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Original Research

Mariannaea camelliae from India: Morphological and Phylogenetic Insights

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Abstract

This study reports *Mariannaea camelliae* isolated as a phylloplane fungus from *Camellia sinensis* leaves collected from Kangra, Himachal Pradesh. The isolate was characterized based on cultural morphology, microscopic features, and molecular phylogeny. Detailed observations of colony morphology on different media, along with conidiophore structure, phialides, and conidia, confirmed its morphological congruence with the type description. Molecular identification using a combined dataset of internal transcribed spacer (ITS), large subunit (LSU), and beta-tubulin gene sequences further supported its identity. Phylogenetic analysis using the maximum likelihood (ML) method positioned the isolate firmly within the *Mariannaea* clade, forming a sister clade with *M. camelliae*. This finding expands the known distribution of *M. camelliae* and underscores the ecological significance of phylloplane fungi in tea ecosystems. Understanding its diversity and functional attributes may contribute to sustainable strategies for plant health and disease management in tea cultivation. To our understanding, this is the first report describing a phylloplane *M. camelliae* using an integrated approach of conventional and modern molecular tools from India.

Keywords: Fungi, Nectriaceae, Phylogeny, Phylloplane, Taxonomy, India.

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1. Introduction

Fungi represent a vast and ecologically integral group of organisms adapted to nearly all terrestrial and aquatic ecosystems. They are pivotal in ecosystem stability through critical functions such as nutrient recycling, decomposition of organic matter, establishment of symbiotic associations, and engagement in complex trophic interactions (Park et al., 2020; Lombard et al., 2015; Domínguez-Núñez & Albanesi, 2020). Despite their ecological significance and the burgeoning recognition for biotechnological applications, fungi remain one of the least explored groups globally. Currently, it's estimated that between 2.2 to 3.8 million species of fungi exist, yet a mere fraction—approximately 170,000—are described formally, underscoring a significant taxonomic gap, especially in tropical and subtropical regions believed to harbor a great diversity of yet undescribed taxa (Wendt et al., 2017).

The Indian Himalayas specifically exemplify an underexplored fungal biodiversity hotspot. This region, characterized by its extensive altitudinal range, diverse vegetation, and high levels of endemism, is likely to serve as a rich repository of fungal diversity that remains largely undocumented (Jung et al., 2018). Systematic documentation of these fungi is essential not only for taxonomic and ecological insights but also for identifying species with potential applications in agriculture, forestry, medicine, and industry (Domínguez-Núñez & Albanesi, 2020). Such efforts align with emerging needs for better understanding biodiversity in the face of environmental change (Printzen et al., 2013).

The genus *Mariannaea* G. Arnaud ex Samson (Nectriaceae) constitutes a diverse group of ascomycetous fungi primarily associated with decaying plant materials, soil, and occasionally insects. The taxonomic placement and phylogenetic relationships of *Mariannaea* have been elucidated through modern molecular techniques, revealing its close relationship within hypocrealean fungi, specifically relating it to genera such as *Cosmospora* and *Acremonium* (Gräfenhan et al., 2011; Lombard et al., 2015). The genus is typified by *Mariannaea elegans* (Corda) Samson, characterized by distinct morphological traits, including slimy, one-celled conidia produced from flask-shaped phialides, with the ability to adapt to various ecological niches (Noh & Kim, 2025). Sexual morph has been linked to *Nectria* (Fr.) Fr. and *Cosmospora* Rabenh. in *Nectriaceae* (Samuels & Seifert, 1991), which has been proved to be a monophyletic group by molecular studies (Cai et al., 2009; Lombard et al., 2015). Hu et al. (2016) reviewed the genus and designated an epitype of the type species *M. elegans* with ex-type culture. Currently, around 25 species of *Mariannaea* are recognized, with new species continuously being described and added to this genus (https://www.indexfungorum.org/, accessed on 4th August 2025). These fungi are often isolated from diverse habitats, including soil, decaying bark, and freshwater environments (Cai et al., 2009; Tang et al., 2012). *Mariannaea* species are recognized for their potential in biotechnological applications, particularly due to their ability to produce robust extracellular enzymes. These enzymes, including cellulases and xylanases, are effective in bioremediation and industrial processes such as the bioconversion of lignocellulosic biomass (Luangsa-ard et al., 2005). Furthermore, some members of this genus exhibit entomopathogenic properties, potentially serving as biocontrol agents against pest populations (Park et al., 2021). The ecological roles of *Mariannaea* highlight its importance not only in decomposing organic matter but also in nutrient cycling within ecosystems. Despite its ecological significance, the diversity of *Mariannaea* species, particularly in less explored regions such as the Indian Himalayas, remains understudied.

