Fungal Biology 123 (2019) 18–28

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Occurrence of *Phoma* Sacc. in the phyllosphere of Neogene Siwalik forest of Arunachal sub-Himalaya and its palaeoecological implications

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ARTICLE INFO

Article history:
Received 4 February 2018
Received in revised form
17 August 2018
Accepted 18 October 2018
Available online 10 November 2018

Corresponding Editor: R Balestrinir

Keywords:
Eastern Himalaya
Host–parasite interaction
Leaf compressions
Mio-pleistocene
Palaeoecology
Phomites

ABSTRACT

The present study reports *in situ* occurrence of two new epiphyllous fungal species of *Phomites* (comparable to modern genus *Phoma* Sacc.) on angiospermic leaf remains recovered from the Siwalik sediments (middle Miocene to early Pleistocene) of Arunachal Pradesh, eastern Himalaya. We describe two new species i.e. *Phomites siwalicus* Vishnu, Khan et Bera S, sp. nov. and *Phomites neogenicus* Vishnu, Khan et Bera S, sp. nov. on the basis of structural details of pycnidia. The pycnidium is a globose or slightly lens-shaped, ostiolate with a collar layer consisting of thick walled cells, sunken in leaf cuticle, with one-celled conidiospores and short-ampulliform conidiogenous cells. Host leaves resemble to those of extant *Dipterocarpus* C. F. Gaertn., *Shorea* Roxb. ex C. F. Gaertn. (Dipterocarpaceae), *Dysoxylum* Blume (Meliaceae), and Poaceae Barnhart. *In situ* occurrence of two *Phomites* morphotypes on the said leaf remains suggests a possible host–parasite interaction in the moist evergreen forest of Arunachal sub-Himalaya during Mio-Pleistocene period. The occurrence of *Phomites* in appreciable numbers indicates a humid climate favored by high rate of precipitation during Siwalik sedimentation, which is also consistent with our previously published climatic data obtained from the study of the macroscopic plant remains.

1. Introduction

Phoma Sacc. is a geographically widespread filamentous fungal genus and represents a large number of species of which more than 220 are currently recognized, as only a fraction of the thousands of species described in the literature have been verified *in vitro* (Aveskamp et al., 2008). Species of the genus *Phoma* are ubiquitous in the environment, and occupy numerous ecological niches (Fitt et al., 2006; Aveskamp et al., 2008). The genus comprises opportunists, saprobes and more than 50 % of pathogens occurring in living tissues of animals and plants. They have been reported from other fungi (Hutchinson et al., 1994; Sullivan and White Jr. 2000), lichens (Diederich and Se'rusiaux 2003; Kocourkova, 2000; Zhurbenko and Alstrup, 2004; Hawksworth and Cole, 2004) and angiosperms (Gugel and Petrie, 1992; Fitt et al., 2006). More than 110 species of *Phoma* have been reported as primary plant pathogens and having severe quarantine significance (Aveskamp et al., 2008). In higher plants, the pathogen causes a series of infections such as stem canker in oil seed rape *Brassica napus* L., black blight in potato, black Leg in Brassicaceae and in several *Solanum* species, crown rot in celery and leaf spot in a large number of angiosperm leaves (Gugel and Petrie, 1992; Fitt et al., 2006). Though *Phoma* became a well-established cosmopolitan plant pathogen since being first reported by Saccardo in 1884, the fossil record of these fungi is rather poor. Previously, there were only four records of fossil fungi resembling modern day *Phoma* (Fritel, 1910; Chitaley and Patil, 1972; Singhai, 1974; Watanabe et al., 1999). Fritel (1910) named the fossil analog of *Phoma* as *Phomites* Fritel. *Phomites myricae* Fritel is known from the fossil leaves of Myricaceae from the Palaeocene of France (Fritel, 1910) and *Phomites ebenoxyloni* Chitaley and Patil from the silicified wood of *Ebenoxylon mohgaoensis* Chitaley and Patil (comparable to modern family

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https://doi.org/10.1016/j.funbio.2018.10.007

Ebenaceae) from the Deccan Intertrappean beds of Mohgaonkalan, Madhya Pradesh, India (Chitaley and Patil, 1972). Another fossil-species *Palaeophoma intertrappea* Singhai is known from the Deccan Intertrappean beds (late Cretaceous) of Mohgaonkalan, Madhya Pradesh (Singhai, 1974).

In study of fossil fungi problems regarding their identification and interpretation are faced frequently (Stubblefield and Taylor, 1988). However, application of palaeomycological studies in phylogeny, ecology, stratigraphy and early land plant–fungi interaction (Pirozynski, 1976; Reddy et al., 1982; Banerjee, 1995; Parsons and Norris, 1999; Mitra et al., 2002; Phipps and Rember, 2004) makes it a suitable candidate to understand the occurrence, diversity and assessment of how fungi may have functioned in shaping past ecosystems (Krings et al., 2009). The objective of the present study is to document the occurrence of cosmopolitan *Phoma* spp. in fossil records and to suggest a possible host–parasite interaction in the ancient forests of eastern Himalaya during Siwalik sedimentation (middle Miocene to lower Pleistocene). Previously, the *in situ* occurrences of very few epiphyllous fungi have been recorded from this region (Mitra and Banerjee, 2000; Mitra et al., 2002; Mandal et al., 2011; Vishnu et al., 2017). In this study, we report and describe two new well-preserved fossil epiphyllous fungal species of *Phomites* (comparable to modern plant pathogen *Phoma*) from four compressed angiospermic (three dicots and one monocot) leaf remains of three angiosperm families recovered from the Siwalik sediments of the Arunachal Himalaya (middle Miocene to early Pleistocene). Epiphyllous fungi can serve as environmental indicators, because they generally reflect a humid environment (Dilcher, 1965; Phadtare, 1989; Phipps and Rember, 2004). Based on the comparative studies on the habitat of extant *Phoma* and their respective hosts, reconstruction of palaeoecology during Siwalik sedimentation (Mio-Pleistocene) has been attempted, with an emphasis on the parasitic interaction between the species of fossil fungal form *Phomities* and the recovered angiospermic host plants.

