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Flammulina yunnanensis (Agaricales), a new record from Darjeeling Hills, India

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Abstract

Morphological and phylogenetic studies were carried out on the collected specimen of *Flammulina yunnanensis*. A detailed morphological description along with field images and ITS (internal transcribed spacer region) sequence analyses suggested that the collected specimen is *F. yunnanensis*. It has a hymeniform suprapellis with clavate-shaped terminal elements without ixohyphidia which is a distinguishing feature amongst other species of the genus *Flammulina*. *Flammulina yunnanensis* is recorded for the first time in India.

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INTRODUCTION

The genus *Flammulina* belonging to the family Physalacriaceae (Agaricales) includes 35 species worldwide. Amongst them, *F. velutipes* (Curtis) Singer is a species that is known to be edible with both nutritional and medicinal properties. Earlier, this genus was known to be monotypic with the only type species *F. velutipes,* Arnolds[1] however in 1977 separated *F. ononidis* from *F. velutipes* which confirmed that *Flammulina* is not a monotypic genus. The genus *Flammulina* can be identified on the basis of characteristics such as having glabrous pileus that turns viscid when wet, yellowish lamellae usually with adnate to adnexed lamellae attachment, spores inamyloid; white spore print, and gelatinized pileipellis with pileocystidia. Species of the genus *Flammulina* are quite similar to each other so a detailed microscopic study is required for proper identification of different species. The type of suprapellis, spore characteristic, cheilocystidia shape, and size are important characteristics that have to be noted for the identification of this genus[2]. Species of *Flammulina* are said to be specially distributed in the Northern Hemisphere, however *F. velutipes* are also distributed in Australasia and South America[3,4]. *Flammulina* has not been studied critically in India, and only *Flammulina velutipes* have been reported[5].

RESULTS

Phylogenetic analyses

Aligned sequences of the ITS (internal transcribed spacer region) dataset were 878 sites long. Among these, 623 were conserved sites, 207 variable sites, 95 informative sites, and 109 singletons. The phylogenetic tree obtained from ML (maximum likelihood) and MrBayes analyses almost showed the same topology. So, the Bayesian tree has been displayed (Fig. 1). The phylogenetic analysis of the nrITS (nuclear ribosomal internal transcribed spacer region) sequences dataset placed the Indian collection (OM428205) together with the Chinese collection (DQ486704) with 100% bootstrap support value.

Taxonomy

Flammulina yunnanensis Z.W. Ge & Zhu L. Yang, Fungal Diversity 32: 63 (2008) Fig. 2

Index Fungorum number: IF 512371

Basidiocarp convex to broadly convex in shape, 1.1–2.1 cm in diameter, surface smooth, yellowish grey (4B2), to greyish orange (5B5), centre greyish orange (5B5), to dark orange (5A8), to greyish red (7B6) to reddish orange (7B7), shiny, viscid to subviscid when moist, glabrous, slightly depressed at the disc, pileus margin striate, incurved, crenate. Lamellae sinuate to adnexed, yellowish, up to 3 mm wide, regular, crowded to sub distant, cream to yellowish white (2A2) with lamellulae of four lengths. Stipe 3.5 cm × 0.3 cm, central, yellowish white at apex, brownish at lower parts, equal, hollow, surface smooth. Context white and unchanging. Spore print pure white (1A1).

Basidiospores 5.68–7.58 × 3.79–4.55 μm; Q = 1.4–1.8, Q_m = 1.57, ellipsoid, sometimes oblong, inamyloid, smooth, thin walled, hyaline, with an apicule, germ pore absent. Basidia 21.98–25.01 × 6.06–7.2 μm, clavate in shape, 4–spored, sterigmata 3.03–4.2 μm long. Pleurocystidia ventricose to lageniform, scattered, 31.08–41.70 × 10.61–15.16 μm, hyaline, slightly thick walled. Cheilocystidia similar to pleurocystidia. Hymenophoral trama parallel to somewhat interwoven. Suprapellis 55–81 μm in thickness, somewhat gelatinized, with a hymeniform layer consisting of clavate shaped terminal elements 15.16–23.5 × 5.3–7.58 μm, ixohyphidia absent. Pileocystidia present, 41.69–90.96 × 6.8–10.99 μm, lageniform to ventricose. Clamp connections are present in all tissue.

