DOCUMENTATION OF POLLEN GRAINS OF COMMONLY FOUND MANGROVES OF INDIAN SUNDARBAN USING FOLDSCOPE

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Abstract : **-** Description and key to, mangrove pollen grains from extant taxa are important for palynological study. In the present study, we aim to prepare a pollen morphological catalogue and identification key for most of Indian mangrove flora. The morphology of the Indian mangrove and associated mangrove pollen grains were identified, described and illustrated by using Foldscope. The Foldscope , an ultra-affordable paper microscope was designed to be extremely portable, durable providing an optical quality similar to conventional research microscopes having magnification of 140X and resolution upto 2 micron (Cybulski et al. 2014).For this study, floral samples were collected from the Indian mangrove region and the pollen grains of the sample species were subjected to acetolysis following Erdman's acetolysis method.(Erdtman1960) and mounted on slides for examination using Foldscope.Under the foldscope, pollen grains were identified by observing few simple morphological characteristics such as Size, pore type , exine ornamentation etc.With the obtained data, Pollen morphological catalogue was prepared containing scientific name, plant photograph, pollen photograph, (obtained using foldscope) and the flowering season for each sampled species of Indian mangrove region.This pollen morphological catalogue for Indian mangrove plants would work as a manual for identification of pollen grains of different true mangrove and mangrove associate plants. The keys would be open for future development of the pollen catalogue so that pollen grains of mangroves can be included when available.

Keywords: Mangrove, Indian Sundarban, Pollen, Foldscope.

1. INTRODUCTION

Mangroves on ecological basis can be referred to as an aggregation of trees and shrubs of divergent taxonomy that dominate in tropical and subtropical coastal line having tidal, saline and brackish wetlands. Mangroves are specially adapted to this salty condition and are ecologically dynamic to the changes in sea level. Mangroves play a significant role in protecting the coastal region in the intertidal zones of estuarine ecosystem which continuously receives natural sedimentation (Bayen, 2012).

Mangrove forests are located in the inter-tidal region of the tropical and subtropical regions of the world ranging between the approximate coordinates of 30° N and 30° S latitude. The total area covered by the mangrove forest of the world in 2000 was 137,760 km^2 in 118 countries and territories and 0.7% of the total tropical forest cover of the world is represented by the the total mangrove area The largest stretch of mangroves is found in Asia with 42% followed by Africa with 20%, North and Central America with 15%, Oceania with 12% and South America with 11%.of the total mangrove cover of the worldAlmost 75% of mangrove forests are condensed in mostly 15 countries (Giri2010). India including the island territories is thefourth largest mangrove area in the world (Naskar& Mandal, 1999), has a mangrove cover of about 6,749 km. These mangrove habitats (7˚N,69°E and 23°N89.5°E) consists of 3 distinct zones: (1)East coast habitats with coast line of about 2700 km, facing Bay of Bengal, (2)West coast habitats having a coast line of about 3000 km, along the Arabian sea, and (3)Island Territories having a coastline of about 1816.6 km. In India, the states that occupy vast area of Mangroves are Andhra Pradesh,Andaman and Nicobar Islands, Goa. Gujarat, Kerala, Maharashtra, Odisha, Tamil Nadu, andWest Bengal (Singh, 2012)

The world's largest adjoining mangrove ecosystem, stretching from India to Bangladesh is represented by the Sundarbans. It is composed of hundreds of islands intersected by a network of tidal rivers, creeks and estuaries. Indian Sundarbans is located in the north-east coast of India at the peak of Bay of Bengal,.

UNESCO declared Sundarbans as the World Heritage site in 1987 and in 1987 it was declared as Global Biosphere Reserve. The Government of India in order to ensure protection to this distinctive gene pool of the world, assigned this deltaic complex spreading over 102 islands,as a Biosphere Reserve. The Indian Sundarbans biodiversity comprises of nearly 250 species of fishes , 100 species of vascular plants, , 300 species of birds and various types of reptiles, amphibians and mammals besides varied species of benthic invertebrates, phytoplankton, zooplankton, bacteria, fungi etc. (Gopal and Chauhan, 2006).

Pollen analysis is important for palynological study of the mangrove flora. Palynological analysis depends on proper identification of pollen grains and spores but keys to identification of pollen grains of wide range mangrove taxa are mostly not available.