2. Materials and methods

2.1. Geological framework

The Siwalik strata extends along the Himalayan foothill region of the Indian subcontinent from the Potwar plateau in the north-west to Brahmaputra in the north-east and represents clastic sediments of freshwater molassic facies accumulated in a long narrow foredeep at the south of Himalayas during middle Miocene to lower Pleistocene (Bora and Shukla, 2005). The entire strata is divided into seven sectors from west to east based on their geographical positions (Ranga Rao et al., 1981) and three horizontal strata i.e. lower Siwalik (middle Miocene to late Miocene), middle Siwalik (Pliocene) and upper Siwalik (late Pliocene to early Pleistocene) (Tripathi, 1986; Kumar 1997). The Eastern Himalayan Siwalik sectors are represented by three geographical sectors viz. Darjeeling Foothills, Bhutan and Arunachal Pradesh. For the present investigation, fossil samples were collected from the three consecutive Siwalik strata of Arunachal Pradesh, i.e. lower Siwalik (Dafla Formation; middle to late Miocene) sediments of Pinjoli, West Kameng district; middle Siwalik (Subansiri Formation; Pliocene) sediments of Jule village, Papumpare district and upper Siwalik (Kimin Formation; late Pliocene to early Pleistocene) sediments of Banderdewa, Papumpare district (Figs. 1 and 2). Chirouze et al. (2012) proposed that the Siwalik sedimentation of Arunachal Pradesh took place between 13 and 2.5 Ma on the basis of magnetostratigraphic data. The transition between the lower and middle Siwaliks is dated at about 10.5 Ma and the middle to upper Siwaliks transition is dated at 2.6 Ma.

The lower part of Siwalik sediments is characterized by fine-grained gray sandstones with clay stones and shale containing abundant plant remains. Middle Siwalik is characterized by medium to fine grained sandstone, greenish yellow siltstone and gray silty shale containing plant fossils. Upper part of Siwalik sedimentation is characterized by loosely packed, pebbly, very coarse to fine grained grey sandstones with high limonitisation at places, and is intercalated with claystones and shale containing plant fossils.

2.2. Materials and methods

The leaf compression fossils were collected from the Siwalik strata of Arunachal Pradesh (situated between 26°27′52″ and 29°29′54″ N and 91°29′50″ and 97°24′56″ E) (Fig. 1). Leaves of modern plants growing in the vicinity of the fossil localities were collected and herbarium sheets of the same were prepared for comparisons. Collected samples were properly catalogued, numbered and stored in the herbarium and museum of the Department of Botany, University of Calcutta (CUH). Morphometric studies of the leaf fossils have been made using hand lens

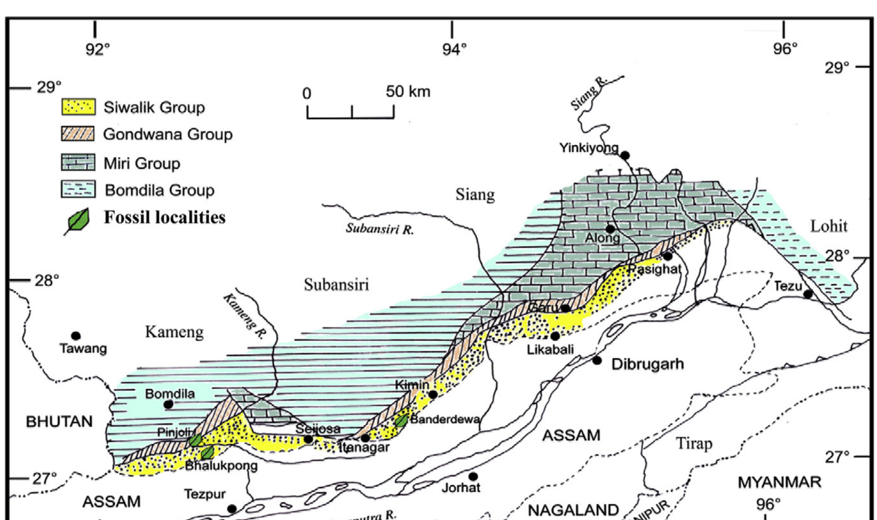

Fig. 1. Geological map of Arunachal foothills (modified after Singh and Tripathi, 1990), line drawing of leaf indicates the fossil localities.

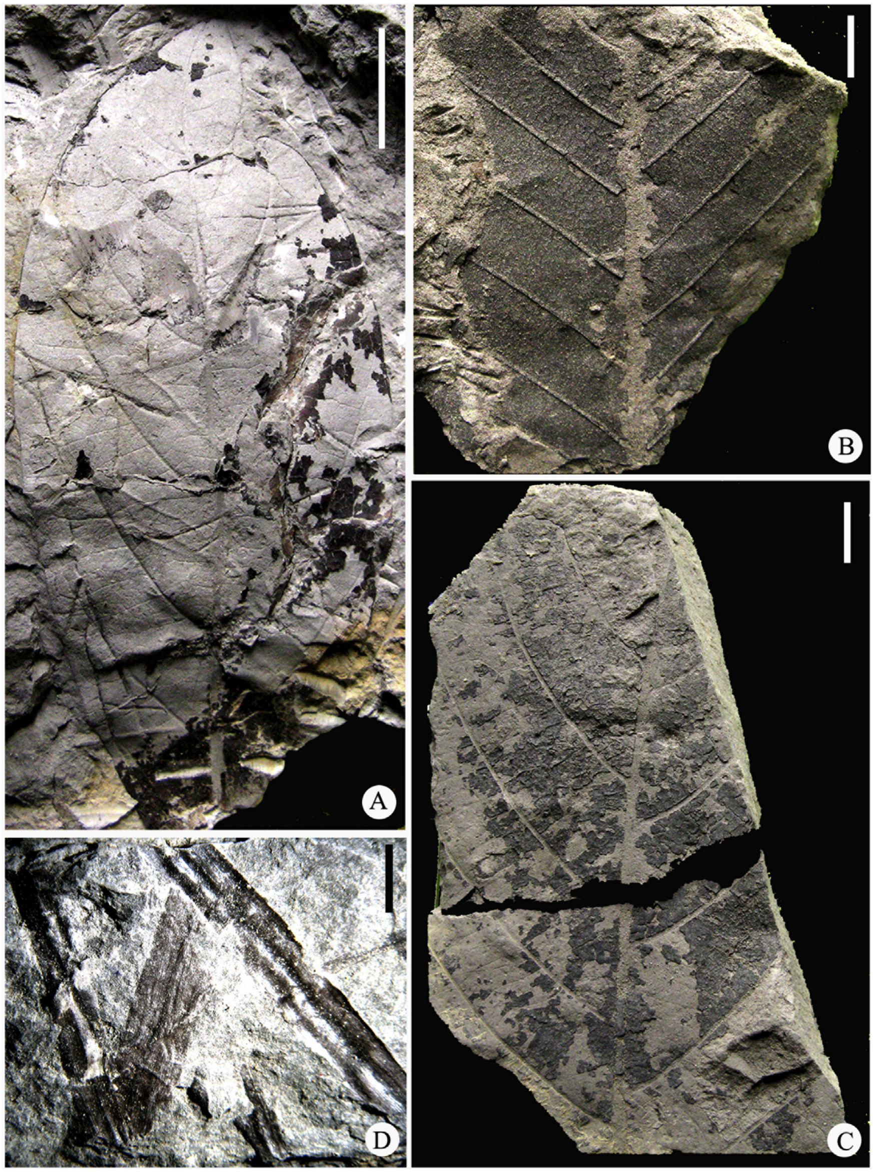

Fig. 2. Fossil leaves recovered from the Siwalik sediments of Arunachal Pradesh. (A) *Dysoxylum raptiensis* Prasad and Awasthi (Meliaceae) (specimen number CUH/PPL/IB7/17) (Scale Bar = 1 cm); (B) *Dipterocarpus siwalicus* Lakhanpal and Guleria (Dipterocarpaceae) (specimen number CUH/PPL/IB7/5) (Scale Bar = 1 cm); (C) *Shorea siwalika* Antal and Awasthi (Dipterocarpaceae) (specimen number CUH/PPL/JU/1) (Scale Bar = 1 cm); (D) *Poacites sivalicus* Sahni (Poaceae) (specimen number CUH/PPL/P/3) (Scale Bar = 1 cm).