Mariannaea camelliae Suwannar. & J. Kumla, initially described from Thailand is known for its association with *Camellia* species leaf litter (Boonmee et al., 2021). This species is morphologically distinguished by verticillately branched conidiophores and chains of conidia (Domínguez-Núñez & Albanesi, 2020). In mycological explorations aimed at documenting foliar fungi in the Northwestern Himalayas, an isolate exhibiting morphological characteristics consistent with *M. camelliae* was recovered, marking its first record in India. The identity of the isolate was further confirmed by phylogenetic characteristics. This record not only broadens the geographical scope of the species but also highlights the rich, yet largely uncharacterized, fungal diversity endemic to the Indian Himalayan region (Park et al., 2020). Such findings are crucial for refining fungal biogeography and enhancing ecological understanding of species distribution patterns in this biodiverse landscape.

The discovery of *Mariannaea camelliae* from Himachal Pradesh, represents a significant addition to the known distribution of this genus from India, further emphasizing the rich and largely undocumented fungal diversity present in the region (Park et al., 2020). This finding not only expands the known geographical range of the species but also underscores the need for systematic exploration and documentation of fungal biodiversity in underrepresented areas.

2. Materials and Methods

Samples were collected from two freshwater streams flowing through scrub jungles in southwest India with differing water qualities and riparian vegetation. Drift conidia of aquatic hyphomycetes in water and accumulated conidia in foam samples were assessed. Colonized aquatic hyphomycetes were assessed in submerged leaf litter and wood (bark and cambium).

2.1 Collection, isolation, and morphological characterization

Tea (*Camellia sinensis*) leaves were collected from Panchrukhi, Kangra region of Himachal Pradesh, India (Figure 1). Samples were collected in sterile polythene bags and transported to the laboratory. The collected tea leaves were surface washed using sterile water, and the washed water was inoculated on potato dextrose agar (PDA) supplemented with antibacterial antibiotics. After 5-7 days of incubation at 25 °C, fungal colonies appearing on agar plates were picked up separately with the help of a stereomicroscope (NIKON SMZ1500 aided with Digi-CAM) and transferred onto fresh PDA plates. The inoculated petri-plates were kept for incubation at 25°C. After 5 days, plates were observed for purity, sporulation and preliminary identification, and then selected colonies were transferred onto 08 different culture media for further studies.

Cultural characteristics were studied on 8-different culture media, viz., Potato dextrose agar (PDA), Rose Bengal agar (RBA), Malt extract agar (MEA), V8 Juice Agar, Sabouraud dextrose agar (SDA), Cornmeal agar (CMA), Czapek dox agar (CDA) and Potato carrot agar (PCA). Methuen handbook of color was referred to for recording colors of the colonies on different agar media (Kornerup & Wanscher 1978). Microscopic details of the fungus in question were observed using lactophenol-cotton blue as staining cum mounting media under Olympus BX53 (Tokyo, Japan) microscope. Measurements and photomicrographs of the fungal structures were taken from Olympus cellSens Standard 3.2 software and Olympus DP74 camera attached with Olympus BX53 microscope. Photomicrographs of the fungal structures were also taken from a Scanning Electron Microscope (Zeiss MA15) Jena, Germany. The pure culture is deposited and accessioned in the National Fungal Culture Collection of India (WDCM 932).

Figure 1. *Camellia sinensis*, the host plant from which *Mariannaea camelliae* was isolated. The host plant was photographed at the field site in Panchrukhi, Kangra, Himachal Pradesh.

2.2 **DNA extraction, PCR amplification, and DNA sequencing**

Genomic DNA was extracted from pure fungal colonies raised on Potato Dextrose Agar (PDA) Petri plates. Following approximately one week of incubation, DNA extraction was performed using a simple, easy, and rapid protocol facilitated by FastPrep®24 tissue homogenizer (MP Biomedicals GmbH, Eschwege, Germany) (Aamir et al., 2015). In brief, 300 mg of fresh mycelia was carefully scraped from the PDA plate and placed in a 2 mL screw cap tube containing a ceramic pestle along with acid-washed fine glass beads [425-600 µm (30-40 U.S. sieve), Sigma].

Subsequently, 1 mL of the lysis buffer, composed of 100 mM Tris HCl (pH 8), 50 mM EDTA (pH 8), and 3% SDS was added to the tube. The homogenization process was performed for 1 minute at 6 M/s twice. Following homogenization, the mixture was centrifuged at 13,000 rpm for 15 minutes at room temperature. The resulting supernatant was carefully transferred into a fresh microcentrifuge tube, and an equal volume of phenol:chloroform: isoamyl alcohol was added. The mixture was shaken well; this was followed by centrifugation at 13,000 rpm for 10 minutes. The upper aqueous layer was transferred into a fresh microcentrifuge tube, and an equal volume of chilled isoamyl alcohol (stored at -20°C) was added. This mixture was incubated for 20 minutes. The DNA precipitated, and the microcentrifuge tube was centrifuged at 13,000 rpm for 10 minutes to pellet down the DNA. The supernatant was discarded, and the DNA pellet was washed with 70% ethanol before being centrifuged at 13,000 rpm for 5 minutes. The pellet was allowed to air-dry and was later dissolved in a 1× TE buffer [10 mM Tris HCl, 1 mM EDTA]. To eliminate RNA, 1 µL of RNase A Solution (20 mg/mL) was added, followed by vortexing, and incubation at 37°C for 30 minutes. The quality and integrity of the

extracted genomic DNA were assessed using 0.8% agarose gel electrophoresis (Sigma-Aldrich, USA) prepared in 1× TAE buffer (0.4 M Tris acetate, 0.01 M EDTA) containing 0.5 µg/mL ethidium bromide.

