Development of a Comprehensive Pollen and Spore Catalog Using Advanced Laboratory Techniques: Case Study of Contemporary Dried and Fresh Flower Specimens from Bangladesh

Abstract

This study focuses on the preparation of dry and fresh flower samples for pollen and spore observation using advanced laboratory techniques, identification of morphological characteristics, and the development of a pollen catalog that provides valuable insights into ecology and pollen morphology for different taxa. This research contributes to the field of palynology, an essential discipline in stratigraphy and biostratigraphy, by creating a detailed pollen catalog that enhances our understanding of paleoenvironmental conditions, depositional history, and potential hydrocarbon presence. Twenty-six fresh and dried flower samples from Chattogram, Cox's Bazar, and Dhaka Districts have been analyzed using acetolysis techniques. Pollen morphologies were examined at 400x and 1000x magnifications through transmitted and fluorescence light microscopy, and high-resolution imaging of one species was further enhanced with Field Emission Scanning Electron Microscopy (FESEM). Pollen morphology documented for twenty-five species across twelve families, including Fabaceae, Asteraceae, Lamiaceae, Solanaceae, Poaceae, Rubiaceae, Campanulaceae, Melastomataceae, Malvaceae, Verbenaceae, Oleaceae, and Heliotropiaceae as well as one spore from the Lygodiaceae family. The resulting catalog provides valuable ecological insights by highlighting morphological features and taxonomic classifications. It is an essential resource for researchers and professionals involved in Holocene pollen studies. This work contributes to the understanding of palynology and underscores the importance of advanced analytical technologies in reconstructing the prehistoric environment of Bangladesh. The data gathered is crucial for comprehending the ecological evolution of the world's largest delta.

Keywords: Pollen Catalog, Palynology, Laboratory Techniques, Acetolysis, Contemporary Flower.

1 Introduction

Palynology is the scientific study of plant spores, pollen, and various microscopic organic remains, spanning both extant and fossilized specimens. Integrating plant sciences with geological disciplines like stratigraphy, historical geology, and paleontology, palynology plays a crucial role in reconstructing past environments (Encyclopaedia Britannica 2017). Coined by Hyde and Williams in 1944 (University of Arizona 2015), the field includes research on pollen morphology, often utilizing electron microscopy, and the examination of organic microfossils, or palynomorphs, within ancient deposits like coal. These palynomorphs, encompassing both plant and animal microstructures are resilient due to their composition, primarily sporopollenin and chitin, which withstand decay except through oxidation (University of Arizona 2015).

Vegetation responds to ecological shifts and human activities, leaving pollen traces that can be preserved as fossils in the subsurface for thousands, or even millions of years. Palynology is a highly effective method for reconstructing past environments, as the resilient outer shells of pollen grains, or exines, are distinct to each species and can endure favorable conditions for millennia. By analyzing these preserved grains, palynologists can identify historical plant species and detect broader environmental patterns (Bercovici and Vellekoop 2017; Birks and Birks 1980; The University of Texas 2018). Pollen analysis offers insights into vegetation dynamics, environmental changes, and human influence over time. These studies provide insights into ancient deposition and diagenetic processes, aiding resource recovery, particularly in fossil fuel exploration (Kennedy, 1998). This makes pollen and spores invaluable to professionals across fields, including geology, ecology, paleoecology, medicine, and agriculture. Stratigraphic palynology, in particular, examines palynomorphs identification, distribution, and abundance to correlate sedimentary sequences of any age, providing essential chronological data. Pollen and spores are also key indicators of climate change.

A major challenge for stratigraphic palynological research in Bangladesh is the absence of a comprehensive pollen catalog, which is essential for accurate identification and correlation. To address this, it has become urgent to develop such a catalog. Recognizing pollen grains requires practice rather than reliance on books, making it crucial for beginners to work under the guidance of an experienced colleague and establish a "pollen herbarium" for reference. Standardizing preparation methods, including embedding media, enhances consistency in research. The acetolysis method is strongly recommended for cleaning samples by removing extraneous material to enhance preparation quality (Faegri and Iversen 1989; Florida Tech 2014; Erdtman 1969). The development of a pollen catalog using modern dried flower specimens from the National Herbarium and fresh specimens from Cox’s Bazar and Dhaka district represents a crucial step forward for palynological research in Bangladesh. This research directly supports Sustainable Development Goals (SDGs) 13, 14, and 15—addressing climate action, life below water, and life on land—as well as SDG 4 on quality education. For this study, dried samples sourced from the Bangladesh National Herbarium in Mirpur were categorized by area, with only those from Chattogram district analyzed. Fresh flower samples were additionally collected from Cox's Bazar and Dhaka districts for comprehensive analysis.

This investigation focuses on the morphological characteristics of pollen and spores, particularly those of different plant groups and species. The main objective of this analysis is to study the current pollen of different taxa, their morphology, ecology and establishment of a complete sample processing technique for pollen analysis of dry and fresh flower samples. By doing so, a pollen catalog is aimed to be created to clarify the present palynological environment. During the Holocene period, most of the biota was similar to the current flora and fauna, which enables us to identify the ancient palynological zone and paleo-environment.

2 Description of The Sampling Area

A total of twenty-six flower samples, both dried and fresh, were collected and analyzed for this study. Twelve dried samples, all from the Chattogram district, were obtained from the Bangladesh National Herbarium in Mirpur. Additionally, thirteen fresh samples were gathered from the coastal area of Cox's Bazar, along with one from Dhaka district (Fig. 1).

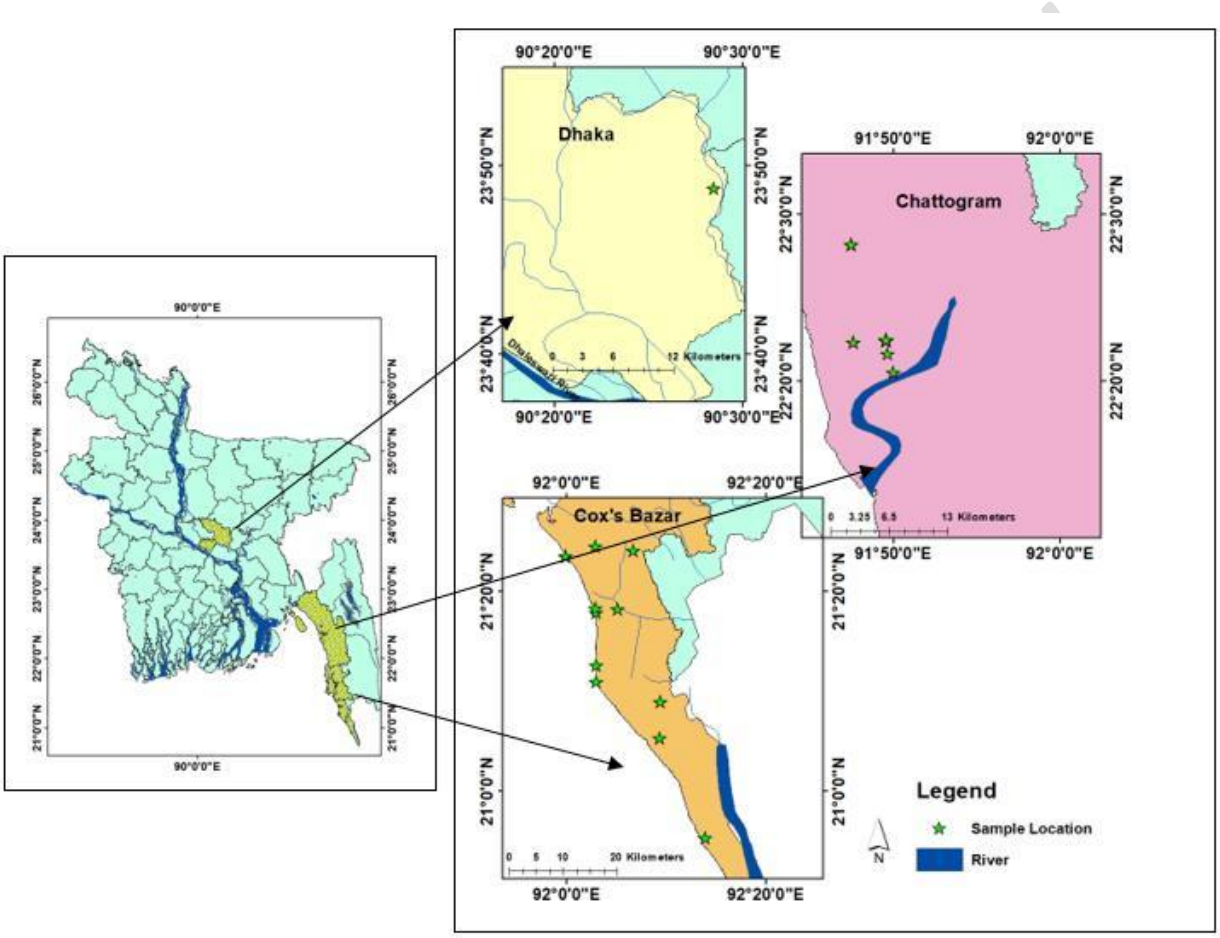


Fig. 1 Location map of the dried and fresh flower samples in three different districts.