and stereo binocular microscope (Zeiss Stemi SV11, magnified into 100 and 400). The fossil leaves (Fig. 2A–D) in majority are identified by comparing them with previously published records of fossil leaves, while some of them were identified by comparing them with herbarium sheets kept at Central National Herbarium (CAL), Shibpur, Howrah; Botanical Survey of India, Itanagar Field Station, Arunachal Pradesh; Department of Botany, CUH and with modern leaves collected from the forests adjacent to the fossil exposures as well.

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 % hydrofluoric acid followed by oxidation with 50 % nitric acid and repeated washing after treating with 2–5 % potassium hydroxide). The cuticles were then dehydrated in an alcohol series (30 %, 50 %, 70 %, 90 %, 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 cuticle with epiphyllous fungi. For scanning electron microscopy the alcohol dried samples were adhered to a stub (12.5 mm in diameter) and coated with a thin layer (20–30 nm) of conducting medium i.e. gold by vacuum evaporation or ion sputtering for further studies.

The epiphyllous fungi associated with leaf epidermal tissue were observed and photographed under transmitted light compound microscope with photographic attachment (Zeiss Axioskop 2 HAL100) and SEM (Zeiss EVO 40). Measurements presented here are taken with the help of image analyzing kit attached to light microscope after comparing the already calibrated scale using video master software. Prepared slides were deposited in the Museum of Department of Botany (Palaeobotany-Palynology Section), CUH.

For the reconstruction of palaeoclimate, quantitative study i.e. CLAMP (Climate Leaf Analysis Multivariate Program) analysis was also done on the fossil flora of Arunachal sub-Himalaya (Khan et al., 2014a).

3. Results

Sub Division: Pezizomycotina O.E. Erikss. and Winka.
Class: Dothideomycetes O.E. Erikss and Winka.
Order: Pleosporales Luttr. Ex M.E. Barr.
Family: Pleosporaceae Nitschke.
Fossil-genus: ***Phomites*** Fritel.
MycoBank number: MB21242.

Diagnosis: Pycnidium, dark brown, globose, ostiolate, partially sunken in leaf cuticle, single or in groups; ostiole distinct, surrounded by collar of thick-walled cell; conidiophores thread-like, arising from the inner wall of reproductive structure.

Fossil-species: ***Phomites siwalicus*** Vishnu, Khan et Bera S, sp. nov (Fig. 3).

Host plant: *Dysoxylum raptiensis* Prasad and Awasthi (Fig. 2A)

Specific diagnosis: Pycnidium dark brown to blackish, globose, ostiolate, partially sunken in leaf cuticle, mostly single, 100–140 μm; ostiole distinct, almost round, centrally located, 8–12 μm in diameter, well-developed collar around ostiolae; conidiospores light yellow in colour, almost oval, one-celled, 8–10 μm in diameter.

Description: Pycnidia, hypophyllous, dark brown to blackish, globose, ostiolate, partially sunken in leaf cuticle, mostly single, 100–140 μm in diameter; ostiole distinct, round, 8–12 μm in diameter, surrounded by a well-developed collar consisting of dark-brown to blackish, thick-walled cells; conidiospores scattered near the vicinity of pycnidium, 8–10 μm in diameter, almost oval, one-celled, light yellow in colour; conidiophores lacking; conidiogenous cells visible in ruptured pycnidium.

Holotype: Specimen number: CUH/PPL/IB7/17/PH_1; Type locality: road cuttings along the Itanagar-Banderdewa road in Papumpare