Partial gene sequences of isolates were determined for three gene markers, i.e., ITS, LSU, and *β-tubulin*. The primer sets used to amplify particular gene regions are ITS-5 (5'-GGAAGTAAAAGTCG-TAACAAGG-3') and ITS-4 (5'- TCCTCCGCTTATTGATATGC-3') for amplifying and sequencing Internal transcribed spacer (ITS) (White et al., 1990); LR-OR (5'-ACCCGCTGAACTTAAGC-3') and LR-7 (5'-TACTACCACCAAGATCT-3') for amplifying and sequencing 28S large subunit of the nrDNA (LSU) (Vilgalys et al., 1990; Vilgalys et al., 1994); Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') for amplifying and sequencing of *β-tubulin* (Glass & Donaldson, 1995).

PCR was carried out in a 25 µL reaction using 12.5 µL 2x Invitrogen Platinum SuperFi PCR Mastermix, 2 µL template DNA (10–20 ng), 1.5 µL 10 pmol primer, 5 µL 5x GC enhancer and H_2O (Sterile Ultra-Pure Water, Sigma, St. Louis, MO, USA), with the volume made to 25 µL. The conditions of the thermo-cycling involved: for the ITS gene region, an initial denaturation at 94 °C for 5 min, 35 cycles of 1 min at 94 °C, 30 s at 52 °C, 1 min at 72 °C, and lastly an extension at 72 °C for 8 min; for LSU, 5 min denaturation at 95 °C, 35 cycles of 60 s at 95 °C, 50 s at 52 °C, and 1.2 min at 72 °C, with a final 8 min extension at 72 °C; for *β-tubulin* gene region, 2 minutes denaturation at 94°C, 40 cycles of 35 seconds at 94°C, 55 seconds at 52°C and 2 minutes at 72°C with a final 10 minutes extension at 72°C.

The PCR amplicons were purified with an ALPHAGEN Biotech Ltd. (Taiwan) PCR purification kit as per the manufacturer's instructions. Purified PCR products of all marker genes were checked on 1.2% agarose gel electrophoresis stained with 0.5 µg/mL ethidium bromide. They were further subjected to a sequencing PCR reaction using a BigDye®Terminator v3.1 Cycle Sequencing Kit, as per the manufacturer's instructions. Briefly, the sequencing PCR reaction of 20 µL included 4 µL of 5× sequencing buffer, 2 µL of BigDye™ Terminator premix, 4 µL of primer (5 pmol), 4 µL of the purified amplicon and H_2O (Sterile Ultra-Pure Water, Sigma), with the volume made to 20 µL. Thermal cycling conditions consisted of an initial denaturation at 96°C for 3 minutes, followed by 30 cycles of 94°C for 10 seconds, 50°C for 40 seconds, and 60°C for 4 minutes. The BigDye® terminators and salts were removed using the BigDye Xterminator® Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's instructions. After performing cycle sequencing with BigDye™ terminators, 80 µL of SAM™ Solution and 20 µL of XTerminator™ solution were added to each tube. The mixture was vortexed for 30 minutes and then centrifuged at 10000 rpm for 30 seconds. The supernatant was transferred to a 96-well microplate, the module was selected, and the run was set up. The sequence was elucidated using the Applied Biosystems SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences obtained were submitted to NCBI GenBank.

2.3 **Phylogenetic analysis**

To determine the evolutionary relationship of this taxon, three ITS, LSU and *β-tubulin* gene regions were used to compare with the existing species under the genus *Mariannaea*. The sequences of the related authentic strains were retrieved from NCBI. A phylogenetic tree was constructed, in which 30 sequences of species belonging to genus *Mariannaea* were aligned with sequences of these taxa. They were used to construct the phylogenetic tree. *Stachybotrys echinatus* CBS 344.39, *Stachybotrys chartarum* CBS 129.13, and *Nectria balansae* A.R. 4446 were selected as the outgroup taxa. The strains used for the construction of the phylogenetic

tree, along with their accession numbers and other details, are tabulated in Table 1. Each gene region was aligned individually with MAFFT v. 6.864b (Katoh & Standley, 2013). The alignments were adjusted and manually checked with Aliview (Larsson, 2014).

