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Understanding *Astrocystis bambusae* (Fam. Xylariaceae) in India: Detailed Morphology and First Molecular Data from the Western Ghats

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ABSTRACT

This study documents the first record of *Astrocystis bambusae* (Henn.) Laessøe & Spooner (Fam. Xylariaceae) from the Western Ghats, a global biodiversity hotspot in India. Detailed morphological observations are provided based on the Indian collection, expanding the taxonomic understanding of the species. Molecular phylogenetic analyses, incorporating ITS, rpb2, and tub2 gene regions, provide the first DNA sequence data for both *A. bambusae* and the genus *Astrocystis* representing an Indian isolate. Together, these findings establish a foundational framework for understanding Indian microfungi based on a polyphasic taxonomic approach.

Keywords: Ascomata; Bamboo; Karnataka; Xylariaceae

INTRODUCTION

The family Xylariaceae was introduced by Tulasne and Tulasne (1863) as an uncertain rank "Xylariei" and later elevated to family status by Miller (1928). The family stands out as an intriguing group, well-recognized for their diversity, global distribution, ecological activities, and the remarkable novel compounds they produce (Suwannasai *et al.*, 2023). The members of the family have traditionally been classified based on stromal form, stromal colour, and ascospore shape and dimensions (Samarakoon *et al.*, 2023). The family is one of the largest and most diverse ascomycetous families with 42 genera and over 800 species (Hyde *et al.*, 2024). *Astrocystis* is one of the prominent genera of the family Xylariaceae.

The genus *Astrocystis* Berk. & Broome was established by Berkeley and Broome (1873) with *A. mirabilis* Berk. & Broome as its type species, collected from dead culms of bamboo. Morphologically, this genus is typically characterized by uni- or occasionally multi-peritheciate stromata development, often beneath the host cuticle or on the surface; relatively short stipe asci; and ascus apical apparatus that are relatively small, amyloid, and inverted hat-shaped (Ju and Rogers, 1990; Laessøe and Spooner, 1994; Li *et al.*, 2022). For a long time, the scientific reliability of the genus *Astrocystis* was uncertain and the genus was mainly considered a synonym of *Rosellinia* De Not. (Ju and Rogers, 1990). Later, Laessøe and Spooner (1994) reassessed both genera and strongly recommended that *Astrocystis* be treated as a separate genus, along with new species and generic concepts for those genera. During this study, Laessøe and Spooner (1994) transferred *Rosellinia bambusae* Henn. to the genus *Astrocystis* and proposed a new combination *Astrocystis bambusae* (Henn.) Laessøe & Spooner. Over the years, the genus *Astrocystis* along with the species *A. bambusae* have gained widespread recognition and acceptance worldwide (Habib *et al.*, 2025; Li *et al.*, 2022; Li *et al.*, 2024; Petrini, 2023; Wu *et al.*, 2021).

Currently, *Astrocystis* comprises 43 epithets worldwide (Index Fungorum, 2026). Historically, based on morphological characteristics, only three species of *Astrocystis* have been documented in different volumes of 'Fungi of India' (Butler and Bisby, 1931; Sarbhoy *et al.*, 1986; Bilgrami *et al.*,

1991; Jamaluddin *et al.*, 2004; Manoharachary *et al.*, 2022).

In the course of the present investigation, the authors collected several noteworthy specimens of *Astrocystis*. To accurately determine their taxonomic placement, detailed morphological characterization along with multigene phylogenetic analyses (ITS, rpb2 and tub2) have been carried out, which led to the identification of the species as *A. bambusae*. This paper comprises the first detailed morphological and molecular characterization of *A. bambusae* based on an Indian collection.

MATERIALS AND METHODS

Sample Collection and Morphological Characterization

During a survey of various forest zones within the Western Ghats of India, several fungus-infested bamboo samples were collected. The samples were placed in paper bags and transported to the laboratory for isolation and morphological examination.

