

## First report of *Asterophora lycoperdoides* (Bull.) Ditmar from dry deciduous forest of Chotanagpur Plateau

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Received : 31.07.2024

Accepted : 28.10.2024

Published : 30.12.2024

A parasitic fungicolous fungus *Asterophora lycoperdoides* was observed and documented for the first time in forest floor of Ajodhya hills under Chotanagpur Plateau. The hilly forest of this region is covered by deciduous trees and the forest floor shows a luxuriant floristic macrofungal diversity. This unique fungus grows on the mature fruitbody of *Russula albonigra*. Detailed morpho-anatomical characterization and intergenomic evolutionary study of the fungus based on 18S rRNA sequencing support that the fungal isolate SRAM-220721 (accession number OR185473) has very close relatedness with *Asterophora lycoperdoides*. Genomic sequence was submitted to NCBI.

**Keywords** : *Asterophora lycoperdoides*, Chotanagpur Plateau, Ayodhya hills, Molecular identification

### INTRODUCTION

Fungi are the second-most diverse group of organisms (O'Brien *et al.* 2005) and macro fungi are important element of nature due to their ecosystem services and human welfare. The fungicolous macrofungi *Asterophora lycoperdoides* belongs to the family Lyophyllaceae under the division Basidiomycota. Interestingly, the parasitic *Asterophora lycoperdoides* chooses Russulaceae members as its hosts, and the cluster of basidiomes grows on the host's fruit bodies (Wright 2006). It was reported from only four states of India, Orissa, Madhya Pradesh, Sikkim, and West Bengal. In India *Asterophora lycoperdoides* was initially reported from Pine forest of Orissa (Dhancholia and Sinha 1987). Rai and Acharya (2010) reported from the Sikkim Himalaya, Sharma *et al.* (2007) reported from the dense tropical forest of Madhya Pradesh and Thapa *et al.* (2020) reported from the Darjeeling District of West Bengal.

### MATERIALS AND METHODS

#### Collection site

*Asterophora lycoperdoides* developed on an overmature blackened basidiocarp of *Russula albonigra* was collected during monsoon period from the forest floor of Saldi 'Jaherthan' or sacred grove (Latitude 23.234630N, Longitude 86.05881E) in Bagmundi CD block, Purulia, West Bengal (Fig. 1). This sacred grove is dominated by *Shorea robusta*, *Diospyros melanoxylon*, *Semecarpus anacardium* and *Terminalia chebula* plants and located ten km north west from Ayodhya hill top.

#### Morpho-anatomical study

Macromorphological characteristics were noted observing fresh specimens in their natural habitat. All the microscopic features such as the nature of the mycelia, hymenial structure, basidiospores and chlamydospores were examined under bright field microscope (Leica DM 3000 LED) and SEM (JEOL JCM-6000 Plus Benchtop) using 5% KOH and Congo red stain. The length of terminal or intercalary chlamydospores was measured

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separately apical appendage to basal appendage end.

### Molecular characterization and phylogenetic analysis

Genomic DNA was extracted from dried powder sample of *Asterophoralycoperdoides*. For qualitative investigation of extracted DNA, gel electrophoresis was carried out and amplified with ITS1 and ITS4 primer (White *et al.* 1990). Purified PCR product was sequenced following Sanger sequencing method (Kshirsagar *et al.* 2020). BLASTn database was used to analyze the average nucleotide identity (ANI) and to compare the relatedness with isolated fungal sequence and submitted to NCBI database (Table 1). The 18S rRNA sequence of SRAM-220721 (OR185473) and other closely related *Asterophora* sequences with one out group (*Russula albonigra*) were aligned by ClustalW program of MEGA 11. Phylogenetic analysis was performed by neighbor joining (NJ) method of MEGA 11 with 1000 bootstraps value (Razaq *et al.* 2012).

## RESULTS AND DISCUSSION

### Taxonomy

The fruit bodies are small, stipitate with a convex pileus the width of which is normally greater than its height. Volva and annulus are absent. Young pileus is 18-35 mm diameter and appears orbicular when viewed from the top. Pileus colour of immature fruit body is white (unicolour) but later the central region of the cap become brownish (bicolour) at maturity (Fig. 2A). Pileal surface is dry, margin incurved in young stage but spreads at maturity. Flesh of the upper surface of the pileus are breaking up to a brown powdery mass of stellate chlamydospores (Fig. 2 B, C, D) measuring 15.5-20.5 × 15.2-19.3 μm. Stipe fragile or cartilaginous, equal at young, when mature clavate to bulbous, centric, cylindrical. Hyphae of stipe interwoven, 2-3 μm wide, thin walled with tapering ends (Fig. 2E), stipe typically fragile or cartilaginous. The base of lamellae is adnexed with stipe at young stage but free at maturity. Gill face smooth. The hymenial layer is composed of closely packed basidia and cystidia. Basidia

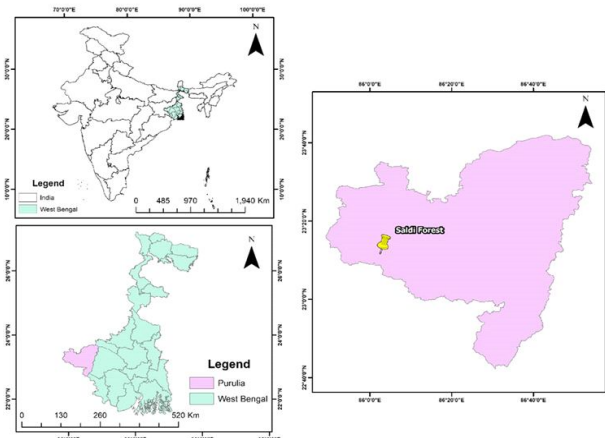


Fig. 1: Location map of the collection site.