Further, alignments were concatenated using MEGA11 (Tamura et al., 2021) and used for the phylogenetic analyses. The best substitution model was figured using ModelFinder (Kalyaanamoorthy et al., 2017). Later, the Windows version IQ-tree tool v.1.6.12 (Nguyen et al., 2015) was used to construct the phylogenetic tree. Bootstrap analyses of 1000 replicates assessed the reliability of the branches. The constructed phylogenetic tree was visualized in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/ accessed on 30th July 2025).

Table 1. GenBank accession numbers and other details of the isolates used for the phylogenetic study.

Sr. no.	Scientific name	Strain	Source	Location	GenBank Accession Nos.		
					ITS	LSU	*β-tub*
1	*Mariannaea chlamydospora*	LC1715^{T}	Submerged wood	China	KX986134	KX986141	KX986147
2	*M. elegans*	CBS 217.73A^{T}	*Pinus sylvestris*, decayed bark	Netherlands	KX986132	KX986139	KX986145
3	*M. camptospora*	CBS 209.73^{T}	Forest soil	Netherlands	MH860663	MH872365	AY624245
4	*M. macro-chlamydospora*	FKI-4735^{T}	Soil	Japan	AB855777	AB855782	-
5	*M. lignicola*	LC1791^{T}	Submerged wood	China	KX986136	KX986143	KX986149
6	*M. lignicola*	LC1792	Submerged wood	China	KX986137	KX986144	KX986150
7	*M. submersa*	MFLU 19-0549	Submerged wood	Thailand	MT496744	MT496752	-
8	*M. submersa*	MFLU 19-0542^{T}	Submerged wood	Thailand	MT496743	MT496751	-
9	*M. pinicola*	CBS 745.88^{T}	*Pinus* sp.	Venezuela	MH862152	MH873845	KM232011
10	*M. samuelsii*	CBS 125515^{T}	-	Guatemala	MH863675	MH875139	KM232015
11	*M. samuelsii*	CBS 746.88	Bark	Jamaica	MH862153	MH873846	KM232014
12	*M. humicola*	CBS 740.95^{T}	Soil	Brazil	KM231755	KM231619	KM232012
13	*M. aquaticola*	MFU090223^{T}	Submerged wood	Thailand	GQ153834	GQ153833	-
14	*M. aquaticola*	MFU090224	Submerged wood	Thailand	GQ153836	GQ153835	-
15	*M. cinerea*	LC 1766^{T}	Submerged wood	China	KX986135	KX986142	KX986148
16	*M. superimposita*	MFLUCC 17-0465^{T}	-	-	MK828662	MK828227	-
17	*M. superimposita*	CBS 124559	Soil	Japan	AB855781	AB855786	-

18	*M. superimposita*	CBS 113472	Soil	Japan	AB85 5780	AB85 5785	-
19	*M. catenulatae*	CBS 491.62^{T}	Wood	Venezuela	KM23 1752	KM23 1617	KM23 2009
20	*M. dimorpha*	HMAS 266564^{T}	Rotten bark	China	KF767 353	KJ002 443	-
21	*M. camelliae*	CMU 329^{T}	*Camellia sinensis*	Thailand	NR_1 75622	NG_0 88070	-
22	*M. camelliae*	NFCCI 6135	*Camellia sinensis*	India	PX071 450	PX071 451	PX09 3732
23	*M. koreensis*	NIBRFGC00 0512616^{T}	Soil	Republic of Korea	OR83 5254	OR83 5270	OR84 1123
24	*M. koreensis*	DUCC15750	Soil	Republic of Korea	PQ533 829	PQ533 831	PP111 899
25	*M. atlantica*	URM 8146^{T}	Soil	Brazil	MN15 1372	MN15 1398	-
26	*M. fusiformis*	LC 1701^{T}	Submerged wood	China	KX98 6133	KX98 6140	KX98 6146
27	*M. elegans var. punicea*	CBS 239.56^{T}	-	Zaire	AY62 4201	AY52 6489	AY62 4244
28	*M. terricola*	URM 92163^{T}	Soil	Brazil	MK10 1011	MK10 1012	-
29	*Stachybotrys echinatus*	CBS 344.39	-	-	KU84 6145	-	KU84 6247
30	*Stachybotrys chartarum*	CBS 129.13	-	-	KM23 1858	MH86 6145	KM23 2127
31	*Nectria balansae*	A.R. 4446	*Coronilla* sp.	France	HM48 4552	GQ50 5996	HM48 4607

Ex-type isolates/vouchers marked as ^{T}

3. Results

3.1 *Taxonomy*

Mariannaea camelliae N. Suwannarach & J. Kumla, Fungal Diversity 111:182, 2021(Figs. 2-4).

