Two new species of *Phomites* Fritel from the phyllosphere of Siwalik forest of Arunachal sub-Himalaya

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Two new species of *Phomites* Fritel from the phyllosphere of Siwalik forest of Arunachal sub-Himalaya

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In situ occurrence of two new species of *Phomites* (comparable to modern plant pathogen *Phoma* Sacc.) have been reported on two angiosperm fossil leaf remains recovered from the upper Siwalik (late Pliocene to early Pleistocene) sediments of Arunachal Pradesh, eastern Himalaya. On the basis of morpho-anatomical details of fruiting bodies i.e. pycnidia, new fungal species i.e. *Phomites arunachalensis* sp. *nov*. and *Phomites macarangensis* sp. *nov*. recovered from leaf cuticles comparable to modern *Amherstia* Wall. (Fabaceae) and *Macaranga* Thouars (Euphorbiaceae) have been proposed. Occurrence of these epiphyllous fungal morphotypes indicates a warm and humid tropical climate with high rate of precipitation during Plio-Pleistocene time in the study area.

Key words: Epiphyllous fungus, *Phomites,* leaf compressions, Plio-Pleistocene, Palaeoecology, host-fungus interaction, Arunachal sub-Himalaya

INTRODUCTION

Phoma Sacc., a filamentous fungal genus, is a well-established cosmopolitan plant pathogen (Gugel and Petrie, 1992; Fitt *et al*. 2006) and represents a large number of species which are ubiquitous in the environment, and occupy numerous ecological niches (Aveskamp *et al*. 2008). At present different species of modern Phoma are being reported from different host genera of diverse families (Thaung, 2008; Zhang *et al*. 2015). Earlier fossil fungi comparable to modern *Phoma* was published under the legitimate genus *Phomites* (Fritel 1910). So far, there are only two records of fossil fungi of *Phomites* (Fritel, 1910; Chitaley and Patil, 1972) such as *Phomites myricae* known from the compressed leaves of Myricaceae from the Palaeocene of France (Fritel, 1910) and *Phomites ebenoxyloni* from the silicified wood of Ebenaceae from the Deccan Intertrappean beds of Madhya Pradesh, India (Chitaley and Patil, 1972).

In the present communication, we report and describe two new fossil fungal species of *Phomites* from the adaxial cuticular surface of two dicotyledonous leaf remains recovered from the Siwalik sediments (middle Miocene to early Pleistocene) of the Arunachal Himalaya. Further, reconstruction of palaeoecology during upper Siwalik sedimentation (Plio-Pleistocene) is also attempted with emphasis on the exo-parasitic interaction between the fossil fungal morphs and their hosts.

MATERIALS AND METHODS

The leaf compression fossils were collected from the upper part of the Siwalik strata (Kimin Formation; late Pliocene to early Pleistocene) of Papumpare district of Arunachal Pradesh (26°27'52'' and 29°29'54'' N and 91°29'50'' and 97°24'56'' E). Upper Siwalik is characterized by loosely packed, pebbly, very coarse to fine grained grey sandstones and is intercalated with claystones and shale containing plant fossils (Kumar, 1997).

The fossil fungi were extracted from the compressed fossil leaves following the standard maceration technique by Kerp and Krings (1999) with little modification (treatment with 48% Hydro Fluoric acid followed by oxidation with 50% Nitric

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acid and repeated washing after treating with 2-5% Potassium Hydroxide). The cuticles were then dehydrated in an alcohol series (30%, 50%, 70%, 90%, and 100%) and stored in 70% alcohol for further study. For light microscopic study the samples were fixed in glass slides using 2% polyvinyl alcohol followed by mounting in Euperol to observe the cuticles with epiphyllous fungi. The fungal remains were photographed under transmitted light compound microscope (Zeiss Axioskop 2) with photographic attachment. Both collected fossil leaf specimens and prepared slides are kept at the Herbarium cum Museum of the Department of Botany, University Calcutta (CUH).