The fungi-infested stems were first observed with a stereomicroscope. Further, thin sections of ascomata were cut with a sterile blade and transferred to drops of lactophenol on slides and microscopic details were recorded. A few slides were stained with Cotton Blue to observe hyaline structures. Finally, the slides were sealed with DPX to preserve for a long duration. All microscopic observations were made with Olympus CX41 microscope (Olympus Corporation, Tokyo, Japan) and photographs were taken with the attached microscopic digital camera (Olympus DP22). The digital photo plate was made with the help of Adobe Photoshop CS5 (Adobe Inc., California, USA). The dried material was then deposited at the Herbarium of Botanical Survey of India, Western Regional Centre, Pune (Acronym: BSI).

Single Spore Isolation and Cultural Characterization

The content inside the ascomata was picked with a sterile needle and placed in sterile distilled water to imbibe. The spore suspension was then transferred to PDA (potato dextrose agar, Himedia, Maharashtra, India) plates supplemented with antibiotics (Senanayake *et al.*, 2020). The plates were incubated at room temperature and inspected for germinating spores after 12 h. Germinated ascospores were then transferred to fresh PDA plates to obtain pure cultures. Colony characteristics were studied using a stereomicroscope, and the growth was measured after 4 weeks. The living culture was then submitted to the National Fungal Culture Collection of India (NFCCI), MACS-Agharkar Research Institute, Pune. Duplicate cultures were also maintained at the Mycological Research Laboratory of Botanical Survey of India, Western Regional Centre, Pune.

DNA Extraction and PCR Amplification

To extract fungal DNA, fresh mycelia were scraped from one-month-old cultures on PDA plates and transferred to 1.5 ml screw-capped microcentrifuge tubes. DNA extraction was performed using the HiPurA® Fungal DNA Purification Kit (Himedia, Maharashtra, India) according to the manufacturer's instructions. Polymerase chain reactions (PCR) were carried out for three loci, namely internal transcribed spacer regions (ITS), partial RNA polymerase II second largest subunit (rpb2), and partial beta-tubulin (tub2). The primers and PCR conditions were followed as outlined by Li *et al.* (2022). Amplification reactions were performed using a total volume of 40µl containing 20µl of Hi-PCR® REDy MasterMix (Himedia, Maharashtra, India), 2µl of both forward and reverse primers, 12µl of nuclease-free molecular-grade double-distilled water (ddHO), and 4µl of template DNA. The PCR products were then purified using HiPurA® PCR Product Purification Kit (Himedia, Maharashtra, India) according to the manufacturer's instructions and visualized using agarose gel electrophoresis. The PCR-purified DNA was sequenced by Barcode Biosciences in Bangalore, India, using the same PCR primers.

Phylogenetic Analysis

The forward and reverse sequences were visualized and assembled in BioEdit (Hall, 1999) to make consensus sequences. The sequences were then subjected to BLASTn searches against the core

nucleotide collection of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 25 January 2026) database using the loci ITS, rpb2, and tub2 to identify the closest matching sequences in the database. Additional sequences used in the phylogenetic tree were downloaded from GenBank based on the closest hits and recent publications (Li *et al.*, 2022; Li *et al.*, 2024). Three raw datasets (ITS, rpb2, and tub2) were created separately. All the datasets were aligned individually using the online version of the multiple sequence alignment program MAFFT v. 7 (https://mafft.cbrc.jp/alignment/software/; Katoh and Standley, 2013) with default settings. The alignment files were then checked and trimmed manually with the conserved motifs using BioEdit (Hall, 1999).

Three separate aligned and trimmed datasets were then concatenated into a multi-locus dataset using MESQUITE (Maddison and Maddison, 2023). The Maximum Likelihood analysis was conducted using the RAxML GUI 2.0 (Edler *et al.*, 2021), employing the General Time Reversible with the proportion of invariable sites and gamma distribution (GTR+G+I) model with 1,000 bootstrap replications. Bayesian inference analysis was performed using MrBayes v.3.2.7 on CIPRES (Ronquist *et al.*, 2012) using GTR+G+I as the best-fit model for all loci. Four simultaneous Markov chain Monte Carlo (MCMC) chains for 2,000,000 generations were conducted with trees sampled every 100th generation. The first 25% of trees were discarded as the burn-in phase, and the remaining were used to calculate the posterior probability by the majority consensus rule. Finally, the phylograms were visualized in FigTree v.1.4.0 (Rambaut, 2018). The GenBank accession numbers of the sequences used in this study are listed in **Table 1**.