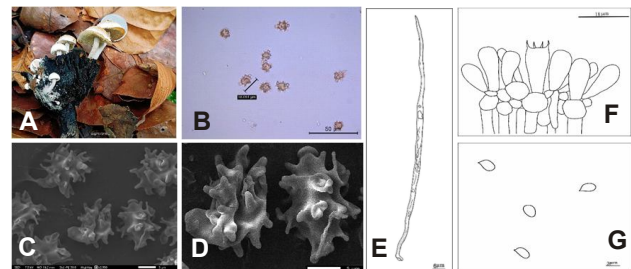


Fig. 2. (A) *Asterophora lycoperdoides* growing on the mature fruit body of *Russulaalbonigra*(B) Chlamydospores(C) SEM of chlamydospores(D) Closer SEM image of Chlamydospores(E) Mycelia of stipe (F) Arrangement of Basidia and Cystidia in hymenium layer(G) Basidiospores

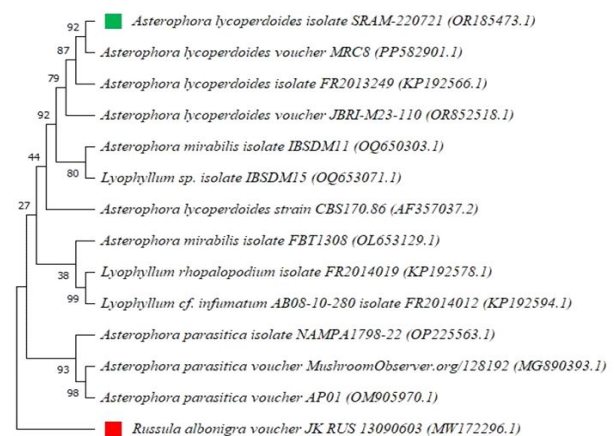


Fig.3: Phylogenetic tree generated with ITS sequences of *Asterophora lycoperdoides* and its related species by NJ method (1000 bootstrap value and other default parameters) using Mega 11 software.

are broadly clavate, four sterigmata and position slightly above the cystidia. Basidiospores 3.2-4.2 × 2.9-3.2 μm, ellipsoid and smooth (Fig. 2 G).

### ITS sequences and their phylogeny

Genome size of SRAM-220721 (OR185473) was determined 684 bp with a GC content of 43%. BLASTn inferred that SRAM-220721 (OR185473)

**Table1** : Fungal species and their accession numbers used to prepare phylogram

Name	Reference name (Voucher/Isolate/Strain name)	Gene bank accession number	Source (Country Name)
<b><i>Asterophora lycoperdoides</i></b>	<b>SRAM-220721</b>	<b>OR185473</b>	<b>India</b>
<i>Asterophora lycoperdoides</i>	MRC8	PP582901	India
<i>Asterophora lycoperdoides</i>	FR2013249	KP192566	France
<i>Asterophora lycoperdoides</i>	JBRI-M23-110	OR852518	South Korea
<i>Asterophora lycoperdoides</i>	CBS170.86	AF357037	Switzerland
<i>Asterophora parasitica</i>	128192	MG890393	USA
<i>Asterophora parasitica</i>	NAMPA 1798-22	OP225563	USA
<i>Asterophora parasitica</i>	AP01	OM905970	Netherland
<i>Asterophora mirabilis</i>	IBSDM11	OQ650303	India
<i>Asterophora mirabilis</i>	FBT1308	OL653129	Australia
<i>Lyophyllum</i> sp	IBSDM15	OQ653071	India
<i>Lyophyllum rhopalopodium</i>	FR2014019	KP192578	France
<i>Lyophyllum infumatum</i>	FR2014012	KP192594	France
<b><i>Russula albonigra</i></b>	<b>JK RUS 13090603</b>	<b>MW172296</b>	<b>Germany</b>

found to be firmly clustered with *Asterophora lycoperdoides* voucher MRC8 (PP582901) and showed 100% similarity. Phylogenetic tree based on NJ method revealed that *Asterophora lycoperdoides* voucher MRC8, *Asterophora lycoperdoides* SRAM-22071 and *Asterophora lycoperdoides* FR2013249 share with the same branch of the phylogenetic tree. Branch representing *Asterophora lycoperdoides* SRAM-220721 (OR185473) showed strong bootstrap value 92% (Table 1, Fig. 3).

## CONCLUSION

*Asterophora lycoperdoides* is very rare and it has been reported first time from dry deciduous forest of Chotanagpur Plateau. As was identified for the first time in this area over the span of a four-year survey of the macrofungi in forests region of Purulia, the host fungus *Russula albonigra* is regularly seen in ectomycorrhizal connection with *Shorea robusta* root, while *Asterophora lycoperdoides* is rarely hosted by it.

## DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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