Known distribution: Yunnan, southwestern China[6].

Material examined: INDIA, West Bengal, 6th mile Lava, Kalimpong, Caespitose, lignicolous on cultivated *Cryptomeria* tree in India, 14th June 2019, coll. Thapa A, Tamang J, CUH AM762.

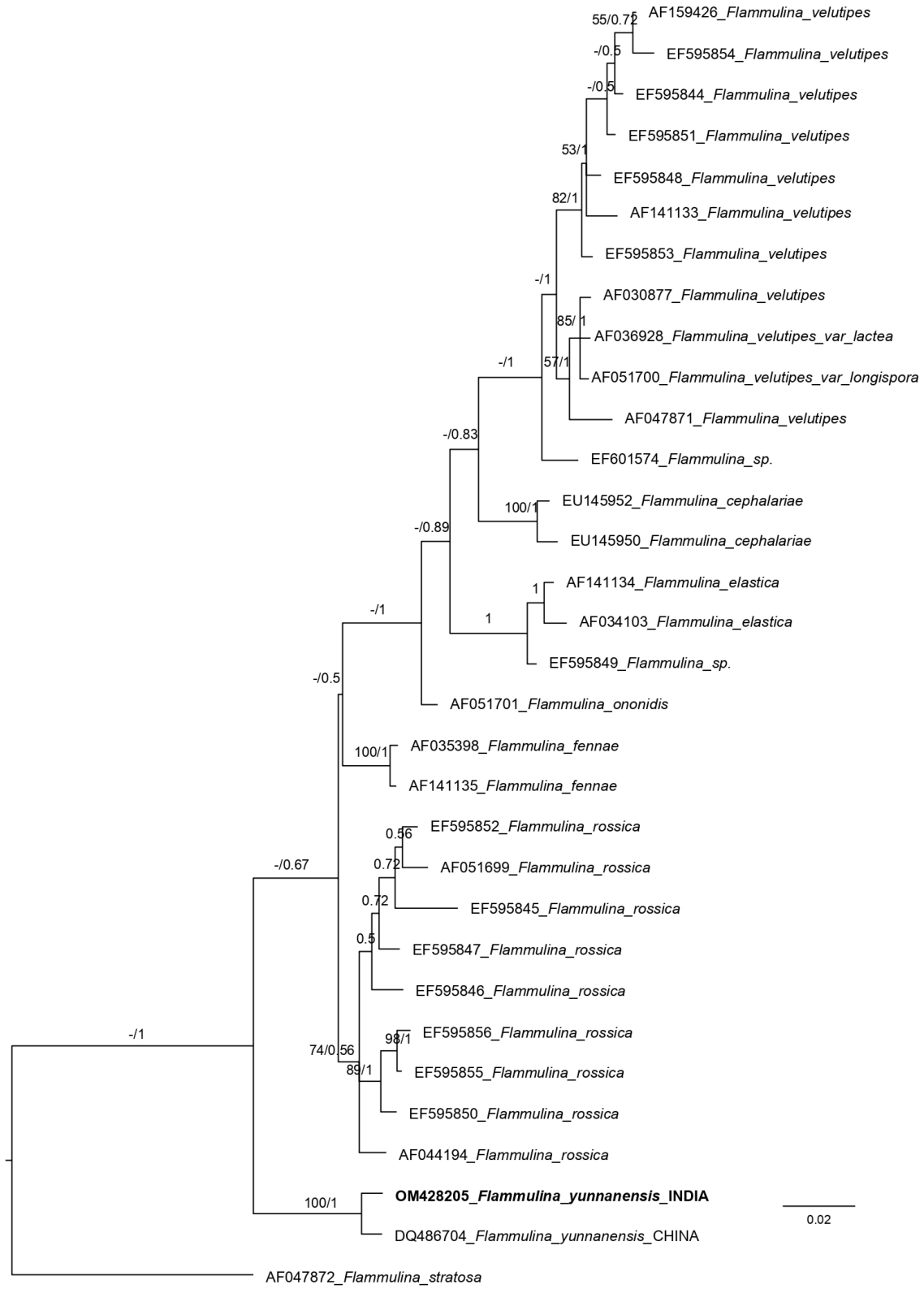

Fig. 1 Phylogenetic tree generated from Bayesian analyses (MrBayes) based on an ITS sequence dataset. Maximum likelihood bootstrap support values equal to or greater than 50% and Bayesian posterior probabilities equal to or greater than 0.50 are indicated on the nodes. The tree is rooted with *Flammulina stratosa* (AF047872).