Table 1: Names, strain numbers, reported countries, and GenBank accession numbers of the taxa used in the phylogenetic analyses, along with references

Name of Fungus	Strain Number	Country of Collection	GenBank Accession Numbers			Reference
			ITS	RPB2	TUB2	
Astrocystis bambusae	ATCC 66430	Taiwan	AY908998	–	–	Peláez *et al*. (2008)
Astrocystis bambusae	GMB0700	China	PP146578	–	PP209113	Li *et al.* (2024)
Astrocystis bambusae	MFLU 24-0522	Thailand	PQ844814	PQ855789	PQ855790	Rathnayaka *et al.* (2025)
Astrocystis bambusae	MFLUCC 14-1219	Thailand	KP401580	–	KP401587	Sir *et al.* (2015)
Astrocystis bambusae	HAST 89021904	China	GU322449	GQ844836	GQ495942	Hsieh *et al.* (2010)
Astrocystis bambusae	**NFCCI 6163**	**India**	**PX563294**	**PX512411**	**PX512412**	**This Study**
Astrocystis bambusicola	MFLUCC 17-0127 (T)	Thailand	NR_158350	MF467946	–	Hyde *et al*. (2017)
Astrocystis cocoes	GMB0037	China	MW732441	MW755333	MW755339	Wu *et al*. (2021)
Astrocystis concavispora	MFLUCC 14-0174	Italy	KP297404	KP340532	KP406615	Daranagama *et al.* (2015)
Astrocystis dinghuensis	GMB0704 (T)	China	PP133237	PP198070	PP197684	Li *et al.* (2024)
Astrocystis guizhouensis	GMB0705 (T)	China	PP133239	PP198072	PP197682	Li *et al.* (2024)
Astrocystis heterocyclae	GMB0706 (T)	China	PP153340	PP198074	PP197680	Li *et al.* (2024)
Astrocystis liuzhouensis	GMB6229 (T)	China	PQ874044	PQ826936	PQ864007	Habib *et al*. (2025)
Astrocystis mirabilis	HAST 94070803	China	GU322448	GQ844835	GQ495941	Hsieh *et al*. (2010)
Astrocystis multiloculata	GMB0033 (T)	China	MW732439	MW755330	MW755336	Wu *et al.* (2021)
Astrocystis pseudomirabilis	GMB0122 (T)	China	ON471845	ON462000	ON461996	Li *et al*. (2022)
Astrocystis sichuanensis	GMB0708 (T)	China	PP153343	PP198076	PP197678	Li *et al.* (2024)
Astrocystis sublimbata	HAST 89032207	China	GU322447	GQ844834	GQ495940	Hsieh *et al.* (2010)
Astrocystis tessellati	GMB0120 (T)	China	ON471849	ON462003	ON461994	Li *et al.* (2022)
Astrocystis thailandica	MFLUCC 15-0009 (T)	Thailand	KU246224	–	–	Li *et al.* (2016)
Xylaria hypoxylon	CBS 122620 (T)	Sweden	KY610407	KY624231	KX271279	Sir *et al*. (2016)
Xylaria polymorpha	MUCL 49884	France	KY610408	KY624288	KX271280	Wendt *et al*. (2018)
Note: Newly generated sequences are in bold, and the type sequences are mentioned as "T" in brackets.						

RESULTS

Phylogenetic Analysis

A MegaBLAST search of our isolate of *Astrocystis bambusae* NFCCI 6163 showed pairwise similarity with *A. bambusae* HAST 89021904: 99% identities (558/567 with 2 gaps) in the ITS region, 100% identities (923/923 with no gaps) in the rpb2 locus, and 100% identities (714/714 with no gaps) in the tub2 locus.