RESULTS AND DISCUSSION

Systematic Description of epiphyllous fungi
Sub Division: Pezizomycotina
Class: Dothideomycetes
Order: Pleosporales
Family: Pleoporaceae
Genus: *Phomites* Fritel, 1910
Diagnosis: Pycnidia are pale brown to dark brown in color, globose to sub-globose in shape and ostiolate. They are sunken singly or in groups in leaf cuticle. The central ostiole is distinct and surrounded by a layer of thick-walled cells. Pycnidial wall consists of brown and thread-like conidiophores.

Species: *Phomites arunachalensis* sp. *nov*. (Fig. 1A, B)
Host plant: cf. *Amherstia* sp. (Fabaceae)

Specific diagnosis: Pycnidium is dark brown, globose, 180-200 μm in size, ostiolate and sunken singly in leaf cuticle. The ostiole is distinct, round and 22 μm in diameter. It is located towards the periphery and surrounded distantly by a collar which is 10-22 μm in thickness.

Description: Pycnidium is hypophyllous, dark brown, globose in shape, 180-200 μm in size, ostiolate and sunken singly on the adaxial surface of the leaf cuticle. The ostiole is distinct, round in shape, 22 μm in diameter and located towards the periphery of pycnidium. It is surrounded distantly by a collar (10-22 μm in thickness) consisting of dark-brown to blackish and thick-walled cells. Collar and ostiole are 30-40 μm apart. Conidiospores are not found. Conidiogenous cells line in the inner wall of the pycnidial cavity.

Holotype: CUH/PPL/IB7/35/PH1

Etymology: The specific epithet '*arunachalensis*' is chosen in reference to the Siwalik sediments of Arunachal foothills from where fossil leaf compression with discussed fruit body remains of *Phomites* was recovered.

Species: *Phomites macarangensis* sp. *nov*. (Fig. 1C, D)
Host plant: cf. *Macaranga* sp. (Euphorbiaceae)

Specific diagnosis: Pycnidium is dark brown, sub-globose, 50-60 μm in size, ostiolate and sunken singly in leaf cuticle. The ostiole is distinct, almost

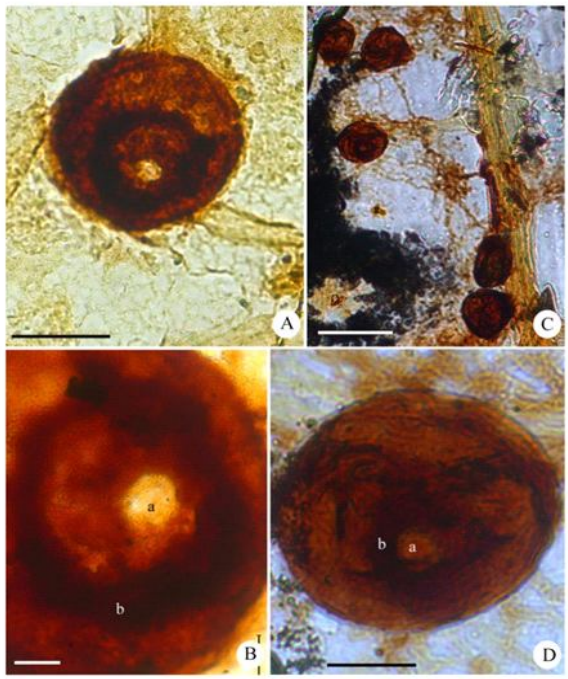

Fig. 1: Light micrographs of *Phomites* (A, B) *Phomites arunachalensis* sp. *nov*. (holotype CUH/PPL/IB7/35/PH1): (A) single mature pycnidium with an ostiole (Scale Bar = 100 μm); (B) ostiolar opening (marked by 'a') with a layer of collar (marked by 'b') in magnified view (Scale Bar = 20 μm); (C, D)*Phomites macarangensis* sp. *nov.* (holotype CUH/PPL/IB7/41A/PH1): (C) scattered dark, brown pycnidial fruit bodies on the adaxial surface (Scale Bar = 100 μm); (D) single mature fruit body showing an ostiole (marked by 'a') and thicker cells in collar (marked by 'b') (Scale Bar = 20 μm).

round and 8-10 μm in diameter. It is located towards the periphery and surrounded closely by a layer of collar which is 5-10 μm in thickness.