DISCUSSION

Flammulina yunnanensis is distinguished from other species of the genus by its morphological characteristics of small ellipsoid basidiospores, hymeniform suprapellis with clavate shaped terminal elements without ixohyphidia. Considering morphological features, the description of our Indian collected

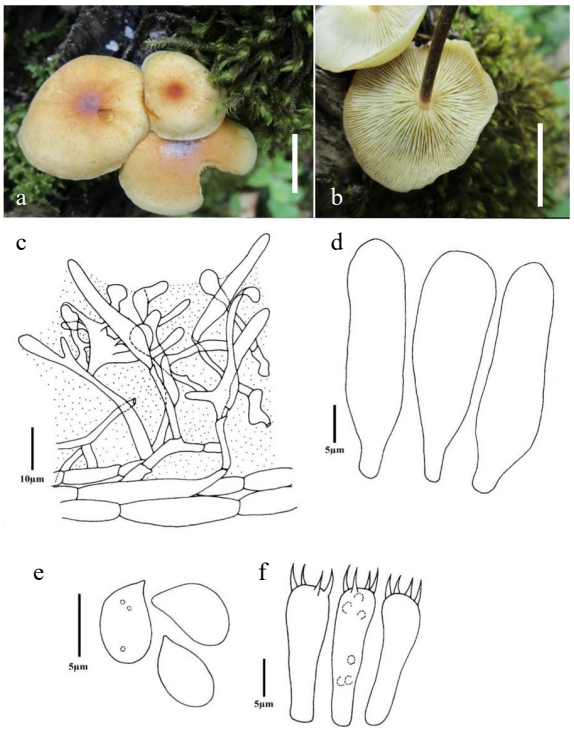

Fig. 2 *Flammulina yunnanensis* (CUH AM762). (a), (b) Habit *in situ* (Scale bars = 10 mm), (c) Pileipellis, (d) Cheilocystidia, (e) Basidiospores, (f) Basidia.

specimen matches the holotype reported from Yunnan, southwestern China[6]. The Indian collection was found on the trunk of the living *Cryptomeria* tree but the Chinese collection is reported to be found on the dead trunk of fagaceous plants and other broadleaved trees.

The morphological identification of *F. yunnanensis* is well supported by the phylogenetic analyses. *Flammulina yunnanensis* has been originally described from Yunnan, China and there is no record of its occurrence in other parts of the world. Thus, *F. yunnanensis* is reported for the first time in this study in an alternative location.

MATERIALS AND METHODS

Specimen and morphological description

The specimen was collected during a field visit in the month of June 2019 from Darjeeling Hills, India. The morphological description of the specimen is based on the field data sheet and color image of the basidiocarp. Basidiocarps were carefully dried using a drier and preserved using self–indicating silica gel for further studies at a laboratory. Colour codes were designated as per Kornerup & Wanscher[7].

Micro-morphological details were observed from the dried specimens by making free hand sections using 5% KOH and staining with Congo red. Melzer's reagent was used to stain basidiospores. For basidiospores, the abbreviation 'Q_m' denotes the average Q of all spores. The specimen was preserved following Pradhan et al.[8] and deposited to the Calcutta University Herbarium (CUHAM762).

Table 1. A list of *Flammulina* species used in the molecular phylogenetic analyses with GenBank accession numbers.