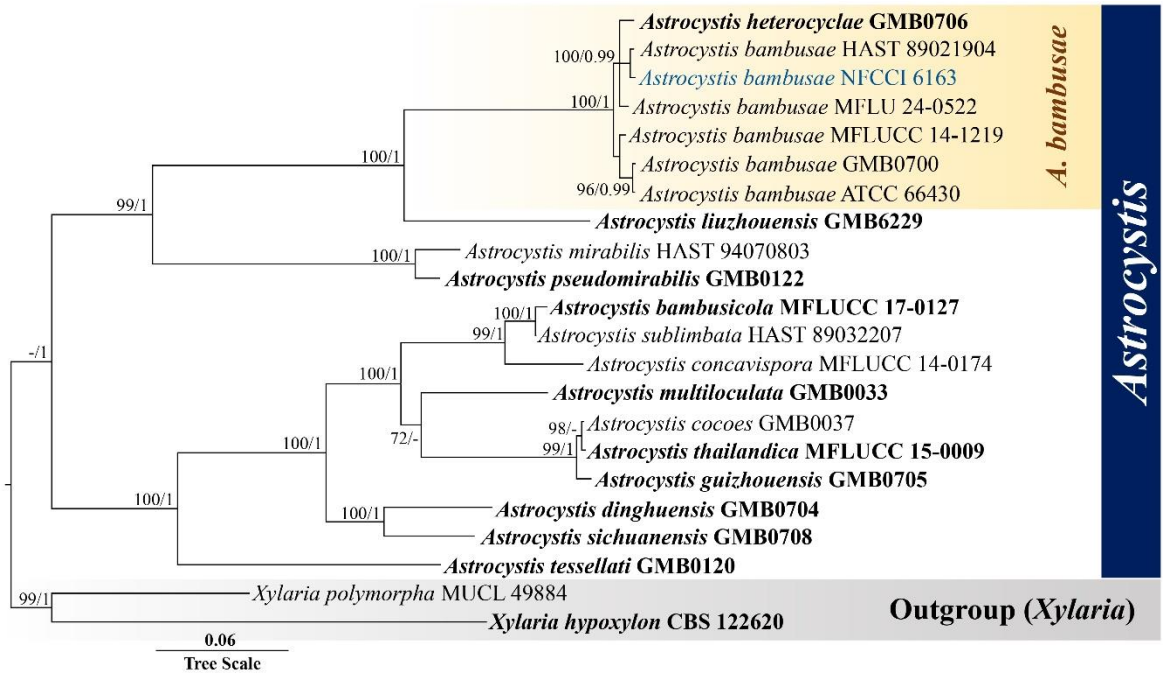


Figure 1. The best phylogram was generated from combined ITS, rpb2 and tub2 sequence data representing *Astrocystis*. Bootstrap support values for maximum likelihood (ML) ≥ 70% and Bayesian posterior probabilities (BPP) ≥ 0.90 are provided at the nodes. The isolate for the new record is in blue. Type strains of other species are in bold black. The scale bar indicates 0.06 substitution changes.

The combined ITS, rpb2 and tub2 dataset included 22 isolates from the genus *Astrocystis*, including an isolate consisting of a new report from India and two outgroups selected from the genus *Xylaria* (**Figure 1**). The final alignment consisted of 3,272 characters in total, including gaps (ITS: 1–603, rpb2: 604–1,728, and tub2: 1,729–3,272). The combined dataset had 1523 distinct alignment patterns with 35.74% gaps. The best-scoring tree with a final optimization likelihood of -16471.22 is shown in Fig. 1. Estimated base frequencies of the best ML tree (α = 1.485, and tree length = 1.479) were A = 0.232, C = 0.287, G = 0.239, T = 0.242. The substitution rates were: AC = 1.562138, AG = 4.571947, AT = 1.338429, CG = 1.256527, CT = 6.873094, GT = 1.00000. The Bayesian tree had the same topology as the ML tree. The average standard deviation of split frequencies was 0.001375 after 2,000,000 generations of runs.

The phylogram showed that the Indian isolate of *Astrocystis* has been clustered inside the clade of

Astrocystis bambusae with strong support (100% ML / 1.00 BPP). Phylogenetic analysis **(Figure 1)** strongly recommends the conspecificity of the Indian isolate (NFCCI 6163) with the other isolates of *A. bambusae* reported throughout the world.