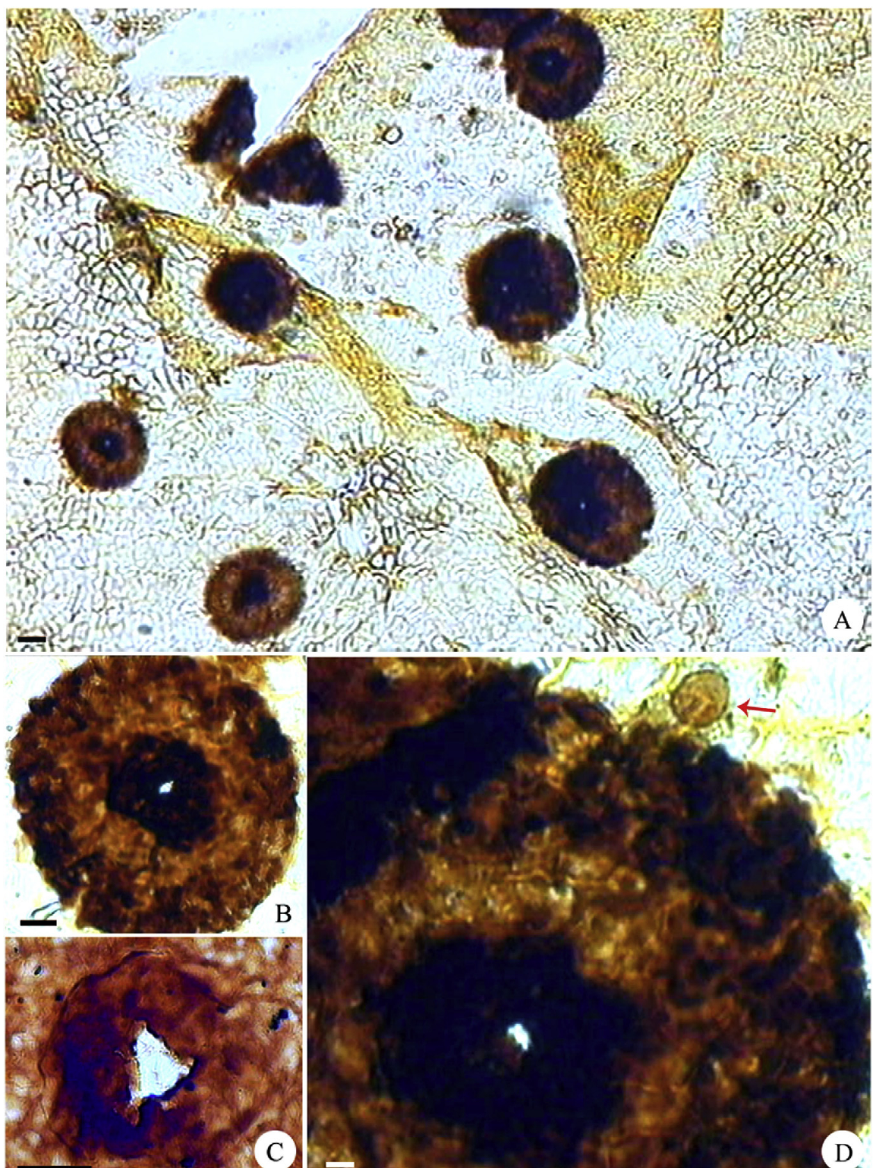

Fig. 3. Light micrographs of *Phomites siwalicus* Vishnu, Khan et Bera S, sp. nov. (CUH/PPL/IB7/17/PH_1) recovered from the leaf cuticle of *Dysoxylum raptiensis*. (A) Scattered dark, brown pycnidia on the adaxial surface (Scale Bar = 20 μm); (B) Single mature pycnidium showing central ostiole and thicker cells in collar (Scale Bar = 20 μm); (C) Ostiolar opening of the pycnidium in magnified view (Scale Bar = 10 μm); (D) Pycnidium with a pycnidiospore (marked by arrow) (Scale Bar = 10 μm). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

district, Arunachal Pradesh; Type horizon: upper part of the Siwalik succession of sediments (Kimin Formation: late Pliocene to early Pleistocene); Collecting date: 21/05/2008; Collectors: Mahasin Ali Khan and Subir Bera; Repository: Specimen is kept at the herbarium and museum of the Department of Botany, CUH.

Etymology: The specific epithet '*siwalicus*' is chosen in reference to the Siwalik deposits from where fossil leaves with discussed fruiting body remains of *Phomites* were recovered.

Fossil-species: ***Phomites neogenicus*** Vishnu, Khan et Bera S, sp. nov (Fig. 4–7).

Host plants: *Dipterocarpus siwalicus* Lakhanpal and Guleria (Fig. 2B); *Shorea siwalika* Antal and Awasthi (Fig. 2C); *Poacites sivalicus* Sahni (Fig. 2D).

Specific diagnosis: Pycnidium light brown to dark brown, globose or slightly lens-shaped with centrally projecting 2–4 notches, ostiolate, partially sunken in leaf cuticle, single or in groups, 120–200 μm; ostiole almost round, mostly located towards periphery, 10–30 μm in diameter, collar around ostiolae.

Description: Pycnidia, light brown to dark brown, globose or slightly lens-shaped with 2–4 notches towards the centre, ostiolate, partially sunken in leaf cuticle, single or in groups, 120–200 μm in diameter; ostiole distinct, almost round, 10–30 μm in diameter, surrounded by an irregular collar of dark-brown to blackish thick-walled cells; conidiospores and conidiophores lacking; short-ampulliform conidiogenous cells visible in ruptured pycnidium and lining in the inner wall of the pycnidial cavity.

Holotype: Specimen number: CUH/PPL/IB7/5/PH$_1$; Type locality: Road cuttings along the Itanagar-Banderdewa road in Papumpare district (lying between longitude 92°40′E and 94°21′E and latitude 26°55′N and 28°40′N), Arunachal Pradesh (situated between longitude 91°29′E and 97°24′E and latitude 26°27′N and 29°29′N); Type horizon: Upper part of the Siwalik succession of sediments

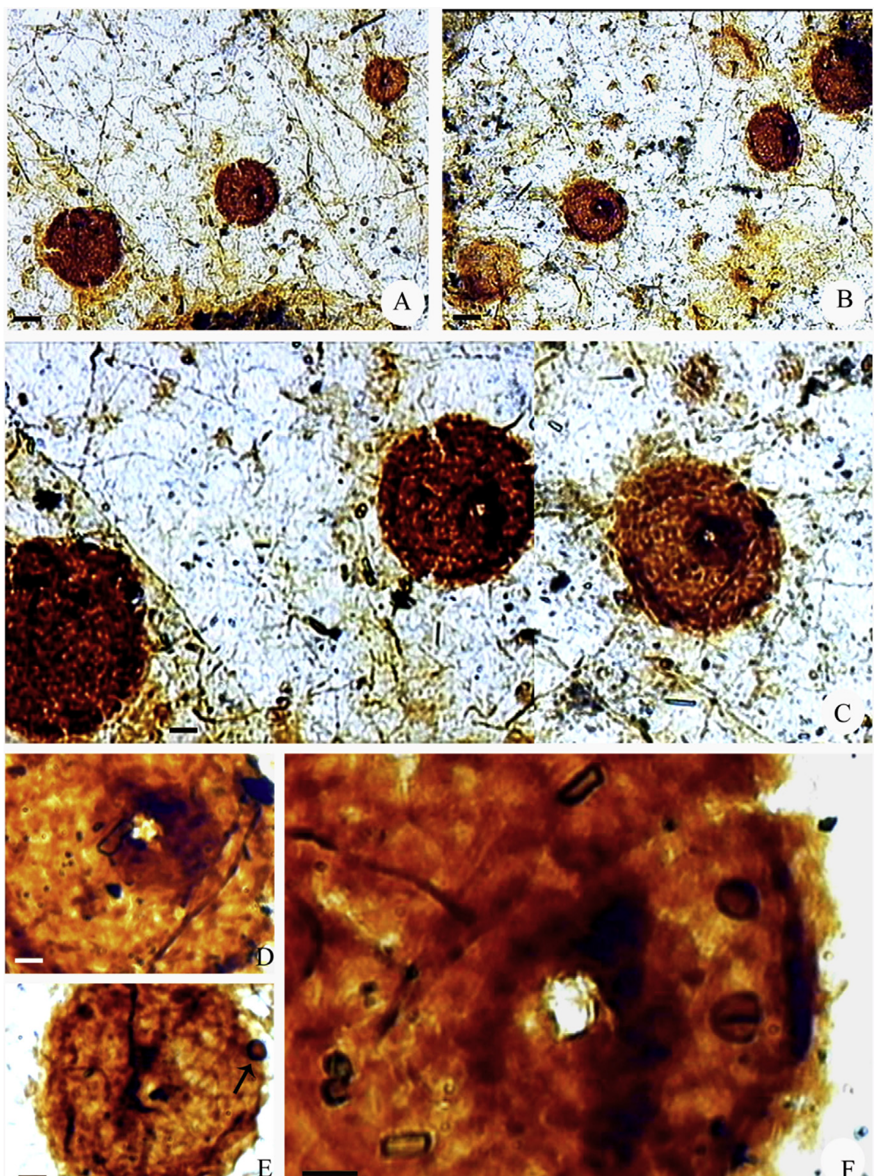

Fig. 4. Light micrographs of *Phomites neogenicus* Vishnu, Khan et Bera S, sp. nov. (Holotype CUH/PPL/IB7/5/PH$_1$) recovered from the leaf cuticle of *Dipterocarpus siwalicus*. (A, B) Scattered dark, brown pycnidia (Scale Bar = 50 μm); (C) Enlarged view of pycnidia (Scale Bar = 30 μm); (D) Single mature pycnidium showing a central ostiole (Scale Bar = 15 μm); (E, F) Pycnidium with an ostiolar opening (Scale Bar = 15 μm). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