Four dried flower samples from Pahartali Upazila and eight from Chattogram City in southeastern Bangladesh have been analyzed. This hilly region extends into India, bordered by the Karnaphuli River to the south, the Bay of Bengal to the west, and the Halda floodplain to the east. Chattogram lies along the tectonically active Chittagong-Tripura folded belt (Guha 1978; Alam et al. 1990). The soil is primarily brown sandy silt and clay silt, with coastal areas in the south consisting of tidal silt clay. The region experiences a tropical monsoon climate with warm, humid conditions, monthly temperatures between 26.0 to 31.7°C (max) and 14.1 to 25.4°C (min), and rainfall ranging from 8 mm in January to 760 mm in July (Chittagong City Corporation 2015; Bangladesh Bureau of Statistics 2022).

Thirteen fresh flower samples have been collected from Cox's Bazar District, bordered by Chattogram to the north, the Bay of Bengal to the south and west, and Bandarban to the east. Major rivers in the region include the Matamuhuri,

Bakkhali, Reju Khal, Naf River, Maheshkhali Channel, and Kutubdia Channel (Cox's Bazar District 2018). Situated in the youngest structural Indo-Burman Range, the area is predominantly low hills, with flood plains and coastal plains as the main landforms (Kresic 2006; Nahin et al. 2019). The landform types in this region are classified into two categories: flood plains and coastal plains (or deltas). The tropical monsoon climate features high temperatures, heavy rainfall, and high humidity, with annual temperatures averaging between 32.8°C (max) and 15.4°C (min). The area receives an average annual rainfall of 3,524 mm, with monthly rainfall ranging from 5 mm in January to 961 mm in July (Bangladesh Bureau of Statistics 2022).

One fresh flower sample has been collected from the Bashundhara area in Dhaka, the capital of Bangladesh, located centrally and surrounded by the Buriganga, Balu, Turag, and Tongi Khal rivers. Dhaka is situated within the southern half of the Madhupur Tract and Floodplain area (Morgan and McIntire 1959). The city experiences a tropical monsoon climate with hot, wet, and humid conditions, an annual average temperature of 26.25°C, and monthly temperatures ranging from 13.1°C in January to 33.8°C in April. Rainfall averages 8 mm in January and 388 mm in July (Bangladesh Bureau of Statistics 2022).

According to Bangladesh's bioecological zonation map, the Chattogram and Cox's Bazar districts fall under four zones: Coastal Plains, Offshore Islands, Sandy Beach/Sand Dunes, and Chattogram Hills/CHTs (Nishat et al. 2002). The Coastal Plain zone features noncalcareous gray floodplain soils (non-saline), while the Sandy Beach/Sand Dunes zone includes Cox's Bazar's extensive sandy beaches and dunes. The Chattogram Hills/CHTs zone is rich in brown hill soils. Dhaka city is part of the Brahmaputra-Jamuna Flood Plain zone, characterized by noncalcareous gray to dark gray floodplain soils.

3 Methodology

All collected samples (Fig. 2c) were treated in the laboratory using various techniques to prepare temporary or permanent slides for microscopic observation. Taxonomical and locational information, along with other relevant details, were recorded, and an inventory was prepared for laboratory analysis (Table 1).

Table 1 Taxonomical information of the observed pollen species.

Species	Kingdom	Class	Order	Family	Sample Condition and Collected from	Upazila
Leucas aspera (Willd.) Link	Plantae	Angiosperms	Lamiales	Lamiaceae	Dry, N.H	Hathazari
Borreria laevis (Lam.) Griseb.			Gentianales	Rubiaceae	Dry, N.H	Pahartali
Solanum sp.			Solanales	Solanaceae	Dry, N.H	Hathazari
Ocimum americanum L.			Lamiales	Lamiaceae	Dry, N.H	Hathazari
Lygodium sp. Sw.		Polypodiopsida	Schizaeales	Lygodiaceae	Dry, N.H	Chattogram
Caesalpinia pulcherrima (L.) Sw.		Angiosperms	Fabales	Fabaceae	Dry, N.H	Chattogram
Lobelia trigona Thwaites, 1860			Asterales	Campanulaceae	Dry, N.H	Chattogram

Synedrella nodiflora (L.) Gaertn.	Asterales	Asteraceae	Dry, N.H	Chattogram
Solanum torvum Sw.	Solanales	Solanaceae	Dry, N.H	Chattogram
Physalis angulata L.	Solanales	Solanaceae	Dry, N.H	Pahartali
Vernonia cinerea (L.) Less.	Asterales	Asteraceae	Dry, N.H	Chattogram
Desmodium triflorum (L.) DC.	Fabales	Fabaceae	Dry, N.H	Hathazari
Melastoma malabathricum L.	Myrtales	Melastomataceae	Fresh, Location	Ukhiya
Lathyrus japonicus Willd.	Fabales	Fabaceae	Fresh, Location	Ukhiya
Chromolaena odorata (L.) R.M.King & H.Rob.	Asterales	Asteraceae	Fresh, Location	Teknaf
Butea monosperma (Lam.) Kuntze	Fabales	Fabaceae	Fresh, Location	Ukhiya
Clerodendrum infortunatum L.	Lamiales	Lamiaceae	Fresh, Location	Ramu
Hibiscus rosa-sinensis L.	Malvales	Malvaceae	Fresh, Location	Ramu
Saccharum spontaneum L.	Poales	Poaceae	Fresh, Location	Dhaka
Lantana involucrata L.	Lamiales	Verbenaceae	Fresh, Location	Cox's Bazar
Lathyrus pratensis L.	Fabales	Fabaceae	Fresh, Location	Cox's Bazar
Heliotropium indicum L.	Boraginales	Heliotropiaceae	Fresh, Location	Cox's Bazar
Vernonia patula (Aiton) Merr.	Asterales	Asteraceae	Fresh, Location	Ramu
Jasminum angustifolium (L.) Willd.	Lamiales	Oleaceae	Fresh, Location	Ramu
Tripidium arundinaceum (Retz.) Welker, Voronts. & E.A.Kellogg	Poales	Poaceae	Fresh, Location	Ramu
Spatholobus parviflorus (Roxb. ex G.Don) Kuntze	Fabales	Fabaceae	Fresh, Location	Teknaf

**N.H: National Herbarium.

For both dry and fresh flowers, the acetolysis method is an effective technique for preparing pollen samples (Fig. 2a). Initially, powdered dry flower samples were sieved through an 80-mesh sieve (180 microns) and dehydrated with 5 ml of glacial acetic acid. Subsequently, 5 ml of an acetolysis solution (9:1 acetic anhydride and concentrated sulfuric acid solution) was added to the samples using a pipette. The samples were then placed in a water bath at temperatures below 70°C, heated to 100°C, and left in the bath for 1–2 minutes after turning it off, with periodic stirring. The mixture was centrifuged at approximately 1800 rpm for 1 or 2 minutes, and the liquid was decanted. The samples were again dehydrated with 5 ml of glacial acetic acid and thoroughly washed with distilled water. For chlorination, 5 ml of glacial acetic acid was added along with 1 to 3 drops of a saturated sodium or potassium chlorate solution and 1 to 2 drops of concentrated HCl. The chlorination mixture was centrifuged, decanted, and the samples were washed with distilled water. Then the samples were transferred to small vials, stirred, centrifuged, and the supernatant was decanted. Vials were inverting onto clean filter paper to remove excess liquid, a 50% aqueous-glycerin mixture and aqua safranin were added. Following centrifugation for 2–3 minutes and the supernatant was decanted. Slides were prepared by using one drop of glycerol and the sample (Fig. 2b, d). After Waiting for some time to distribute the liquid evenly under the cover slide, the edges of the cover slide were sealed with transparent nail polish or white acrylic paint (Fig. 2e). The slides were then ready for microscopic observation. Additionally, one processed sample was dried and stored for Field Emission Scanning Electron Microscope (FESEM) analysis.