Description: Pycnidium is hypophyllous, dark brown, sub-globose in shape, 50-60 μm in size,

Table 1: Comparative details of fossil species of *Phoma*

Fossil fungal morphs	Pycnidium						
	Shape	Size (μm)	Ostiole			Collar	
			Shape	Size (diameter)	Position	Position in relation to ostiole	Thickness
Phomites arunachalensis sp. *nov.*	Globose	180-200 μm	Round	22 μm	Towards periphery	Distant	10-22 μm
Phomites macarangensis sp. *nov.*	Sub-globose	50-60 μm	Almost round	8-10 μm	Towards periphery	Close	5-10 μm

ostiolate and sunken singly on the adaxial surface of the leaf cuticle. The ostiole is distinct, almost round in shape, 8-10 μm in diameter and located towards the periphery of fruit body. It is surrounded closely by a layer of collar (5-10 μm in thickness) consisting of dark-brown to blackish and thick-walled cells. Conidiospores are totally absent. Conidiophores are inconspicuous and conidiogenous cells line in the inner wall of the pycnidial cavity.

Holotype: CUH/PPL/IB7/41A/PH1

Etymology: The specific epithet *'macarangensis'* is chosen in reference to the host plant leaf cuticle of *Macaranga* from where fruit body remain of *Phomites* was recovered.

The pycnidia of aforesaid fossil fungal species are yellowish brown to dark brown to blackish, globose or slightly lens-shaped, ostiolate with a collar layer consisting of thick walled cells, and interwoven network of hyphae forming pseudoparenchymatous tissue and sunken in leaf cuticle singly. These morpho-anatomical features of pycnidial fruit bodies show similarity with those of present day common plant pathogenic anamorph *Phoma* Saccard. Previously published fossil fungi under the genus *Phomites* (Fritel, 1910) resembling modern day Phoma are incomplete and their photographs are not clear enough for comparison with our present fungal morphs. Since pycnidia described here are morphologically distinguishable (Table 1) and are from different host plant leaf cuticles, we describe these fungal morphs as two new species under the legitimate genus *Phomites.*

Epiphyllous fungi are regarded as reliable environmental indicators, because they generally reflect a warm and humid environment with high rainfall (Lange, 1978, 1980; Wells and Hill, 1993). So, the recovery of epiphyllous fungal species of *Phomites* indicates a tropical warm and humid climate with high rate of precipitation during the Siwalik sedimentation (Kimin formation: Plio-Pleistocene). This interpretation is also consistent with earlier published climate data obtained from the study of the macroscopic plant remains (Khan *et al.* 2011, 2014a,b, 2016, 2017a,b) as well as the study of few epiphyllous fungal remains from the same sedimentary strata(Das *et al*. 2007; Khan *et al.* 2015; Vishnu *et al*. 2017).

In situ occurrence of two fungal morphotypes of *Phomites* on angiospermous leaf remains suggests a possible host-parasite interaction in the ancient Siwalik forest of Arunachal sub-Himalaya during Mio-Pleistocene period. In the present study, two species of *Phomite*s i.e. *Phomites arunachalensis* and *Phomites macarangensis* are reported from the two angiosperm host families viz. Fabaceae and Euphorbiaceae respectively. At present, extant *Phoma* is known to be associated with the members of the diverse angiosperm families including above-said families (Thaung,2008; Zhang *et al*. 2015). This suggests that this epiphyllous fungus still continues to be associated with the same host families since the Plio-Pleistocene time. A similar host choice of *Phoma* since Plio-Pleistocene also suggests a general host-parasite co-evolution in the Siwalik ancient forests of Arunachal sub-Himalaya.

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