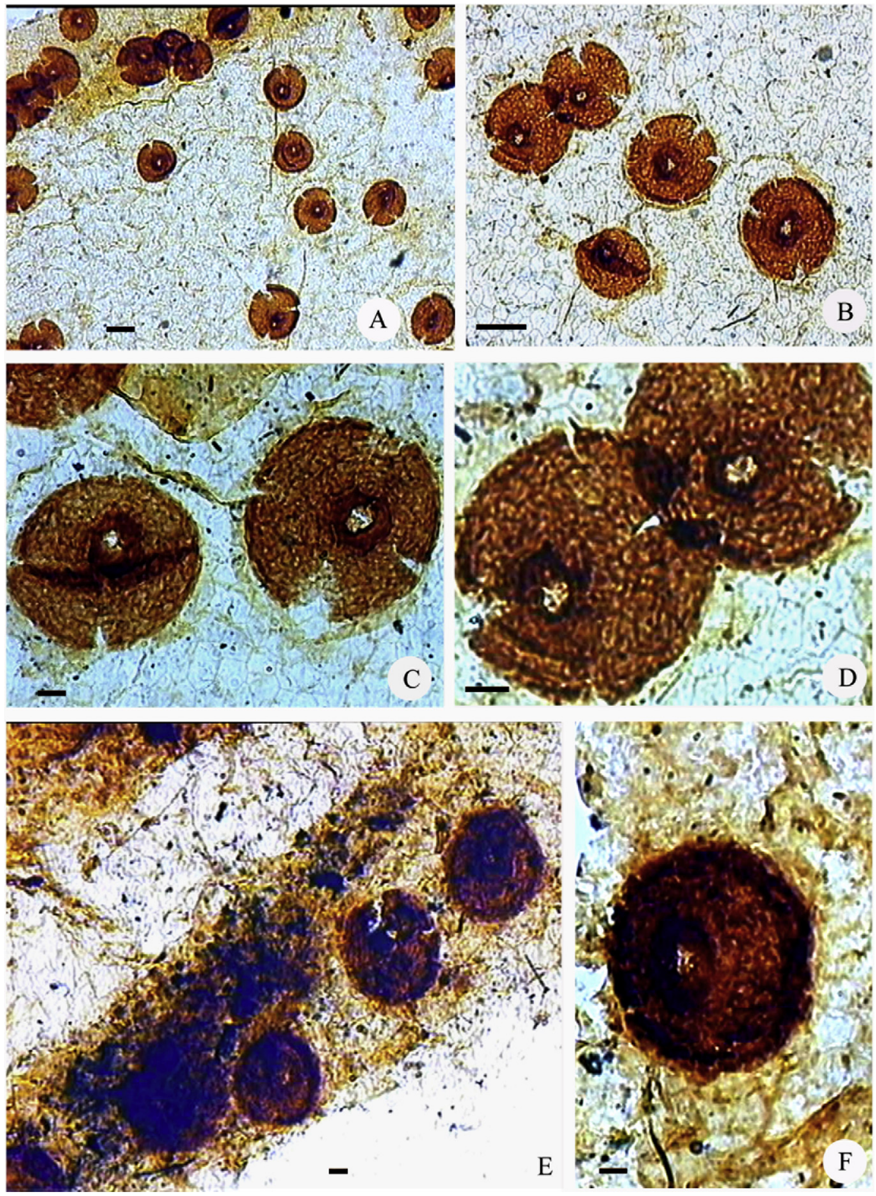

Fig. 5. Light micrographs of *Phomites neogenicus* Vishnu, Khan et Bera S, sp. nov. (Paratype CUH/PPL/JU/1/PH_1) recovered from the leaf cuticle of *Shorea siwalika* (A–D) and *Poacites miocenicus* sp. nov. (Paratype CUH/PPL/P/3/PH_1) recovered from the leaf cuticle of *Poacites sivalicus* (E–F). (A, B) Scattered dark, brown pycnidia (Scale Bar = 100 μm); (C, D) Enlarged view of pycnidia showing central ostioles (Scale Bar = 25 μm); (E) Scattered dark, brown pycnidia (Scale Bar = 10 μm); (F) Enlarged view of one pycnidium showing a central ostiole (Scale Bar = 10 μm). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

(Kimin Formation: late Pliocene to early Pleistocene); Collecting date: 21/05/2008; Collectors: Mahasin Ali Khan and Subir Bera; Repository: Specimen is kept at the herbarium and museum of the Department of Botany, University of Calcutta (CUH).

Paratypes: Specimen number: CUH/PPL/JU/1/PH_1; Locality: road cuttings to the Jule village in Papumpare district, Arunachal Pradesh; Horizon: middle part of the Siwalik succession of sediments (Subansiri Formation: Pliocene); Specimen number: CUH/PPL/P/16/PH_1; Locality: road cuttings to the South of Pinjoli area in West Kameng district (lying between longitude 91°31′E and 92°40′E and latitude 26°54′N and 28°01′N), Arunachal Pradesh; Horizon: Lower part of the Siwalik succession of sediments (Dafla Formation: middle to late Miocene).

Etymology: The specific epithet refers to the stratigraphical occurrence (Neogene deposits) of the discussed fruiting bodies of *Phomites*.Table 1.

4. Discussion

4.1. Comparisons

The morpho-anatomical features (globose or slightly lens-shaped with definite ostiolar openings and interwoven network of hyphae forming pseudoparenchymatous tissue, with some scattered one-celled conidiospores) of pycnidial fruit bodies of aforesaid fossil fungal species show similarity with those of present day common plant pathogenic fungus *Phoma* Saccardo of family Pleosporaceae. At present different species of *Phoma* are reported from different angiosperm leaves (Table 2).