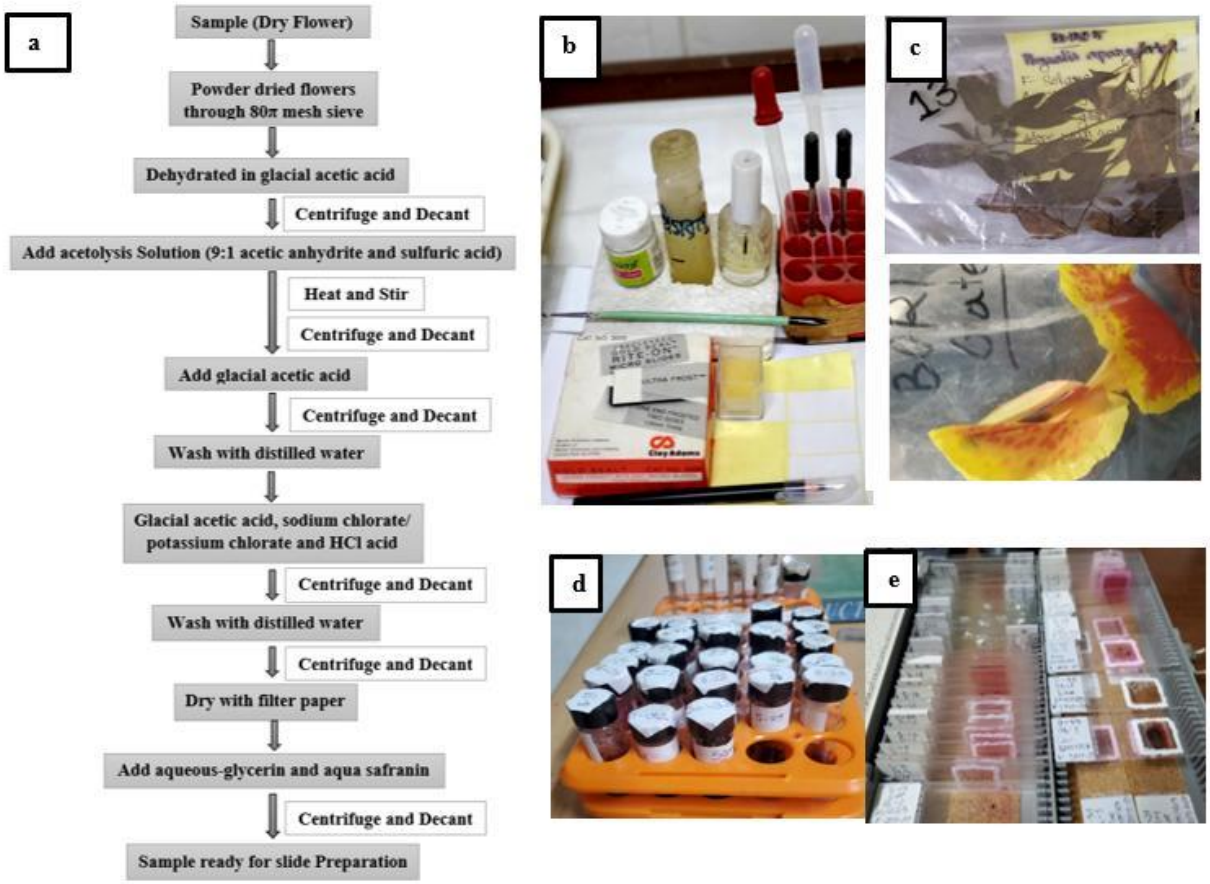


Fig. 2 a) Laboratory technique flow chart b) Slide preparation kits c) Collected samples stored in sample bags with proper labeling. d) Prepared samples in vials e) Temporary slides for microscopic observation.

Following slide preparation, pollen and spore samples were examined at 400x and 1000x magnifications using a Carl Zeiss Axio Lab.A1 microscope equipped with fluorescence. Images were captured using an Axiocam 105 color camera, with scaling and processing conducted through ZEN 2.3 lite software. Observations documented detailed morphological characteristics in a data sheet. Fluorescence light imaging was used to enhance visibility, though some samples showed diminished fluorescence, limiting their clarity under this mode (Hoyle et al. 2018). Additionally, one species was observed under a Carl Zeiss Field Emission Scanning Electron Microscope (FESEM) to capture high-magnification images with detailed morphology. Characteristics from these data sheets were cross-referenced with existing internet records.

4 Results and Discussions

4.1 Taxonomy and Pollen Morphology

This report presents pollen analysis from 25 species across 12 families, plus a spore from the Lygodiaceae family. It includes six species from Fabaceae, four from Asteraceae, three each from Lamiaceae and Solanaceae, two from Poaceae, and one species each from Rubiaceae, Campanulaceae, Melastomataceae, Malvaceae, Verbenaceae, Oleaceae, and Heliotropiaceae.

Scientific Name: *Leucas aspera* (Willd.) Link (GBIF Secretariat 2022**).**
Common Name: Common Leucas, Thumba.
Local Name: Shetadron, Dhulfi, dulpi, Danda-kalas.
Category: Indigenous.
Habit: Herb.
Pollen morphology: Prolate-spheroidal shape in equatorial view and circular-spheroidal shape in polar view, Tricolpate type, Reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 3; a-c).
Pollen size: Equatorial size 26 µm (13.0 µm- 30.9 µm) and polar size 20 µm (19.23 µm- 22.25 µm).

Scientific Name: *Borreria laevis* (Lam.) Griseb. (GBIF Secretariat 2022**).**
Common Name: Button weed.
Local Name: Not yet Known.
Category: Indigenous.
Habit: Herb.
Pollen morphology: Oblate -Spheroidal shape in equatorial view and circular-spheroidal shape in polar view, Tricolporate/Pentacolporate type, Reticulate-scabrate ornamentation with monad unit and dicot grain arrangement (Fig. 3; d-f).
Pollen size: Equatorial size 30 µm (25 µm- 32.03 µm) and polar size 24.33 µm (22 µm- 28.28 µm).

Scientific Name: *Solanum sp.* L. (GBIF Secretariat 2022**).**
Common Name: Divine nightshade.
Local Name: Not yet Known.
Category: Not yet Known.
Habit: Shrub.
Pollen morphology: Sub prolate shape in equatorial view and Triangular (Convex) shape in polar view, Tricolporate type, Psilate ornamentation with monad unit and dicot grain arrangement (Fig. 3; g-i).
Pollen size: Equatorial size 23.50 µm (20.32 µm- 26.88 µm) and polar size 23.31 µm (22.85 µm- 23.85 µm).

Scientific Name: *Ocimum americanum* L. (GBIF Secretariat 2022**).**
Common Name: American basil, lime basil, or hoary basil.
Local Name: Baro-tulsi.
Category: Indigenous.
Habit: Herb.