Taxonomic Treatment

Astrocystis bambusae (Henn.) Laessøe & Spooner, Kew Bull. **49(1)**: 13 (1994) [1993]. **Figure 2**

Description: Saprobic on dead bamboo culms, visible as black raised lump on the host. **Sexual Morph:** Stromata 0.3–0.6 mm diam., 0.2–0.4 mm high, scattered or gregarious, solitary, superficial, black, hexagonal prism-shaped, top blunt, containing one ascoma, with a circle of black tissue at the bottom. Perithecia 0.2–0.5 mm diam., 0.1–0.35 mm high, comprising black, fragile,

carbonaceous tissue. Ostioles at the same level as stromatal surface, papillate, darker than stromata. Asci 95–130 × 5.0–7.5 µm ($\overline{x}$ = 115±2.5 × 6.5±0.75 µm, n = 30), 8-spored, unitunicate, cylindrical, short pedicellate, persistent, apically rounded, with amyloid inverted hat-shaped to somewhat cuneate apical apparatus, blue stained with Cotton Blue, 2.5–5.0 µm high × 1.5–2.5 µm wide ($\overline{x}$ = 4.0 × 1.9 µm). Ascospores 9–13.5 × 4–6 µm ($\overline{x}$= 12±1.0 × 4.5±0.5 µm, n = 50), uniseriate, unicellular, equilateral ellipsoid, with rounded ends, smooth, hyaline when immature, dark brown at maturity, aseptate, with a straight prominent germ slit nearly full-length, lacking appendages, and rarely with hyaline sheath. **Asexual Morph:** Undetermined.

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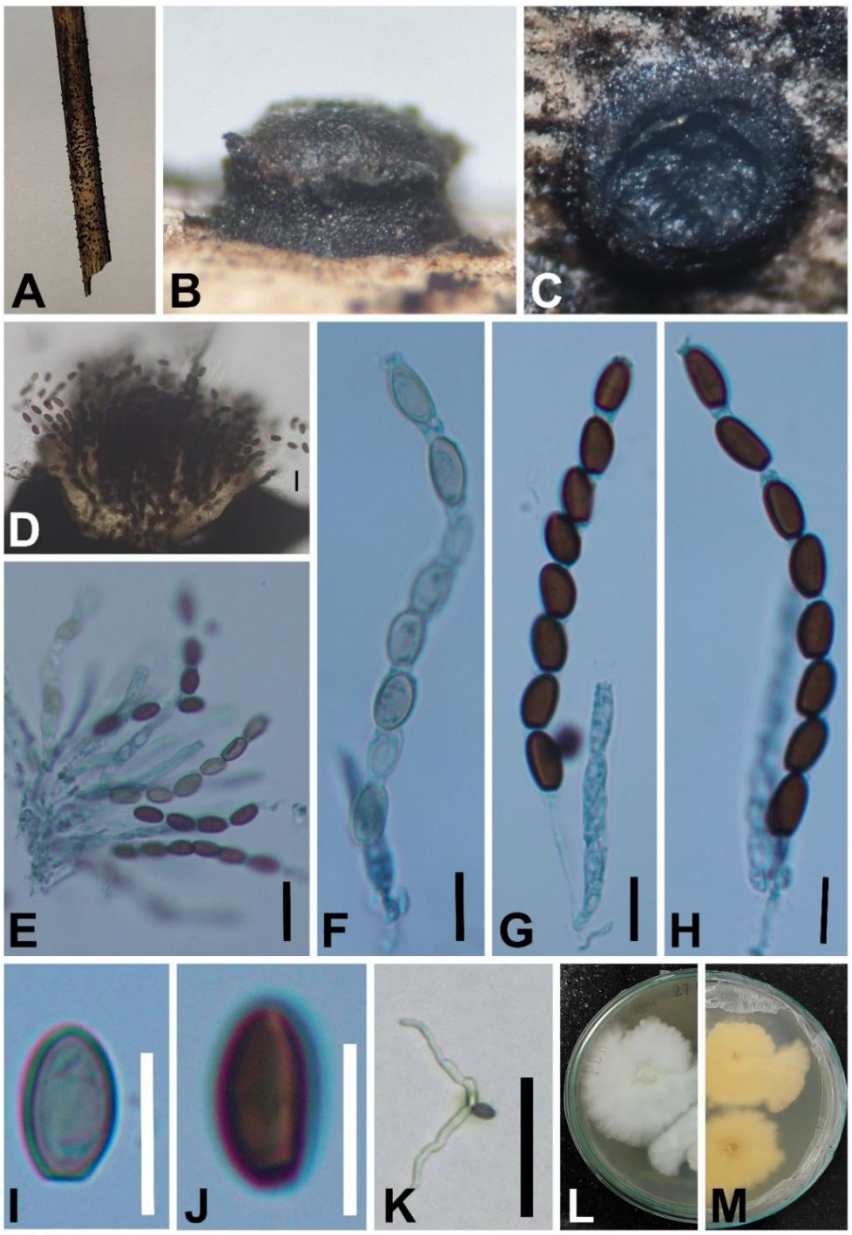