Previously fossil fungi comparable to modern *Phoma* was placed under the order Sphaeropsidales of Fungi imperfecti (Kalgutkar and Jansonius, 2000) and published under the fossil genera i.e., *Phomites* (Fritel, 1910), *Palaeophoma* (Singhai, 1974) and *Archephoma*

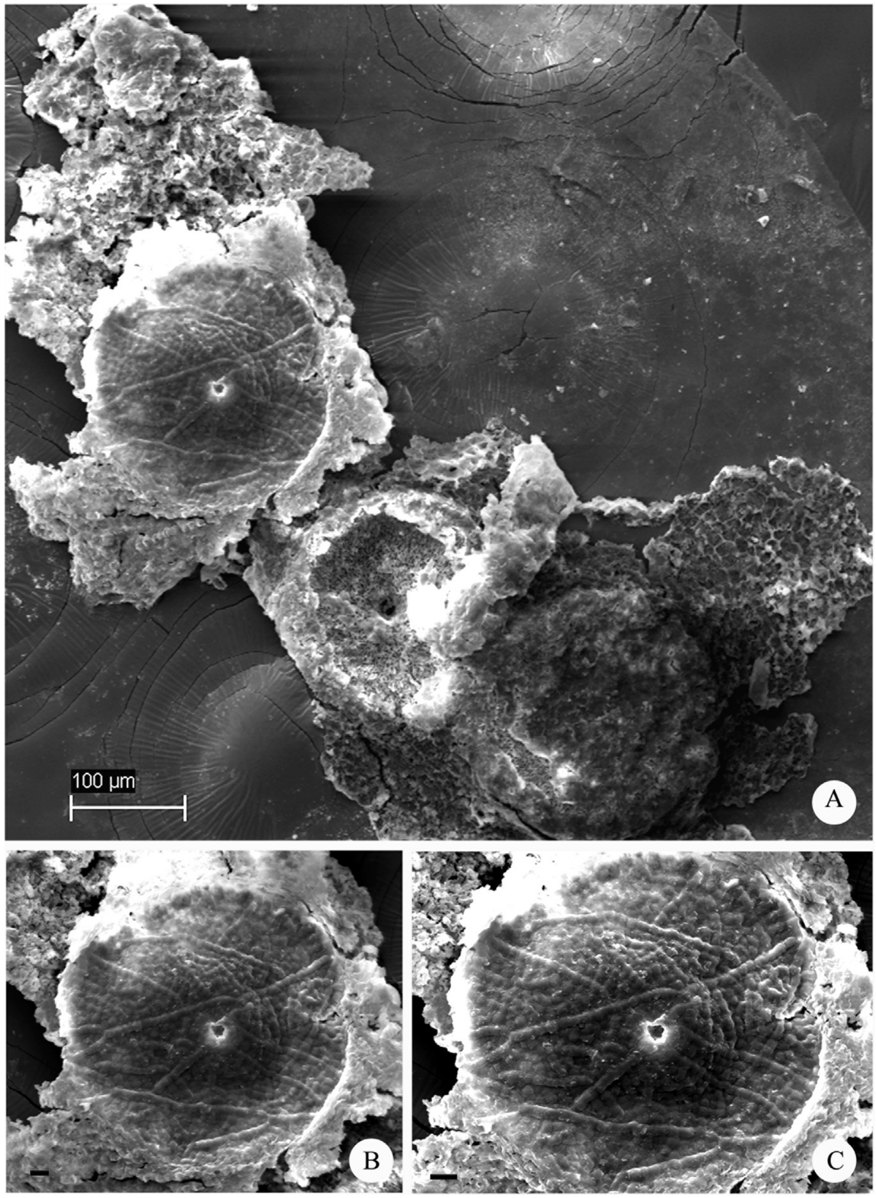

Fig. 6. SEM photographs of *Phomites neogenicus* Vishnu, Khan et Bera S, sp. nov. (Holotype CUH/PPL/IB7/5/PH_1) recovered from the leaf cuticle of *Dipterocarpus siwalicus*. (A) Pycnidia on the leaf surface (Scale Bar = 100 μm); (B) Pycnidium showing interwoven network of hyphae (Scale Bar = 10 μm); (C) Enlarged view of pycnidium showing interwoven network of hyphae (Scale Bar = 10 μm).

(Watanabe et al., 1999). So far there are only four records of fossil fungi resembling modern day *Phoma* (Fritel, 1910; Chitaley and Patil, 1972; Singhai, 1974; Watanabe et al., 1999). However, these fossil fungal forms are incomplete and their photographs are not clear enough for comparison. As morpho-anatomical structure of pycnidia described here are quite distinguishable (Table 3), we describe these pycnidia as two new fossil-species under the legitimate genus *Phomites*. Fossil-species *Phomites neogenicus* sp. nov. differs from *Phomites siwalicus* sp. nov. in having larger pycnidia with narrower collar around ostiolae. However, *Phomites siwalicus* is characterized by more strongly developed collar around ostiolae.

4.2. Taxonomy of phoma

The genus *Phoma* has generally been considered by most modern mycologists to be taxonomically problematic not only due to ambiguous morphological criteria, but also due to uncertain phylogenetic affinities. In recent years, the anamorphic genus *Phoma* has been subdivided into nine sections based on morphological characters, and included teleomorphs in *Didymella* spp., *Leptosphaeria* spp., *Pleospora* spp. and *Mycosphaerella* spp., suggesting the polyphyly of the genus (Gruyter et al., 2012). Furthermore, extensive work on the taxonomy of *Phoma* has been done by Boerema et al. (2004), using culturing techniques and morphological data. This led to the publication of a handbook entitled "*Phoma* Identification Manual" (Boerema et al., 2004) providing keys and descriptions of more than two hundred taxa at specific and infra-specific level, but many *Phoma* species still remain to be identified (Gruyter et al., 2012). Systematics of modern *Phoma* is based mainly on cultural characteristics and molecular studies (Hawksworth and Cole, 2004; Diederich et al., 2007). Cultural isolation of the fungi is very important to understand their anamorph and teleomorph differentiation and morphology. In case of fossil study there is no scope of isolation of fungi in culture and