Host/distribution: *Camellia sinensis* from Palampur, Himachal Pradesh, India

Description: Hyphae hyaline, septate, smooth-walled, sometimes constricted near septa, 1.8–5.7 ($\bar{x}$=3) µm wide. Conidiophores hyaline, septate, smooth-walled, erect, macronematous, mononematous, sometimes branched, bearing 1–4 phialides, 54–198 × 1.45–2.95 ($\bar{x}$=112 × 2) µm. Phialides hyaline, smooth-walled, flask-shaped, 9.35–25 × 1.45–3.1 ($\bar{x}$=16 × 2.25) µm. Conidia hyaline, aseptate, smooth-walled, globose to subglobose, produced in imbricate chains 2.5–5.5 × 2.1–4.5 ($\bar{x}$ = 3.75 × 3.1) µm; Q=1–1.65, Qm=1.24. Chlamydospores when present, hyaline, smooth and thick-walled, terminal to intercalary, produced in chains, globose to ellipsoidal or lageniform, sometimes irregular, 8.3–20.4 × 5.05–12.45 ($\bar{x}$ = 13.3 × 8.7) µm.

Material examined: INDIA, Himachal Pradesh, Kangra, Palampur, Panchrukhi, *Camellia sinensis* leaves, S. Rana, 1st November 2024, NFCCI 6135.

GenBank numbers: ITS = PX071450, LSU = PX071451, *β-tubulin* = PX093732.

Culture characteristics: After 7 days of incubation at 25 °C, colonies on SDA reached 26–30 mm diameter, bit raised and velvety, having uniform to irregular filamentous edges; front orange grey (5B2), reverse greyish orange (5B4). On MEA, colonies reached 35 mm in

diameter, white to off-white, fluffy and velvety, slightly raised with uniform circular filamentous edges; front white (4A1), and reverse pale yellow (4A3). On PDA colonies reached 35 mm in diameter, white to off-white, fluffy and velvety with slightly raised texture and filamentous uniform to irregular edges; the front was dull yellow (3B3), and the reverse reddish yellow (4A7). On RBA, colonies measured 16 mm, also white to off-white, fluffy, velvety, slightly raised, with uniform and irregular filamentous edges; front white (10A1), and the reverse pastel red (10A4). On V8 juice agar, colonies reached 27 mm, showing a white to off-white fluffy texture, slightly raised, velvety with filamentous and irregular edges; front white (6A1), and reverse orange, grey (6B2). On Czapek Dox Agar, colonies measured 28 mm, light grey to white with a dense cottony, fluffy and velvety texture, having a smooth, well-defined edge; front white (6A1), reverse light brown (6D4). On CMA, colonies reached 29 mm in diameter, pale white to light grey with a fine, central dense cottony and radiating appearance, and a circular edge; front white (1A1), reverse pale yellow (1A3). On PCA, colonies measured 39 mm, white to off-white, fluffy, velvety, slightly raised with uniform and irregular filamentous edges; front white (5A1), reverse orange, grey (5B2).