The present study aims at identification, description and illustration of the morphology of the Indian mangrove and the associated mangrove pollen grains by using Foldscope.The development in palynological study would lead to rapidly increasing numbers of pollen types as well as to strongly revised and new pollen keys, and to the ample availability of photographic reference material. The challenges of interdisciplinary and globally oriented studies would stimulate a stronger international integration of palaeoecological research and the development of regional and global databases with the help of the pollen morphological catalogue of Indian mangrove floras.

2. MATERIALS AND METHODS

2.1. Study Area

As represented in Figure 1(Fig 1), for the present study, floral samples were collected from different region Indian Sundarban which includes regions of Samshernagar (24.3847° N, 91.8977° E), Jharkhali (22.0306° N, 88.7013° E), Mousuni(21˚56'16" N, 88˚ 12' 43") and Bhagabatpur (25.7667° N, 85.7084° E). Floral sampling sites are shown in Fig 1.

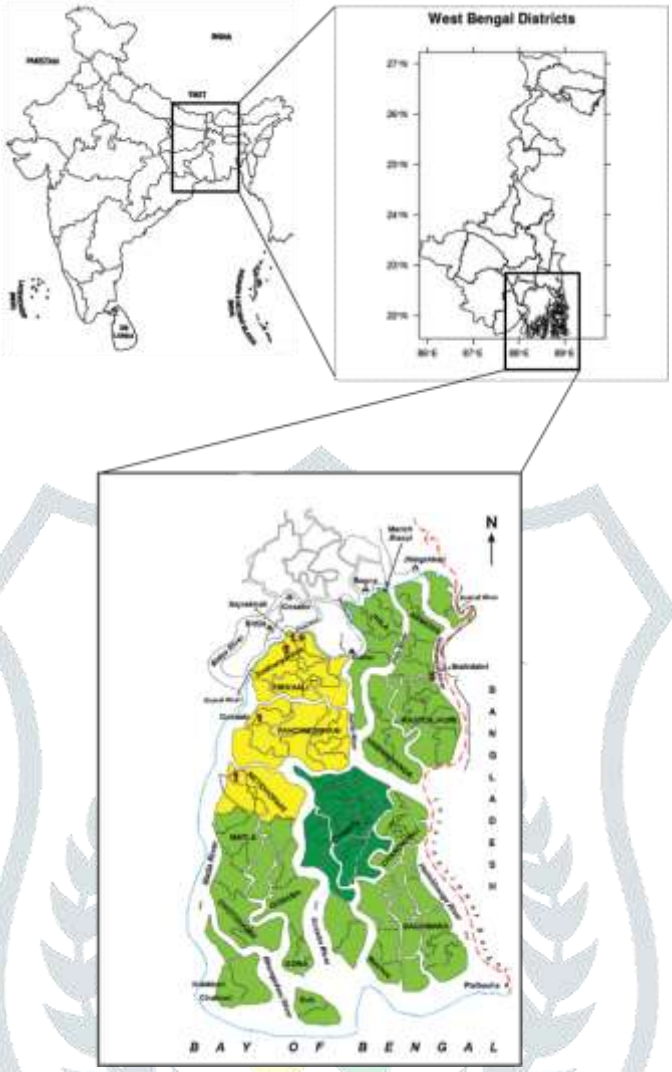


Fig 1: Sampling locations at Indian Mangrove region illustrated in above map: Samshernagar, Jharkhali, Mousuni and Bhagabatpur (adapted from Chowdhury.et al. 2016).

2.2. Microscopic study of pollen grains using Foldscope

2.2.1. Collection of flower samples

The pollen grains of the floral samples were collected from the mature anthers of the fresh flowers of the trees and shrubs growing around Indian mangrove region during their peak flowering period (Table 1 and 2). To remove the other floral impurities, the pollen grains were dried at 37˚C, mildly crushed and passed through sieves of different sizes (150,240,300 and 400 mesh/cm^2) successively. The purity of the isolated pollen grains was checked under the Foldscope. The batches used during the work contained <1% non –pollen grains impurities.