Figure 2: *Astrocystis bambusae* (BSI F-PWL-2006): A. Ascostroma on the host. B–C. Appearance of ascostroma under stereo microscope: Side view (B) and Top view (C). D. Vertical section of Ascostroma. E. Asci in a bunch. F–H. Asci at different stages of maturity: immature ascus (F), mature ascus with short stipe (G) and mature ascus with reverse hat-shaped apical apparatus (H). I–J. Different stages of Ascospores: immature (I) and mature with longitudinal germ slit (J). K. Germinating ascospore on PDA. L–M. Culture on PDA: Obverse view (L) and Reverse view (M). Scale Bars: D, E = 20 µm; F–J = 10 µm; K = 100 µm

Material examined: INDIA, Goa, Mhadei Wildlife Sanctuary, Savordem, on bamboo culms, 26/02/2022, Rashmi Dubey, BG-35-a (BSI F-BG-35a); Goa, Bicholim, On way to Mhadei, on bamboo culms, 27/02/2022, Rashmi Dubey, BG-64 (BSI F-BG-64); Karnataka, Kodagu District, Pushpagiri Wildlife Sanctuary, Somavarapete taluka, Beedhali, 12.65761° N, 75.70135° E, alt. 1119 m., on bamboo culms, 06/03/2025, *Sayantan Jash*, PWL-2006.1 (BSI F-PWL-2006; living culture NFCCI 6163).

GenBank: ITS = PX563294; rpb2 = PX512411; tub2 = PX512412

Distribution: AFRICA: Ghana (Laessøe and Spooner, 1994). ASIA: China (Ju and Rogers, 1990; Li *et al.*, 2024); India (Laessøe and Spooner, 1994; Present Study); Philippines (Ju and Rogers, 1990); Taiwan (Peláez *et al*., 2008); Thailand (Rathnayaka *et al*., 2025; Sir *et al*., 2015). SOUTH AMERICA: Guyana (Ju and Rogers, 1990).

Notes: *Astrocystis bambusae* is typically characterized by solitary, superficial, black, hexagonal prism-shaped ascostroma with papillate ostiole, short pedicellate, unitunicate asci with inverted hat-shaped apical apparatus and uniseriate, aseptate, equilateral ellipsoid ascospores with a straight, prominent germ slit. The morpho-anatomical description, including the shape and size of ascostroma, asci and ascospores of our collection, aligns with the published authentic description of *A. bambusae* (Ju and Rogers, 1990; Li *et al.*, 2024; Petrini, 2023). In the phylogenetic analysis, it also clustered within the species clade of *A. bambusae* with strong statistical support (100% ML / 1.00 BPP).

DISCUSSION

The type isolate of *A. bambusae* was collected from the Philippines and later it expanded its geographic distribution to Africa and South America (Ju and Rogers, 1990). Currently, the species is distributed in all three tropical continents of the Earth but is restricted to bambusoid monocotyledons as its host (Laessøe and Spooner, 1994; Petrini, 2023). There are no published reports or morphological descriptions of *A. bambusae* from India, except six dried Indian herbarium specimens deposited in the Fungarium of Royal Botanic Garden, Kew (Catalogue Numbers: K-M000474275, K-M000400078, K-M000380747, K-M000419629, K-M000934559, K-M000934560), which includes the collection of A. Pandey and R.C. Rajak from Jabalpur, Madhya Pradesh, India (Laessøe and Spooner, 1994). However, no published report or detailed morphological description of *A. bambusae* based on an Indian collection has been traced to date. Apart from this, there is no molecular sequence data available in GenBank for the Indian *Astrocystis*. Henceforth, this study presents the first record of *Astrocystis bambusae* from the global biodiversity hotspot of the Western Ghats, along with the first detailed morphological description of the species based on an Indian collection, and the first molecular data generated for the genus *Astrocystis* from an Indian isolate.

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