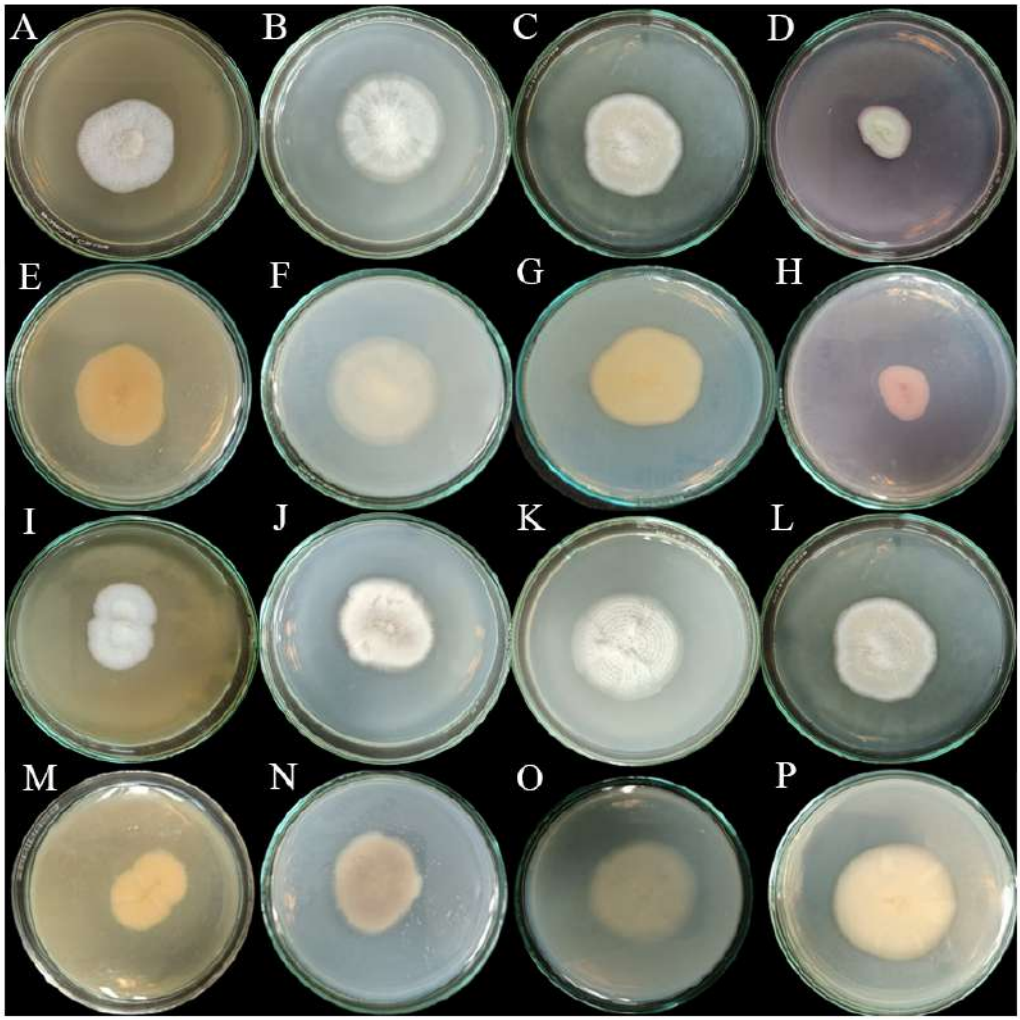

Figure 2. Colonies of *Mariannaea camelliae* after 7 days of incubation on SDA (A, E); MEA (B, F); PDA (C, G); RBA (D, H); V8 Juice agar (I, M); Czapek Dox agar (J, N); CMA (K, O); (PCA) L, P; (front view of colonies—A, B, C, D, I, J, K, L); reverse view of colonies—E, F, G, H, M, N, O, P).

Notes: The present isolate is identified as *Mariannaea camelliae* based on morphological characters and phylogenetic analysis. This isolate produced similar whorls of phialides in verticils on long conidiophores as reported earlier by Boonmee et al. (2021) for ex-type *Mariannaea camelliae* TBRC13889. In the present isolate, conidia produced were aseptate, hyaline, smooth-walled, and produced in imbricate chains as reported in its type species (*Mariannaea camelliae* TBRC13889). Moreover, the current isolate was found to make a sister clade in the phylogenetic tree based on ITS, LSU, and *β-tubulin* with the reported *Mariannaea camelliae* TBRC13889.

