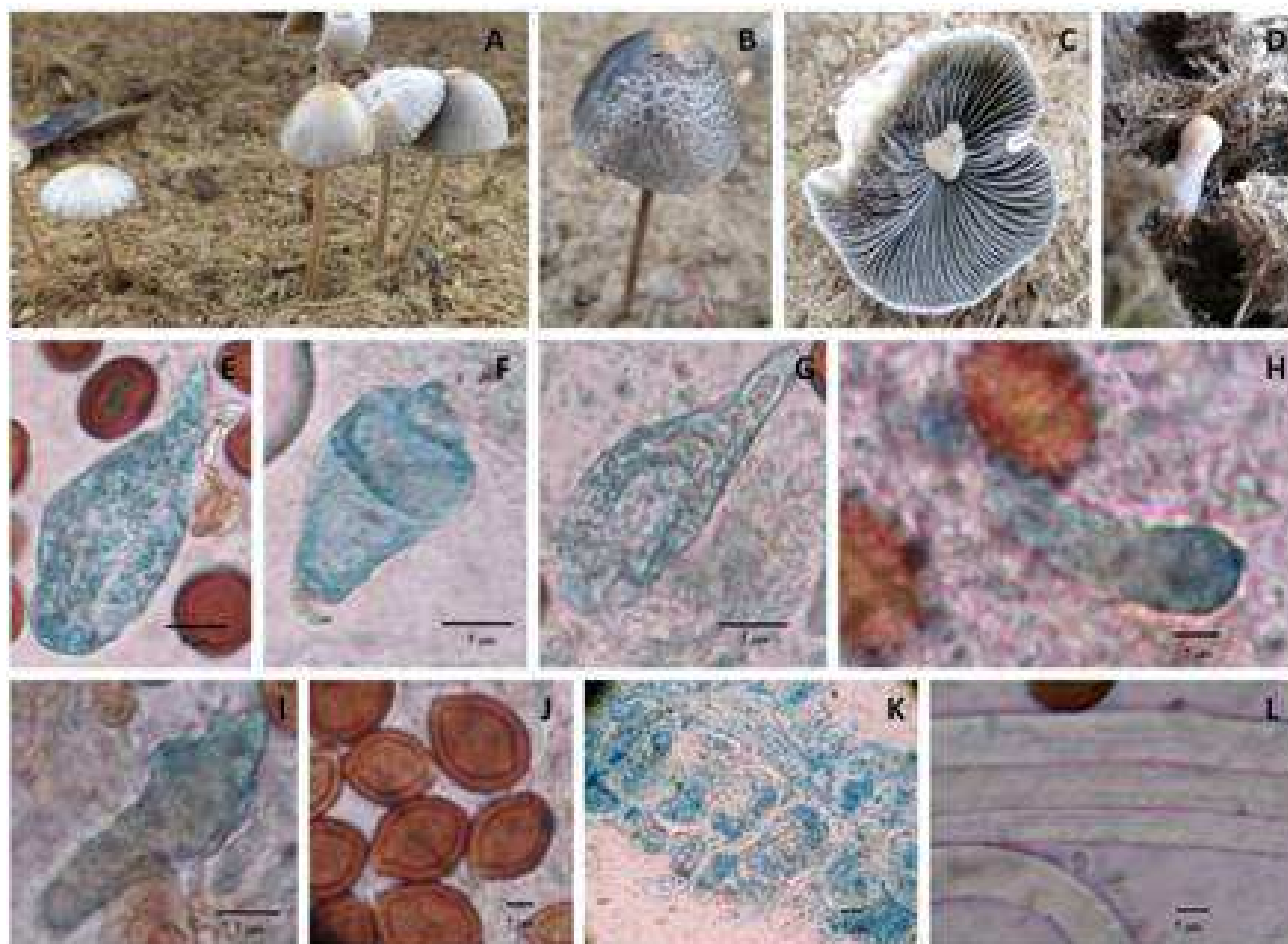


Figure 2. *Panaeolus foeniseccii*. A - Basidiomes growing in natural habitat; B - dried basidiome; C - Lower side of the cap; D - Immature basidiome; E - I - Cheliocystidia; J - Basidiospores; K - Pileus cuticle; L - Parallel arrangement of hyphae in stipe.