Table 1: list of true mangrove plants collected from Indian Sundarbans

Sl. No.	Scientific Name	Family	Local Name	Habit
1	*Avicennia alba* (Forssk.) Vierh.	Avicenniaceae	Kalobaen	Tree
2	*Avicenniaofficinalis* L.	Avicenniaceae	Jatbaen	Tree
3	*Avicennia marina* (Forssk.) Vierh.	Avicenniaceae	Pyarabaen	Tree
4	*Aegiceras corniculatum* (L.) Blanco	Myrsinaceae	Khalsi	Tree
5	*Bruguiera gymnorhiza* (L.) Lam.	Rhizophoraceae	Kankra	Tree
6	*Ceriops tagal*(Perr.)C.B. Rob.	Rhizophoraceae	Mat Goran	Tree
7	*Excoecaria agallocha*L.	Euphorbiaceae	Genwa	Tree
8	*Kandelia candel*(L.)Druce	Rhizophoraceae	Kripa	Tree
9-	*Nypa fruticans*Wurmb	Arecaceae	Golpata	Tree
10	*Sonneratia caseolaris*(L.) Engl.	Sonneratiaceae	Chak Keora	Tree
11	*Sonneratia apetala*Buch.-Ham.	Sonneratiaceae	Tok keora	Tree
12	*Sonneratia alba*Sm.	Sonneratiaceae	Keora	Tree

Table 2: list of mangrove associate plants collected from Indian Sundarbans

Sl. No.	Scientific Name	Family	Local Name	Habit
1	*Acanthus ilicifolius* L.	Acanthaceae	Hargoza	Shrub
2	*Caesalpinia crista* L.	Caesalpiniaceae	Shingrilata	Shrub
3	*Derris scandens* (Roxb.)Benth.	Fabaceae	Noalota	Climber
4	*Heliotropium curassavicum* L.	Boraginaceae	Nona hatisur	Herb
5	*Hibiscus tilliaceus*L.	Malvaceae	Sea hibiscus	Shrub
6	*Ipomoea pes-caprae*(L.) R. Br.	Convolvulacaea	Chhagalknuri	Creeper
7	*Thespesia populnea*(L.) Sol. ex Corrêa	Malvaceae	Paras	Tree

2.2.2. Foldscope

The Foldscope , an ultra-affordable paper microscope developed by Manu Prakash and his team at Standford School of Medicine was designed to be extremely portable, durable providing an optical quality similar to conventional research microscopes having magnification of 140X and resolution upto 2 micron(Cybulski et al. 2014).Foldscope was prepared by merging principles of optical design with origami enabling high-volume fabrication of microscopes from 2D media. Flat compact design of the Foldscope was obtained by using flexure mechanism via folding.

2.2.3. Acetolysis of pollen grains and Foldscope Microscopy preparation

Pure pollen grains were obtained by passing through several meshes. 1ml of acetolysis mixture (acetic anhydride and concentrated sulphuric acid in 9:1 ratio) was added to 100mg of pollen grains. After heating and centrifugation at 500g for 10 minutes, pellet was washed with distilled water. Then 5-6 drops of glycerin and equal amount of water was added to it (Erdtman 1960). Pollen grains were pelleted off by centrifugation and mounted on a slide for analysis underFoldscope.

2.3. Preparation of pollen morphological catalogue

Pollen morphological catalogue for the Indian mangrove floral species was prepared with the available data containing the (1)scientific name, (2) common name, (3) plant photograph,(4) pollen grain photograph of the sample species (using foldscope), (5) the flowering period for each species of the Indian Mangrove region.

3. RESULTS AND DISCUSSION

With the data obtained from microscopic analysis of the pollen grains using foldscope, a pollen morphological catalogue was prepared as represented in Table no. 3 and 4.Mangrove pollen grains identification from diverse taxa and their illustration are very significant for palynological study.In the present study 19 floral species of Indian Sunderban have been studied of which 12 were true mangroves namely, *Avicennia alba*, *Avicennia officinalis*, *Avicennia marina*, *Aegiceras corniculatum*, *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Excoecaria agallocha*, *Kandelia candel*, *Nypafruticans*, *Sonneratia caseolaris*, *Sonneratia apetala*, *Sonneratia alba* and 7 species, *Acanthus illicifolius*, *Thespesia populnea*, *Derris scandens*,, *Hibiscus tiliaceous*, *Ipomoeapes-carpae*, *Heliotropium curassavicum*, *Caesalpinia crista* were associate Indian mangroves. Pollen grains were observed, analyzed and morphologically characterized using Foldscope as a microscopic tool.Pollen morphological characters observed under foldscope revealed that most of the pollen grains of the sampled true mangroves plants were with monad grain arrangement tri-colporate pores

and reticulate surface ornamentation while the associate mangrove sample plants revealed that the pollen grains were mostly with monad grain arrangements, tri-colporate pores and echinate and psilate surface ornamentation.