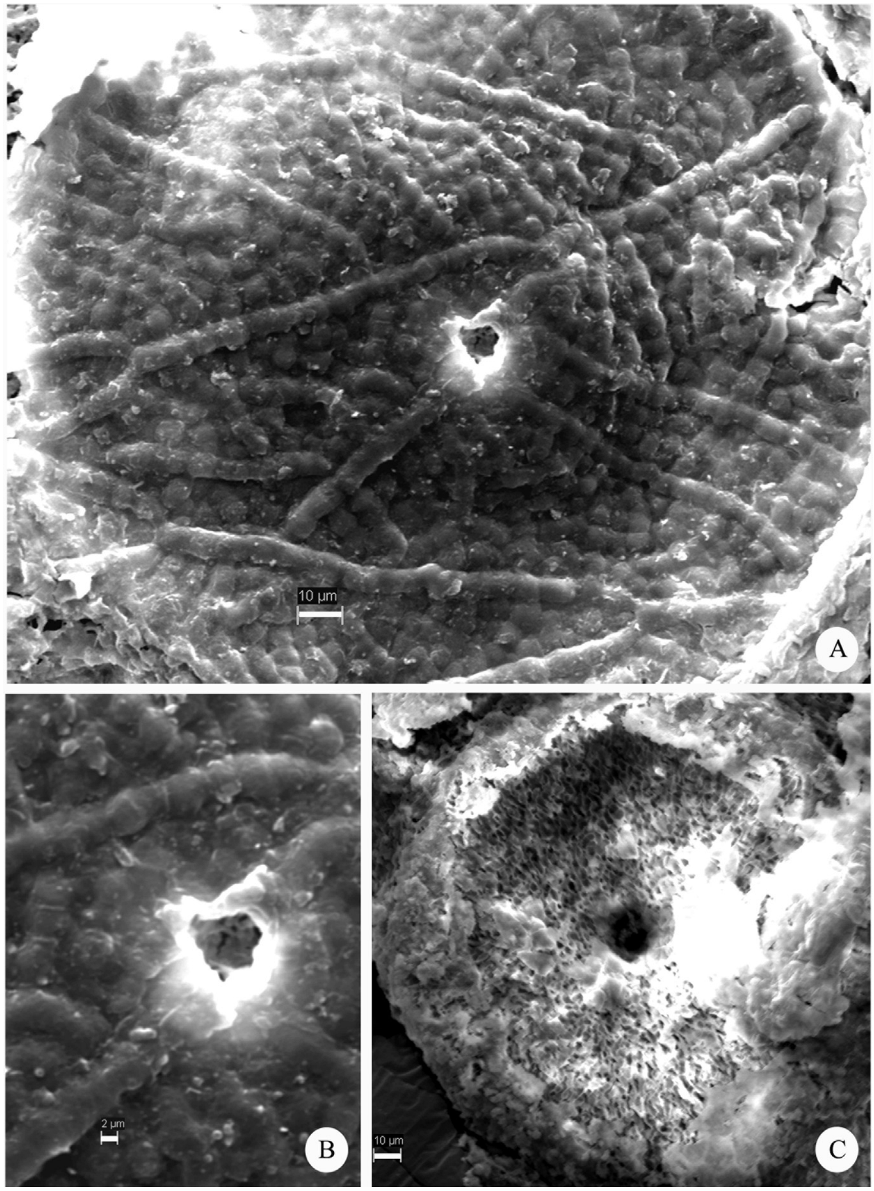

Fig. 7. SEM photographs of *Phomites neogenicus* Vishnu, Khan et Bera S, sp. nov. (Holotype CUH/PPL/IB7/5/PH_1) recovered from the leaf cuticle of *Dipterocarpus siwalicus*. (A) A pycnidium showing pseudoparenchymatous tissue like interwoven network of hyphae (Scale Bar = 10 μm); (B) A prominent ostiolar opening of the pycnidium (Scale Bar = 2 μm); (C) Ruptured pycnidium showing internal cellular details (Scale Bar = 10 μm).

therefore it is difficult to assign the fossil fungal form into certain anamorph with a definite teleomorph, if no associated sexual stage is preserved in the fossil.

In the previous description of the fossil form of *Phomites*, Fritel (1910) described it as an imperfect fruiting structure while Chitaley and Patil (1972) described it as a silicified pycnidium with conidiospores. But photographs of the earlier described forms were indistinct. So, in the present perspective, as no teleomorphic connection is found in *Phomites* or *Phomities* is a validly published fossil fungal genus, we describe well preserved fruiting body remains as two new fossil-species under the legitimate genus *Phomites*.

4.3. Palaeoecological implications

Epiphyllous fungi have the potential to provide important information about past climate and habitat (Lange, 1978, 1980; Wells and Hill, 1993). Some epiphyllous fungi grow in the humid tropics and subtropics (Dilcher, 1965; Phadtare, 1989; Tripathi, 2001; Phipps and Rember, 2004; Shi et al., 2010). So, the present record of *in situ* epiphyllous fungal forms and their respective hosts may be utilized in interpreting palaeoclimate in the Siwalik strata of Arunachal Pradesh, eastern Himalaya. The discovery of an appreciable number of well-preserved fungal fruit bodies of *Phoma* indicates a humid climate favored by high rate of precipitation during the Mio-Pleistocene time in the ancient forests of Arunachal sub-Himalaya, consistent with the evidence from previously published fossil plants and the current host plants. At present, the area experiences a prolonged rainy season and remains under warm, humid but a comparatively drier climate for the major part of the year (Champion and Seth, 1968; Hazra et al., 1996).

The recovered host fossil leaves are comparable to modern day taxa *Dipterocarpus* sp., *Shorea* sp. (Dipterocarpaceae), *Dysoxylum* sp.

Table 1
Generalised lithotectonic succession in Arunachal Pradesh (after Joshi et al., 2003).

NORTH		
Gondwana Group		Carbonaceous shale, sandstone and coal
	Main Boundary Faults	
Lower Siwalik (Dafla Formation)		Well indurated sandstone, shale and siltstone with plant fossils
	Thrust	
Middle Siwalik (Subansiri Formation)		Weakly indurated sandstone and shale and siltstone with plant fossils
	Thrust	
Upper Siwalik (Kimin Formation)		Sandstone and claystone/shale with plant fossils fossil wood
	Main Frontal Fault	
Assam Alluvium/ Quaternary deposits		
SOUTH		

(Meliaceae), and the family Poaceae (Fig. 3, Table 2). The current distribution of modern equivalent taxa and physiognomic characters of recovered fossil leaves clearly suggests that tropical evergreen forests were present in Arunachal Pradesh under a tropical warm, humid condition with a high rate of precipitation during the Siwalik sedimentation (Miocene to Pleistocene). Several records of mega plant fossils confirm this interpretation (Khan et al., 2011; Khan and Bera, 2014; Khan et al., 2014a, 2014b; Khan et al., 2015, Khan et al., 2016, 2017a, 2017b, 2018a, 2018b). Our CLAMP parameters (MAT, mean annual temperature, 23.7°–25.4 °C ± 2.8 °C; CMMT, cold month mean temperature, 16.9°C–21.3 °C ± 4 °C and a WMMT, warm month mean temperature, 27.9–28.2 °C ± 3.3 °C and a week monsoonal climate with growing season precipitation, GSP, 174–198 cm ± 92 cm) are also in conformity with the interpretation following present study of climatic conditions in Arunachal sub-Himalaya during Siwalik sedimentation. CLAMP scoresheets, CLAMP high resolution gridded meteorological calibration dataset and CLAMP leaf physiognomic calibration dataset are available as supplementary materials (Supplementary materials; Tables S1, S2, S3, S4, S5).