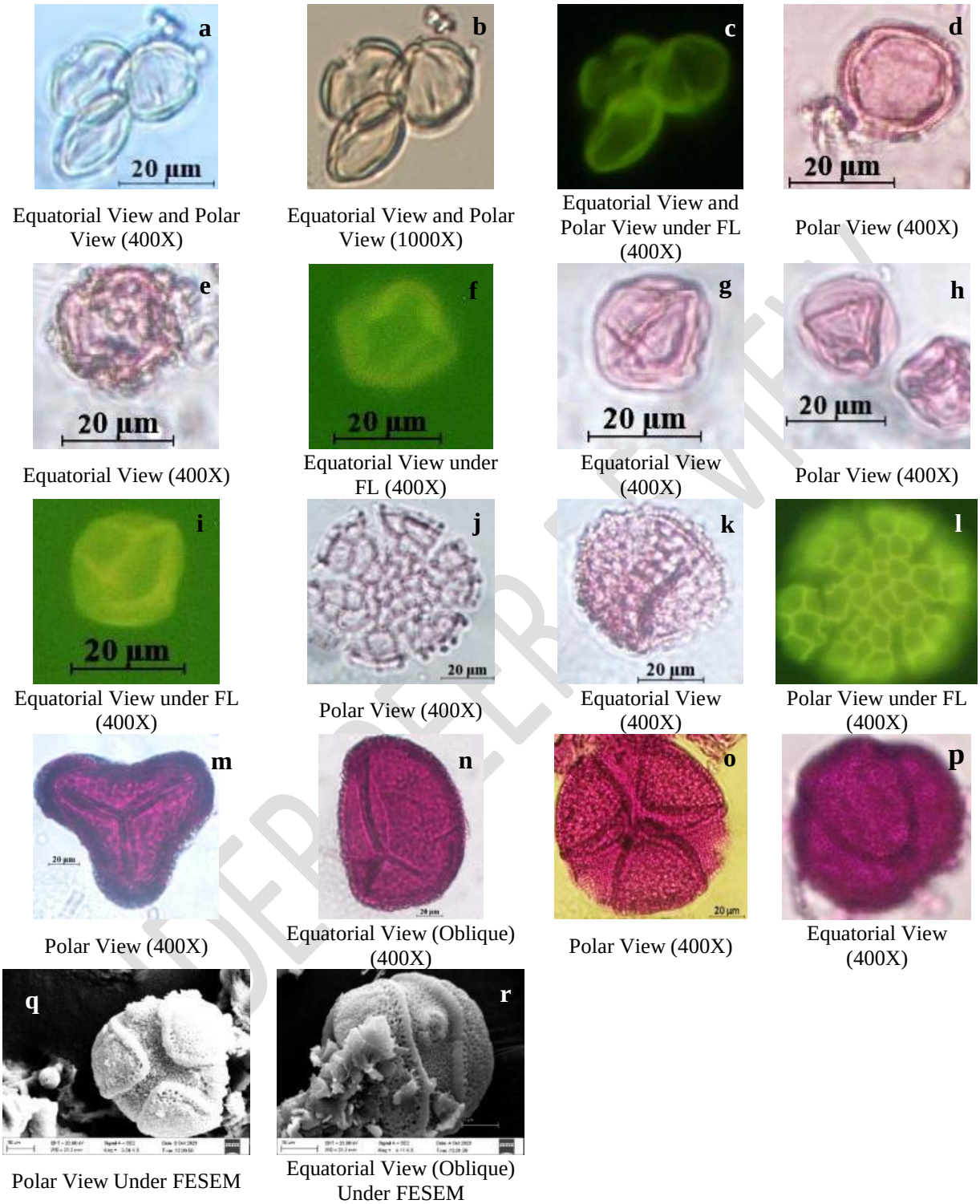


Fig. 3 a-c *Leucas aspera* (Willd.) Link; d-f *Borreria laevis* (Lam.) Griseb.; g-i *Solanum sp.* L.; j-l *Ocimum americanum* L.; m-n *Lygodium sp.* Sw.; o-r *Caesalpinia pulcherrima* (L.) Sw. FL: Fluorescence Light Microscope; FESEM: Field Emission Scanning Electron Microscope.

Pollen morphology: Sub-prolate to Oblate shape in equatorial view and circular-spheroidal shape in polar view, Stephanocolpatae/ Zonocolpate type, Reticulate-bireticulate ornamentation with monad unit and dicot grain arrangement (Fig. 3; j-l).
Pollen size: Equatorial size 50.7 µm (49.5 µm- 60.38 µm) and polar size 71.05 µm (53.13 µm- 75.39 µm).

Scientific Name: *Lygodium sp.* Sw. (GBIF Secretariat 2022**).**
Common Name: Climbing Fern.
Local Name: Lata Dhekia.
Category: Indigenous.
Habit: Fern.
Pollen morphology: Prolate to sub-prolate shape in equatorial view and Triangular to circular shape in polar view, Trichotomosulcate type, Rugulate ornamentation with monad unit and dicot grain arrangement (Fig. 3; m-n).
Pollen size: Equatorial size 132 µm (107.4 µm-140 µm) and polar size 121.3 µm (94.72 µm- 131.8 µm).

Scientific Name: *Caesalpinia pulcherrima* (L.) Sw. (GBIF Secretariat 2022**).**
Common Name: Barbados flower-fence, Peacock Flower, Paradise Flower.
Local Name: Radhachura.
Category: Exotic.
Habit: Shrub.
Pollen morphology: Sub-prolate shape in equatorial view and Circular (convex) shape in polar view, Tricolporate type, Reticulate/Verrucate ornamentation with monad unit and dicot grain arrangement (Fig. 3; o-r).
Pollen size: Equatorial size 80.97 µm (71.89 µm- 82.93 µm) and polar size 101 µm (97.15 µm- 106.47 µm).

Scientific Name: *Lobelia trigona* Thwaites, 1860 (GBIF Secretariat 2022**).**
Common Name: Chickweed Lobelia.
Local Name: Badnali.
Category: Indigenous.
Habit: Herb.
Pollen morphology: Sub-Prolate shape in equatorial view and Trilobate to round (convex) shape in polar view, Tricolporate type, Reticulate-Rugulate ornamentation with monad unit and dicot grain arrangement (Fig. 4; a-b).
Pollen size: Equatorial size 26.87 µm (24.98 µm- 27.32 µm) and polar size 27.04 µm (22.04 µm- 28.15 µm).

Scientific Name: *Synedrella nodiflora* (L.) Gaertn. (GBIF Secretariat 2022**).**
Common Name: Node weed, Cinderella weed.
Local Name: Relanodi.
Category: Exotic.
Habit: Herb.
Pollen morphology: Circular shape in equatorial view and circular-spheroidal shape in polar view, Tricolporate type, Echinate ornamentation with monad unit and dicot grain arrangement (Fig. 4; c-d).
Pollen size: Equatorial size 31.75 µm (27.34 µm- 35.25 µm) and polar size 24.61µm (23.17 µm- 29.23 µm).

Scientific Name: *Solanum torvum* Sw. (GBIF Secretariat 2022**).**
Common Name: Turkey berry, Devil's fig, Pea eggplant.
Local Name: Tit Begun.
Category: Exotic.
Habit: Shrub.
Pollen morphology: Prolate-Spheroidal shape in equatorial view and circular-spheroidal shape in polar view, Tricolporate type, Psilate, Scabrate or Microverrucate ornamentation with monad unit and dicot grain arrangement (Fig. 4; e-g).
Pollen size: Equatorial size 25.39 µm (23.59 µm- 26.98 µm) and polar size 22.85 µm (20.69 µm- 26.02 µm).