3.2. Phylogenetic Analysis

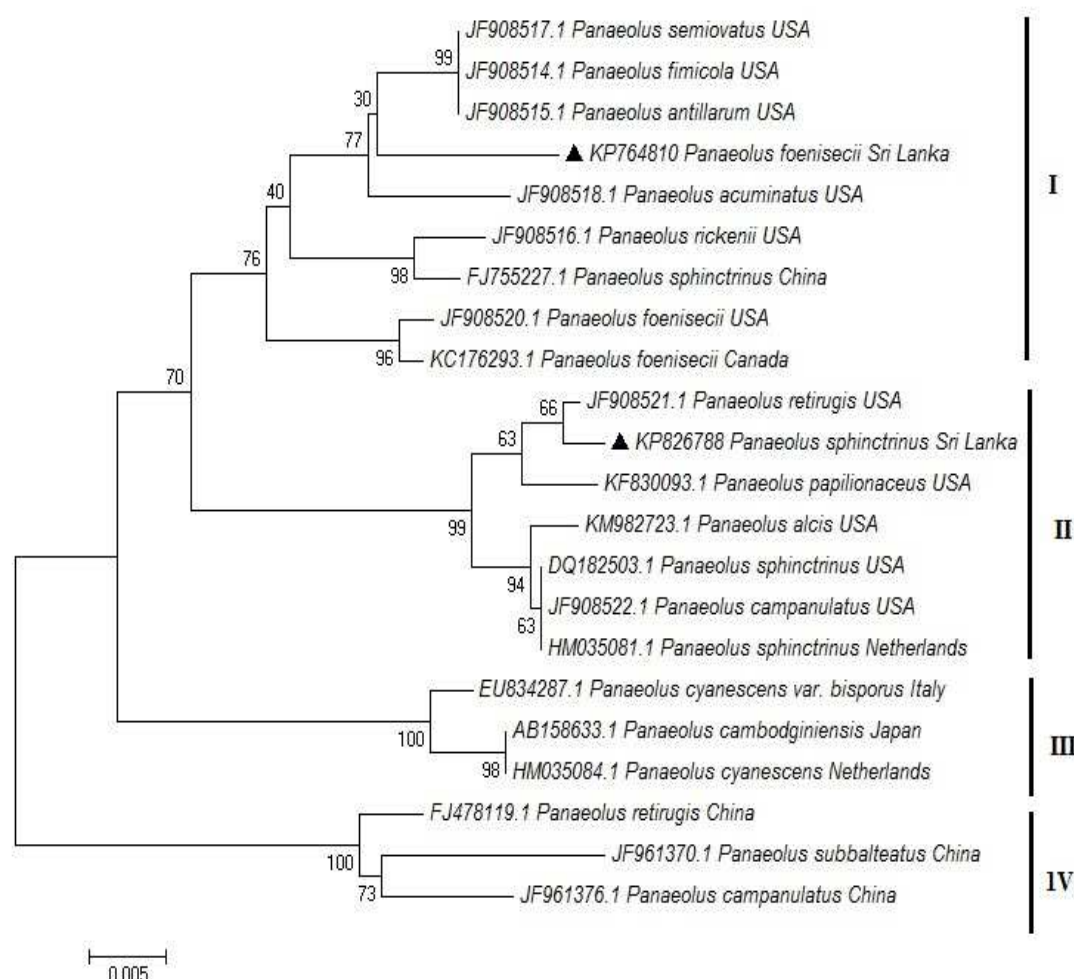


Figure 3. Phylogenetic relationship of *P. sphinctrinus* and *P. foeniseeii* (▲) from Sri Lanka with closely related species of same genus reported from several other countries. Bootstrap values based on 1000 replicates are shown next to the branches. Twenty two nucleotide sequences are involved in this analysis. A total number of 537 positions were there in the final data set after eliminating all positions containing gaps and missing data.

4. Conclusion

The dimensions of microscopic structures (basidiospores, basidia, pileocystidia and caulocystidia) and size of fruiting body in *P. sphinctrinus* from Sri Lanka is much lower than the same species reported from India and Pakistan [6, 14]. Since *P. sphinctrinus* was collected from a dry zone forest reserve in Sri Lanka and the difference in geographical areas could account for these lesser dimensions. Same pattern was observed in *Fulvifomes fastuosus* recorded from Sri Lanka where the microscopic structures were much smaller when compared to the same species from China [17]. However microscopic features of *P. foeniseeii* from Sri Lanka are in agreement with characteristics reported by Kuo 2002 [16] for the same species.

Molecular characterization of the two species from Sri Lanka was performed using 20 sequences of closely related species of same genera reported from different areas of the world. According to the phylogenetic analysis 4 major clusters can be observed (Fig. 3). As these sequences are

intraspecifically different, two sequences of same species might behave in a totally different way. According to reference [14] another reason for this behavior could be the misidentification of species as there are close similarities of species in *Panaeolus* genus. However *P. sphinctrinus* from Sri Lanka is clustered together with the same species reported from USA and Netherlands in clade II while *P. foeniseeii* from Sri Lanka is clustered together with same species reported from USA and Canada in clade I (Fig. 3).

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