Species	Collections	Location	Substrate	GenBank accession #
F. elastica	TENN 56057	Austria: Vienna	On *Salix alba*	AF034103
F. elastica	TENN 54689	Netherlands	On *Salix*	AF141134
F. elastica	HKAS 52018	Germany: Marburg	EF595849	
F. fennae	Th.Kuyper 2220	Netherlands: Utreght, Breukelen	AF141135	
F. fennae	TENN 54172	Switzerland: Canton Graubunden	On *Alnus incana*	AF035398
F. ononidis	TENN 54743	Germany	–	AF051701
F. rossica	I. Bulakh	Russia: Terr. Primorsk	–	AF051699
F. rossica	TENN 54169	United States: Alaska	On *Salix*	AF044194
F. rossica	HKAS 46076	China: Tibet, Changdu	On *Salix*	EF595845
F. rossica	HMJAU 20588	China: Jilin, Zuojia	–	EF595847
F. rossica	HKAS 43699	China: Tibet, Leiwuqi	On *Salix*	EF595846
F. rossica	HKAS 45970	China: Tibet, Changdu	On *Salix*	EF595850
F. rossica	HKAS 32154	China: Sichuan, Xiangcheng	On *Salix*	EF595856
F. rossica	HKAS 32155	China: Sichuan, Daocheng	On *Picea*	EF595855
F. rossica	HKAS 7930	China: Jilin, Baihe	In *Betula* forest	EF595852
F. sp.	HKAS 51191	China: Tibet, Mozhugongka	On the base of a dead trunk	EF601574
F. stratosa	TENN 56240	New Zealand: South Island	–	AF047872
F. yunnanensis	HKAS 32774	China: Yunnan, Lushui	In forest with *Schima* trees	DQ486704
F. velutipes	TENN 56008	Canada: British Columbia.	–	AF141133
F. velutipes	TENN 54748	Netherlands: Prov. Zeeland	–	AF036928
F. velutipes	K 28262	United Kingdom: Surrey, Ham	–	AF030877
F. velutipes	TENN55402	United States: California	On *Lupinus arboreus*	AF047871
F. velutipes	TENN 56028	United States: Michigan	–	AF051700
F. velutipes	HKAS 49485	China: Yunnan, Kunming	Cultivated	EF595844
F. velutipes	HKAS 51962	China: Hubei, Wuhan	On *Broussonetia papyrifera*	EF595848
F. velutipes	HKAS 47767	China: Hunan, Changsha	On *Broussonetia papyrifera*	EF595853
F. velutipes	HKAS 47768	China: Hunan, Changsha	On *Broussonetia papyrifera*	EF595854
F. velutipes	HKAS 51988	China: Jilin, Changbai Mt.	On *Betula platyphylla*	EF595851
F. velutipes	FH DH97 –080	China: Sichuan, Gongga	On dead hard wood	AF159426
F. cephalariae	SEST05120701	Spain	–	EU145952
F. cephalariae	SEST04111402	Spain	–	EU145950
F. yunnanensis	CUH AM762	India: Darjeeling hills	On *Cryptomaria*	OM428205

DNA extraction and PCR amplification

DNA was isolated using an XcelGen Fungal gDNA Mini Kit following the protocol of the manufacturer. ITS1 and ITS4 primer pair[9] were used for the rDNA amplification. PCR product purification was performed using QIAquick® Gel Extraction Kit (QIAGEN, Germany). Sequencing was done on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using the same primer pairs used for the amplification of the rDNA ITS region. BioEdit v.7.0.5 software was used for editing the newly generated sequence of *F. yunnanensis* and given for BLAST search (NCBI). A new generated sequence of *F. yunnanensis* was deposited in Genbank with accession number OM428205.

Sequence alignment and phylogenetic analyses

The nrITS sequence of *F. yunnanensis* along with the dataset of Hughes et al.[10] and Ge et al.[6] downloaded from GenBank was aligned using Mega v.7.0. The final ITS dataset (Table 1) consisted of 32 samples of *Flammulina,* where *Flammulina stratosa* was designated as an outgroup referring to the previous studies[9,10].

Maximum likelihood (ML) analysis in RAxML HPC2 v. 8.2.12[11] used the best fit nucleotide substitution model by jModelTest2 on XSEDE using CIPRES web portal. Bayesian analyses of this dataset were also estimated in MrBayes v.3.2.7[12]. The initial run of 10^6 generations using Metropolis Coupled Monte Carlo Markov (MCMC) chains was carried out as described by Vishal et al.[13]. Among 10,001 samples, a total of 7,501 trees were used to calculate the Bayesian posterior probability. Maximum Likelihood bootstrap (MLBS) and Bayesian posterior probabilities (pp) values over 50% and 0.50 respectively are considered in the phylogenetic tree.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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