4.4. Host-fungi interaction

Epiphyllous fossil fungi preserved along with other plant material are both parasitic and saprophytic in nature (Taylor and Osborn, 1996). The development of the fungal fruiting bodies depends on the nutrient and growth-regulating substances in the host plants (Maheshwari, 2006), and the fungi may produce some

Table 2
Current records of some modern *Phoma* spp.

Family	Genus	References
Aceraceae	*Acer saccharum, A. negundo*	Zhang et al. (2015)
Amaranthaceae	*Amaranthus*	Zhang et al. (2015)
Annonaceae	*Miliusa, Annona*	Thite and Kulkarni (1976); Zhang et al., (2015)
Apiaceae	*Saposhnikovia*	Zhang et al. (2015)
Apocynaceae	*Cynanchum*	Zheng et al. (2004)
Asperagaceae	*Sansevieria*	Zhang et al. (2015)
Asteraceae	*Atractylodes, Dendranthema, Cirsium*	Zhang et al. (2015)
Basellaceae	*Basella*	Zhang et al. (2015)
Combretaceae	*Terminalia*	Shivas and Alcorn (1996)
Dipterocarpaceae	*Dipterocarpus, Shorea*	Singh et al. (2004)
Elaeagnaceae	*Elaeagnus*	Zheng et al. (2004)
Euphorbiaceae	*Jatropha, Ricinus*	Thaung (2008)
Fabaceae	*Bauhinia, Caragana, Desmodium, Glycine, Pisum*	Zhang et al. (2015)
Lauraceae	*Cinnamomum*	Thaung (2008)
	Lindera	Zhang et al. (2015)
	Cinnamomum	Thaung (2008)
Moraceae	*Ficus*	Paulus et al., (2006); Zheng et al., (2004)
	Morus	Aghapour et al. (2009)
Poaceae	*Oryza, Coix Saccharum, Triticum, Zea, Sorghum, Panicum*	Thaung (2008); Boerema (1976); Gruyter et al., (2009); Zhang et al., (2015)
Rosaceae	*Cotoneaster, Eriobotrya, Prunus, Sanguisorba*	Zhang et al. (2015)
Rubiaceae	*Mitragyna*	Pharamat (2009)
	Mitragyna, Coffea	Pharamat (2009), Zhang et al., (2015)
Rumiceae	*Rumex*	Zhang et al. (2015)
Solanaceae	*Physalis*	Zhang et al. (2015)
Vitaceae	*Parthenocissus*	Zhang et al. (2015)

Table 3
Comparative details of fossil species of *Phoma*.

Fossil-species	Pycnidium					Conidiospore		
	Shape	Size (μm)	Ostiole			Shape	Size (μm)	No of cell
			Shape	Size	Position			
Phomites siwalicus Vishnu, Khan et Bera S, sp. nov.	Globose	100–140 μm	Round	8–12 μm in diameter	Centrally located	Oval	8–10 μm in diameter	One
Phomites neogenicus Vishnu, Khan et Bera S, sp. nov.	Globose or slightly lens-shaped	120–200 μm	Round	10–30 μm in diameter	Mostly towards periphery	Not found		

substances, which can protect the host plants from natural enemies (Carroll, 1988).

The fungal fruit body remains of *Phoma* described here represents the first evidence of fungi associated with diverse angiosperm leaves from Siwalik Foreland Basin. This discovery is important because documented evidence of this fungal form residing on and in the leaves of angiosperms, one of the dominant plant groups in Siwalik during the Mio-Pleistocene time, is generally scarce. We are aware of only one previous report on fungal *Phoma* associations with angiosperm Myricaceae leaf from Palaeocene sediments of France (Fritel, 1910). In our study, epiphyllous fructifications of extant *Phoma* are recovered from four angiosperm leaf remains belonging to three families collected from the Siwalik sediments (Mio-Pleistocene) of Arunachal foothills, eastern Himalaya. Consequently, *Phoma* is established as a widely distributed leaf infecting fungi since middle Miocene in the tropical ancient forest of the eastern Himalayan Siwalik sector. Forest ecosystems are among the more complex and heterogeneous terrestrial environments where plant pathogenic and parasitic fungi play an important role in the shaping of tropical tree communities (Gilbert, 2002; Gallery et al., 2007). Parasitic fungi that infect leaves or grow on leaves may be pathogenic or biotropic. Modern *Phoma* is a leaf pathogen and causes many disease symptoms (Aveskamp et al., 2008) but in only some of our specimens faint leaf spots were recognized as infection symptoms.

In the present study, the fossil fungal form *Phomites* is reported from the three angiosperm host families viz. Dipterocarpaceae, Meliaceae, and Poaceae. At present, extant *Phoma* is known to be associated with the members of all the above mentioned families except in Meliaceae (Singh et al., 2004; Thaung, 2008; Boerema, 1976; Gruyter et al., 2009; Pharamat, 2009; Zhang et al., 2015). However, a similar host choice of *Phoma* since middle Miocene in the tropic suggests a general host parasite co-evolution in the Siwalik ancient forests of Arunachal sub-Himalaya. In co-evolutionary host parasite systems, each species constitutes an ever changing environment to which its opponent has to adapt where the evolutionary potential depends on mutation, migration and recombination (Gandon and Michalakis, 2002). The discontinuation of similar association at present may be the consequence of the ever changing climate towards the cooling events. Primary biotic responses against changing environment are variable in different organisms which can be persistent *in situ* occurrence at tolerant limits, or migration to regions of tolerance limits or extinction (Davis et al., 2005). In the present perspective gradual and consistent shift in environment in the eastern Himalayan foothill region may lead to the persistent host preferences at tolerant limits for *Phoma* in majority of cases, whereas the deviation may be due to the changes in different interacting parameters of inherent nature such as genetic variability, changes in chemical nature, changes in cell wall thickness or presence or absence of growth factors (Caroll and Wicklow, 1992; Khardori, 1989).

Acknowledgements

This work was financially supported by the Department of Science and Technology, Government of India, New Delhi [grant number: SR/S4/ES-67/2003] and University Grants commission, New Delhi [F. No. 31–178/2005(SR)]. We acknowledge the UGC-CAS VII Department of Botany, University of Calcutta for necessary facilities. We would like to convey our sincere gratitude to Dr. R. M. Kalgutkar, Calgary, Alberta for his precious suggestion on the nomenclature of the fossil fungus. Special thanks are due to Geological Survey of India for helping us during the SEM studies of fossil fungi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2018.10.007.

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