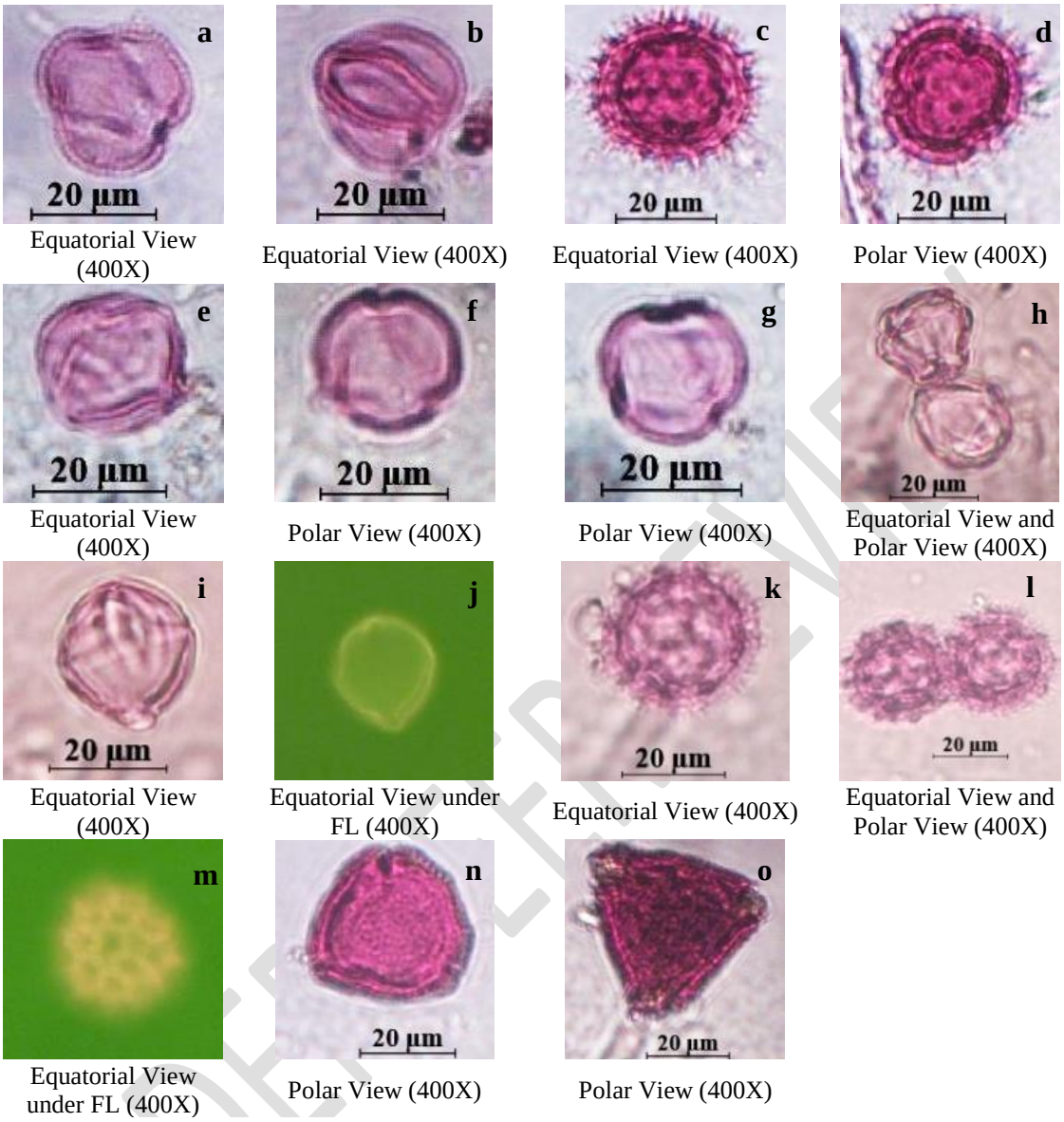


Fig. 4 a-b *Lobelia trigona* Thwaites, 1860; c-d *Synedrella nodiflora* (L.) Gaertn.; e-g *Solanum torvum* Sw.; h-j *Physalis angulata* L.; k-m *Vernonia cinerea* (L.) Less.; n-o *Desmodium triflorum* (L.) DC. FL: Fluorescence Light Microscope.

Scientific Name: *Physalis angulata* L. (GBIF Secretariat 2022**).**
Common Name: Cutleaf ground cherry, Dog Tomato, Cold Cherry.
Local Name: Potka.
Category: Exotic.
Habit: Herb.
Pollen morphology: Prolate-Spheroidal shape in equatorial view and circular-spheroidal shape in polar view, Tricolporate type, Micro-reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 4; h-j).
Pollen size: Equatorial size 26.67 µm (22.0 µm- 29.10 µm) and polar size 24.50 µm (20.57 µm- 28.23 µm).

Scientific Name: *Vernonia cinerea* (L.) Less. (GBIF Secretariat 2022**).**
Common Name: Little ironweed.
Local Name: Kukshim, Shyalmutra.
Category: Exotic.

Habit: Herb.
Pollen morphology: Circular-Spheroidal shape in equatorial view and circular-spheroidal shape in polar view, Tricolporate type, Echinate, Lophate[1] ornamentation with monad unit and dicot grain arrangement (Fig. 4; k-m).
Pollen size: Equatorial size 33.16 µm (32.21 µm- 35.20 µm) and polar size 27.88 µm (27.04 µm- 31.18 µm).

Scientific Name: ***Desmodium triflorum*** (L.) DC. **(**GBIF Secretariat 2022**).**
Common Name: Three-flower beggar weed, Tick clover.
Local Name: Kudaliya.
Category: Indigenous.
Habit: Herb.
Pollen morphology: Sub-prolate shape in equatorial view and Triangular shape in polar view, Tricolporate type, Gemmate, Verrucate or Scabrate ornamentation with monad unit and dicot grain arrangement (Fig. 4; n-o).
Pollen size: Equatorial size 56.8 µm (44.3 µm- 60.2 µm) and polar size 43.74 µm (35.25 µm- 48.63 µm).

Scientific Name: ***Melastoma malabathricum*** **L. (**GBIF Secretariat 2022**).**
Common Name: Indian-rhododendron.
Local Name: Dantranga, Ban-tezpata.
Category: Indigenous.
Habit: Shrub.
Pollen morphology: Prolate to Sub-prolate shape in equatorial view and Circular-Spheroidal shape in polar view, Tricolporate, Heteroaperturate, Pseudocolpus type, Verrucate, Scabrate, Gemmate, or Striate ornamentation with monad unit and dicot grain arrangement (Fig. 5; a-d).
Pollen size: Equatorial size 20.45 µm (15.05 µm- 22.23 µm) and polar size 21.43 µm (20.32 µm- 23.43 µm).

Scientific Name: ***Lathyrus japonicus*** **Willd. (**GBIF Secretariat 2022**).**
Common Name: Beach Pea.
Local Name: Not yet Known.
Category: Exotic.
Habit: Herb.
Pollen morphology: Prolate shape in equatorial view and Triangular-spheroidal shape in polar view, Tricolporate type, Reticulate, Perforate or Psilate ornamentation with monad unit and dicot grain arrangement (Fig. 5; e-g).
Pollen size: Equatorial size 25.0 µm (20.44 µm- 26.72 µm) and polar size 26.80 µm (18.83 µm- 31.15 µm).

Scientific Name: ***Chromolaena odorata*** **(L.) R.M.King & H.Rob. (**GBIF Secretariat 2022**).**
Common Name: Christmas bush.
Local Name: Bon Motmotia.
Category: Exotic.
Habit: Shrub.
Pollen morphology: Prolate-spheroidal shape in equatorial view and Triangular-spheroidal shape in polar view, Tricolporate type, Microechinate, Verrucate or Scabrate ornamentation with monad unit and dicot grain arrangement (Fig. 5; h-k).
Pollen size: Equatorial size 21.65 µm (16.51 µm- 26.13 µm) and polar size 17.39 µm (15.14 µm- 18.48 µm).

Scientific Name: ***Butea monosperma*** **(Lam.) Kuntze. (**GBIF Secretariat 2022**).**
Common Name: Flame-of-the-forest, Dhak, Palash, and Bastard Teak.
Local Name: Polash.
Category: Indigenous.
Habit: Tree.
Pollen morphology: Sub-prolate shape in equatorial view and Circular-spheroidal shape in polar view, Tricolporate, Zonocolporate type, Micro-reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 5; l-o).
Pollen size: Equatorial size 33.47 µm (27.21 µm- 35.21µm) and polar size 33.51µm (27.64 µm- 34.58 µm).

[1] Lophate: pollen wall with coarse meshed pattern formed by lophae and lacunae.