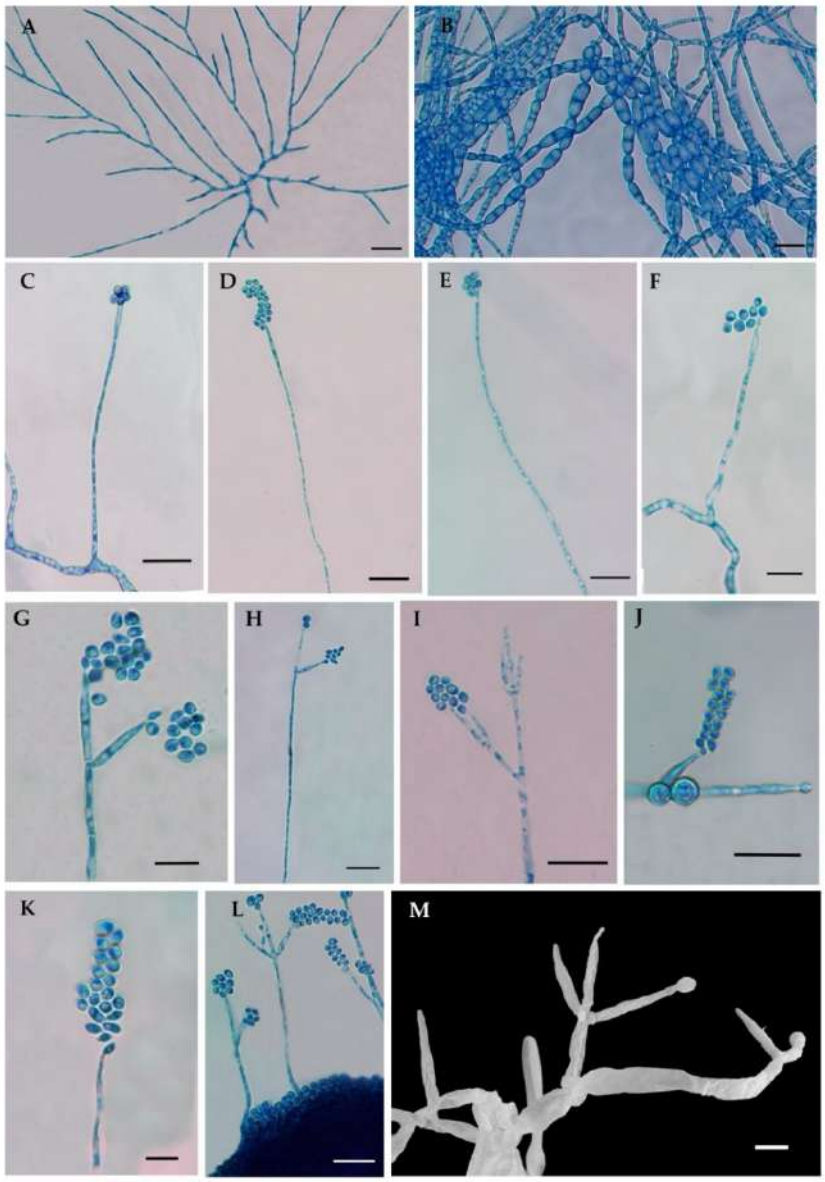

Figure 3. Morphological characters of *Mariannaea camelliae* NFCCI 6135 under an Olympus BX53 (Tokyo, Japan) microscope. A. Hyphae; B. Chlamydospores; C–F. Conidiophores bearing conidia; G–H, L. Branched conidiophores bearing conidia; I. Conidiophores and phialides; J–K. Conidia produced in imbricate chains; M. Branched conidiophores bearing conidia under Scanning Electron Microscope—Scale bars: A–E, H–L = 20 µm; F, G = 10 µm, D = 2 µm.

3.2 *Phylogenetic analysis*

The ITS, LSU, and *β-tubulin* gene regions were used to determine this isolate's identity. The phylogenetic tree in which 31 sequences of species belonging to *Mariannaea* were compared; the concatenated file contained sequence data with 1772 columns, 487 distinct patterns, 203 parsimony-informative, 126 singleton sites, and 1443 constant sites. TNe+I+G4 was chosen as the best-fit model according to the Bayesian Information Criterion as per ModelFinder. Based on the above model, the phylogeny was carried out using the Maximum Likelihood Method. The rate parameters were A-C: 1.00000, A-G: 2.36445, A-T: 1.00000, C-G: 1.00000, C-T: 6.84888, G-T: 1.00000; base frequencies were A: 0.250, 0.250, G: 0.250, and T: 0.250; the proportion of invariable sites was 0.631, and gamma shape alpha was 0.782. The log-likelihood of the consensus tree was -5483.891 (Figure 4). Tree branches were tested based on 1000 ultrafast bootstrap support replicates (UFBoot) and with an SH-like approximate likelihood ratio test (SH-like aLRT) with 1000 replicates. The combined phylogenetic tree generated using ITS, LSU, and β-tubulin sequence data nested this isolate in a unique, distinct, and well-supported clade in the genus *Mariannaea*, which forms a sister clade with *Mariannaea camelliae* CMU 329 (Ex-type) supported with good SH-like aLRT and ultrafast bootstrap (UFBoot). The phylogenetic inference based on multiple gene regions supported by morphology, phylogeny this isolate is identified and documented here as *Mariannaea camelliae* N. Suwannarach & J. Kumla.

Discussion

The identification of *Mariannaea camelliae* from the phylloplane of *Camellia sinensis* leaves in Kangra, Himachal Pradesh, expands our understanding of fungal biodiversity associated with tea plants, marking its distinction from previous findings that reported this species in leaf litter of *C. sinensis* (Trilaksana & Saraswati, 2016). The phylloplane is a vital microhabitat for a variety of microorganisms, including fungi, which play crucial roles in plant health and ecosystem functionality. Fungal communities residing on the leaf surface can help inhibit the growth of phytopathogens, contribute to nutrient cycling, and enhance plant stress resilience (Zhang et al., 2019; Yang et al., 2013).

In this study, we identified the isolate using cultural characteristics, microscopic characteristics as well as phylogenetic analysis. We conducted the molecular phylogenetic analysis of *Mariannaea camelliae* using the maximum likelihood (ML) method, employing a combined dataset of internal transcribed spacer (ITS), large subunit (LSU), and beta-tubulin sequences. The ML approach is widely recognized for its accuracy in inferring phylogenetic relationships, particularly in complex datasets (Mehrishi et al., 2020). This analysis provides a clear picture of the evolutionary relationships of *M. camelliae* within its genus and related taxa while emphasizing the importance of molecular data in resolving phylogeographic questions that morphological analyses alone may not adequately address (Aardra et al., 2023). The inclusion of diverse sequence data enhances the robustness of the phylogenetic tree, allowing for more reliable interpretations of evolutionary dynamics within the clade containing *Mariannaea camelliae* (Ullah et al., 2022). Literature suggests that utilization of combined molecular datasets greatly improves phylogenetic resolution and accuracy, especially when dealing with taxa that exhibit morphological conservatism (Gilbert & Rossie, 2007). The successful implementation of the ML method in this context underscores its utility in documenting fungal diversity and understanding their ecological roles in specific environments.