Table3: pollen morphological catalogue of some Indian Sundarban flora-

Scientific Name of the plants (Common name)	Picture of the flower	Picture of the pollen (acetolysis method)	Characteristics of the pollen grain	Flowering Period
Avicennia alba (Kala baine)			unit-monad, pore-tricolporate, surface ornamentation-reticulate	February- June
Avicennia officinalis (Jatbaen)			unit-monad, pore-tricolporate, surface ornamentation-reticulate	April- August
Avicennia marina (Pyarabaen)			Unit-monad, Pore-tricolporate, Surface ornamentation- reticulate	June-September
Aegiceras corniculatum (Khalsi)			Unit-monad, Pore-tricolporate, Surface ornamentation- reticulate	July-March
Bruguiera cylindrica (Bokul Kankra)			unit-monad, pore-tricolporate, surface ornamentation-reticulate	January- May
Bruguiera gymnorrhiza (Kankra)			unit-monad, pore-tricolporate, surface ornamentation-reticulate	December-April

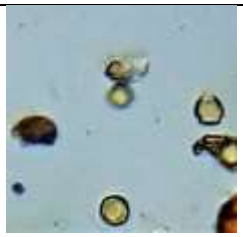
Scientific Name of the plants (Common name)	Picture of the flower	Picture of the pollen (acetolysis method)	Characteristics of the pollen grain	Flowering Period
Ceriops tagal (Moth goran)			unit-monad, pore-tricolporate, surface ornamentation-psilate	June -January
Excoecaria agallocha (Genwa)			unit-monad, pore-tricolporate, surface ornamentation-reticulate	March-October
Kandelia candel (Garia)			unit-monad, pore-tricolpate, surface ornamentation- psilate	March-July
Nypa fruticans (Golpata)			unit-monad, pore-monosulcate, surface ornamentation- echinate	Round the year
Sonneratia caseolaris (Chak Keora)			unit-monad, pore-triporate, surface ornamentation-reticulate	February- August
Sonneratia apetala (Tok Keora)			unit-monad, pore-triporate, surface ornamentation-reticulate	January- April
Sonneratia alba (Keora)			unit-monad, pore-triporate, surface ornamentation-reticulate	January-April

Table 4: pollen morphological catalogue of some Indian mangrove associate flora

Scientific Name of the plants (Common name)	Picture of the flower	Picture of the pollen under Foldscope	Characteristics of the pollen grain	Flowering Period
Acanthus ilicifolius (Hargoza)			unit-monad, pore-tricolpate, surface ornamentation-reticulate	Round the year
Caesalpiniacrista (Shingrilata)			unit-monad, pore-tri-margocolpate, surface ornamentation-reticulate	January- March
Derris scandens (Kalilata)			unit-monad, pore-tricolporatee, surface ornamentation-reticulate	January-June
Heliotropicum curassavicum (Hatisur)			unit-monad, pore-tricolporate, surface ornamentation-psilate	January-March
Hibiscus tiliaceous (Sea hibiscus)			unit-monad, pore-pantoporate, surface ornamentation-echinate	October-June
Ipomoea pes-caprae (Kalmi lata)			unit-monad, pore-pantoporate, surface ornamentation-echinate	Round the year
Thespesia populnea (Paras)			unit-monad, pore-pantoporate, surface ornamentation-echinate	January-March

4. CONCLUSION

The morphology of the Indian Sundarbans mangrove and associated mangrove pollen grains were identified, described and illustrated by using Foldscope. Pollen morphological catalogue was prepared which could serve as a identification tool for pollen grains during future palynological study of the Indian Mangrove flora. The development in palynological study using Foldscope would lead to fast increasing numbers of pollen grains database as well as to strongly revised and new pollen keys for the morphological identification of pollen grains of the Indian Mangroves and its associates.The study would also lead to the ample availability of photographic reference material. The constraints of interdisciplinary global based studies would stimulate a stronger integration of palaeoecological

research and upliftment of universal databases with the help of the pollen morphological catalogue of Indian mangrove floras. The pollen morphological catalogue would remain open for further development and up-gradation of the current data

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