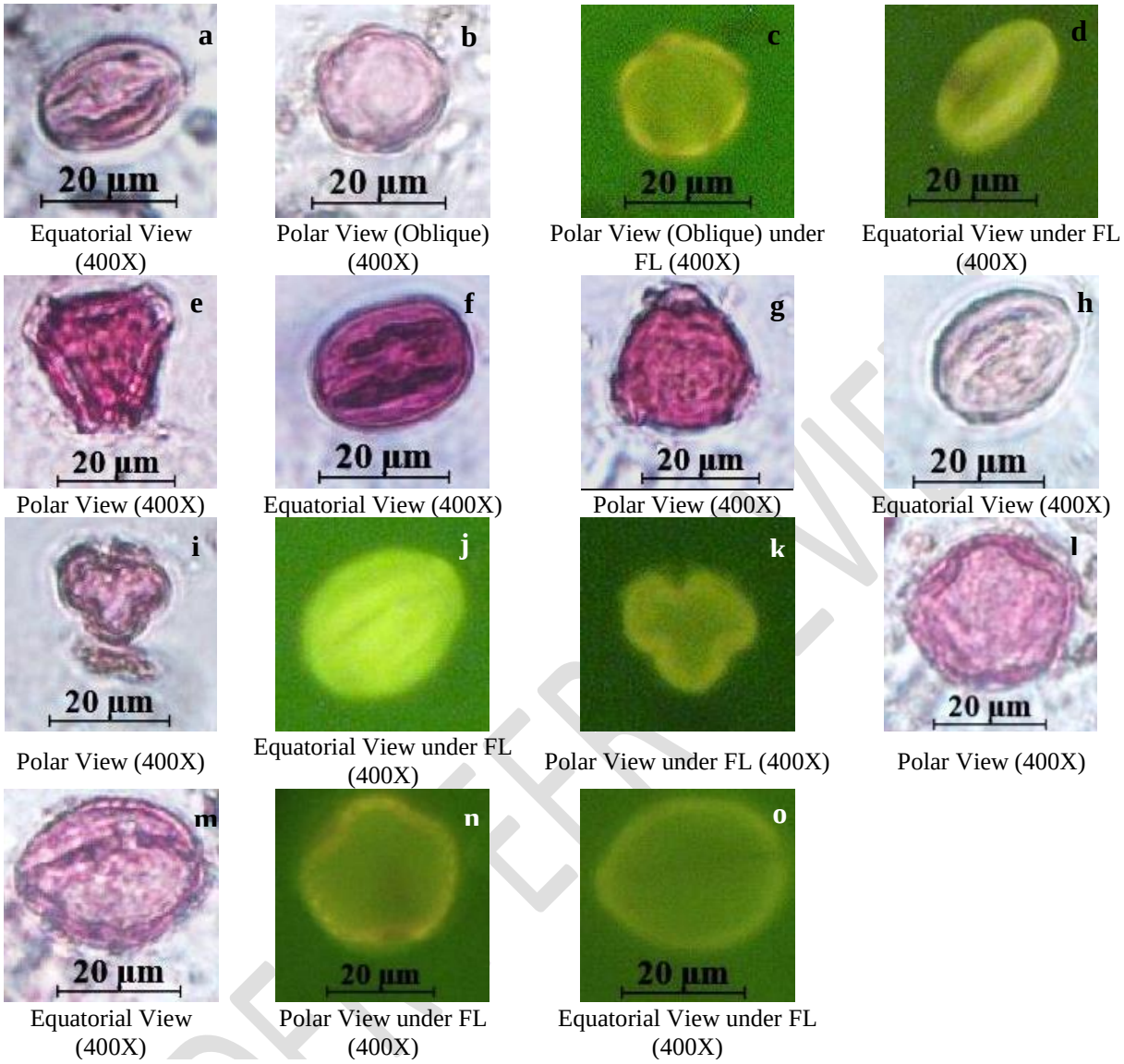


Fig. 5 a-d *Melastoma malabathricum* L.; e-g *Lathyrus japonicus* Willd.*;* h-k *Chromolaena odorata* (L.) R.M.King & H.Rob.; l-o *Butea monosperma* (Lam.) Kuntze. FL: Fluorescence Light Microscope.

Scientific Name: *Clerodendrum infortunatum* L. (GBIF Secretariat 2022**).**
Common Name: Hill glory bower.
Local Name: Bhant, Ghentu.
Category: Indigenous.
Habit: Shrub.
Pollen morphology: Sub-prolate shape in equatorial view and Circular, Outline-lobate[2], Sunken apertures shape in polar view, Tricolpate type, Echinate-Microreticulate ornamentation with monad unit and dicot grain arrangement (Fig. 6; a-d).
Pollen size: Equatorial size 60.99 µm (46.22 µm- 72.98 µm) and polar size 54.26 µm (35.69 µm- 64.40 µm).

Scientific Name: *Hibiscus rosa-sinensis* L. (GBIF Secretariat 2022**).**
Common Name: Chinese hibiscus, China rose.

[2] Lobate: Outline in polar view of a pollen grain with bulged inter-apertural areas (mainly in dry pollen grains).

Local Name: Jaba.
Category: Exotic.
Habit: Shrub.
Pollen morphology: Circular-spheroidal shape in equatorial view and Circular-spheroidal shape in polar view, Pantoporate type, Echinate ornamentation with monad unit and dicot grain arrangement (Fig. 6; e-f).
Pollen size: Equatorial size 129.17 µm (102.49 µm- 156.43 µm) and polar size 128.02 µm (107.56 µm- 131.62 µm).

Scientific Name: *Saccharum spontaneum* L. (GBIF Secretariat 2022**).**
Common Name: Wild sugarcane, Kans grass.
Local Name: Kash Ful.
Category: Indigenous.
Habit: Herb.
Pollen morphology: Circular shape in equatorial and polar view, Monoporate type with annulus[3], Scabrate ornamentation with monad unit and Monocot grain arrangement (Fig. 6; g-i).
Pollen size: Equatorial size 43.46 µm (36.42µm- 45.29 µm).

Scientific Name: *Lantana involucrata* L. (GBIF Secretariat 2022**).**
Common Name: Buttonsage.
Local Name Sada Lantana.
Category: Indigenous.
Habit: Shrub.
Pollen morphology: Prolate shape in equatorial view and Triangular shape in polar view, Tricolporate type, Psilate, Perforate ornamentation with monad unit and dicot grain arrangement (Fig. 6; j-k).
Pollen size: Equatorial size 42.57 µm (34.10 µm- 46.46 µm) and polar size 40.39 µm (35.83 µm- 42.48 µm).

Scientific Name: *Lathyrus pratensis* L. (GBIF Secretariat 2022**).**
Common Name: Meadow Pea, Meadow Vetchling.
Local Name: Not yet known.
Category: Exotic.
Habit: Herb.
Pollen morphology: Prolate shape in equatorial view and Triangularly convex shape in polar view, Tricolporate type (aperture peculiarities: margo[4]), Psilate, Perforate ornamentation with monad unit and dicot grain arrangement (Fig. 6; l-n).
Pollen size: Equatorial size 38.31 µm (18.13 µm- 45.59 µm) and polar size 26.72 µm (18.76 µm- 30.30 µm).

Scientific Name: *Heliotropium indicum* L. (GBIF Secretariat 2022**).**
Common Name: Indian heliotrope.
Local Name: Hantishur.
Category: Exotic.
Habit: Herb.
Pollen morphology: Prolate shape in equatorial view and Lobate shape in polar view, Heterocolpate type, Gemmate, Scabrate, Faintly Reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 7; a-c).
Pollen size: Equatorial size 47.59 µm (37.29 µm- 54.64 µm) and polar size 35.19 µm (27.43 µm- 38.88 µm).

Scientific Name: *Vernonia patula* (Aiton) Merr. (GBIF Secretariat 2022**).**
Common Name: Kukshim.
Local Name: Kukshim Thankuni.
Category: Exotic.
Habit: Herb.
Pollen morphology: Circular-spheroidal shape in equatorial view and Circular-spheroidal shape in polar view, Tricolporate type, Echinate, Lophate ornamentation with monad unit and dicot grain arrangement (Fig. 7; d-e).
Pollen size: Equatorial size 39.62 µm (33.45 µm- 43.50 µm).

[3] Annulus: Ring like wall thickening surrounding a porus or ulcus, width: 1.5 – 2.1 µm.
[4] Margo: Exine area with different ornamentation bordering a colpus/colporus.

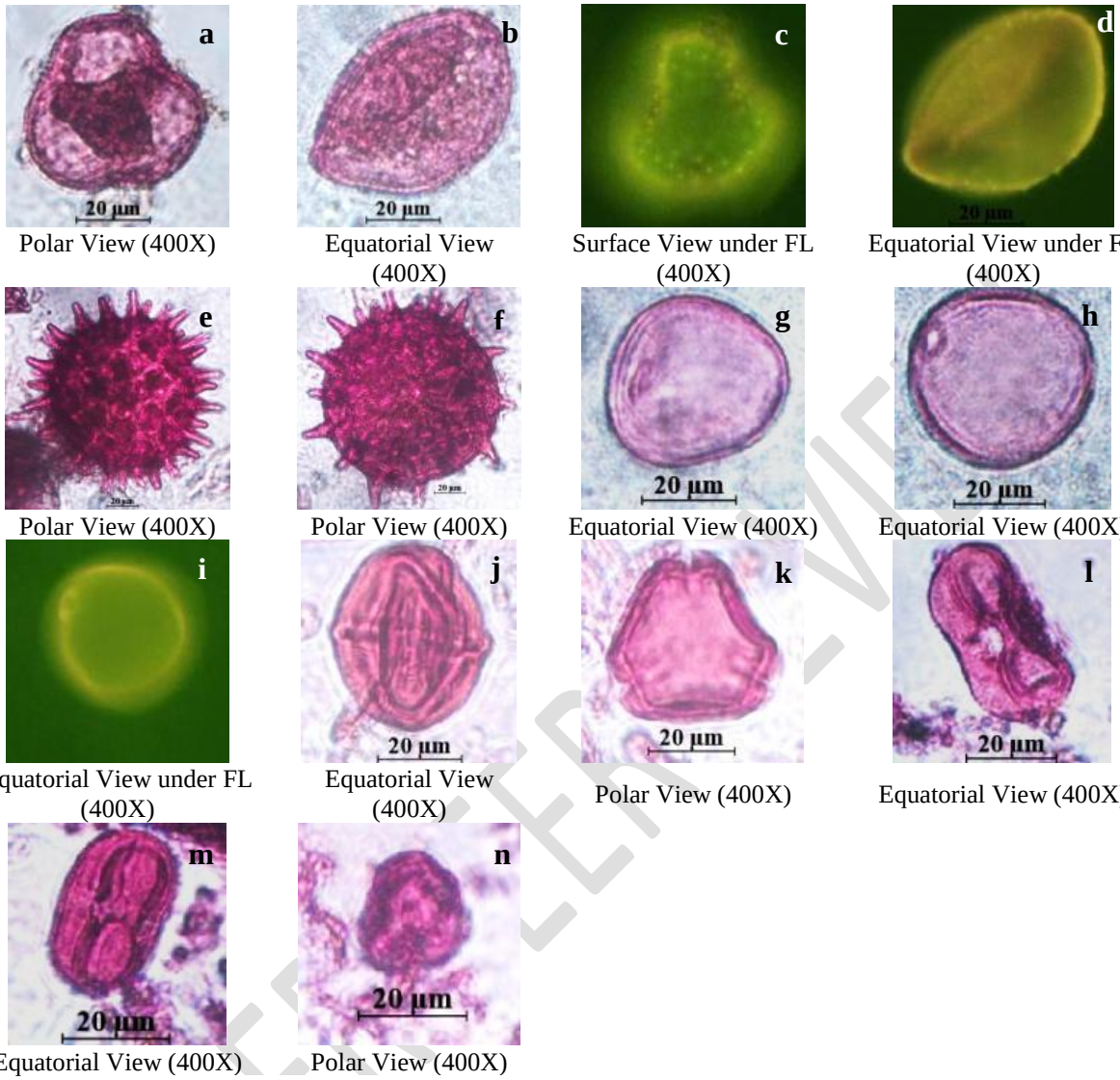
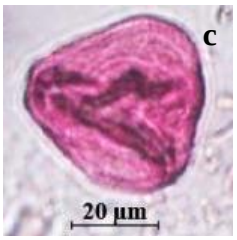