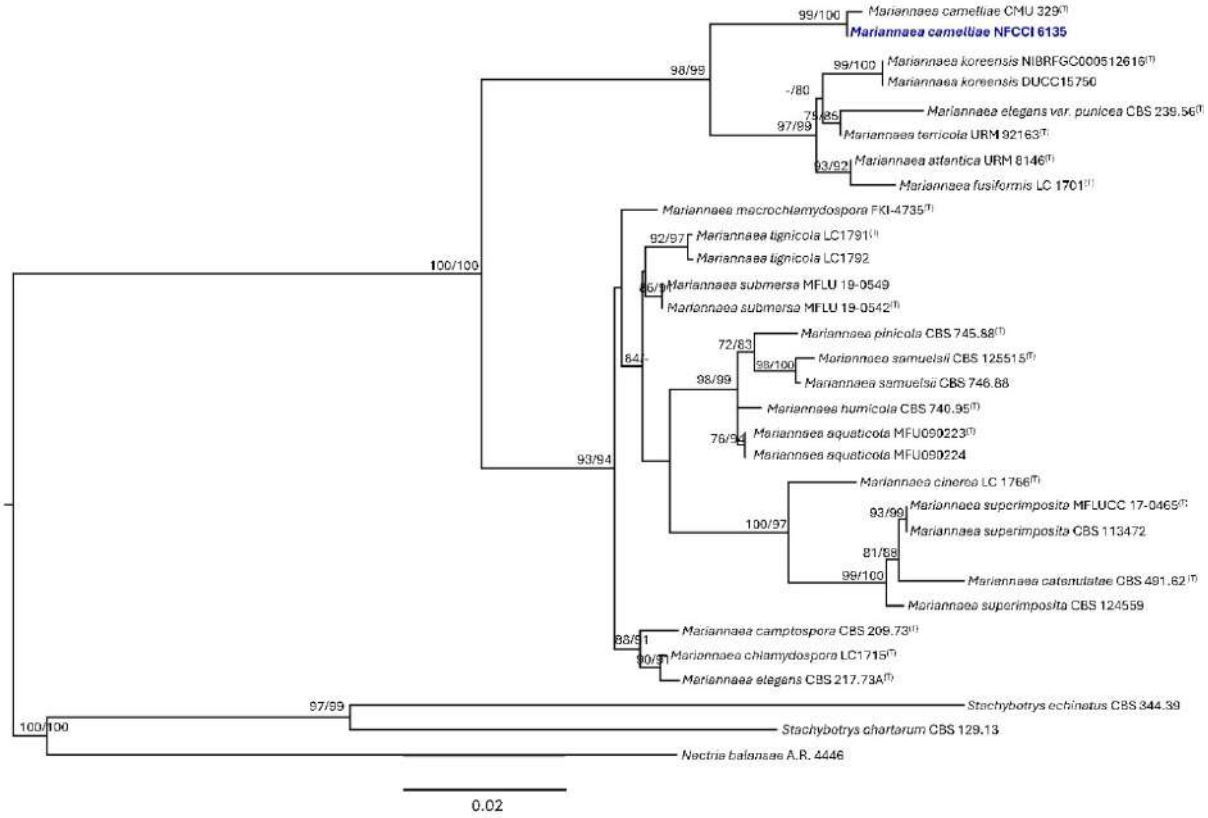

Figure 4. Molecular phylogenetic analysis of *Mariannaea camelliae* NFCCI 6135, generated by the maximum-likelihood (ML) method based on combined ITS, LSU, and *β-tubulin* sequence data. The taxon isolated in this study is represented in blue bold.

Moreover, the relationship of *M. camelliae* with *C. sinensis* further supports the hypothesis that specific fungal species can adapt and thrive in particular niche environments, extending previous understandings of its ecological roles as observed in litter habitats (Trilaksana & Saraswati, 2016). The unique adaptations of *M. camelliae* to its environment may provide insights into fungal interactions with plants and their overall contributions to ecosystem services in tea cultivation, including resource stability and pest management for *C. sinensis*. Looking forward, further exploration into the biochemical properties of *M. camelliae* could unveil novel/interesting natural products with potential applications in agriculture and related sectors. Fungi are known producers of bioactive compounds, and identifying such capabilities in *M. camelliae* could provide sustainable alternatives to synthetic chemicals in agricultural practices (Mizobata et al., 2023). This underscores the importance of conserving fungal biodiversity within agroecosystems, where such microorganisms may become invaluable partners in sustainable farming strategies. In conclusion, the isolation and subsequent phylogenetic analysis of *Mariannaea camelliae* from *Camellia sinensis* in Kangra not only enriches the catalogue of Indian fungal diversity but also exemplifies the intricate ecological relationships between fungi and plants. The insights gained from this research reinforce the necessity of continued exploration, characterization, documentation, and conservation of indigenous fungal resources to harness potential ecological and biotechnological benefits.

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