Fig. 6 a-d *Clerodendrum infortunatum* L.; e-f *Hibiscus rosa-sinensis* L.; g-i *Saccharum spontaneum* L.*;* j-k *Lantana involucrata* L.; l-n *Lathyrus pratensis* L. FL: Fluorescence Light Microscope.

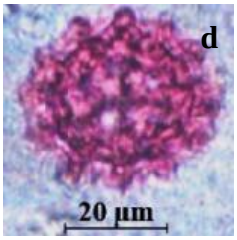
Scientific Name: *Jasminum angustifolium* (L.) Willd. (GBIF Secretariat 2022**).**
Common Name: Wild Jasmine.
Local Name: Mollika, Rai Belly.
Category: Indigenous.
Habit: Climbing Shrub.
Pollen morphology: Prolate shape in equatorial view and Lobate shape in polar view, Tricolporate type, Gemmate, Reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 7; f-h).
Pollen size: Equatorial size 51.72 µm (44.56 µm- 55.41µm) and polar size 44.76 µm (37.34 µm- 54.88 µm).

Equatorial View (400X)

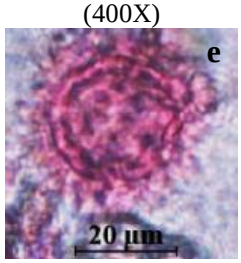
Equatorial View (400X)



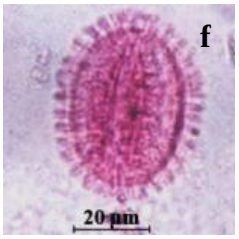
Polar View (Oblique) (400X)



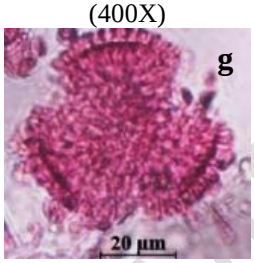
Equatorial View (400X)



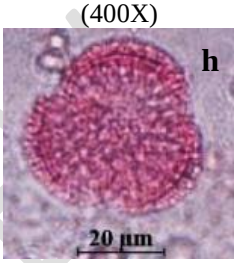
Equatorial View (400X)



Equatorial View (400X)



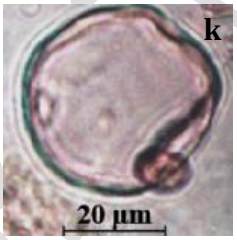
Polar View (400X)



Polar View (400X)

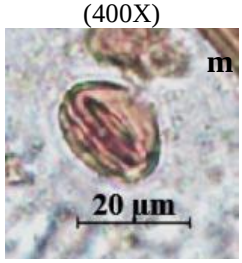
Equatorial View (400X)

Equatorial View (400X)

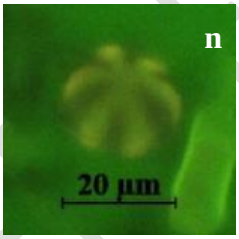


Equatorial View (400X)

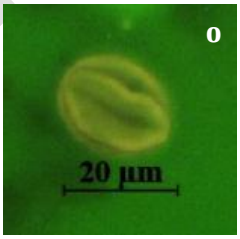
Polar View (400X)



Equatorial View (400X)



Polar View (Oblique) under FL (400X)



Equatorial View under FL (400X)

Fig. 7 a-c *Heliotropium indicum* L.; d-e *Vernonia patula* (Aiton) Merr.; f-h *Jasminum angustifolium* (L.) Willd.; i-k *Tripidium arundinaceum* (Retz.) Welker, Voronts. & E.A.Kellogg; l-o *Spatholobus parviflorus* (Roxb. ex G.Don) Kuntze. FL: Fluorescence Light Microscope.

Scientific Name: *Tripidium arundinaceum* (Retz.) Welker, Voronts. & E.A.Kellogg (GBIF Secretariat 2022**).**
Common Name: Hardy Sugar Cane.
Local Name: Not yet known.
Category: Exotic.
Habit: Herb.
Pollen morphology: Circular-spheroidal shape in both equatorial and polar view, Monoporate (pore with annulus[5]) type, Psilate, Reticulate ornamentation with monad unit and Monocot grain arrangement (Fig. 7; i-k).
Pollen size: Equatorial size 33.82 µm (29.64 µm- 37.95 µm).

[5] Annulus: Ring like wall thickening surrounding a porus or ulcus, width: 1.8 – 2.22 µm

Scientific Name: *Spatholobus parviflorus* (Roxb. ex G.Don) Kuntze (GBIF Secretariat 2022**).**
Common Name: Bando Lata, Palas-climber.
Local Name: Goalia lata, Sal lata, Pan lata.
Category: Indigenous.
Habit: Climbing Shrub.
Pollen morphology: Prolate-spheroidal shape in equatorial view and Circular-spheroidal shape in polar view, Hexacolporate type, Faintly Reticulate ornamentation with monad unit and dicot grain arrangement (Fig. 7; l-o).
Pollen size: Equatorial size 20.0 µm (18.04 µm- 21.93 µm) and polar size 19.36 µm (16.25 µm- 20.40 µm).

4.2 Advanced Laboratory Techniques

According to Faegri and Iversen (1989), acetolysis treatment is the most effective method for removing extraneous materials from fresh and dry flower samples. This process enhances the clarity of microscopic slides, making it easier to identify the morphological characteristics of pollen and spores. The technique is widely recognized for its ability to isolate pollen and spores while preserving their structural details for accurate identification and analysis.

The laboratory procedure for preparing a pollen and spore catalog involves several carefully executed steps. Initially, samples are washed repeatedly with glacial acetic acid to dehydrate the material and prepare it for further treatment. Next, an acetolysis solution (mixture of acetic anhydride and concentrated sulfuric acid) is applied to dissolve unwanted organic materials, leaving the pollen and spores intact. Following the acetolysis step, the samples undergo a chlorination treatment using a mixture of glacial acetic acid, a saturated solution of sodium or potassium chlorate, and concentrated hydrochloric acid. This step further cleans the samples by removing any remaining debris or pigments that may interfere with visual analysis. Finally, microscopic slides are prepared by mounting the cleaned samples in a glycerin solution, often stained with safranin for visibility, and sealed to prevent contamination and dehydration. This advanced systematic approach is essential for the accurate identification and cataloging of pollen and spore species. Microscopic studies using transmitted and fluorescence light enhance the visibility of morphological characteristics, providing a unique method for catalog preparation. Additionally, detailed morphological analysis is achieved through high-magnification imaging of selected species using FESEM. Previous studies on pollen herbarium collection have utilized advanced acetolysis methods and transmitted light microscopy (Haque and Rahman 2015; Haque et al. 2020; Haque et al. 2024a; Haque et al. 2024b); however, this research uniquely applies fluorescence light microscopy and FESEM to enhance visibility along with systematic acetolysis technique.

4.3 Ecological Significance

Information on the ecological significance of the studied species has been described from various online documents and websites.

Leucas aspera (Willd.) Link, is an annual herb growing 15-60 cm tall, found across India, Bangladesh, Myanmar, and other Southeast Asian countries. It thrives in grassy plains, arable crops, and open dry soils at elevations up to 500 meters (Srinivasan 2011; Ken Fern 2014). *Borreria laevis (Lam.) Griseb.*, grows as an annual/perennial herb herb up to 25 cm in height, native to tropical America but now pantropical. It grows in altered environments like roadsides and farms, thriving in sunny or lightly shaded areas, often on hard soils at elevations up to 2,000 meters (Morton 1981; Ken Fern 2014). *Solanum sp. L.*, is a diverse genus of flowering plants including economically important crops like potatoes, tomatoes, and eggplants. Solanum species vary in growth habits, including both annuals and perennials (Armando 2001).

Ocimum americanum L., is an erect, perennial, aromatic plant native to tropical Africa and Asia, often cultivated and found in fields, roadsides, and open waste places. It thrives in sunny, wind-sheltered areas, preferring temperatures of

22–30°C and rainfall between 700–2,000 mm, at elevations up to 2,000 meters (Ken Fern 2014). *Lygodium sp.*, a genus of about 40 fern species native to tropical regions, used for fiber in the Philippines. It grows in forests and damp thickets at elevations up to 1,000 meters (Christenhusz and Chase 2014; Ken Fern 2014). *Caesalpinia pulcherrima*, a shrub or small tree, native to tropical regions, valued for its colorful flowers. It grows in well-drained soils, tolerating a variety of conditions, including clay and salty soils, and can survive temperatures as low as -8°C (Gillman and Watson 2011; Floridata 2014; Missouri Botanical Garden 2015).

Lobelia trigona Thwaites, is a decumbent annual herb with 3-winged stems, commonly found in wet rice fields, ditches, and moist deciduous forests. Native to South and Southeast Asia, it thrives in moist environments at various elevations (Cook 1996; Sasidharan and Sivarajan 1996). *Synedrella nodiflora* (L.) Gaertn., an erect, ephemeral herb from tropical America, now widespread in tropical and subtropical regions. It grows in disturbed habitats with sufficient soil and air moisture, favoring fertile soils with high organic content (Wang 1990; Holm et al. 1997). *Solanum torvum Sw.*, is an evergreen shrub, 1-4 meters tall, native to the West Indies and now widespread in the tropics. It grows in moist areas at elevations up to 1,600 meters, tolerating a wide range of temperatures (12 - 35°c) and rainfall (700 - 4,200mm). It is used locally as food and medicine (Ken Fern 2014; Manandhar 2002).

Physalis angulata L., is an annual herb from the Americas, widely introduced to tropical and subtropical regions. It grows 50 cm to 2 m tall, often found in debris, fields, and coastal sands, favoring nitrate-rich soils (Ken Fern 2014; Fernald 1950; Hall et al. 1991; Raju et al. 2007). *Vernonia cinerea* (L.) Less., a fast-growing annual herb, native to Africa and Asia, commonly found in disturbed areas, roadsides, and pastures in tropical regions (USDA-ARS 2013). It thrives in full sun and prefers sandy-loam soils (Randall 2012; Holm et al. 1997). *Desmodium triflorum* (L.) DC., is a mat-forming, annual to perennial herb, growing 8-50 cm long and found at elevations up to 1,000 meters. It thrives in moist, well-drained soils in grasslands, roadsides, and pastures, tolerating a wide range of temperatures (14 - 32°c) and rainfall (1,200 - 5,000mm) conditions, including heavy grazing (Ken Fern 2014). *Melastoma malabathricum* L., a spreading shrub or small tree, native to tropical Asia and Polynesia, growing up to 5 m tall. It flourishes in disturbed sites, secondary forests, and plantation areas, preferring fertile, well-drained soils from sea-level up to 3,000 meters (Adams, 1972; Ken Fern 2014; Bodkin 1991).

Lathyrus japonicus Willd., is a perennial plant growing up to 0.6 m, thriving in well-drained, moist soils on coastal beaches, dunes, and salt marshes (PFAP 2023). *Chromolaena odorata* (L.) R.M.King & H.Rob., a widely distributed tropical shrub considered one of the world's worst weeds. Native to tropical Central and South America, it invades open habitats such as forest clearings and roadsides. A plant of the warm and humid tropics and subtropics, where it is found at elevations up to 1,000 meters and can tolerate wide range of temperature (16 - 38°c) and rainfall (1,500 - 5,000mm) (Ken Fern 2014). *Butea monosperma* (Lam.) Kuntze., is a slow-growing, deciduous tree reaching 5-15 m, growing at low to moderate elevations up to 1,500 meters. It thrives in dry tropical climates and tolerates various soil types, temperature range (4 - 49°c) and rainfall (450 - 4,500 mm) (Ken Fern 2014). *Clerodendrum infortunatum* L., an erect shrub or small tree growing 1-5 m tall, often cultivated for its aromatic flowers. Found in thickets and village groves, it prefers moist, humus-rich soils, grows at elevations from sea-level up to 500 meters (Ahmed 2011; Ken Fern 2014).

Hibiscus rosa-sinensis L., is an evergreen shrub or small tree (1-4 m) grown globally as an ornamental. Likely native to eastern Asia, it thrives in tropical climates, providing food, medicine, and dye. Found from sea level to 3400 m and can tolerates 725 to 2500 mm rainfall (Phillips and Rix 1998). *Saccharum spontaneum* L., a perennial tropical grass found across Asia, Africa, and the Mediterranean. It thrives in moist soils along riverbanks and wastelands, growing

up to 5 m. Originating in India, it is aggressive on heavy, moisture-retentive soils (CABI 2023; Sen 1981; Balyan et al. 1997). *Lantana involucrata* L., is a 3-meter evergreen shrub found in tropical forests, often used for food, medicine, and hedges. It grows in disturbed areas, at elevations from near sea level to 1,200 meters and thrives in rainfall between 750-3000 mm annually (Ken Fern 2014). *Lathyrus pratensis* L., a cold-hardy, herbaceous perennial with climbing stems, growing 30-120 cm. Native to Eurasia and North Africa, it grows in grasslands and hedgerows, preferring full sun and moist soils (Huxley 1992).

Heliotropium indicum L., is an erect, coarse annual herb up to 1.5 m tall with a taproot, found in tropical and subtropical regions. It thrives in wet, fertile soils near rivers, lakes, and roadsides, typically below 1000 m. *Vernonia patula* (Aiton) Merr., a stiff, erect annual plant growing 12-45 cm tall. It is commonly found near sea level in coastal vine thickets (Australian Tropical Rainforest Plants 2023). *Jasminum angustifolium* (L.) Willd. is a slender, evergreen shrub or vine native to Peninsular India, Sri Lanka, and the Andaman Islands, found in scrub jungles and seasonally dry tropical areas. *Tripidium arundinaceum* (Retz.) Welker, Voronts. & E.A.Kellogg, a monocot, tall, perennial grass, native to tropical Asia, up to 4 m high, found along streams, riversides, and dry sandy soils (Welker and Kellogg 2004). *Spatholobus parviflorus* (Roxb. ex G.Don) Kuntze, a large climbing plant growing up to 25 meters, found in various habitats across the Indian subcontinent, Indochina, and Southeast Asia, commonly in sunny places on slopes and riverbanks; at elevations from 200 - 2,000 meters (Brandis 1906).

5 Conclusion

This catalog comprehensively analyzes twenty-six dried and fresh flower samples from Chattogram, Cox's Bazar, and Dhaka Districts, providing detailed information on taxonomy, pollen morphology, and ecology. Included are descriptions of one spore from the Lygodiaceae family and pollens from twenty-five species across twelve families: Fabaceae, Asteraceae, Lamiaceae, Solanaceae, Poaceae, Rubiaceae, Campanulaceae, Melastomataceae, Malvaceae, Verbenaceae, Oleaceae, and Heliotropiaceae, all highlighting their ecological significance.

The study benefits from refined pollen sample processing techniques following acetolysis methods for both dried and fresh samples, enhancing the depth of palynological analysis. These methods along with transmitted and fluorescence light microscopy aid researchers in exploring the history of plant communities, habitats, and environmental conditions based on identified plant groups and species. This catalog also serves as a reference tool for academicians and professionals in Holocene pollen studies, facilitating paleoenvironmental reconstruction.

This catalog can be expanded in future studies to deepen the understanding of regional habitats, with a well-established methodology for pollen sample preparation contributing valuable insights for ongoing botanical and palynological research. In conclusion, this catalog is a substantial contribution to botany and palynology, offering insights that may advance our understanding of plant communities and their ecological contexts.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing Interests

The authors declare that there are no competing interests related to this research.

Consent to Publish

All authors whose names appear on the submission give their consent to publish this paper if it is accepted.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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